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Thermodynamics of Vinyl Ethers. X.* The Stabilizing Effect of β-Phenyl Substitution on α,β-Unsaturated Ethers

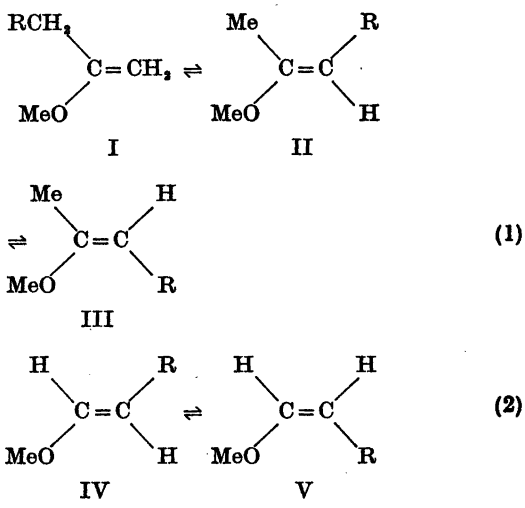
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The effect of β-phenyl substitution on the thermodynamic stability of α,β-unsaturated (olefinic) ethers has been studied by means of chemical equilibration of suitable isomeric compounds. The stabilizing energy of a β-phenyl substituent was obtained as 15 ± 2 kJ mol^{-1}. The destabilizing methyl-phenyl *cis* interaction energy across a double bond was evaluated as 7.5 ± 1.5 kJ mol^{-1}.

Almost forty years ago Kistiakowsky and coworkers [1] started their investigations of the enthalpies of hydrogenation of unsaturated compounds, including a number of variously substituted ethenes. The large amount of thermochemical data obtained shed much light on the factors affecting the stability of olefinic compounds. It was found, for instance, that if one or several of the hydrogen atoms of ethene were replaced by alkyl groups [2] or unsaturated groups, such as vinyl or phenyl groups,[3] considerably less heat was liberated in the hydrogenation of the ethylenic bond than was produced in the hydrogenation of ethene itself. This was regarded as indicative of the enhanced stability of the ethylenic system due to the particular substituent involved. The enthalpy of hydrogenation of the ethylenic linkage of styrene (phenylethene) was about 18 kJ mol^{-1} more positive than that of ethene in the gas phase at 355 K,[1,3] which shows that in olefins a phenyl group linked to the double bond causes a stabilization of about 18 kJ mol^{-1}.

The previous studies of this series have revealed that the double bond of a vinyl ether is not as effectively stabilized by alkyl groups as that of an "ordinary" olefin. Hence it was of interest to find out whether the same phenomenon could be observed if the double bond of a vinyl ether were substituted by a phenyl group. For that purpose, thermodynamics of interconversion of the vinyl ethers I – V were investigated (R = Ph).



On going from I to II (or from I to III), a phenyl group is transferred from a nonconjugated allylic position to a site where conjugation with the vinyl group is possible. The value of $\Delta H°$(g) for this reaction (I→II), corrected by the value of the destabilizing Me – Ph *cis* interaction energy, is taken to represent the stabilizing energy of β-phenyl substitution in vinyl ethers. The value of the Me – Ph *cis* interaction energy was determined by compar-

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ing the changes in standard enthalpy for reactions II→III and IV→V in cases R=Ph and R=Me. Thermodynamic data for the case R=Me have been published previously in this series.

EXPERIMENTAL

Syntheses. I, II and III. A 45 % yield of a mixture of I, II and III (11 % I, 74 % II and 15 % III) was obtained when a mixture of benzyl methyl ketone (0.20 mol), trimethyl orthoformate (0.25 mol) and methanol (50 cm^3) was slowly distilled in the presence of a small amount of *p*-toluenesulfonic acid,[4] first at atmospheric, finally at reduced pressure. The mixture was collected at 359 to 365 K at 1.0 kPa. *IV and V.* A 65 % yield of a mixture of IV and V (64 % IV, 36 % V) could be collected at 481 to 483 K at 99.1 kPa when 0.17 mol of the dimethyl acetal of 2-phenylacetaldehyde (obtained from Aldrich) were fractionated from a catalytic amount of *p*-toluenesulfonic acid. *NMR spectra.* The NMR spectra were recorded on a 60 MHz Perkin Elmer R 10 spectrometer at 307 K. Carbon tetrachloride was used as solvent and TMS as internal standard. *I*: τ 6.69 ($-CH_2-$) 6.56 ($-CH_3$), 6.19 (H *trans* to MeO), 6.12 (H *cis* to MeO), 2.8 (aromatic protons), $J(=CH_2)$ 1.7 Hz; *II*: τ 8.07 ($CH_3-C=$), 6.46 (CH_3-O-), 4.50 ($H-C=$), 2.9 (aromatic protons); *III*: τ 8.07 ($CH_3-C=$), 6.41 (CH_3-O-), 4.77 ($H-C=$), 2.9 (aromatic protons); *IV*: τ 6.43 (CH_3-O-), 4.23 (H *cis* to MeO), 2.97 ($H-C\lesssim_O$), 2.9 (aromatic protons), J_{vic} 13.5 Hz; *V*: τ 6.34 (CH_3-O-), 4.82 (H *trans* to MeO), 3.97 ($H-C\lesssim_O$), 2.9 (aromatic protons), J_{vic} 7.2 Hz.

Determination of normal boiling temperatures. The normal boiling temperatures of the compounds studied were determined by the gas-chromatographic method described earlier.[5] *I, II and III*: reference curve (compound, boiling temperature at 101.3 kPa, relative retention time): 1-methoxycyclohexene, 417.1 K, 0.238; 1-ethoxycyclohexene, 435.0 K, 0.327; 1-propoxycyclohexene, 455.9 K, 0.518; 1-cyclopentoxycyclopentene, 479.7 K, 1.000. The relative retention times of I, II and III were 0.735, 1.036 and 1.304 corresponding to normal boiling temperatures of about 469.7, 480.2 and 485.2 K, respectively. *IV and V*: the retention times of IV and V were equal implying that the boiling temperatures of the two isomers are also equal. Therefore, the thermodynamic functions of isomerization of IV to V are equal in the vapor and liquid phases,[5] and hence the boiling temperatures need not be known.

Table 1. Values of the mean equilibrium constant K and its standard error for the isomerization reactions involving the compounds: 2-methoxy-3-phenylpropene (I), (*E*)-2-methoxy-1-phenylpropene (II), (*Z*)-2-methoxy-1-phenylpropene (III), (*E*)-1-methoxy-2-phenylethene (IV), and (*Z*)-1-methoxy-2-phenylethene (V). The number of independent determinations is denoted by n and the letters A, B, C, and D refer to the various initial mixtures used (see text). Solvent: cyclohexane.

Temp. [a] K	Initial mixture	n	K_1 (II/I)	K_2(III/I)	K_3(III/II)	K_4(V/IV)
295.2	A	2	14.8 ± 0.4	2.25 ± 0.05	0.153 ± 0.002	
311.2	A	2	11.6 ± 0.1	2.13 ± 0.04	0.185 ± 0.004	
326.2	A	2	10.2 ± 0.2	2.10 ± 0.05	0.207 ± 0.002	
336.2	A	3	9.40 ± 0.10	2.09 ± 0.05	0.223 ± 0.001	
354.2	A	9	7.31 ± 0.07	1.74 ± 0.03	0.238 ± 0.005	
355.2	B	2	7.55 ± 0.08	1.92 ± 0.04	0.253 ± 0.001	
373.2	A	9	6.83 ± 0.09	1.80 ± 0.03	0.268 ± 0.006	
388.2	A	4	6.09 ± 0.16	1.84 ± 0.05	0.302 ± 0.001	
395.2	C	3	5.54 ± 0.06	1.74 ± 0.03	0.317 ± 0.009	
404.2	A	3	5.25 ± 0.06	1.68 ± 0.07	0.322 ± 0.042	
423.2	A	2	4.82 ± 0.01	1.65 ± 0.02	0.343 ± 0.004	
423.2	B	2	4.73 ± 0.01	1.68 ± 0.02	0.355 ± 0.005	
295.2	D	2				0.485 ± 0.001
326.2	D	4				0.498 ± 0.007
354.2	D	6				0.528 ± 0.007
375.2	D	2				0.533 ± 0.006
396.2	D	2				0.544 ± 0.001
407.2	D	4				0.551 ± 0.004

[a] Accurate to ± 0.5 K.

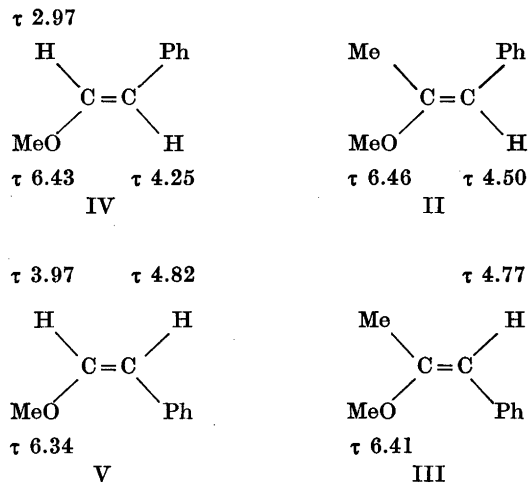
Equilibration experiments. Prior to the equilibrations, the substrates were purified by preparative GLC (the column contained 10 % SE-30 on Chromosorb G), if possible. However, IV and V could not be separated owing to the similar retention times. Three different fractions of I, II and III were thus available for the equilibrations: (A): 11 % I, 74 % II and 15 % III; (B): 100 % II; (C): 75 % I, 21 % II and 3 % III. The equilibrations of I, II and III were conducted in cyclohexane with iodine as catalyst. The total substrate concentration was 1.2 mol dm^{-3} and the catalyst concentration 0.006 mol dm^{-3}. The equilibrated samples were analyzed by GLC by means of a 4 m column containing 10 % SE-30 on Chromosorb G. Peak separation was good. The relative retention times of I, II and III were 0.57, 0.78 and 1.000, respectively. Column temperature was kept at 388 K. Under these conditions, partial isomerization of the samples injected was found to occur unless sufficient amounts (about 5 μl) of diethylamine were injected into the column immediately before injection of the equilibrated sample.

The equilibration of IV and V was conducted in the neat liquid with mercuric acetate as catalyst. Catalyst concentration was 0.24 mol dm^{-3}. The synthetic product (denoted by D in Table 1) containing 64 % of IV and 36 % of V was used as the initial mixture for the equilibrations. The peaks of IV and V were satisfactorily separated in the analytical column used (an 8 m column containing 10 % Carbowax 20 M on Chromosorb G), when long retention times (148 min for IV, 154 min for V) were used. These compounds are much less reactive than I, II and III, and hence no isomerization was found to take place during the analyses (column temperature 423 K). However, prior to analysis the equilibrated samples were diluted with an equal volume of diethylamine to maintain alkaline conditions in the column. Equilibrium was considered having been reached when the isomer ratio remained constant in successive samples taken over sufficiently extended periods of time. The relative peak areas were determined by the cut-and-weigh method.

Additional details of the practical performance of the equilibrations are found in the previous papers of this series.

CONFIGURATIONAL ASSIGNMENT OF THE GEOMETRIC ISOMERS

The *Z* and *E* forms [6] of 1-methoxy-2-phenylethene (V and IV, respectively) are easily identified, since the value of the vicinal coupling constant between the olefinic protons in the *Z* form of a disubstituted ethene is known to be about a half of that in the *E* form.[7] In the present case, the coupling constants were 7.2 and 13.5 Hz; hence the compound possessing the larger coupling constant was taken as the *E* isomer (IV). On the other hand, configurational assignment of the geometric isomers of 2-methoxy-1-phenylpropene (II and III) is more complicated. Kresge and Chen [8] have prepared the same pair of isomers and they observed that one of the isomers gave a methyl group NMR signal at τ 8.07 and the other at τ 8.27. From this they concluded that the former or the more volatile isomer should be *β*-methoxy-*trans*-*β*-methylstyrene, *i.e.* (*Z*)-2-methoxy-1-phenylpropene (III). In support of this assignment they gave some synthetic evidence. Kresge and Chen did not specify the possible solvent used in the NMR experiment but we have not been able to detect any difference in the positions of the methyl signals derived from the two isomers when the spectra were taken in CCl_4 solution. Moreover, it appears that in compounds of the type studied in this work, the isomer having methyl *trans* to phenyl does not always give a methyl group NMR signal at a lower field than the other geometric isomer, contrary to the assumption of Kresge and Chen. Thus Rummens and de Haan [9] have published NMR data for the geometric isomers of 1-phenylpropene: in the neat liquid the isomer having Me *trans* to Ph gave a methyl group signal about 0.03 ppm *upfield* from that of the *cis* form. On the basis of the following reasoning it appears that the configurational assignment of the geometric isomers of 2-methoxy-1-phen-



Scheme 1.

ylpropene, proposed by the above authors, is erroneous.

First, the NMR data reveal some interesting features. In the presentation in Scheme 1, the structures of the geometric isomers of 2-methoxy-1-phenylpropene have been drawn with the assumption that the more volatile isomer has the *E* configuration (II). On going from IV to V, the signal due to the β-proton (the proton bound to the same carbon as Ph) undergoes a shift of about 0.6 ppm to a higher field. A somewhat smaller shift in the same direction is expected on going from II to III, since replacement of a hydrogen atom in ethene or a substituted ethene by a methyl group causes a larger upfield shift in the position of the signal due to the proton *trans* to the group introduced. If the *E* and *Z* forms of 2-methoxy-1-phenylpropene are taken as proposed above, the shift in question is about 0.3 ppm to a higher field, in agreement with expectation. If the configurational assignment suggested by Kresge and Chen were correct, the signal of the β-proton would be removed about 0.3 ppm *downfield* on going from the *E* form to the *Z* form, which is hardly acceptable in view of the about 0.6 ppm *upfield* shift in the case IV→V.

Considering the two *Z* forms V and III, the signal of the β-proton is found at about τ 4.8 in each case. This is reasonable, since replacement of the α proton in V by a methyl group has no effect on the spatial orientation of the phenyl group in a *trans* position relative to the proton replaced. Moreover, in each *E* − *Z* isomer pair studied, the signal of the methoxy group of the *E* form is found at a somewhat higher field than that of the *Z* form, although the difference in the positions of the signals is very small.

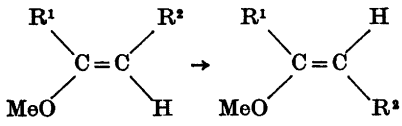
Further evidence for the configurational assignment proposed above is obtained from the equilibration data. It has been shown that of the geometric isomers of an α,β-dialkylsubstituted vinyl methyl ether the one having the higher entropy value is the *Z* form.[4,5] In the present case, the entropy of the less volatile isomer is about 7.3 J K^{-1} mol^{-1} higher than that of the other geometric isomer, and hence we are apt to regard the former as the *Z* form.

RESULTS

The primary results of the equilibration experiments are shown in Table 1. From the values of the mean equilibrium constant at various temperatures, the values of $\Delta G°$, $\Delta H°$ and $\Delta S°$ at 298.15 K for each isomerization reaction involved were calculated as described previously.[10] The results are collected in Table 2. Finally, the values of $\Delta H°$ and $\Delta S°$ in the ideal gas state at 298.15 K were evaluated from the liquid phase data and the normal boiling temperatures (Table 3).[5]

DISCUSSION

Let us consider the changes in standard enthalpy and standard entropy accompanying reaction (3), for which experimental data are given in Table 4. In the case R^2=Me, the



(3)

Table 2. Values of the thermodynamic functions $\Delta G°$, $\Delta H°$ and $\Delta S°$ for isomerization reactions involving the compounds given in Table 1. The solvent is cyclohexane and the temperature 298.15 K. The errors are twice the standard errors.

Reaction	$\Delta G°$/kJ mol^{-1}	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
I →II	−6.51±0.10	−9.0±0.5	−8.4±1.2
I →III	−2.00±0.12	−2.5±0.6	−1.8±1.4
II →III	4.50±0.08	6.5±0.4	6.6±1.0
IV→V	1.80±0.04	1.2±0.2	−2.1±0.5

Table 3. Values of the thermodynamic functions $\Delta H°$ and $\Delta S°$ in the gas phase at 298.15 K for the isomerization reactions involving the compounds mentioned in Table 1.

Reaction	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
I →II	−7.3±0.6	−6.8±1.3
I →III	0.0±0.6	0.5±1.5
II →III	7.3±0.5	7.3±1.1
IV→V	1.2±0.4	−2.1±0.7

Table 4. Experimental data for reaction (3).

R^1	R^2		$\Delta H°$(g)/kJ mol^{-1}	$\Delta S°$(g)/J K^{-1} mol^{-1}	Remarks
H	Me		0.43	2.9	Ref. 11
Me	Me		9.85	13.4	Ref. 4
		Increase	9.4	10.5	
H	Ph		1.2	−2.1	This work
Me	Ph		7.3	7.3	This work
		Increase	6.1	9.4	

increase in $\Delta H°$(g) on going from R^1 = H to R^1 = Me is about 9.4 kJ mol^{-1}, while the increase is only about 6.1 kJ mol^{-1} in the case R^2 = Ph. This shows that the Me − Ph *cis* interaction is about 9.4 − 6.1 = 3.3 kJ mol^{-1} higher than the Me − Me interaction, which is obtained as 4.2 kJ mol^{-1} from the difference in the standard enthalpies of (*Z*)-2-butene and (*E*)-2-butene.[12] Hence the Me − Ph *cis* interaction energy may be evaluated as 3.3 + 4.2 = 7.5 kJ mol^{-1} with an estimated accuracy of ± 1.5 kJ mol^{-1}. This result is in slight disagreement with a reported enthalpy difference (about 4.2 kJ mol^{-1}) between the geometric isomers of 1-phenylpropene.[13] On the other hand, theoretical calculations [14] suggest a value of about 11 kJ mol^{-1} for the same enthalpy difference. It is of interest that the same calculations [14] of the relative enthalpies of the geometric isomers of 2-phenyl-2-butene show that the isomer with methyl *cis* to phenyl should have an enthalpy about 3.3 kJ mol^{-1} higher than that of the other isomer with the two methyl groups in a *cis* position. Since the difference 3.3 kJ mol^{-1} can be regarded as arising from the difference between Me − Ph and Me − Me *cis* interaction energies, the value of the former is obtained as 3.3 + 4.2 = 7.5 kJ mol^{-1}, which is exactly equal to that achieved in this study.

The value of the Me − Ph *cis* interaction energy, 7.5 ± 1.5 kJ mol^{-1}, is reasonable in the light of the values of the Me − Me and Ph − Ph *cis* interaction energies. The former is known to be about 4.2 kJ mol^{-1},[12] while the values reported for the Ph − Ph *cis* interaction energy are somewhat scattered. Thus Kistiakowsky and Smith [15] obtained the enthalpy difference between gaseous (*Z*)- and (*E*)-stilbene as about 12 kJ mol^{-1}, and Fischer *et al.*[16] reported the enthalpy of the *Z* form to be 12.1 ± 1.2 kJ mol^{-1} higher than that of the *E* form in methylcyclohexane. From the "selected values" of Cox and Pilcher,[17] the value of $\Delta H°$(g) in (*E*)-stilbene → (*Z*)-stilbene is obtained as about 16 kJ mol^{-1}, if the standard enthalpy of formation of crystalline (*E*)-stilbene is combined with the enthalpy of vaporization data reported by Morawetz.[18] Hence the most probable value of the Ph − Ph *cis* interaction energy seems to be 12 to 16 kJ mol^{-1}, *i.e.* about twice the value of the Me − Ph *cis* interaction energy.

The stabilizing effect of β-phenyl substitution (excluding destabilizing *cis* interactions) is now obtained as about 15 kJ mol^{-1}, with an estimated error of ± 2 kJ mol^{-1}, since the observed enthalpy change for I → II, −7.3 kJ mol^{-1}, is a composite of the true stabilizing effect of the β-phenyl substituent and the destabilizing *cis* interaction energy, 7.5 kJ mol^{-1}. This figure (15 kJ mol^{-1}) may be compared with the stabilizing effect of β-phenyl substitution on an "ordinary" olefinic double bond. As mentioned previously, a phenyl group stabilizes the double bond of ethene by about 18 kJ mol^{-1} (as determined from enthalpies of hydrogenation), while the group parameters of Benson *et al.*[19] suggest an enthalpy change of about −21 kJ mol^{-1} for the reaction 3-phenylpropene → 1-phenylpropene (neglecting steric corrections), which corresponds to the reaction I → II (R = Ph) with the methoxy groups replaced by hydrogen atoms. Hence in vinyl ethers, a β-phenyl substituent causes a stabilization 3 to 6 kJ mol^{-1} lower than that brought about by the same substituent on ordinary olefins. Qualitatively, this result compares well with the weak stabilizing power of alkyl groups on the double bond of vinyl ethers: the enthalpy change, 0.45 kJ mol^{-1},[4] for 2-methoxy-1-butene → (*E*)-2-methoxy-2-butene is about 9 kJ mol^{-1} more positive

than that (-8.4 kJ mol^{-1}, Ref. 12) for the reaction between the corresponding olefins, 1-butene→(*Z*)-2-butene. These facts are explainable in terms of the reduced double-bond character of the ethylenic system of vinyl ethers, which is a result of electron delocalization involving the lone-pair electrons of the oxygen atom and the π-electrons of the double bond:

$$-\ddot{\underset{\cdot\cdot}{O}}-C=C \longleftrightarrow -\overset{\oplus}{\underset{\cdot\cdot}{O}}=C-\overset{\ominus}{C}:$$

The entropy change ΔS° (g) in the reaction I→II (R = Ph) is -6.8 J K^{-1} mol^{-1}. The group parameters of Benson *et al.*[19] predict an entropy change of about -13.8 J K^{-1} mol^{-1} for the reaction between the corresponding hydrocarbons, 3-phenylpropene → (*Z*)-1-phenylpropene (neglecting *cis*-correction). According to the API values,[13] the entropy of (*Z*)-1-phenylpropene is about 3.4 J K^{-1} mol^{-1} higher than that of the *E* isomer. Taking this figure to represent the value of the *cis*-correction term in (*Z*)-1-phenylpropene, the calculated entropy change for 3-phenylpropene → (*Z*)-1-phenylpropene is obtained as $-13.8+3.4=-10.4$ J K^{-1} mol^{-1}, which is about 4 J K^{-1} mol^{-1} more negative than the value of ΔS°(g) in I→II. The reason for this difference is not clear.

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Ozonation of Carbodiimides

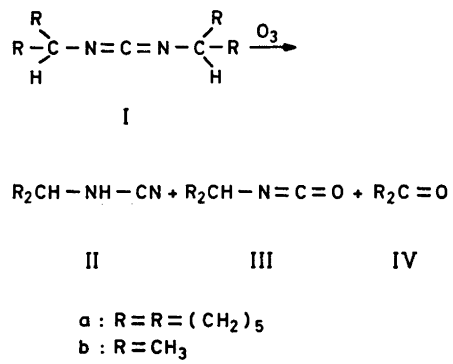
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A series of *N,N'*-disubstituted carbodiimides has been ozonized. Ozone consumption was not quantitative, but gradually two mol equivalents could be absorbed. Main products from the reactions were the correspondingly substituted ketones, isocyanates, cyanamides, and molecular oxygen. Minor amounts of nitrocyclohexane, *N,N'*-dicyclohexyl urea, and carbon dioxide were obtained from *N,N'*-dicyclohexyl carbodiimide, and unidentified peroxy functions were observed to an extent of 10 – 15 % from all carbodiimides. As the products indicate both carbon-nitrogen single and double bond cleavage, different reaction paths are incorporated in the proposed reaction scheme.

The reaction of cumulated unsaturated three-atom systems with ozone has not been studied extensively until recently. Ketenes yielded α-lactones as primary products,[1] simple allenes have been shown to give ozonolytic products, *viz.* carbonyl compounds and carbon monoxide,[2] sterically hindered allenes gave in addition products where the carbon-carbon bonds in the allene systems are intact.[3,4] Recently, a report has appeared that shows that ketenimines (both simple and sterically hindered) yielded cleavage products (carbonyl fragments and isocyanides) with no sign of compounds with the carbon chain intact.[5] The present paper will deal with the reaction of ozone with carbodiimides and since the last possible three-atom heterocumulene, an isocyanate, is shown to be a product, its reaction with ozone is touched upon.

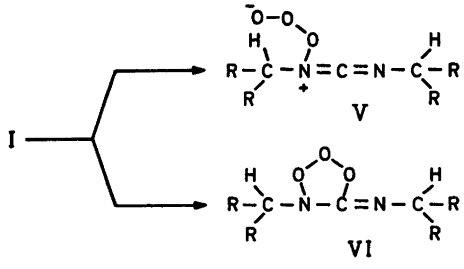
The main products from the ozonation of carbodiimides (I) (of which *N,N'*-dicyclohexyl and *N,N'*-diisopropyl carbodiimides are studied most extensively) are alkyl cyanamides (II), isocyanates (III), carbonyl compounds (IV), and molecular oxygen (Table 1).



Scheme 1.

It is obvious that the observed products must originate from more than one reaction path. Stoichiometrically, it seems reasonable that the cyanamides and the carbonyl compounds are formed in one of these paths, while the isocyanates must have been formed in another reaction.

In one of the most frequent utilizations of carbodiimides, *viz.* the formation of peptide bonds, Khorana [6] suggests a mechanism starting with a nucleophilic attack of a carboxylate



Scheme 2.

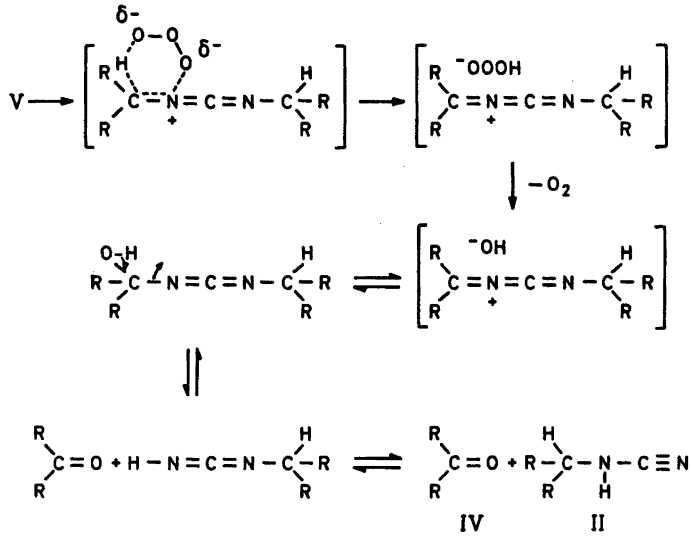
Table 1. Ozonation of *N,N'*-dialkyl carbodiimides $R_2CH-N=C=N-CHR_2$.

Compound [b]	R + R = $(CH_2)_5$					R = Me		
	GLC[a] Column	MeOAc Found mM	Calc.[b] mM	CCl_4 Found mM	Calc.[b] mM	GLC[a] Column	$(COOEt)_2$ Found mM	Calc.[b] mM
Starting material (*x*)	A	7.5	6.1	7.9	6.8	B,C	1.9	1.6
O_3 consumed (*y*)		11.0	7.3	9.0	8.1		2.0	2.2
O_2 evolved (*z*)		7.5	5.2	6.3	6.2		1.7	1.6
R_2CO(*u*)	A,B	3.0	3.4	4.6	4.8	B,C	1.2	1.3
$R_2CHNHCN$ (*a*)	D	2.8		4.3		D	1.0	
R_2CHNCO (*b*)	A,B	1.0		0.8		B,C	0.3	
R_2CHNO_2 (*c*)	A,B	0.4		0.3			0	
Peroxy comp. (*d*)		1.5		1.4			0.3	
Urea (*e*)		0.8		0.3			0	
a+*b*+*d*+*e*		6.1		6.8			1.6	
CO_2		0.2–0.3		n.c.[c]			n.c[c]	

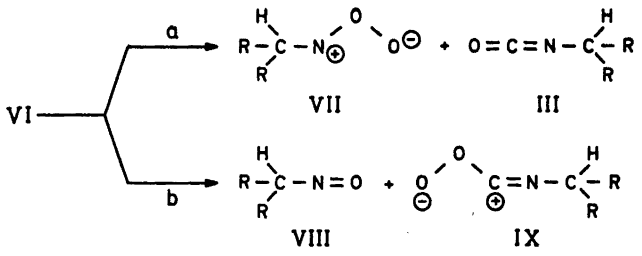
[a]See experimental section for description. [b]See Scheme 7. [c]Not checked.

anion on the central carbon atom, true enough, after activating this position by protonation of one of the imide nitrogen atoms. This indicates that ozone, whose ability to react only as an electrophile is no more questioned, will approach the carbodiimide function at the lone pair of one of the nitrogen atoms. The next step of the reaction may then be the formation of an adduct V or a five membered ring VI (Scheme 2).

There seems to be no way to distinguish between this description of the early phase of the reaction and the π-complex theory very well substantiated by Bailey [7] for the olefinic double bond-ozone reaction except that one emotionally would prefer the first explanation when the more polar carbon-nitrogen double bond is approached by ozone. However, at this point the problem seems more semantic in nature. More important is the further fate of the carbodiimide-ozone primary adducts.



Scheme 3.

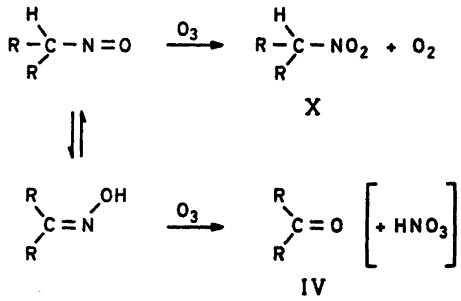


Scheme 4.

In adduct V the relative strength of the nitrogen–oxygen *versus* the oxygen-oxygen bond is of considerable importance. As discussed earlier [9] this should depend on the basicity of the nitrogen atom. As this must be rather low in carbodiimides the strength of the nitrogen-oxygen bond is rather weak, but strong enough to weaken the α-carbon-hydrogen bond (Scheme 3).

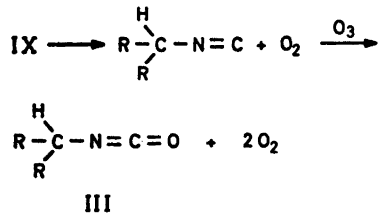
The first two equilibria involved are analogous to those in the Mannich reaction. Monosubstituted carbodiimides are unstable relative to their tautomers, the cyanamides.[9] As seen from Table 1, the yields of ketones IV are somewhat higher than those of the cyanamides II. Since the cyanamides are found rather unreactive towards ozone compared to the carbodiimides and since the other reaction path primarily involves the carbon-nitrogen double bond forming intermediate VI, analogous to the one proposed for ozonolysis of simple allenes, the excess of ketones IV must have been formed through extensive rearrangement of VI or through secondary reaction of some primary products with ozone. The latter reaction is obvious from the amount of ozone absorbed in reaction. In principle, intermediate VI could break down in two ways (Scheme 4).

An entity like VII would most likely rearrange to the corresponding nitro compound. Thus, one should expect path *a* to give equal amounts of isocyanates and nitro compounds, which is not observed. On the other hand, if path *b* is preferred several things may happen. Firstly, nitroso compounds should react with ozone to give nitro-compounds. In addition they rearrange easily to oximes (provided an α-hydrogen is available) which again react easily with ozone with good yields of correspondingly substituted carbonyl compounds.[10] (Scheme 5).



Scheme 5.

Path *b* is analogoues to the main reaction way proposed for simple allenes,[2] but a similar breakup of intermediate IX to give nitroso compound VIII and carbon monoxide does not take place, as the latter is not a reaction product. However, IX may give molecular oxygen and isonitriles, which upon ozonation give good yields of isocyanates [11] (Scheme 6).



Scheme 6.

The existence of the all-carbon analogue of IX is indicated very strongly by Hartzler [12] by observing the formation of an α-hydroxy ester by ozonolysis of an allene in ethanol.

None of the reaction paths so far proposed do explain the formation of 4 – 10 % disubstituted ureas. This would necessitate the presence of

Table 2. Reactivity of carbodiimides $R_1-N=C=N-R_2$ with ozone.[a]

	R_1	R_2	Consumed O_3 (mmol)	NMR-position of α-hydrogen[b]
1.	*t*-Bu	*t*-Bu	1.6	–
2.	*t*-Bu	Me	2.2	2.92
3.	*t*-Bu	Et	3.3	3.20
4.	*t*-Bu	i-Prop.	6.7	3.48
5.	*t*-Bu	MeOOC–CH(Me)–	5.6	3.87
6.	i-Prop.	i-Prop.	7.9	3.47
7.	Cyclohexyl	Cyclohexyl	10.6	3.1

[a]10 mmol carbodiimide dissolved in 25 ml MeOAc, 18 mmol O_3 passed through the solution at −40°C. [b]Chemical shift in ppm (δ-values in CCl_4) relative to tetramethylsilane.

water and/or acids, and as the solvents used were carefully predried, these reagents must have been formed during ozonation. As mentioned above, the ozone absorption is not quantitative and as ethyl acetate is found to yield carboxylic acids upon ozonation,[13] urea formation in this solvent is understandable. Oxidation of carbon tetrachloride by ozone should not give any hydrogen-containing species capable of initiating urea formation.[6] However, the nitric acid formed (Scheme 5) may be responsible for the urea in this solvent.

From Table 1 it can be seen that the reaction path outlined in Scheme 3 is more important than any other. Thus factors influencing the rate of this reaction, may have great effect on the ozone consumption. In fact, *N,N'*-di-*tert*-butyl carbodiimide, where no α-hydrogen is available reacts very sluggishly with ozone and no cyanamide is detected. A further comparison of reactivity of several carbodiimides towards ozone is given in Table 2.

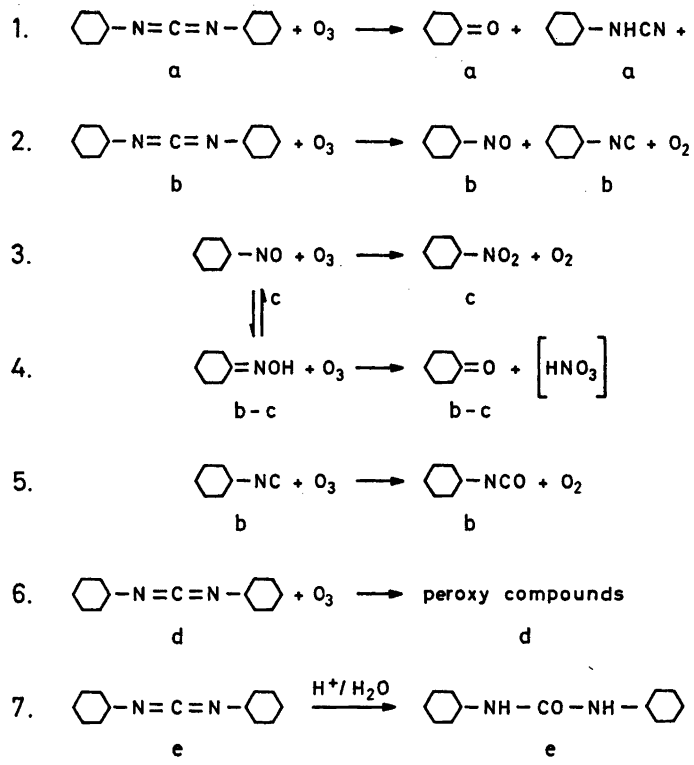
The first four entries in Table 2 show that decreased electron density at the α-hydrogen (as indicated by their resonance position in NMR) is linked in a qualitative manner to the ozone absorption. The increase in reactivity going from compound 4 to 6 merely reflects that there are two α-hydrogens in 6. That compound 5, having the α-hydrogen with lowest electron density, reacts slower than 4, must mean that the primary attack on the nitrogen lone-pair is reduced inductively by the ester group. Why compound 7 should react faster than 6 is not easy to explain. Steric effects may come into play here.

The apparent discrepancy in the amounts of ozone used and oxygen produced compared to consumption of starting material when ozonizing in methyl acetate (Table 1) is probably due to reaction with the solvent. This reaction, however, cannot be solely responsible for the peroxidic content (~20 %) as this is found to be of same order of magnitude in carbon tetrachloride where solvent attack is normally considered slow. Treatment of the ozonized solution with triphenyl phosphine did not increase the amount of any of the compounds in Table 1, although triphenyl phosphine oxide was formed to the same extent as the peroxidic content. This indicates severe breakdown of some species to lower molecular weight peroxy compounds.

A summary of the reaction schemes is given in Scheme 7 (using *N,N'*-dicyclohexyl carbodiimide as an example.

From these sequences can be obtained:
Consumed starting material: $x=a+b+d+e$
Consumed ozone: $y=a+b+c+(b-c)+b+d$
Production of oxygen: $z=a+b+c+b$
Produced cyclohexanone: $u=a+(b-c)$
where a is the yield of cyanamide, b the yield of isocyanate, c the yield of nitro compound, d the total peroxydic content, and e the yield of urea. The results are entered in Table 1.

Examination of the figures in Table 1 indicates that only 80–85 % of the starting materials have been accounted for (including unknown peroxy compounds). Some of this discrepancy may be due to systematic errors in the analytical procedures used. However, as such error may go both ways, the actual reasons must be sought elsewhere. The authors feel that the real causes



Scheme 7.

may be twofold. Firstly, the ozone reactions with the starting materials are not quantitative. Thus primary products may react further at rates comparable with those of the primary reaction; secondary reaction that it not accounted for by the postulated reaction schemes. Even secondary reaction products may react with ozone, being a powerful oxidizing agent, to give low volatility products not separable in GLC from solvent peaks. Secondly, some of the reaction products, *viz.* the cyanamides and the isocyanates are known to dimerize and, indeed, in some cases, especially using copper columns both dimers and trimers of cyanamides were formed in the gas chromatograph detected on a combination instrument GLC–MS. If further oligomerization were to take place during ozonization, they might be of too low volatility to be detected. In view of these analytical difficulties, the authors feel confident that the present results give a fairly good description of the reaction of carbodiimides with ozone.

EXPERIMENTAL

General. The ozone source was a Welsbach T23 Ozone Generator giving 0.77–0.80 mmol ozone/min. Oxygen analyses were performed using a Beckman F3 oxygen analyzer as described in the literature.[14]

Gas liquid chromatography (GLC) analyses were performed on Varian Aerograph, model 1520, using column A: 2.4 m × 3 mm copper column with 20 % SE30 on Chromosorb WHMDS (60–80 mesh); column B: 2.4 × 3 mm copper column with 10 % PEG 4000 on Chromosorb WHMDS (60–80 mesh); column C: 2.4 m × 3 mm copper column with 10 % SE52 on Chromosorb WHMDS (60–80 mesh) and on Hewlett-Packard 5700 A, using column D: 4.5 m × 3 mm glass column with 10 % OV17 on Chromosorb WHMDS (60–80 mesh). The gas chromatographs were equipped with Disc integrators on the recorder systems for quantitative analyses.

Materials. Solvents used were pure and anhydrous. *N,N'*-Dicyclohexyl and *N,N'*-diisopropyl carbodiimide were commercially available (Koch-Light Laboratories Ltd. and Aldrich Chem. Co., Inc., respectively) and were distilled

prior to use. The other carbodiimides were synthesized according to standard procedures.

The proper isothiocyanates were prepared from the amines,[15] and the disubstituted thioureas were formed on reaction between these isothiocyanates and the proper amines.[16] Treatment of the thiourea with yellow mercuric oxide yielded the carbodiimides.[16]

N-t-Butyl-N'-(1-methoxycarbonyl)ethyl thiourea was synthesized accordingly from alanine methylester and *t*-butyl isothiocyanate. M.p. 84.0–84.5 °C (CCl_4–pentan), yield 85 %. (Found: C 48.90; H 8.08; N 12.54. Calc. for $C_9H_{18}N_2O_2S$: C 49.51; H 8.31; N 12.83). MS: *m/e* 218 (M^+) NMR (δ values, CCl_4): 1.48 (s, 9 H); 1.49 (d, 3 H, $J \sim 8$ Hz), 3.75 (s, 3 H) 5.02 (q, 1 H, $J \sim 8$ Hz) 6.5–6.8 (m, broad, 2 H) IR (KBr): 3300 (s), 1740 (s).

N-t-Butyl-N'-(1-methoxycarbonyl)ethyl carbodiimide. B.p. 92.5–93.0 °C/10 mmHg. Yield 85 %. (Found: C 58.16; H 8.62; N 14.98. Calc. for $C_9H_{16}N_2O_2$: C 58.57; H 8.69; N 15.20). MS: *m/e* 184 (M^+). NMR (δ values, CCl_4): 1.30 (s, 9 H); 1.40 (d, 3 H, $J \sim 8$ Hz); 3.73 (s, 3 H); 3.87 (q, 1 H, $J \sim 8$ Hz). IR (liq.-film) 2110 (s), 1790 (s).

Standards for GLC, cyanamides,[17] isocyanates,[18] nitro compounds[19] were synthesized according to literature.

Ozonation of carbodiimides. In a typical run, 2.06 g (10 mmol) *N,N'*-dicyclohexyl carbodiimide was dissolved in 25 ml methyl acetate and 18 mmol ozone (with nitrogen as carrier gas [14]) passed through the solution at −40 °C (ozonation in carbon tetrachloride at 0 °C and in diethyl oxalate at −40 °C). The exit gases were analyzed for oxygen,[14] carbon monoxide,[3] and carbon dioxide. Approximately 10 mmol of ozone were absorbed by the solution. Qualitative and quantitative analyses were run on GLC (columns and results, Table 1). Urea estimation was done gravimetrically by letting a definite volume of reaction solution stand under dry condition for one week and correcting for estimated solubility. Peroxide contents were determined in a conventional way with sodium iodide in acetonitrile with perchloric acid present or by reaction with triphenyl phosphine to give the phosphine oxide, both estimated quantitatively on Column D.

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Bis(*gem*-dimethyl)-substituted Cyclic Diynes

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Bis-*gem*-dimethyl-substituted 16- and 18- membered cycloalkynes have been synthesized. The importance of the relative positions of the *gem*-dimethyl groups for cyclization yields and conformations is discussed.

Conformational problems have earlier been investigated by use of *gem*-dimethyl substitution of large rings.[1] For further studies 16- and 18-membered cyclic hydrocarbons with *gem*-dimethyl groups in two positions were wanted. The easiest route to such compounds has been found to go over the corresponding cyclic diynes. These cyclic diynes have now been prepared. Also of interest were the conformational consequences of the combination of *gem*-dimethyl groups and triple bonds in cyclizations and in cyclic compounds.

For the cyclization the method of Wotiz *et al.* and Dale *et al.*[2,3] was used, the reaction between α,ω-dibromoalkanes and the disodium salt of α,ω-diynes in liquid ammonia. The dibromides used in the cyclizations were first synthesized in eight (and nine) steps from β,β-dimethylglutaric acid, the *gem*-dimethyl-substituted nonadiyne in five steps from the same source.

The cyclization reactions were slow in liquid ammonia at −30 °C, and at least five days were needed. The concentration in the cyclization step was *ca.* 0.1 M; however, the poor solubility in liquid ammonia makes it likely that the actual concentrations of dissolved reactants were lower.

The results of the cyclizations to two 16- and one 18-membered rings are shown in Table 1. The isolation of the cyclic diynes from the reaction mixture was difficult and the methods that had to be used and the loss of material differed for the three cyclic diynes. The yields are therefore given both as weight of isolated cyclic product and as cyclic product present in the reaction mixture determined by gas chromatography, the last values most probably being closest to the real cyclization yields.

5,5,13,13-Tetramethylcyclohexadeca-1,8-diyne. The most probable conformation of 5,5-dimethylnona-1,8-diyne before cyclization has

Table 1. Yields in cyclizations of bis-*gem*-dimethyl-substituted dibromides with terminal diynes.

Cyclic product	Reactants		Reaction time, d	Yield of cyclic product, %	
	Dibromide	Diyne		Isolated	In reaction mixture
5,5,13,13-Tetramethyl-cyclohexadeca-1,8-diyne	1,7-dibromo-4,4-dimethyl-heptane	5,5-dimethyl-nona-1,8-diyne	5½	15	34
10,10,13,13-Tetramethyl-cyclohexadeca-1,5-diyne	1,10-dibromo-4,4,7,7-tetra-methyldecane	hexa-1,5-diyne	5	13	40
12,12,15,15-Tetramethyl-cyclooctadeca-1,7-diyne	»	octa-1,7-diyne	6	17	33

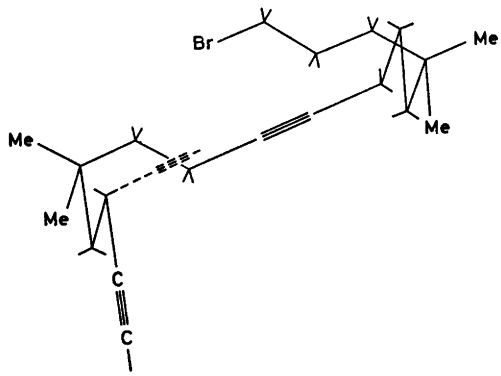


Fig. 1. Conformations in cyclization of 5,5-dimethylnona-1,8-diyne and 1,7-dibromo-4,4-dimethylheptane.

been described earlier [4,5] and is seen in Fig. 1 together with the conformation of 1,7-dibromo-4,4-dimethylheptane most favourable for cyclization.[5] There is some distance between the reaction centres and the yield was 15 %.

Cyclization with the same *gem*-dimethyl substituted diyne, but without dimethyl groups on the dibromide chain was tried earlier,[5] but resulted in no cyclic diyne. This is in accordance with the even longer distance between the reaction centres in this case, because there is no *gem*-dimethyl group that causes a bend on the carbon chain.

The best conformation of the unsubstituted cyclic hexadecadiyne described earlier by Dale *et al.*[6] with parallel acetylenic bonds is not likely for the 5,5,13,13-tetramethylcyclohexadeca-1,8-diyne, because of the space-demanding *gem*-dimethyl groups. It is also difficult to find other low-energy conformations for the compound, and this explains why it is a liquid and does not crystallize even after cooling to −100 °C.

10,10,13,13-Tetramethylcyclohexadeca-1,5-diyne. The most probable conformation of the 4,4,7,7-tetramethyl substituted carbon chain in cyclizations has been discussed earlier.[7] Of the two conformations of hexa-1,5-diyne (*anti* and *gauche*) only the *gauche* form has to be considered in cyclizations. In Fig. 2 is shown how well these conformations of the reactants fit together for an almost strainfree compact conformation of the 10,10,13,13-tetramethylcyclohexadeca-1,5-diyne.

As shown in Table 1 the cyclization yield of this reaction (determined by gas chromatography) was the highest found in these cyclizations.

The fact that the compound is crystalline, m.p. 50 °C, is also in good agreement with the possibility of having a low energy conformation. The enthalpy and entropy of melting were $\Delta H = 4.5$ kcal/mol and $\Delta S = 14.0$ e.u. The infrared spectra were taken of the crystals in potassium bromide and dissolved in carbon disulfide, Fig. 3. Although the spectra are not identical in the two phases, the absorption bands of the crystalline state are strong also in the spectrum of the solution, indicating that the crystal conformer is a main component also in solution.

12,12,15,15-Tetramethylcyclooctadeca-1,7-diyne. For octa-1,7-diyne the *+gauche, anti, +gauche* form seems best fitted for cyclization, because here the bonds are bent so as to bring the end groups on the same side of the molecular chain. With the most probable conformation of 1,10-dibromo-4,4,7,7-tetramethyldecane, mentioned above and shown in Fig. 2, the reacting end groups will be in reasonably close contact for the final cyclization. A suggestion for a somewhat open conformation of the cyclic compound with retention of the conformations of the reactants is shown in Fig. 4. However, the

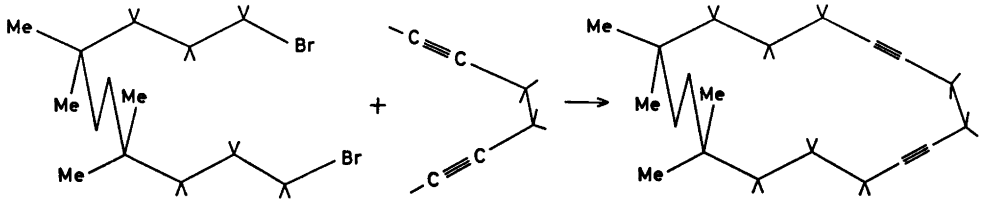


Fig. 2. Conformations in cyclization of 1,10-dibromo-4,4,7,7-tetramethyldecane with hexa-1,5-diyne.

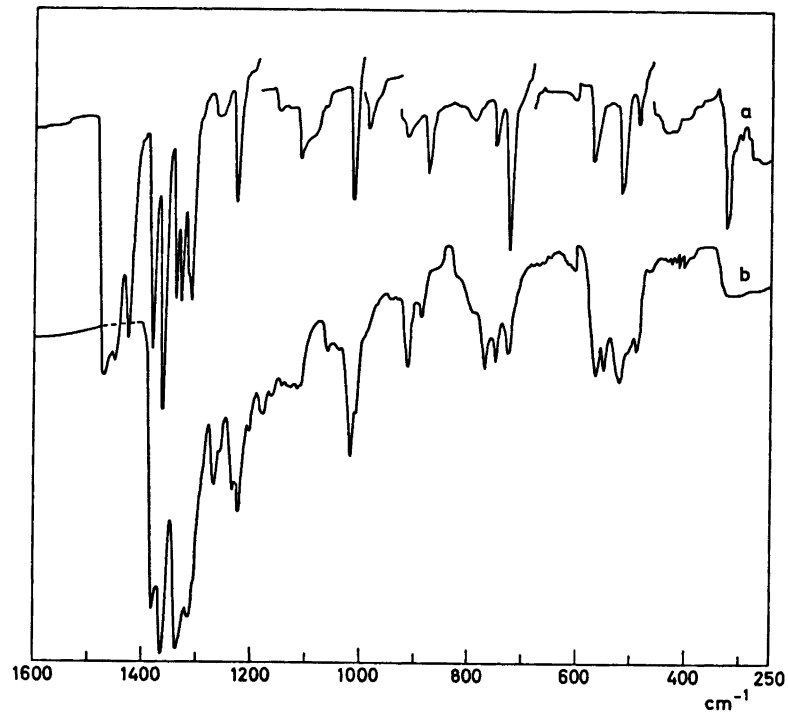


Fig. 3. IR-spectra of 10,10,13,13-tetramethylcyclohexadeca-1,5-diyne in the crystalline state in KBr below 0 °C (a), and in carbon disulfide solution (b).

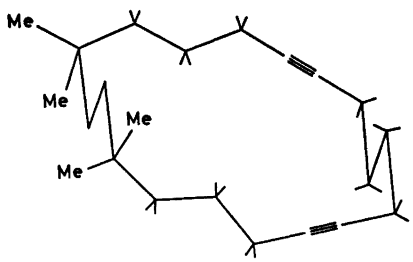


Fig. 4. Conformation of 12,12,15,15-tetramethylcyclooctadeca-1,7-diyne.

cyclic diyne is a liquid and does not crystallize by cooling to −100 °C, which indicates that one preferred conformation does not exist for this compound.

EXPERIMENTAL

5,5,13,13-Tetramethylcyclohexadeca-1,8-diyne. 1,7-Dibromo-4,4-dimethylheptane was first synthesized in 8 steps[8] and 5,5-dimethylnona-1,8-diyne in 4 steps from β,β-dimethylglutaric acid.[4]

Sodium (4.6 g = 0.2 mol) was dissolved in liquid ammonia (1 l) at −30 °C in the presence of iron(III) nitrate as catalyst.[9] The solution was stirred for 1 h and 5,5-dimethylnona-1,8-diyne (14.8 g = 0.1 mol) added dropwise. After another hour of stirring 1,7-dibromo-4,4-dimethylheptane (28.6 g = 0.1 mol) was added slowly and the ammonia solution refluxed at −30 °C for 5½ d by use of an acetone/solid carbon dioxide condenser. Ether (250 ml) was added and the ammonia permitted to evaporate. Undissolved residue was dissolved in water, the water layer was extracted 3 times with ether, all ether extracts were dried with magnesium sulfate. After filtration the ether was distilled off and the unreacted 5,5-dimethylnona-1,8-diyne also removed by distillation. The residue was chromatographed on a column with basic alumina. Elution with benzene-pentane 1:10, gave *5,5,13,13-tetramethylcyclohexadeca-1,8-diyne* (4.08 g = 15 %). Mol.w. 272. (Mass spectrometry). B.p. 148 − 154 °C/0.04 mmHg.

The product was unstable and elemental analysis could only be made of the fully hydrogenated product.[10]

4,4,7,7-Tetramethyldecane-1,10-diol. 4,4,7,7-Tetramethylsebacic acid[11] was synthesized in 7 steps from β,β-dimethylglutaric acid.

Lithium aluminium hydride (11.4 g = 0.3 mol)

was suspended in dry ether (2 l). 4,4,7,7-Tetramethylsebacic acid (51 g=0.2 mol) dissolved in dry ether (800 ml) was added dropwise to the vigorously stirred solution. After 39 h of stirring at room temperature and 2 h of refluxing excess of hydride was destroyed by addition of water. Hydrolysis was carried out with 10 % sulfuric acid (600 ml). After separation of the ether layer the aqueous layer was extracted 3 times with ether. The combined ethereal extracts were washed with sodium hydrogen carbonate solution, dried with magnesium sulfate and filtered. The ether was removed, and the residue distilled, fraction 134 – 138 °C/0.05 mmHg was *4,4,7,7-tetramethyldecane-1,10-diol*. (Found: C 72.94; H 12.80. Calc. for $C_{14}H_{30}O_2$: C 72.98, H 13.13). After repeated treatment with lithium aluminium hydride, of the residue from vacuum distillation, the total yield was 66 % (30.6 g).

1,10-Dibromo-4,4,7,7-tetramethyldecane. 4,4,7,7-Tetramethyldecane-1,10-diol (29.7 g=0.12 mol) was treated at 120 °C with hydrogen bromide until absorption was complete. The product was extracted with light petroleum, the extract washed with water, with some drops of concentrated sulfuric acid and finally with sodium hydrogen carbonate solution. After drying with calcium chloride, filtration and removal of solvent the dibromide was isolated by vacuum distillation. Fraction, b.p. 150 – 152 °C/0.2 mmHg was *1,10-dibromo-4,4,7,7-tetramethyldecane* (36.1 g=85 %). (Found: C 47.59; H 7.73; Br 44.71 Calc. for $C_{14}H_{28}Br_2$: C 47.23; H 7.93; Br 44.89).

10,10,13,13-Tetramethylcyclohexadeca-1,5-diyne. Sodium (2.3 g=0.1 mol) was dissolved in liquid ammonia (1 l) as described above. After 1 h of stirring freshly distilled hexa-1,5-diyne (Farchan Research Laboratories) (3.9 g=0.05 mol) was added dropwise. Stirring was continued, and after another hour 1,10-dibromo-4,4,7,7-tetramethyldecane (17.8 g=0.05 mol) was added slowly. The solution was stirred and the ammonia refluxed for 5 d. The product was isolated as described above for 5,5,13,13-tetramethylcyclohexadeca-1,8-diyne. Recrystallization from ethanol gave *10,10,13,13-tetramethylcyclohexadeca-1,5-diyne* (1.75 g=13 %). Mol.w. 272 (mass spectrometry). (Found: C 87.94; H 11.88. Calc. for $C_{20}H_{32}$: C 88.16; H 11.84).

12,12,15,15-Tetramethylcyclooctadeca-1,7-diyne. As described above, sodium (1.98 g=0.09 mol) was dissolved in liquid ammonia (0.7 l) and stirred for 1 h. Octa-1,7-diyne [12] (4.55 g=0.045 mol) was added dropwise. After another hour of stirring 1,10-dibromo-4,4,7,7-tetramethyldecane (15.3 g=0.045 mol) was added slowly and the solution refluxed at −30 °C for 6 d. Ether was added (300 ml) and the ammonia permitted to evaporate. Then water was added, (250 ml) and the water layer extracted 3 times with ether. All ether extracts were washed successively with 2 N hydrochloric acid, 10 % sodium hydrogen carbonate and water, dried with magnesium sulfate, filtered and the ether distilled off. Unreacted octa-1,7-diyne was removed by distillation and the rest vacuum-distilled at low pressure. Fraction b.p. 154 – 156 °C/0.35 mmHg was *12,12,15,15-tetramethylcyclooctadeca-1,7-diyne* (2.14 g=17 %). Mol.w. 300 (mass spectrometry). (Found: C 87.98; H 11.87. Calc. for $C_{22}H_{36}$: C 87.92; H 12.08).

Repeated distillations were necessary because the boiling points of the dibromide and the cyclic diyne were close. Column chromatography was impossible because the retention time of the two compounds with pentane was the same. For the elemental analyses isolation by gas chromatography was necessary.

The infrared spectra were recorded in a Perkin-Elmer Grating Infrared Spectrophotometer 457. For the calorimetric measurements a Perkin-Elmer Differential Scanning Calorimeter IB was used.

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NMR-Studies of the Interaction of Metal Ions with Poly-(1,4-hexuronates). III. Proton Magnetic Resonance Study of the Binding of Lanthanides to Methyl α-D-Gulopyranoside in Aqueous Solution

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The influence of some paramagnetic lanthanide ions on the PMR spectra of methyl α-D-gulopyranoside in aqueous solution has been studied. Analysis of the induced shift curves indicated that a 1:1-complex was formed. The induced shift ratios for Eu^{3+} and Pr^{3+} were not equal for all protons, thus suggesting significant contact shifts or a complex of non-axial symmetry. By carrying out an approximate correction for asymmetry, the best fit of calculated and observed shifts was obtained with the lanthanide ion located near the *ax-eq-ax* sequence of the three oxygen functions at C_1, C_2, and C_3.

The exploration of possible metal-ion binding-sites in sugar molecules has recently been intensified [1–4] with the main aim of obtaining a better understanding of the rather unique ion-binding properties of polyuronides.[5]

The effect of certain inorganic salts on the position of the equilibrium at the anomeric centre of a number of reducing sugars in aqueous solution has been studied.[2] It was suggested that pyranose rings possessing an *ax-eq-ax* sequence of three contiguous hydroxy groups offer a particularly good binding site.[2] LIS(lanthanide induced shift)-measurements [4] indicated that methyl α-D-galactopyranosiduronic acid provides a similar, triangularly-shaped binding site, composed of the axial hydroxy group at position 4, the equatorial carboxy group, and the ring oxygen. The induced shifts of both $Eu(NO_3)_3$ and $Pr(NO_3)_3$ could be accounted for remarkably well by a pseudo-contact mechanism assuming axial symmetry of the complex.[6]

These results suggests that the use of lanthanides as probes for evaluating binding sites in metal-sugar complexes has a great potential. However, in the general case the interpretation of the results may not be so simple as for α-D-galactopyranosiduronic acid. The recent LIS-results of Angyal [7] on methyl β-D-hamamelopyranoside (containing the *ax-eq-ax*-sequence) seemed not to be related to the equation [8] for pseudocontact shifts in axially symmetrical complexes:

$$\Delta\nu/\nu_0 = K(3\cos^2\theta - 1)/r^3 \qquad (1)$$

This may suggest that the interpretation of LIS-data for neutral sugar derivatives may represent a more difficult problem than for uronic acid derivatives, and we will here present LIS-results on α-D-gulopyranoside, another neutral sugar derivative containing the *ax-eq-ax* sequence of two hydroxy groups and one OCH_3 group.

RESULTS AND DISCUSSION

The 60 MHz ^1H NMR-spectrum before and after the addition of $Eu(NO_3)_3$ to a molar ratio (Eu^{3+}/sugar) of 2.9 is given in Fig. 1. The assignments were confirmed by double-resonance experiments, and the size of the coupling constants indicates that no change in conformation ($^4C_1$) occurs during the addition of lanthanide. The shifts induced by $Eu(NO_3)_3$ and $Pr(NO_3)_3$ are given in Fig. 2. Use of $Pr(ClO_4)_3$

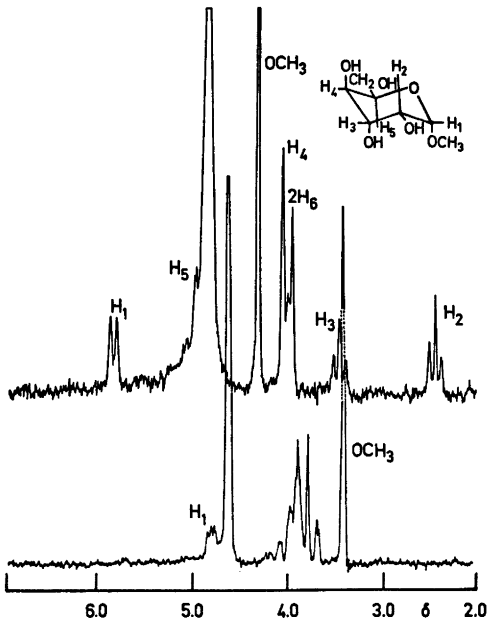


Fig. 1. The 60 MHz ^{1}H NMR-spectrum of methyl α-D-gulopyranoside in D_2O before (a) and after (b) the addition of europium nitrate to a molar ratio of 2.9 (Eu^{3+}/sugar). $J_{1,2}=J_{2,3}=J_{3,4}=3.5$ Hz, $J_{4,5}=1.0$ Hz and $J_{5,6}=6.0$ Hz.

instead of $Pr(NO_3)_3$ yielded identical results. The initial slopes, and the ratios between the initial slopes for the two sets of experiments, are given in Table 1.

According to Bleaney *et al.*,[9] the ratios between pseudocontact shifts at different proton sites in a complex of axial symmetry should be

Table 1. Initial slopes, k, and slope ratios from Figs. 2 and 3.

Proton	k_{Eu} Hz	k_{Pr} Hz	k_{Pr}/k_{Eu}
H_1	57	−57	−1
H_2	−96	23.5	−0.24
H_3	−30	34	−1.13
H_4	~0	1	−
H_5	54	−45.5	−0.84
H_6	12	−12	−1
$O-CH_3$	48	46	−0.95

independent of the lanthanide ion, provided the geometrical structure of the complex is retained. Table 1 shows that this is the case for all protons except H_2. The marked deviation for this proton may accordingly be due to (1) the presence of more than one type of complex in solution, the relative proportions of which may be different for different cations, (2) a large contact-contribution to the H_2-shift, (3) lack of axial symmetry in the complex, or (4) different geometry of the Eu^{3+} and the Pr^{3+}-sugar complexes. These possibilities must, therefore, be considered separately.

(1). A test for stoichiometry proposed by Job and applied by Roth *et al.*[10] on LIS-problems has been used. The difference $(\delta_1-\delta_2)$ between the induced shifts of the H_1- and the H_2-protons was measured at a series of substrate, $[S]_0$, and $Eu(NO_3)_3$-concentrations, $[Eu]_0$. In Fig. 3 the plot of $[S]_0$ $(\delta_1-\delta_2)$, where $\delta_1-\delta_2$ was measured at a constant $([S]_0+[Eu]_0)=0.5$ (M), against $[Eu]_0/([S]_0+[Eu]_0)$, is given. The

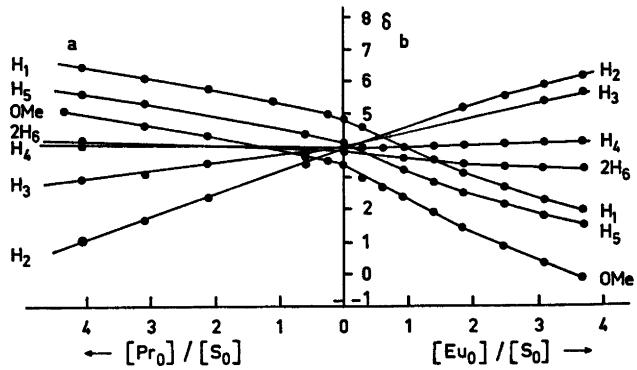


Fig. 2. a. Induced shifts, δ, relative to dioxane against the molar ratio of europium nitrate to substrate $[Eu]_0/[S]_0$ at $[S]_0=0.39$ M. b. Same data as in a for praseodymium nitrate. $[S]_0=0.282$ M.

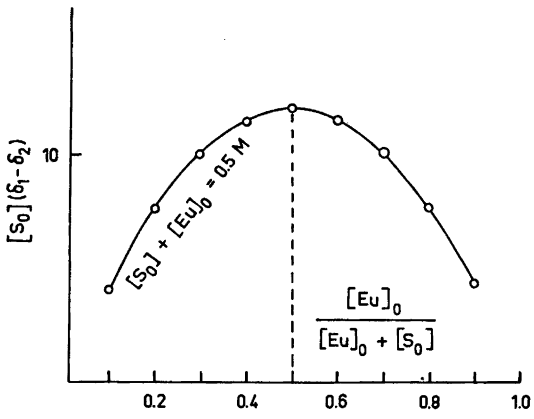


Fig. 3. Job's method used on the complex formation between α-methyl-D-gulopyranoside and europium nitrate.

maximum of the curve at $[Eu]_0/([S]_0+[Eu]_0)=0.5$ indicates, according to Roth *et al.*,[10] and in agreement with earlier suggestions,[1] that a complex of 1:1 stoichiometry is the dominating species. Initial slopes are, therefore, proportional to the bound shifts, Δ, and may be used in attempting to position the Ln-ion in the complex.

(2). An automatic search program,[6] applying eqn. (1), was used to find the best position of the Ln^{3+} ions and the magnetic axis for the two sets of data in Table 1, in order to establish whether contact-shift contributions appeared more probable for the Eu or the Pr-complexes. The results in Table 2 clearly indicate that both the lanthanide-ions are located at the $O_1(ax)-O_2(eq)-O_3(ax)$-site, but the data for the Pr-complex yield distances which are chemically more reasonable within the accuracy of 0.3 Å suggested [13] for this type of analysis. The distance between the position of the Pr^{3+} and the Eu^{3+} ion was found to be 1.1 Å. It appears, therefore, that a significant contact contribution to the H_2-shift in the Eu-complex could explain the difference between the two sets of data. Because the tendency for contact-shift contributions [11,14] is larger for the Eu^{3+} than for the Pr^{3+} ion, this possibility cannot be ruled out. At present, it seems difficult to estimate the contact shift contribution for the various protons in methyl α-D-gulopyranoside. We therefore want to demonstrate that the observed LIS may be explained on the basis of an asymmetric complex.

(3). Since in a 1:1 Ln-sugar complex the ligand field is most probably less than axially symmetrical, it seems possible that some asymmetry in the magnetic properties of the complex could account for the results. The equation for pseudocontact shifts in complexes of rhombic magnetic symmetry is, according to Bleaney *et al.*,[9]

$$\frac{\Delta\nu}{\nu_0} = A\frac{3\cos^2\theta-1}{r^3} + D\frac{\sin^2\theta\cos 2\phi}{r^3} \qquad (2)$$

The first term in eqn. (2) is seen to be equivalent to the axially symmetrical situation, and the task is, therefore, to see if it is possible to find a position of the axis of asymmetry giving a large value, for the second term in eqn. (2), for the H_2-proton relative to all the other protons.

The geometric part of the second term was

Table 2. Observed relative shifts and "best fit" calculation assuming axial symmetry of the complexes. The sign convention in the calculations is the same as that used previously.[6]

Proton	Eu-data Obs.	Calc.	Pr-data Obs.	Calc.
H_1	1.0	1.03	1.0	1.05
H_2	−1.68	−1.60	−0.41	−0.42
H_3	−0.53	−0.81	−0.60	−0.62
H_4	0.01	0.08	0.03	0.05
H_5	0.95	0.97	0.80	0.82
H_6	0.21	0.33	0.21	0.30
Distance to	O_1, O_2, O_3 (Å) 2.2, 1.5, 3.3		1.7, 1.9, 2.5	
Mean square deviation	1.7×10^{-2}		1.2×10^{-3}	

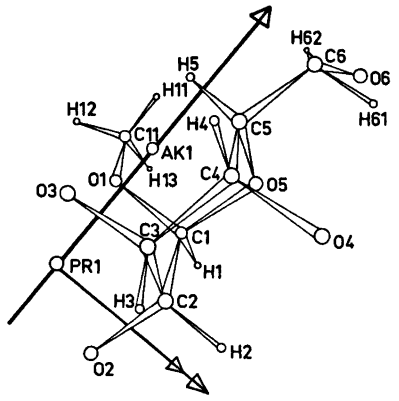


Fig. 4. Computer drawing of Pr^{3+}-sugar complex as obtained from the asymmetry calculations in Table 3. The symmetry axis is given by a single and the asymmetry axis by a double arrow. PR1: the praseodymium ion (not to scale); AK1: an auxilliary point introduced to define the direction of the symmetry axis.

calculated by keeping the direction of the symmetry axis and the Ln position as indicated from the Eu^{3+}-data, and by rotating the asymmetry axis in small steps. For a certain direction of the asymmetry axis (see Fig. 4) the relative values 0.006, 1.0, 0.090, 0.020, 0.012, and 0.040 for the protons H_1, H_2, H_3, H_4, H_5, and H_6, respectively, were obtained. Very similar values were found for the Pr^{3+} site. By assuming that $D_{Pr}/D_{Eu} = -A_{Pr}/A_{Eu} = 1$, the two sets of data could be corrected, and the search procedure applying eqn. (1) could be carried out. The results in Table 3 show that the corrected data now give very similar positions of the Eu^{3+} and the Pr^{3+} ion. The distance between the two sites is 0.3 Å. Somewhat disturbing is the fact that the distance from the Ln^{3+} ion to O_3 is as high as 3 Å. However, taking into account that the present calculation must be regarded only as a first, crude correction for asymmetry, the results strongly suggest that both sets of data can be accounted for by eqn. (2), *i.e.* without assuming any contact shift contributions to the observed shifts.

(4). In the above treatment we have assumed that the Pr^{3+}- and the Eu^{3+}-sugar complexes have an equivalent geometry. Induced shifts have also been obtained for $Er(NO_3)_3$, $Yb(NO_3)_3$, and $Ho(NO_3)_3$ (unpublished results). The three sets of shift curves were, as expected, very similar (Ho^{3+} giving shifts of opposite signs compared to Er^{3+} and Yb^{3+}), but markedly different from the Eu and Pr data. Most interesting was a very large induced shift for the HDO-proton, which was observed in all three cases. The corresponding shift was very small in the case of Eu^{3+} and Pr^{3+}. It seems therefore that both the chemistry and the geometry may vary among the lanthanide-sugar complexes. The similarity of the Er^{3+}, Yb^{3+}, and Ho^{3+} results on one hand, and the Pr^{3+} and Eu^{3+} results on the other, suggests, however, that this change occurs in a discontinuous manner along the lanthanide series and that the LIS-approach is valuable when proper account is taken of the possibility of magnetic asymmetry in the complexes and contact shift contributions.

Table 3. Observed relative shifts corrected for asymmetry (see text) and "best-fit" calculation applying the equation for axial symmetry.

Proton	Eu-data Corrected	Calc.	Pr-data Corrected	Calc.
H_1	1.0	1.03	1.0	1.04
H_2	−1.01	−0.98	−1.05	−1.03
H_3	−0.45	−0.61	−0.64	−0.73
H_4	0.01	0.05	−0.03	−0.06
H_5	0.87	0.87	0.79	0.81
H_6	0.23	0.33	0.19	0.31
Distance to	O_1, O_2, O_3 (Å)			
	1.8, 1.8, 3.2		1.7, 1.85, 3.0	
Mean square deviation				
	5.5×10^{-3}		3.2×10^{-3}	

Note added in proof. In a recent paper by Angyal; Greeves and Pickles (*J. Chem. Soc. Chem. Commun* (1974) 589) it has been demonstrated on the basis of LIS-experiments with *epi*-inositol and 1,6-anhydro-β-D-allopyranose that the contact interaction is greatest when the bonds connecting the proton and the cation form a planar zig-zag arrangement. A preliminary analysis of their data suggests that a similar, planar arrangement of $Ln^{3+}-C_2-H_2$ in methyl α-D-gulopyranoside may cause a considerable contact shift contribution to the H_2-shift (see Table 1).

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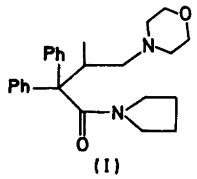
Crystal Structures of Synthetic Analgetics. III. Dextromoramide Bitartrate

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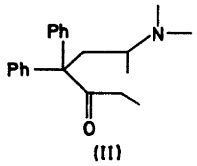
Department of Chemistry, University of Oslo, Oslo 3, Norway

The molecular and crystal structure of dextromoramide (+)-bitartrate has been determined by X-ray methods. The crystals are orthorhombic, space group $P2_12_12_1$ with unit cell dimensions $a=7.976(3)$ Å; $b=17.024(5)$ Å; $c=20.046(5)$ Å. The structure was determined by direct methods and refined to a conventional R of 0.053 for 1909 observed reflections.

The morpholine moiety is in *anti* position relative to the quaternary carbon atom, the nitrogen atom being engaged in a comparatively strong N−H···O hydrogen bond of 2.76 Å to the ionized end of the bitartrate ion. Thus there is no interaction between the amino group and the carbonyl group. The pyrrolidine ring has the Cγ-*exo* conformation, Cγ being 0.61 Å out of the plane through the other atoms of the ring. The amide group is not strictly planar as the torsional angle about the C′−N bond is 6.4°. An extensive network of hydrogen bonds form molecular layers normal to the z-axis whereas the layers are connected through van der Waals forces only.



(I)



(II)

Moramide (I) is a tertiary amide related to methadone (II) and among the more potent analgetics.[1] The present structure determination was carried out as a part of the investigation on morphine-like analgetics being in progress in this laboratory.[2-3] Of the two isomers of moramide the *dextro* form is by far the most active. Thus analgesic activity resides in the same enanthiomer as in the non-narcotic propoxyphene.[1]

EXPERIMENTAL

Single crystals of dextromoramide (+)-bitartrate were obtained from the commercially available compound by recrystallization from ethanol. The crystals are prisms with orthorhombic symmetry. Systematically absent reflections $h00$, $0k0$, and $00l$ for odd indices are compatible with space group $P2_12_12_1$. Unit cell dimensions were determined on a Syntex $P\bar{1}$ diffractometer with graphite crystal monochromated Mo$K\alpha$-radiation ($\lambda=0.71069$ Å).

A crystal of dimensions 0.5 mm × 0.2 mm × 0.2 mm was employed for the collection of three-dimensional intensity data. The ω autocollection program was applied with a scan rate of 3° min^{-1}. The scan range was $2\theta(\alpha)\pm0.5°$ and the background were counted 0.7 times the scan time. The intensities of three standard reflections were measured periodically during the collection of data. They showed no systematic variation. E.s.d's. in the intensities were taken as the square root of the total counts with a 2 % addition for instrumental instability.

A total of 2946 independent reflections were recorded within the limit of 0.59 for $\sin\theta/\lambda$; 1909 had a net count larger than $2\sigma_I$.

The data were corrected for Lorentz and polarization effects, and for secondary extinction.

All calculations were performed on a CDC 6600 computer using the programs described in Ref. 4, except for the phase determination which was done with MULTAN, written by P. Main *et al.*[5] Atomic form factors were those of Hanson *et al.*[6] for O, N, and C and of Stewart *et al.*[7] for H.

CRYSTAL DATA

Dextromoramide (+)-bitartrate, $C_{25}H_{32}N_2O_2 \cdot C_4H_6O_6$, orthorhombic.
$a = 7.976(3)$ Å, $b = 17.024(5)$ Å, $c = 20.046(5)$ Å.
$V = 2721.9$ Å^3, $M = 524.27$, $Z = 4$.
$D_{obs} = 1.29$ g cm^{-3} (flotation), $D_{calc} = 1.28$ g cm^{-3}.
Systematic absences: $h00$, $0k0$, $00l$ for odd indices; space group $P2_12_12_1$.

STRUCTURE DETERMINATION

Normalized structure factors were calculated by means of the K-function.[8] The phase determination was carried out with MULTAN, applying the 443 highest E-values (≥ 1.25). One of the resulting E-maps revealed the positions of all the 39 non-hydrogen atoms. Successive Fourier syntheses, isotropic and anisotropic full matrix least-squares refinement gave an R-factor of 0.09. Approximate positions of all the hydrogen atoms were calculated from stereochemical considerations, taking reasonable hydrogen bonds into account. All the hydrogen atoms were refined isotropically. But the three hydroxyl hydrogen atoms got large B-values and were excluded from further calculations. Hydrogen atoms bonded to the same carbon atom were given common B-values. The refinement finally converged at a conventional R of 0.053 ($R_w = 0.039$).

A complete list of observed and calculated structure factors may be obtained from the author on request. The atomic parameters are given in Table 1 where the anisotropic temperature factor is given by

$$\exp -[B11h^2 + B22k^2 + B33l^2 + B12hk + B13hl + B23kl]$$

E.s.d's in bond lengths and angles are 0.005 – 0.007 Å and 0.3 – 0.5°, respectively.

DISCUSSION

The numbering of the atoms is shown in Fig. 1. Interatomic distances and angles are given in Tables 2 and 3, whereas some torsional angles are listed in Table 4.

According to earlier investigations of (+)-tartaric acid [9–10] the absolute configuration of this isomer is found to be 2*R*,3*R*. The drawings in the present paper depict this optical isomer, referred to as 27*R*, 28*R* with the present numbering. Usually the tartrate ion consists of two planar halves with a dihedral angle of 60°.[11] In the present case, each of the two carboxyl groups, with the addition of C27 and C28, respectively, is planar. However, the two hydroxyl oxygen atoms O5 and O6 are situated 0.14 and 0.26 Å out of the planes, respectively. The dihedral angle between the planes is 65.2°. Ionization of one carboxyl group is obvious from the two equal C – O bond distances C26 – O3 [1.248(5) Å] and C26 – O4 [1.259(5) Å], whereas the other end of the tartrate ion is non-symmetrical.

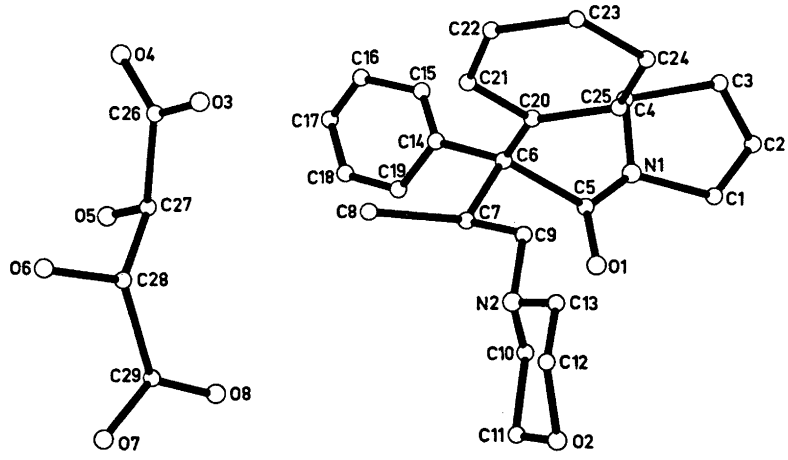


Fig. 1. The numbering of the atoms.

Table 1. Positional and thermal parameters for the heavy (10^4) and the hydrogen atoms (10^3), with e.s.d's in the parentheses.

ATOM	X	Y	Z	B11	B22	B33	B12	B13	B23
C1	-1669(7)	6587(3)	4897(2)	111(11)	21(2)	24(2)	32(8)	2(7)	4(3)
C2	-2774(8)	6836(3)	4647(3)	178(15)	26(2)	46(2)	49(11)	47(11)	6(4)
C3	-1893(8)	6546(3)	5284(3)	193(15)	33(2)	32(2)	-9(11)	79(10)	-29(4)
C4	-1374(7)	5715(3)	5187(2)	122(11)	35(2)	15(1)	11(9)	23(7)	-4(3)
C5	189(6)	5354(3)	4813(2)	75(9)	17(2)	16(1)	-14(8)	-1(6)	-3(3)
C6	1868(6)	4637(2)	4338(2)	85(9)	17(2)	11(1)	3(8)	7(6)	-8(3)
C7	2139(6)	4265(3)	3758(2)	91(9)	21(2)	12(1)	-7(8)	8(6)	8(3)
C8	2716(7)	3431(3)	3888(2)	128(11)	23(2)	16(1)	26(8)	18(7)	-8(3)
C9	3638(6)	4799(3)	3686(2)	111(10)	26(2)	12(1)	-5(8)	21(6)	-4(3)
C10	3468(6)	4771(3)	2356(2)	108(10)	31(2)	14(1)	-4(8)	-5(7)	8(3)
C11	4533(7)	4641(3)	1743(2)	118(11)	44(3)	13(1)	-8(10)	7(7)	3(3)
C12	7889(6)	5801(3)	2275(3)	72(9)	41(2)	21(2)	-3(9)	28(7)	9(4)
C13	6888(6)	5134(3)	2923(2)	92(10)	29(2)	18(1)	-28(9)	8(7)	12(3)
C14	-331(6)	4963(2)	4551(2)	63(9)	16(2)	16(1)	5(7)	3(6)	8(3)
C15	-719(7)	3984(3)	5217(2)	133(12)	28(2)	13(1)	8(8)	8(7)	5(3)
C16	-2851(8)	3423(3)	5388(2)	153(12)	25(2)	16(1)	-13(9)	12(8)	16(3)
C17	-3889(7)	3877(3)	4899(3)	101(11)	26(2)	24(2)	-28(9)	18(8)	4(3)
C18	-2666(6)	3224(3)	4252(3)	85(10)	27(2)	22(2)	-8(8)	18(7)	1(3)
C19	-1355(7)	3717(3)	4872(2)	93(10)	29(2)	12(1)	8(8)	-4(7)	-4(3)
C20	2251(6)	4894(3)	4984(2)	61(9)	26(2)	12(1)	17(8)	21(6)	-1(3)
C21	2985(7)	4328(3)	5319(2)	132(11)	38(2)	18(1)	31(9)	8(7)	1(3)
C22	4078(8)	4542(3)	5822(3)	127(12)	42(3)	22(2)	23(11)	-13(8)	-1(4)
C23	4495(7)	5318(4)	5918(3)	108(11)	56(3)	17(1)	-32(10)	8(7)	-7(4)
C24	3787(7)	5889(3)	5587(3)	119(12)	38(3)	19(2)	-39(10)	-3(8)	-14(3)
C25	2788(7)	5675(3)	5081(2)	109(11)	24(2)	17(1)	1(8)	14(7)	-4(3)
C26	-3982(7)	2642(3)	2293(2)	118(10)	38(2)	18(2)	43(9)	8(7)	8(3)
C27	-2283(6)	2562(3)	2886(2)	77(10)	22(2)	19(2)	8(8)	11(7)	8(3)
C28	-962(7)	2281(3)	2537(2)	121(10)	22(2)	14(1)	4(8)	6(7)	-8(3)
C29	847(7)	2543(3)	2377(3)	115(11)	32(2)	28(2)	-21(9)	-11(8)	-18(3)
N1	-986(5)	5796(2)	4398(2)	97(8)	25(2)	13(1)	14(7)	4(5)	4(2)
N2	4551(5)	4626(2)	2962(2)	96(8)	19(2)	14(1)	4(6)	8(5)	-8(2)
O1	219(4)	5478(2)	3415(1)	162(8)	25(1)	11(1)	6(6)	9(4)	7(2)
O2	5963(5)	5138(2)	1713(2)	151(8)	49(2)	18(1)	-28(8)	24(5)	18(2)
O3	-4185(5)	3133(2)	2756(2)	158(8)	32(1)	22(1)	54(6)	13(5)	-7(2)
O4	-5666(5)	2214(2)	2829(2)	115(8)	53(2)	37(1)	-27(7)	-13(6)	-26(3)
O5	-2292(5)	2686(2)	1443(2)	168(8)	48(2)	14(1)	54(6)	11(5)	-11(2)
O6	-1113(5)	1468(2)	2645(2)	111(7)	28(1)	19(1)	-3(5)	1(5)	6(2)
O7	1984(4)	2818(2)	2487(2)	88(7)	33(2)	32(1)	18(6)	-8(5)	-8(2)
O8	1112(5)	3287(2)	2181(2)	135(8)	38(2)	61(2)	-36(7)	-18(7)	25(3)

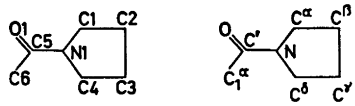
ATOM	X	Y	Z	B	ATOM	X	Y	Z	B
H1C1	-67(6)	694(3)	389(2)	3,7(1,3)	H2C11	495(6)	483(3)	167(2)	4,4(,8)
H2C1	-215(6)	642(3)	366(2)	3,7(,9)	H1C12	798(6)	539(2)	223(2)	3,8(4,2)
H1C2	-385(8)	665(4)	468(3)	6,3(1,1)	H2C12	752(6)	443(2)	225(2)	3,8(,7)
H2C2	-272(8)	745(4)	461(3)	6,3(1,3)	H1C13	556(6)	572(3)	297(2)	3,1(,3)
H1C3	-254(7)	654(3)	573(3)	5,1(1,4)	H2C13	677(6)	497(3)	328(2)	3,1(,9)
H2C3	-87(7)	692(3)	538(2)	5,1(1,8)	HC15	-13(7)	413(3)	561(2)	5,7(1,2)
H1C4	-242(8)	539(3)	515(3)	4,8(2,1)	HC16	-217(7)	337(3)	599(2)	5,5(1,5)
H2C4	-44(8)	556(3)	539(2)	4,5(1,1)	HC17	-392(7)	275(3)	586(3)	6,8(1,5)
HC7	148(6)	425(3)	332(2)	3,3(1,3)	HC18	-335(6)	284(3)	383(2)	4,8(1,3)
H1C8	329(6)	316(3)	349(2)	3,2(1,5)	HC19	-118(5)	382(2)	368(2)	1,5(,9)
H2C8	178(6)	313(3)	397(2)	3,2(2,3)	HC21	276(9)	376(3)	529(3)	6,8(1,8)
H3C8	342(6)	338(3)	423(2)	3,2(,7)	HC22	451(8)	487(3)	615(2)	6,9(1,6)
H1C9	442(6)	475(2)	395(2)	1,9(1,9)	HC23	527(6)	547(3)	532(2)	5,1(1,3)
H2C9	328(6)	539(2)	356(2)	1,9(,7)	HC24	418(5)	648(2)	564(2)	2,2(,9)
H1C10	313(5)	535(2)	241(2)	2,8(1,7)	HC25	232(6)	615(2)	466(2)	5,8(1,1)
H2C10	251(5)	436(2)	233(2)	2,8(,7)	HC27	-193(7)	387(3)	183(3)	3,4(1,6)
HN2	483(7)	488(3)	294(2)	3,9(1,3)	HC28	-129(6)	255(2)	342(2)	2,1(1,8)
H1C11	387(6)	473(2)	132(2)	4,4(1,9)					

Table 2. Bond lengths (Å).

DISTANCE	(A)	DISTANCE	(A)	DISTANCE	(A)
C1 - N1	1,488(6)	C1 - C2	1,519(7)	C2 - C3	1,548(8)
C3 - C4	1,517(7)	C4 - N1	1,482(6)	C5 - N1	1,346(5)
C5 - O1	1,221(5)	C5 - C6	1,579(6)	C6 - C7	1,578(6)
C7 - C8	1,517(6)	C7 - C9	1,526(6)	C9 - N2	1,514(5)
N2 - C10	1,503(6)	C10 - C11	1,502(6)	C11 - O2	1,422(6)
C12 - O2	1,421(6)	C12 - C13	1,508(6)	C13 - N2	1,503(6)
C6 - C14	1,544(6)	C14 - C15	1,397(6)	C15 - C16	1,384(7)
C16 - C17	1,376(7)	C17 - C18	1,388(6)	C18 - C19	1,378(6)
C19 - C14	1,393(6)	C6 - C20	1,555(6)	C20 - C21	1,481(6)
C21 - C22	1,383(7)	C22 - C23	1,376(7)	C23 - C24	1,394(7)
C24 - C25	1,384(7)	C25 - C20	1,391(6)	C26 - O3	1,259(6)
C26 - O4	1,246(6)	C26 - C27	1,536(7)	C27 - O5	1,489(6)
C27 - C28	1,539(7)	C28 - O6	1,489(5)	C28 - C29	1,542(7)
C29 - O7	1,298(6)	C29 - O8	1,211(6)		

To make the comparison with other pyrrolidine residues easier, the conventional nomenclature [12–13] of proline peptides is put into parentheses in the further text.

The puckering of the pyrrolidine ring is revealed by the atom C3 (Cγ) being situated 0.61 Å out of a plane through N1, C1, C2, and C4 (N, Cα, Cβ, Cδ), given in Table 5.



The present envelope conformation is thus similar to that found in most of the earlier reported proline determinations.[13–14] C5(C′) is situated 0.05 Å out of the plane described above and on the opposite side relative to C3 (Cγ). Applying the proposed notation in Ref. 13, the present conformation may be referred to as C3-*exo* (Cγ-*exo*.) Although the atoms N1, C1, C4, C5 (N, Cα, Cβ, Cγ, C′) are coplanar the amide group is not strictly planar and subsequently the non-planarity may be described by a rotation about the C5–N1 (C′–N) amide bond. The torsional angle is 6.4° and is probably due to intramolecular strain, as reported for other amides containing the pyrrolidine ring.[13] This may be caused by the close approaches C4···C6 (Cδ···C1α, 3.09 Å) and C1···O1 (Cα–O, 2.68 Å).

An amide group containing an unsubstituted pyrrolidine ring may be looked upon as both a *cis* and a *trans* peptide. As such a unit, the dimensions of the correspondent standard peptide units [14–16] may indicate the non-symmetrical sp^2-configuration of the nitrogen atom. The rather large difference between the two angles C1–N1–C5 (Cα–N–C′) and C4–N1–C5 (Cδ–N–C′) of 118.4 and 130.3°, respectively, is in agreement with values found in related compounds.[13,17,18] Also the standard values [14–16] imply an opening of the latter bond angle compared to the former. The C5–N1 bond of 1.346(5) Å is somewhat longer than normal for C–N single bonds in peptides (1.325 Å).[14] A corresponding lengthening is also reported by Kartha *et al.*,[18] although that amide group is essentially planar.[18]

As the moramide molecule is highly crowded, with four large groups bonded to C6, the C–C single bonds involving this quaternary carbon atom is long as compared to the normal values.[19] The bond lengths C5–C6 (1.570 Å), C6–C7 (1.578 Å), C6–C14 (1.544 Å), and C6–C20 (1.555 Å) clearly exhibit the increased interatomic distances in this part of the molecule. Inspection of Table 3 shows the deformations of the bond angles around C6, the values varying from 105.1° (C5–C6–C14) to 113.9° (C14–C6–C20). C6 is coplanar with one of the two strictly planar aromatic rings (C20,···,C25), whereas it is 0.07 Å out of the other. This non-planarity and the above mentioned angular deformations are attributed to steric repulsions, as reported for propoxyphene HCl [2] and methadone.[3,20]

The conformation of the propylamine chain

Table 3. Bond angles (°).

ANGLE	(°)	ANGLE	(°)
C1 - N1 - C4	111,2(4)	N1 - C1 - C2	104,4(4)
C1 - C2 - C3	102,6(5)	C2 - C3 - C4	103,4(4)
C3 - C4 - N1	101,9(4)	C1 - N1 - C5	118,4(4)
C4 - N1 - C5	130,3(4)	N1 - C5 - O1	120,3(4)
N1 - C5 - C6	119,7(4)	O1 - C5 - C6	119,8(4)
C5 - C6 - C7	106,8(3)	C5 - C6 - C14	105,1(4)
C5 - C6 - C20	112,1(4)	C7 - C6 - C14	110,4(3)
C7 - C6 - C20	109,8(4)	C14 - C6 - C20	113,8(3)
C6 - C7 - C8	114,5(4)	C6 - C7 - C9	109,8(4)
C8 - C7 - C9	110,5(4)	C7 - C9 - N2	115,1(4)
C9 - N2 - C10	112,5(4)	C9 - N2 - C13	109,1(3)
C13 - N2 - C10	108,8(3)	N2 - C10 - C11	108,9(4)
C10 - C11 - O2	113,8(4)	C11 - O2 - C12	109,9(4)
O2 - C12 - C13	111,5(4)	C12 - C13 - N2	110,8(4)
C6 - C14 - C15	123,9(4)	C6 - C14 - C19	119,4(4)
C14 - C15 - C16	121,4(5)	C15 - C16 - C17	120,2(5)
C16 - C17 - C18	120,8(5)	C17 - C18 - C19	118,9(5)
C18 - C19 - C14	122,8(4)	C19 - C14 - C15	116,5(4)
C6 - C20 - C21	120,1(4)	C6 - C20 - C25	121,9(4)
C20 - C21 - C22	121,8(5)	C21 - C22 - C23	120,6(5)
C22 - C23 - C24	119,3(5)	C23 - C24 - C25	120,3(5)
C24 - C25 - C20	121,8(5)	C25 - C20 - C21	117,9(5)
O3 - C26 - O4	127,8(5)	O3 - C26 - C27	115,8(5)
O4 - C26 - C27	116,4(5)	C26 - C27 - O5	109,8(4)
C26 - C27 - C28	111,1(4)	O5 - C27 - C28	118,9(4)
C27 - C28 - O6	112,8(4)	C27 - C28 - C29	111,1(4)
O6 - C28 - C29	113,4(4)	C28 - C29 - O7	114,7(5)
C28 - C29 - O8	120,1(5)	O7 - C29 - O8	125,1(6)

Table 4. Torsional angles (°).

DIHEDRAL ANGLE	( )	DIHEDRAL ANGLE	( )
C1 - C2 - C3 - C4	-40,3(6)	C1 - N1 - C5 - O1	-9,5(7)
C4 - N1 - C5 - O1	173,4(5)	C1 - N1 - C5 - C6	174,8(4)
C4 - N1 - C5 - C6	-2,2(7)	N1 - C5 - C6 - C14	63,7(5)
N1 - C5 - C6 - C20	-60,5(5)	C5 - C6 - C14 - C15	-118,8(5)
C5 - C6 - C20 - C25	-14,5(6)	C15 - C14 - C6 - C7	135,2(5)
C21 - C20 - C6 - C7	-73,7(5)	C14 - C6 - C7 - C8	-48,3(5)
C20 - C6 - C7 - C8	77,5(5)	C14 - C6 - C7 - C9	-172,7(4)
C20 - C6 - C7 - C9	-46,9(5)	C6 - C7 - C9 - N2	-166,5(4)
C7 - C9 - N2 - C10	65,2(5)	C7 - C9 - N2 - C13	-174,8(4)
C9 - N2 - C10 - C11	176,8(4)	C9 - N2 - C13 - C12	-177,8(4)
N2 - C10 - C11 - O2	-59,1(5)	N2 - C13 - C12 - O2	56,8(5)
C10 - C11 - O2 - C12	69,1(5)	C11 - O2 - C12 - C13	-57,9(5)
O4 - C26 - C27 - O5	5,7(7)	C26 - C27 - C28 - C29	-155,8(4)
O5 - C27 - C28 - O6	-46,2(6)	O7 - C29 - C28 - O6	-12,5(6)
O3 - C26 - C29 - O7	119,7(6)		

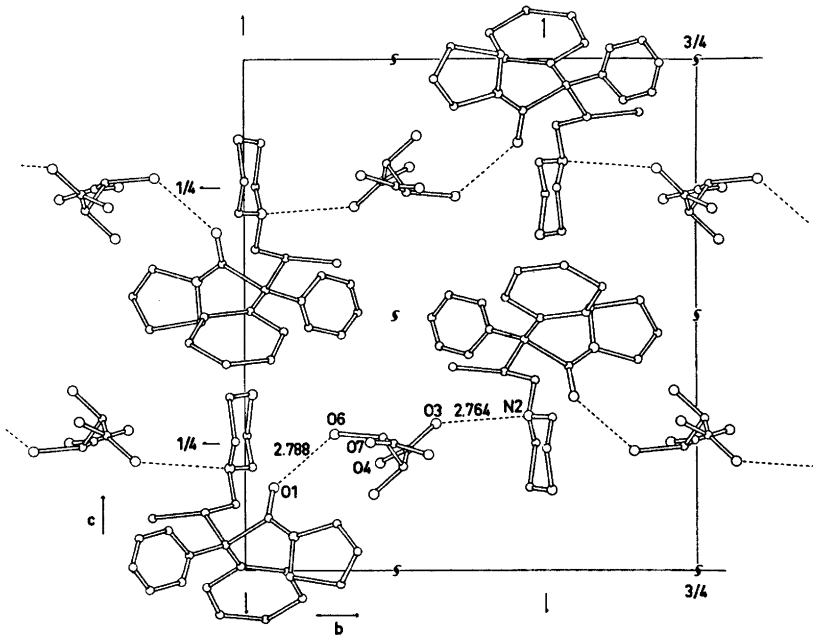


Fig. 2. The crystal structure as seen along the *a*-axis [the intermolecular distance O7···O4 (i) is 2.544 Å].

Table 5. Least square planes and deviations (Å) of the individual atoms.

Equations:

Plane A (pyrrolidine ring)
$(0.0966a + 0.0335b + 0.0142c)\ R - 7.605 = 0$
Plane B (amide group)
$(0.0992a + 0.0322b + 0.0136c)\ R - 7.261 = 0$

Plane A	Deviations	Plane B	Deviations
N1 (N)	−0.024	N1 (N)	−0.014
C1 (C^{α})	0.027	C1 (C^{α})	0.005
C2 (C^{β})	−0.016	C4 (C^{δ})	0.005
C3* (C^{γ})	0.606	C5 (C^{1})	0.006
C4 (C^{δ})	0.017	C6* (C^{α}_{1})	0.111
C5* (C′)	−0.047	O1* (O)	−0.136

Atoms with an * do not define the plane.

is nearly staggered, the dihedral angle C6 − C7 − C9 − N2 being −166.5°. The extension of this chain is to some extent attributed to the hydrogen bonds in the crystal — more precisely the comparatively strong hydrogen bond of 2.764 Å which links the morpholino nitrogen atom N2 to the ionized end of the bitartrate ion. The morpholine ring has the chair conformation, and the acidic proton on N2 is in axial position. The average C − N2+ distance of 1.507 Å is normal for C − N single bonds with a protonated nitrogen atom.

The three-dimensional molecular arrangement is shown in Figs. 2 and 3. Although the hydroxyl hydrogen atoms are not located, the distances and angles given in Table 6 support the assumption that the contacts O7···O4 (2.544 Å) and O6···O1 (2.788 Å) are hydrogen bonds. Fig. 2 depicts the layers normal to the *z*-axis whereas the extension of each single layer is shown in Fig. 3. This figure also visualizes the infinite chains of bitartrate ions along the *a*-axis. Each organic anion is additionally connected to two different moramide moieties.

A cyclic conformation similar to that found in methadone [3,20] is not preferred in the present structure. The presence of a hydrogen bonded nitrogen atom in the propylamin chain results in an overall shape of the present analgetic similar to that reported for methadone HBr [21] and dextropropoxyphene HCl.[2]

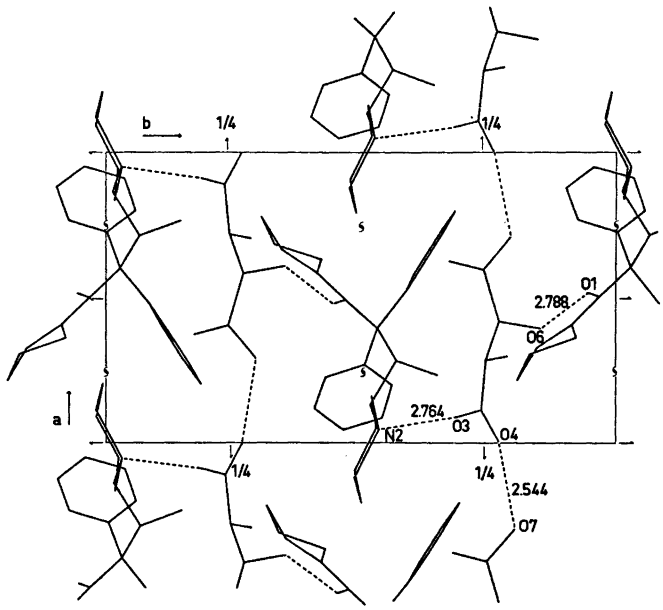


Fig. 3. One single layer of the crystal structure as seen along the *z*-axis.

Table 6. Distances (Å) and angles (°) of the hydrogen bonds. The letters (*i*) and (*ii*) give the symmetry code of the acceptor atom.

Distances		Angles	
O7···O4(*i*)	2.544	C29–O7–O4(*i*)	119.5
		C26(*i*)–O4(*i*)–O7	124.5
O6···O1(*iii*	2.788	C28–O6–O1(*ii*)	117.5
		C5(*ii*)–O1(*ii*)–O6	150.1
N2···O3(*i*)	2.764	C9–N2–O3(*i*)	118.9
		C10–N2–O3(*i*)	103.7
		C13–N2–O3(*i*)	103.0
(*i*) $x+1$ y z		C26(*i*)–O3(*i*)–N2	140.0
(*ii*) $-x$ $y-\frac{1}{2}$ $\frac{1}{2}-z$		N2–HN2–O3(*i*)	163.8

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The Molecular Structure of *trans*-Cyclooctene

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The molecular structure of *trans*-cyclooctene has been determined by gaseous electron diffraction. The molecule was found to have a twist conformation. The values of the principal bond distances (r_a), valence angles and dihedral angles (ω) with estimated uncertainty of 2σ are: $r_{C=C}=1.332$ Å (0.003), $r_{C_3-C_5}=1.499$ Å (0.008), $r_{C_4-C_5}=1.549$ Å (0.002), $r_{C_3-H}=1.110$ Å (0.002), $\angle C=C-C=121.9°$ (0.8), $\angle C_2C_3C_4=104.6°$ (0.4), $\angle C_3C_4C_5=113.8°$ (0.4), $\angle C_4C_5C_6=118.8°$ (0.6), $\angle C=C-H=118.4°$ (2.0), $\angle HCH=106.8°$ (1.7), $\omega(C=C)=136.0°$ (0.4), $\omega(C_2-C_3)=-91.1°$ (0.8), $\omega(C_3-C_4)=54.2°$ (1.0), $\omega(C_4-C_5)=-82.8°$ (0.6), $\omega(C_5-C_6)=111.8°$ (1.0), $\omega(H-C=C-H)=177.5°$ (11.2).

trans-Cyclooctene is a highly strained disymmetric molecule whose structure has been the subject of considerable discussion since the compound was first synthesized by Cope *et al.*[1] in 1953. X-Ray and NMR studies [2,3] on both metal complexes and the dibromocarbene adduct [4] allow the conclusion that the *trans*-cyclooctene molecule exists in a twist conformation. Theoretical calculations by Allinger [5,6] and by Ermer [7,8] support these observations. The only experimental determination of the structure of the free molecule (Gavin,[10] 1973) does, however, indicate that the molecule assumes a chair conformation. In the hope of elucidating this interesting structural problem it was decided to undertake a new electron diffraction study of the *trans*-cyclooctene molecule in the gas phase.

EXPERIMENTAL

The sample of *trans*-cyclooctene used in the present study was kindly provided by Dr. Robert D. Bach, Wayne State University. The electron diffraction data were recorded on the Oslo electron diffraction unit [11] at two different nozzle-to-plate distances (480.78 and 200.78 mm). The exposures were made at room temperature and at an electron wavelength of 0.06458 Å.

Four plates from each of the two camera lengths were selected for analysis which was carried out in the usual way.[12] The range of data obtained from these distances were $1.500 \leq s \leq 18.750$ Å^{-1} with data interval $\Delta s = 0.125$ Å^{-1} and $7.25 \leq s \leq 41.25$ Å^{-1} with $s = 0.25$ Å^{-1} ($s = 4\pi\lambda^{-1} \sin\theta$ where λ is the electron wavelength and 2θ is the scattering angle).

The theoretical counterpart to the experimental molecular intensity function may be calculated as:

$$sM(s) = \sum_{i \neq j} \frac{|f_i(s)||f_j(s)|}{|f_C(s)|^2} \cos\left[\eta_i(s) - \eta_j(s)\right] \sin(sr_{ij}) \exp\left(-\tfrac{1}{2}u_{ij}^2 s^2\right) r_{ij}^{-1}$$

The sum extends over all atom pairs i, j in the molecule. r_{ij} represents an internuclear distance and u_{ij} the corresponding root-mean-square amplitude of vibration. $f_j(s) = |f_j(s)| \exp[i\eta_j(s)]$ is the complex atomic scattering factor of atom j.

Radial distribution (RD) functions were calculated by Fourier inversion of the intensity functions after multiplication with the artificial damping function $\exp(-ks^2)$.

STRUCTURE ANALYSES

All conformations that might be reasonably imagined for the *trans*-cyclooctene molecule, including twist and chair conformations, have C_2 symmetry. In addition to this obvious supposition, the following assumptions about the molecular structure were made: (a) equal $C_{sp^3}-C_{sp^3}$ bond lengths, (b) equal $C_{sp^3}-H$ bond lengths, (c) local C_{2v} symmetry for CH_2 groups, and (d) equal H—C—H valence angles.

When these assumptions were applied, the geometry of a molecular model of *trans*-cyclo-

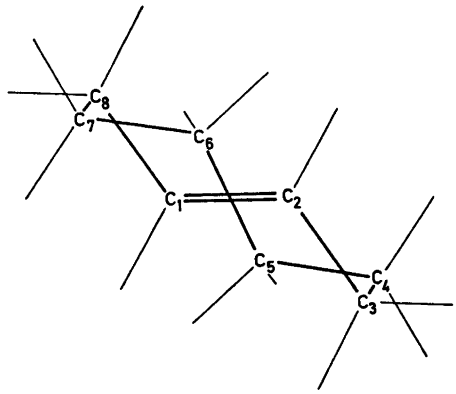


Fig. 1. trans-Cyclooctene. Molecular model which shows the numbering of the atoms.

octene could be completely defined by thirteen parameters. These were chosen as four bond lengths (C=C, C–C, –C–C–, –C–H), five valence angles ($\angle$C=C–C, $\angle C_2C_3C_4$, $\angle C_3C_4C_5$, $\angle$C=C–H, $\angle$HCH) and three dihedral angles (C–C=C–C, $C_1=C_2-C_3-C_4$, H–C=C–H). In addition the difference between the C_3-H_3 and C_1-H_1 distances were treated as a geometrical parameter.

The experimental radial distribution curve is shown in Fig. 3. The peaks at about 1.1 and 1.5 Å represent the carbon hydrogen and carbon carbon bond distances, respectively. The peak at about 2.15 Å contains contributions from non-bonded carbon hydrogen distances over one bond angle. The RD curve beyond 2.3 Å has seven more or less well resolved peaks that might be attributed to non-bonded carbon carbon distances across the ring. Since this region of the RD curve has so many well defined peaks, *trans*-cyclooctene must be a fairly rigid molecule. This hardly surprising observation is supported by the fact that it is possible to isolate optical isomers of the compound.

The structure of *trans*-cyclooctene was studied by least squares refinements of the molecular intensities. Both a twist and a chair model was studied in the early stages of the investigation. It became, however, soon evident that the twist model was clearly superior to the chair model. The following discussion does therefore only refer to the study of the twist conformation of the *trans*-cyclooctene molecule.

In order to calculate a theoretical molecular intensity function for use in the least squares refinements it is necessary to assign values for the vibrational amplitudes (u-values) of all internuclear distances. Starting parameters for all the vibrational amplitudes were obtained by calculating these values from an assumed force field, using Gwinn's method.[13,14] In these calculations Allinger's [5] force constants were applied.

Some of the vibrational amplitudes could be determined experimentally. It was for example possible to obtain experimental u-values for all bond distances except for the C_2-C_3 and the C_1-H_1 bonds. The u-value for the C_2-C_3 bond was determined indirectly by varying this parameter systematically while the geometrical parameters and some of the u-values were refined by least squares. The lowest weighted square error sum was obtained for $u(C_2-C_3)$ equal to 0.0525 Å. This result seems reasonable compared to the experimental value of 0.0521 Å for $u(C_2-C_4)$. The calculated u-values for C_2-C_3 and C_3-C_4 are 0.0492 and 0.0491 Å.

When the theoretical RD curve based on the calculated u-values was compared with the experimental RD curve it was clearly seen that the theoretical curve was more smeared out and had fewer distinct features than the experimental curve. This observation indicated that the calculated vibrational amplitudes for the nonbonded distances were on an average too large.

It is reasonable to assume that the relative magnitudes between the calculated vibrational amplitudes were approximately correct. This problem was therefore studied by systematically reducing all calculated vibrational amplitudes of nonbonded distances by a certain procentage. For each set of u-values constructed in this way ordinary least squares refinements were carried out. The weighted squared error sum and most of the standard deviations of the refined parameters had fairly deep minima for a reduction of the calculated vibrational amplitudes of about 15 %. The reduced calculated vibrational amplitudes for the nonbonded distances were therefore applied in the following. In this connection it should be mentioned that when the u-value for the

Table 1. Final structural parameters and dependent distances obtained for *trans*-cyclooctene. Distances (r_a) and root-mean-square amplitudes (u) in Å, angles in degrees. 2σ are twice the standard deviations.

Bond distances

	r_a	2σ	u	2σ	$u^{calc.}$
$C=C$	1.332	0.003	0.0337	0.0033	0.0408
C_2-C_3	1.499	0.008	(0.0525)		0.0492
C_3-C_4	1.549	0.002	0.0521	0.0022	0.0491 (av.)
C_3-H_3	1.110	0.002	0.0813	0.0023	0.0790
C_1-H_1	1.103		(0.0790)		0.0790

Valence angles and dihedral angles

		2σ			2σ
$\angle C=C-C$	121.9	0.8	$\omega(C-C=C-C)$	136.0	0.4
$\angle C_2C_3C_4$	104.6	0.4	$\omega(C_1=C_2-C_3-C_4)$	−91.1	0.8
$\angle C_3C_4C_5$	113.8	0.4	$\omega(C_2-C_3-C_4-C_5)$	54.2[a]	1.0
$\angle C_4C_5C_6$	118.8[a]	0.6	$\omega(C_3-C_4-C_5-C_6)$	−82.8[a]	0.6
$\angle C=C-H$	118.4	2.0	$\omega(C_4-C_5-C_6-C_7)$	111.8[a]	1.0
$\angle H-C-H$	106.8	1.7	$\omega(H-C=C-H)$	177.5	11.2

Nonbonded CC distances

	r_a	2σ	u^b		r_a	2σ	u^b
C_1C_7	2.412	0.005	0.0658	C_1C_4	3.193	0.006	0.0793
C_1C_3	2.477	0.005	0.0645	C_3C_6	3.450	0.006	0.0847
C_3C_5	2.595	0.005	0.0665	C_3C_8	3.752	0.008	0.0705
C_4C_6	2.668	0.007	0.0682	C_4C_7	3.784	0.007	0.0746
C_1C_6	2.868	0.007	0.0752	C_3C_7	4.052	0.005	0.0842
C_1C_5	3.100	0.010	0.0853				

[a] Dependent angle. [b] The applied vibrational amplitudes are those calculated from an assumed force field and then reduced by 15 %.

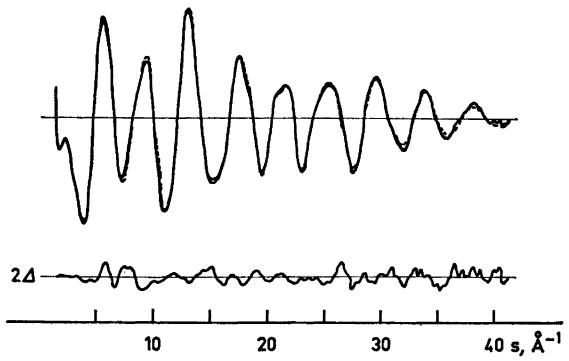


Fig. 2. trans-Cyclooctene. Experimental (——) and theoretical (– – –) molecular intensity functions. The scale of the difference curve is twice the scale of the intensities.

Table 2. trans-Cyclooctene. Results obtained in least-squares refinements of independent parameters when the H−C=C−H dihedral angle (ω_{HH}) is varied systematically. The standard deviations in brackets. Distances (r_a) and root-mean-square amplitudes (u) in Å, angles in degrees.

Parameter	ω_{HH} 160°	170°	180°	190°	200°	220°
r(C=C)	1.3322(18)	1.3322(17)	1.3320(17)	1.3318(17)	1.3318(17)	1.3318(18)
u(C=C)	0.0339(17)	0.0338(17)	0.0337(16)	0.0336(17)	0.0334(17)	0.0333(17)
r(C_2-C_3)	1.4989(39)	1.4992(38)	1.4988(36)	1.4977(36)	1.4967(35)	1.4954(35)
r(C_3-C_4)	1.5491(10)	1.5491(10)	1.5492(9)	1.5494(9)	1.5495(9)	1.5493(8)
u(C_3-C_4)	0.0518(12)	0.0521(12)	0.0521(11)	0.0519(11)	0.0517(11)	0.0512(11)
r(C−H)	1.1097(12)	1.1097(11)	1.1096(11)	1.1095(11)	1.1095(12)	1.1095(12)
u(C−H)	0.0810(12)	0.0811(12)	0.0813(11)	0.0814(12)	0.0814(12)	0.0812(13)
∠C=C−C	121.64(.46)	121.74(.44)	121.94(.42)	122.15(.42)	122.23(.43)	122.17(.44)
$\angle C_2C_3C_4$	104.60(.22)	104.60(.21)	104.63(.21)	104.72(.21)	104.88(.22)	105.39(.23)
$\angle C_3C_4C_5$	113.62(.20)	113.69(.20)	113.79(.20)	113.87(.20)	113.95(.21)	114.33(.25)
∠HCH	105.85(.92)	106.46(.88)	106.75(.86)	106.69(.85)	106.38(.85)	104.90(.87)
∠C=C−H	118.43(1.27)	118.71(1.13)	118.41(.99)	117.68(.90)	116.72(.83)	115.06(.80)
ω(C−C=C−C)	136.45(.43)	136.25(.43)	136.03(.44)	135.98(.47)	136.23(.49)	136.97(.48)
ω(C=C−C−C)	−90.83(.40)	−90.92(.38)	−91.09(.37)	−91.36(.38)	−91.75(.40)	−92.93(.47)
$\sum_i w_i \Delta_i^2$	8.04	7.56	7.47	7.69	7.98	8.42

C_3C_7 distance, which appears as a fairly well isolated peak on the RD curve, is refined in least squares a value of 0.0840 Å is obtained, while 0.0842 Å corresponds to the reduced calculated u-value.

It was possible to refine all geometrical parameters simultaneously in the least squares calculations, except for the difference between the C_3-H_3 and C_1-H_1 bonds. This parameter was determined by the combined trial and error/least squares procedure described above to be 0.007 Å. The final structural parameters obtained for *trans*-cyclooctene are listed in Table 1.

Even if the standard deviation of the H−C=C−H dihedral angle (ω_{HH}) is as large as 5.6° it might seem surprising that it is possible at all to determine this parameter in

Table 3. Correlation matrix for

C=C	C_2-C_3	C_3-C_4	∠C−C=C	$\angle C_2C_3C_4$	ω(1=2)	ω(2−3)	$\angle C_3C_4C_5$
b							
0.0017	0.0037	0.0009	0.445	0.212	0.450	0.395	0.204
1.000	0.565	−0.479	−0.623	−0.340	−0.447	0.390	0.236
	1.000	−0.836	−0.818	−0.589	−0.578	0.564	0.351
		1.000	0.670	0.432	0.454	−0.392	−0.420
				0.416	0.779	−0.616	−0.182
				1.000	0.450	−0.721	0.024
					1.000	−0.314	−0.292
						1.000	−0.384
							1.000

[a] Distances (r) and root-mean-square amplitudes (u) in Å, angles in degrees. [b] Standard deviations from

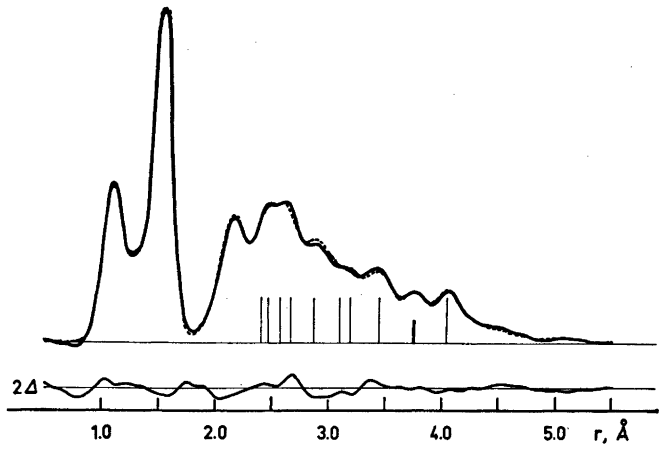


Fig. 3. trans-Cyclooctene. Experimental (——) and theoretical (— — —) radial distribution curves. Artificial damping constant $k = 0.0015$ Å^2. The scale of the difference curve is twice the scale of the RD-curves. The vertical bars indicate the positions of the CC nonbonded distances.

the least squares refinements. As this dihedral angle is very important for the discussion of the structural implications in connection with the CC double bond, the parameter was also studied by a different approach. ω_{HH} was varied systematically between 160 and 220° while the other geometrical parameters were refined by least squares. Coplanar sigma bonds at each of the unsaturated carbon atoms would correspond to ω_{HH} equal to 244° (see Fig. 4). The results are presented in Table 2, and they strongly support the observed value of 177.5° for ω_{HH}.

The theoretical intensity and radial distribution functions calculated from the final parameters for *trans*-cyclooctene (Table 1), are shown in Figs. 2 and 3 together with their experimental counterparts. Table 3 gives the correlation matrix for the parameters of the molecule.

parameters of *trans*-cyclooctene.[a]

C–H	∠HCH	∠C=C–H	$\omega(H_1C_1C_2H_2)$	u(C=C)	$u(C_3-C_4)$	u(C–H)
0.1131	0.875	1.025	5.651	0.0016	0.0011	0.0012
0.191	0.257	−0.046	−0.092	0.113	0.452	0.182
0.130	0.254	0.062	−0.129	0.207	0.860	0.065
−0.097	−0.093	−0.035	0.124	−0.159	−0.672	−0.009
−0.127	−0.354	−0.135	0.280	−0.178	−0.690	−0.082
−0.021	−0.365	−0.006	0.124	−0.116	−0.554	−0.096
−0.079	−0.317	−0.149	0.209	−0.126	−0.491	−0.073
0.045	0.382	0.003	−0.302	0.119	0.521	0.092
0.093	−0.089	−0.096	0.190	0.060	0.279	0.016
1.000	0.354	−0.085	−0.50	−0.068	0.100	0.037
	1.000	0.154	0.163	0.002	0.300	0.168
		1.000	0.014	0.049	−0.002	−0.065
			1.000	−0.043	−0.060	0.056
				1.000	0.038	−0.086
					1.000	0.157
						1.000

least squares.

DISCUSSION

In Table 4 the geometrical parameters determined for *trans*-cyclooctene in the present study are compared with other structural results obtained for this molecule. All the other data refer to theoretical calculations of structure parameters, except those in the last column which are Gavin's [10] electron diffraction data for the twist conformation. Gavin concludes that the molecule apparantly assumes a chair conformation but the only parameters that differ noticeably in his determination of the two conformations, are $\angle C_3C_4C_5$ (chair: 110.9°), $\angle C_4C_5C_6$ (115.3°) and $\omega(C-C=C-C)$ (157.7°).

The present study concludes that the *trans*-cyclooctene assumes a twist conformation. This observation is in agreement with most investigations concerning this molecule and its derivatives.[2-6] The only reported indication of another conformation is Gavin's [10] electron diffraction study. Ermer [8] suggested that possibly Gavin might have had a *trans*-cyclooctene sample which was prepared under non-equilibrium conditions and consisting primarily of the chair conformation. The barrier separating the chair and twist conformations will probably be sufficiently high (*ca.* 125 – 150 kJ mol^{-1}) to prevent rapid interconversion at room temperature. Even if this should be true, there are several inconsistencies between Gavin's results and those calculated for a chair model.[8] They will, however, not be discussed here.

The correspondence between the results obtained for *trans*-cyclooctene in the present study and those calculated by Allinger [6] and by Ermer [8] is remarkably good. The structural parameters show that the strain in the molecule is distributed among the valence angles, torsional angles and the double bond.

Allinger's [5] calculated structure for *trans*-cyclooctene was the first one to indicate a substantial rehybridization of the unsaturated carbon atoms in the ring. Locating the hydrogens in toward the ring, which constitutes a rehybridization of the olefinic carbons, will reduce the angle between the *p* orbitals and thus increase the overlap. The present experimental results confirm Allinger's predictions concerning the structural features in connection

Table 4. Comparison of results obtained for the structure of *trans*-cyclooctene. The numbers in brackets are standard deviations as resulting from least squares refinements. Distances are given in Å and angles in degrees.

	Present study	Allinger [5]	Ermer [7]	Buemi [8]	Gavin [9]
Bond distances					
C=C	1.3321(17)	1.3376	1.338	(1.331) [a]	1.363(8)
C_2-C_3	1.4990(38)	1.4990	1.504	(1.505) [a]	(1.538) [b]
C_3-C_4	1.5491(9)	1.5462	1.541	(1.533) [a]	1.538(3)
Csp^3-H	1.1096(11)	1.0998	1.107		1.107(2)
Valence angles					
$\angle C=C-C$	121.9(.4)	118.9	117.9	117.5	114.9(3.6)
$\angle C_2C_3C_4$	104.6(.2)	104.0	106.4	98.0	101.3(2.0)
$\angle C_3C_4C_5$	113.8(.2)	116.5	115.0	108.8	115.9(2.5)
$\angle C_4C_5C_6$	118.8(.3)	119.7	117.0	114.5	107.7(3.0)
$\angle C=C-H$	118.4(1.0)	120.5	121.9		
$\angle H-C-H$	106.75(.86)	104.5	108.0		114(2.0)
Dihedral angles					
C=C	136.0(.2)	145.0	138.0	−163.0	134.6(6.5)
C_2-C_3	−91.1(.4)	−92.6	−87.0	+64.0	
C_3-C_4	54.2(.5)	46.4	50.0	+32.7	
C_4-C_5	−82.8(.3)	−76.7	−80.6	−96.4	
C_5-C_6	111.8(.5)	110.9	114.8	+115.2	
$H-C=C-H$	177.5(5.6)	170.4	177.6		

[a] Assumed values. [b] Assumed equal to C_3-C_4.

with the double bond.

Evidence supporting the indicated rehybridization of the unsaturated carbon atoms is also found in the 0.8 D dipole moment of *trans*-cyclooctene,[15] as compared to the quite normal 0.4 D moment of the *cis* isomer.

Based on the parameters presented in Table 1, the angle between the p orbitals is found to be about 20°, which should result in a reduction in the overlap between these orbitals of about 12 %. It might therefore seem surprising that the C=C bond is found to be slightly, but not significantly shorter than in ethylene. If, however, the hybridization of the orbitals extending from the carbon atoms of the double bond is roughly evaluated, based on the observed valence angles, the orbitals making up the C_1-C_2 sigma bond are found to have about 36.4 % s-character ($sp^{1.74}$ orbitals). An estimate of the expected shortening in a σ bond when going from sp^2 to $sp^{1.74}$ orbitals is found to be somewhat larger than the expected increase because of non-parallell p-orbitals. The observed CC double bond length seems therefore to be reasonable.

The discussion above weighs heavily on the exact location of the hydrogen atoms at the C=C bond. Even when the large error of ω_{HH} (see Fig. 4) is taken into account the present study shows beyond doubt that the discussed hydrogen atoms really are bent in towards the ring. It would, however, be of great interest to obtain more experimental information concerning this important structural detail. Substituting methyl groups for the two hydrogens in question will probably not cause any drastic structural changes at the unsaturated carbon atoms as no serious additional steric strain is expected for the dimethyl derivative. An electron diffraction study of 1,8-dimethyl-*trans*-cyclooctene is therefore being planned.

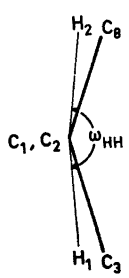


Fig. 4. trans-Cyclooctene. A projection of the dihedral angles at the carbon carbon double bond.

There are not many reported data that are suitable for comparison with the amplitudes of vibrations determined in this study. Gavin [10] has determined the u-values for the non-bonded CC distances to be 0.130 Å for $r < 3.45$ Å, 0.120 Å for $r = 3.45$ Å and 0.090 Å for $r > 3.45$ Å. These amplitudes are almost an order of magnitude larger than those found in the present study, and they are not compatible with the experimental radial distribution curve. His reported u-values for the bond distances are, however, not very different from those determined here.

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Crystal Structure of 3-Ethyladenine

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The crystal structure of 3-ethyladenine has been derived from 1760 reflections measured by counter methods. The value of R is 0.062 and the estimated standard deviations in distances between non-hydrogen atoms are 0.002 – 0.003 Å. The molecular structure is compared with that of 9-substituted purines. The bonds N1 – C6 – C5 – N7, as well as C5 – C4, are left relatively unaltered by the tautomerization, whereas those in the fragment N1 – C2 – N3 – C4 – N9 are changed by 0.02 – 0.03 Å. Hydrogen bonds N6 – H···N7 link the molecules together in dimers.

In the purines the proton may be attached to N1, N3, N7, or N9, corresponding to four fundamental tautomeric forms. Derivatives of all forms are known, and the structure has been established by X-ray analysis for a number of N7- and N9-tautomers. [1,2] For the other forms the geometries have had to be assumed.[3] In the present paper the structure of an N3-derivative, 3-ethyladenine, is reported. The compound is of considerable biological interest, as an appreciable proportion of alkyl groups is attached to adenine N3 by alkylation of DNA.[10] Different molecular mechanisms have been discussed for such alkyl-induced mutations.[11,12]

EXPERIMENTAL. STRUCTURE ANALYSIS

A sample of 3-ethyladenine ($C_7N_5H_9$, M. W. = 163.1) was kindly supplied by Dr. Peter Brookes of the Chester Beatty Research Institute. Unit cell dimensions are $a = 9.007(4)$ Å, $b = 7.139(3)$ Å, $c = 13.806(5)$ Å, and $\beta = 118.58(2)°$. The

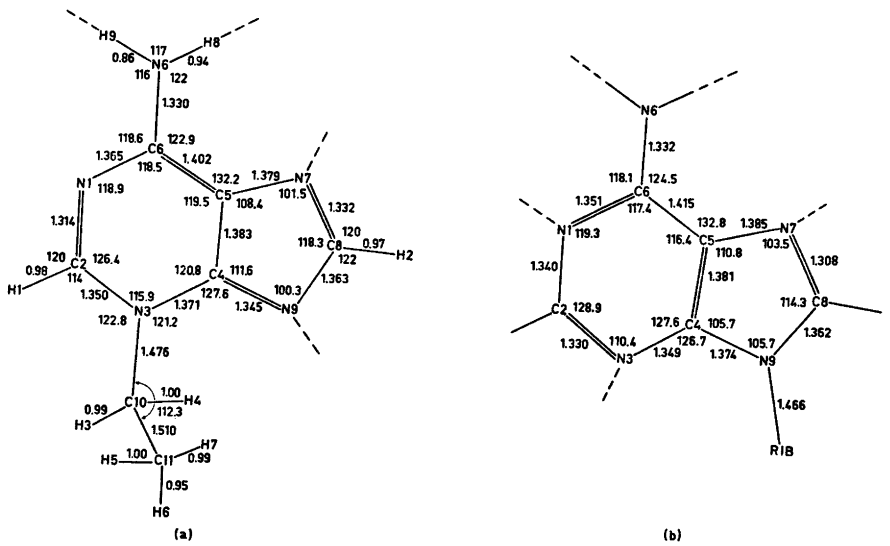


Fig. 1. Bond lengths and bond angles in 3-ethyl-adenine (a) and adenosine (b). E.s.d's in both compounds are about 0.003 Å and 0.2°.

Table 1. Positional and thermal parameters for non-hydrogen atoms (x10^5) with estimated standard deviations. The anisotropic temperature factor is exp $|-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)|$.

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C2	15270	55899	33804	1263	1722	451	578	646	456
	25	28	16	38	44	14	66	38	43
C4	31226	46146	51824	942	1322	401	−75	647	69
	22	25	14	31	37	13	56	33	36
C5	34856	29773	48022	886	1342	375	94	615	19
	21	25	14	30	36	12	55	31	35
C6	28035	26921	36636	933	1569	400	−67	665	−13
	22	26	15	31	40	12	58	33	38
C8	46675	29590	65321	1549	2039	382	1009	627	116
	27	30	17	40	50	14	76	38	45
C10	18663	77953	48622	1538	1268	648	75	1030	115
	27	27	17	40	40	16	64	43	42
C11	7242	76162	53770	2408	1830	1054	1124	2288	342
	34	36	22	57	54	25	91	65	62
N1	17884	40493	29629	1334	1843	421	552	643	169
	20	23	13	33	39	12	55	32	35
N3	21466	59826	44618	1074	1312	470	128	733	131
	19	21	12	29	32	11	48	30	32
N6	30726	11425	32357	1636	1919	385	696	819	−81
	22	23	12	35	38	11	59	33	35
N7	45132	18862	56998	1359	1758	405	771	698	178
	20	23	12	30	36	11	55	30	34
N9	38555	46423	62904	1511	1797	420	646	726	−89
	21	23	13	33	38	12	59	32	35

Table 2. Hydrogen atom coordinates (x10^3) and temperature factors.

Atom	x	y	z	B(Å^2)
H1	83	659	289	2.4
H2	532	252	729	3.8
H3	140	868	423	3.7
H4	298	838	538	4.7
H5	−46	736	480	7.7
H6	70	878	570	5.1
H7	111	662	594	5.0
H8	386	24	367	3.3
H9	282	120	255	3.3

space group is $P2_1/c$. Flotation gave a density of 1.38 g/cm^3, corresponding to four (calc. 3.97) molecules in the unit cell.

Intensity measurements were carried out on a crystal of dimensions $0.4\times0.2\times0.12$ mm using a Picker automatic diffractometer and Mo$K\alpha$ radiation ($\lambda=0.71069$ Å, graphite monochromator). The $\omega/2\theta$ scan technique (rate 2°/min) was employed and 1760 reflections with $2\theta<65°$ were recorded with measurable intensities, using a cut-off limit of 2σ. The intensities of two standard reflections showed no systematic variation during the data collection. Absorption and extinction corrections were not applied.

The structure was solved by direct methods and refined by full matrix least squares calculations to $R=0.062$ ($R_w=0.059$). The weighting scheme was based on standard deviations from counter statistics and an assumed 2 % fluctuation in diffractometer stability. Anisotropic temperature factors were applied to the non-hydrogen atoms, isotropic ones to the hydrogen atoms. Positional and thermal parameters are given in Tables 1 and 2 and the corresponding bond lengths and angles in Fig. 1. Estimated standard deviations lie in the range 0.0022–0.0031 Å for bond lengths and 0.15–0.18° for bond angles involving non-hydrogen atoms and are about ten times as large for those involving hydrogen atoms.

The programs used are described in Ref. 4. The atomic scattering factors were those of Hanson *et al.*[5] for non-hydrogen atoms and of Stewart *et al.*[6] for hydrogen atoms. A table of observed and calculated structure factors will be supplied by the authors on request.

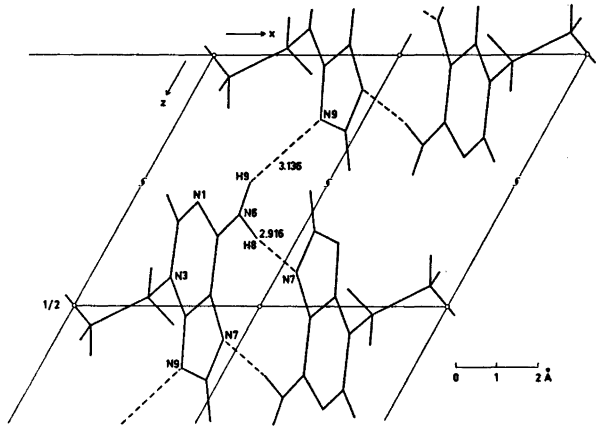


Fig. 2. The crystal structure viewed along *b*.

Table 3. Distances (Å) from the purine plane.

C2	0.002	N3	0.021
C4	−0.003	N7	0.011
C5	0.008	N9	−0.018
C6	0.003	C10	0.119
C8	−0.003	C11	−1.153
N1	−0.019	N6	−0.009

DISCUSSION

In Table 3 distances from the least squares plane through the nine ring atoms are given. It is seen that the purine ring is essentially, but not exactly, planar. The deviations cannot be described in terms of the bending about C4 – C5 characteristic for most purines.[7] The terminal methyl group points away from the plane.

The molecule is in its amino form as shown by the positional and thermal parameters of atoms H8 and H9. This is in agreement with spectroscopic measurements.[9]

For comparison the geometry of a representative N9-tautomer, adenosine,[2] is also shown in Fig. 1. The main difference between the N3- and the N9-tautomer occurs in the internal ring angles at atoms N3 and N9. This is consistent with earlier evidence that bonding of an extra-annular atom to a ring nitrogen atom causes the ring angle to increase.[1,8] In the present structure the ring angle at N3 is 5.5° greater and that at N9 5.4° smaller than in the N9-tautomer. Adjacent ring angles are correspondingly adjusted.

A surprising feature of the structure is that the bonds N1 – C6, C6 – C5, and C5 – C4 differ very little from the corresponding ones in the N9-tautomer, in spite of the fact that the principal valence bond structure is entirely different, as indicated in Fig. 1. The bond N1 – C6 is thus considerably shorter (1.365 Å) than C6 – C5 (1.402 Å) also in the N3-tautomer. Evidently the principal canonical structure does not "contribute" decisively to the electronic structure. A general comparison of the bond lengths in the two tautomers shows that the bonds N1 – C6 – C5 – N7, as well as C5 – C4, are not much altered by the tautomerization, whereas those in the fragment N1 – C2 – N3 – C4 – N9 all are considerably changed (by 0.020 – 0.029 Å). The same holds in a general way for the bond angles.

The geometry found in the present structure analysis differs considerably from that assumed in theoretical calculations on the N3-tautomer of purine.[3]

In the crystal (Fig. 2) the molecules are linked together in pairs across centres of symmetry by N6 – H8···N7 hydrogen bonds of length 2.916 Å. The adenine moieties of a pair are approximately coplanar. The pairs are connected by weak N6 – H9···N9 hydrogen bonds of length 3.136 Å. The methyl groups pack in columns along $(0,y,0)$ and $(0,y,1/2)$, the distances between neighbouring methyl carbon atoms in the columns being 3.61 and 3.93 Å.

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Mass Spectra of Nitro-substituted Aromatic Nitramines: a Novel *ortho*-Effect

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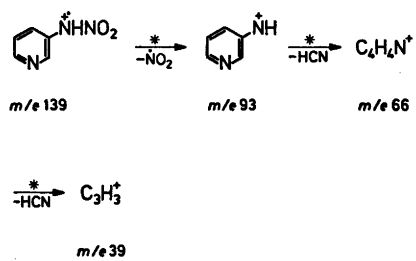
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The mass spectra of nitro-substituted aromatic nitramines have been studied; compounds of this kind carrying neighbouring nitro and nitramino functions exhibit a hitherto unrecognized mass spectrometric proximity-effect. An oxygen atom is transferred from the nitro to the nitramino group, leading after elimination of NO_2 to loss of two NO fragments. The possibility of observing similar interactions in other compounds bearing nitro groups next to nitrogen substituents has been investigated.

ortho-Effects or proximity-effects are frequently observed in the mass spectral decomposition of benzenoid compounds with neighbouring substituents.[1] One of the substituents that frequently take part in *ortho*-interactions is the *nitro* group,[2,3] which has been observed to interact with neighbouring groups in two distinct ways: (i) an oxygen atom of the nitro group may abstract a hydrogen atom from the *ortho* substituent, as is seen by the formation of abundant $[M-OH]^+$ ions in the decomposition of *o*-nitrotoluene [4,5] or *o*-nitroaniline;[6] or (ii) a nitro oxygen atom may migrate to the neighbouring function, as has been observed in the fragmentation of *o*-nitrotoluene,[5] *o*-nitroanisole,[7] *o*-nitrohydrazones,[8–10] *o*-nitrostilbenes,[8,11] *o*-nitrothiobenzoic esters,[12] and *o*-nitrobenzoic amides.[13]*

In connection with studies of the photochemical reactions of aromatic nitramines [14] we have examined also the mass spectral reactions of many of these compounds to see if a correlation between the mass spectral and the photochemical behaviour should exist. The electron impact induced decomposition of a number of simple nitraminopyridines has been reported recently by Wilson and collaborators,[15] and we have been able to confirm that also more highly substituted compounds of this class decompose by the fragmentation reactions described.[15] The inclusion of substituents such as chloro or methyl on the aromatic ring does not change the fragmentation pattern appreciably, but a few additional decomposition reactions are introduced in a quite predictable way. The reactions of *N*-alkyl nitramines (see Fig. 1b) are significantly influenced by the presence of the alkyl group, but the basic decomposition reactions of aromatic nitramines remain unchanged.

The initial reaction of the nitramine molecular ion is elimination of an NO_2 radical, with formation (formally) of a protonated nitrene (see Scheme 1); similarly, the initial photochemical



Scheme 1.

* Schwarz and Bohlmann [13] report that *o*-nitrobenzpiperidide eliminates a piperidinoxyl radical upon electron impact; similar reactions have been observed by the present authors to take place in the fragmentation of the *N*-ethyl- and *N*,*N*-diethylamides of *o*-nitrobenzoic acid.

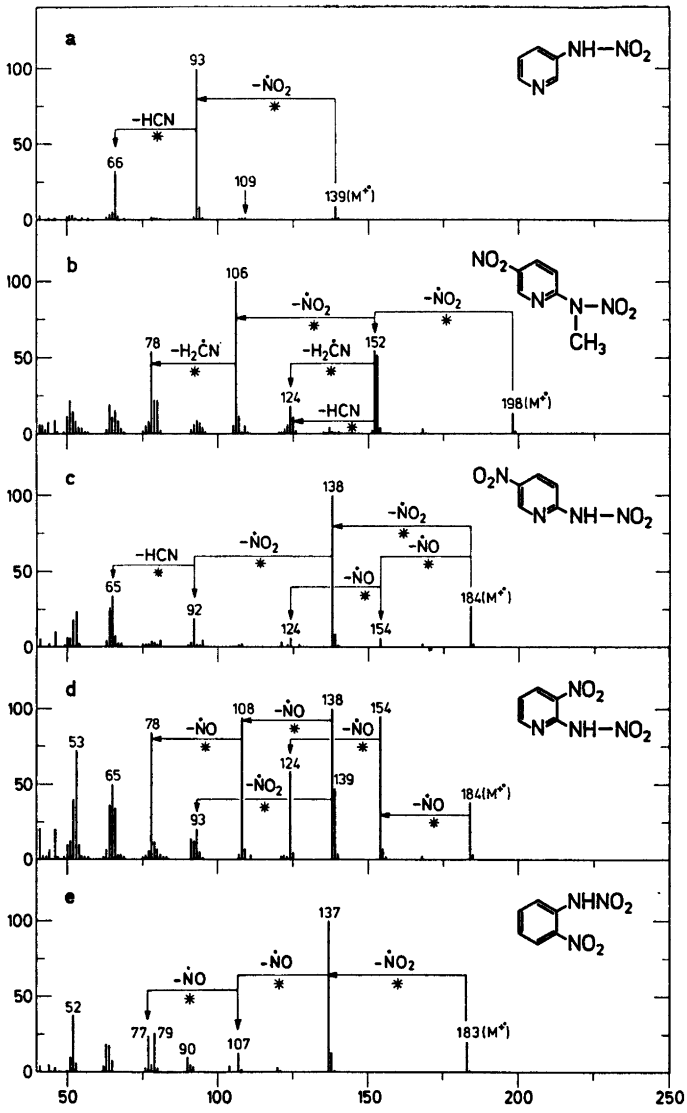
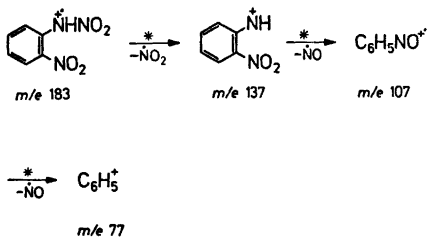


Fig. 1. Mass spectra (at 70 eV) of (a) 3-nitraminopyridine; (b) *N*-methyl-5-nitro-2-nitraminopyridine; (c) 5-nitro-2-nitraminopyridine; (d) 3-nitro-2-nitraminopyridine; (e) 2-nitro-*N*-nitroaniline.

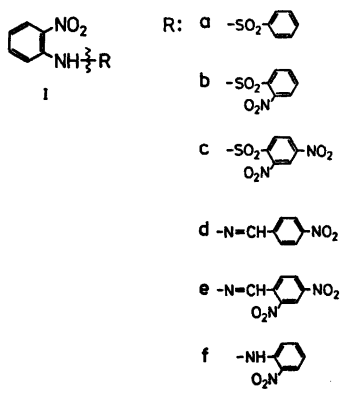
reaction is NO_2 expulsion.[14] In most cases the $[M-NO_2]^+$ ion then decomposes by elimination of another substituent, if present, or by cleavage of the aromatic nucleus (see Fig. 1a). For nitro-substituted nitramines a similar situation exists (Fig. 1c), except when the nitro and nitramino substituents are situated next to each other. In these cases the reactions subsequent to NO_2 loss take a different course (see Scheme 2 and Figs. 1d and 1e). Two NO fragments are eliminated in succession, showing that one of the oxygen atoms of the nitro group has become bonded to the remaining nitrogen atom of the nitramino function. The proximity of the nitro and nitramino substituents has consequences also for other decomposition reactions. Comparison of the spectra of the 5- and 3-nitro-2-nitraminopyridines (Figs. 1c and 1d)



Scheme 2.

shows that NO loss from the molecular ion is much more pronounced in the latter case, where it is followed by loss of another NO fragment. Whether the amide nitrogen atom is lost also in these reactions is not clear; a definitive decision would require isotopic (^{15}N) labelling. A low intensity peak in the spectrum of 2-nitro-*N*-nitroaniline (Fig. 1e) shows that an OH· radical may be lost from the $[M-NO_2]^+$ ion, probably also as a result of an *ortho*-interaction similar to that observed in *o*-nitroaniline.[6]

In an attempt to investigate whether the *ortho*-interaction uncovered in the present work is common to compounds that under electron impact generate ions with nitro groups *ortho* to aromatic -NH+ groups (protonated nitrenes) we have examined the mass spectra of the compounds shown in Scheme 3. Fission of the bond indicated in (I) with a wavy line occurs in the decomposition of the sulfonamides (Ia–c) and hydrazones (Id,e), but always with charge retention in R; 2,2′-dinitrohydrazobenzene (If), likewise does not give an *m/e* 137 peak (*cf.* Scheme 2) but decomposes instead by elimination of a water molecule and formation of nitrophenyldiazonium ions. It is thus not possible yet to assess the general nature of the *ortho*-effect described above.



Scheme 3.

Thermal effects. Wilson and coworkers [15] noted that the nitramines examined by them suffered decomposition if the heated inlet of the mass spectrometer was used for introduction of the samples into the ion source rather than the direct inlet (solid probe). We have observed that not even use of the direct inlet will completely eliminate reactions prior to ionization. In most cases peaks originating from an "impurity" with an apparent molecular ion of mass 45 a.m.u. less than that of the nitramine appeared in the spectra. This contaminant is presumably the parent amine generated by wall reactions of the nitramine. The intensity of the impurity peaks was found to increase with the temperature of the ion source and with the length of time elapsing from introduction of the compound to the spectrum was actually run. Spectra of the pure parent amines [16] support the suggested identity of the contaminant.

$[M+1]^+$ peaks were encountered in the spectra of several compounds; the additional hydrogen atom is "picked up" at the source walls rather than by bimolecular reactions of two nitramine molecules, since the [M + 1] peaks were replaced by [M + 2] peaks upon saturating the spectrometer with D_2O.

EXPERIMENTAL

The mass spectra were recorded on an AEI MS-902 mass spectrometer by Ms. E. Wolff-Jensen. The ion source temperature was kept as low as possible, typically at 100 °C.

The nitraminopyridines examined were prepared as given in the literature.[17] *o*-Nitro-*N*-nitroaniline was prepared by dehydration of *o*-nitroanilinium nitrate according to Hoff.[18] The sulfonamides (Ia–c) were prepared from *o*-nitroaniline and the appropriate sulfonyl chloride, and the hydrazones (Id,e) were prepared by condensation of *o*-nitrophenylhydrazine with the appropriate benzaldehyde. 2,2′-Dinitrohydrazobenzene was obtained from the corresponding azobenzene by reduction with ammonium hydrosulfide as described by Green and Rowe.[19]

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Structural Studies of Metabolic Products of Dopamine. II.* Crystal and Molecular Structure of the 1:1 Complex of 6-Hydroxydopamine Hydrochloride and its Oxidized, *p*-Quinonoid, Form

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The crystal structure of a red oxidation product of 6-hydroxydopamine (3,4,6-trihydroxyphenylethylamine) hydrochloride has been determined by X-ray methods. The red crystals were found to contain the hydrochlorides of both 6-hydroxydopamine and its oxidized, *p*-quinonoid, form. The two species form a 1:1 molecular complex in the crystals. The crystals are monoclinic, space group $P2_1/n$ with $a = 6.815(1)$, $b = 13.108(3)$, $c = 20.365(6)$ Å and $\beta = 95.43(2)°$. The structural parameters are refined to a conventional R-factor of 0.065. Standard deviations in the bond lengths are 0.007 – 0.01 Å and in bond angles 0.5 – 0.7°. The component molecules of the complex are stacked alternately along the *a*-axis. The crystal structure is stabilized through an extensive hydrogen bond system.

6-Hydroxydopamine, a natural oxidation product of dopamine, is known to be a chemical sympathectomic agent.[1] The mechanism of its activity has been extensively discussed and recent theories center on the ease of oxidation of this compound. The products formed during oxidation are considered to be possible neurotoxic agents as well as important intermediates in melanin formation.[1–7]

Investigations have shown that 6-hydroxydopamine readily autoxidizes to form a quinone and hydrogen peroxide.[7] The quinone, which theoretically can have either *o*- or *p*-quinonoid structure, may undergo ring closure to yield indoline and indole derivatives. The mechanism of this intramolecular cyclization is not established and different reaction products are reported.[1–4]

During recrystallization of 6-hydroxydopamine, red crystals appeared.[8] It was assumed that an oxidation had taken place and that the red compound formed was an intermediate in the formation of melanin. Regarding the discussion on the oxidation path of 6-hydroxydopamine, the structure determination of this oxidation product was considered to be of interest.

EXPERIMENTAL

The red crystals formed during recrystallization of 6-hydroxydopamine [8] were mostly found to be twinned, but single crystals could be obtained and were used in the present study.

Oscillation and Weissenberg photographs indicated monoclinic symmetry and the space group was found to be $P2_1/n$.

Unit cell parameters were determined and three-dimensional intensity data collected on a SYNTEX $P\bar{1}$ diffractometer using graphite monochromated Mo$K\alpha$ radiation. Unit cell parameters were obtained from a least-squares treatment of angular measurements of fifteen reflections. Diffraction data for 2364 reflections with $2\theta < 65°$ were recorded using the $\omega - 2\theta$ scanning mode; the scan range was 1.6° and the scan speed varied from 2 to 8° min^{-1} depending on the peak intensity. Background counts were measured at each end of the scan range, the ratio of the summarized background counting time to scan time being 0.7. The 1641 reflections having net intensities greater than

* The first publication in this series appeared in *Acta Chem. Scand. 26* (1972) 2670.

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations for non-hydrogen atoms ($\times 10^4$). The temperature factor is given by $\exp-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}hk + B_{13}hl + B_{23}kl)$.

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl1	7656(3)	2448(1)	890(1)	300(7)	32(1)	19(1)	−1(6)	29(3)	9(1)
Cl2	2469(4)	118(1)	685(1)	325(8)	46(1)	22(1)	−6(6)	24(3)	0(2)
O1A	3526(9)	−414(3)	3991(2)	405(21)	31(3)	22(2)	−52(14)	62(9)	−11(3)
O2A	3824(8)	1081(3)	4857(2)	368(19)	47(3)	13(1)	37(14)	29(8)	−3(3)
O3A	2218(9)	3532(4)	3127(2)	309(19)	63(4)	18(1)	29(15)	7(9)	6(4)
NA	2800(10)	2479(4)	1089(2)	348(22)	44(4)	16(2)	46(20)	48(10)	11(5)
C1A	2410(12)	1768(6)	2888(3)	161(24)	54(6)	12(2)	1(19)	35(11)	8(6)
C2A	2728(12)	772(6)	3103(3)	202(26)	60(6)	13(2)	−33(21)	29(11)	1(6)
C3A	3230(12)	566(5)	3769(3)	229(27)	31(5)	19(2)	−9(19)	36(12)	9(5)
C4A	3325(11)	1355(5)	4217(3)	125(22)	52(6)	10(2)	9(18)	21(10)	10(5)
C5A	3062(11)	2355(6)	4017(3)	179(22)	48(5)	12(2)	−31(20)	10(10)	1(5)
C6A	2554(10)	2535(6)	3351(3)	161(22)	42(5)	20(2)	24(21)	27(11)	12(6)
C7A	1785(12)	1984(6)	2166(3)	194(24)	71(6)	14(2)	−2(22)	24(11)	11(5)
C8A	3470(11)	2354(6)	1813(3)	224(24)	59(6)	15(2)	−45(22)	7(11)	9(5)
O1B	7412(8)	805(4)	2328(2)	350(20)	48(3)	10(1)	0(15)	5(8)	−10(3)
O2B	7738(9)	−838(4)	3092(2)	429(22)	31(3)	16(1)	6(15)	25(9)	−11(3)
O3B	8560(9)	1428(3)	4900(2)	445(21)	42(3)	11(1)	2(14)	5(9)	−3(3)
NB	7631(10)	5043(4)	4062(3)	377(25)	30(4)	23(2)	4(19)	65(11)	6(5)
C1B	7947(10)	2169(5)	3847(3)	122(21)	27(4)	14(2)	6(16)	29(10)	1(5)
C2B	7675(11)	1985(5)	3198(3)	195(24)	26(4)	17(2)	0(18)	27(11)	9(5)
C3B	7651(11)	954(5)	2923(3)	151(24)	44(5)	16(2)	12(18)	22(11)	−3(5)
C4B	7896(11)	72(5)	3390(3)	178(22)	38(5)	17(2)	33(20)	28(11)	9(5)
C5B	8302(11)	247(5)	4040(3)	202(24)	33(5)	14(2)	−44(19)	19(11)	4(5)
C6B	8302(12)	1270(5)	4298(3)	187(25)	36(5)	15(2)	−27(18)	18(11)	1(5)
C7B	7969(11)	3192(5)	4166(3)	185(25)	34(4)	14(2)	−5(17)	14(11)	−4(5)
C8B	7474(12)	4059(5)	3697(3)	214(25)	26(4)	20(2)	12(18)	25(12)	−3(5)

Table 2. Fractional atomic coordinates ($\times 10^3$) with estimated standard deviations for hydrogen atoms. The isotropic thermal parameter is $B = 4.2$ Å^2.

Atom	x	y	z
H2A	259(10)	13(5)	281(3)
H5A	328(11)	294(5)	439(3)
H71A	71(12)	248(6)	208(3)
H72A	118(12)	138(5)	198(3)
H81A	474(11)	180(5)	184(3)
H82A	400(12)	297(5)	191(3)
HO1A	378(13)	−44(6)	433(4)
HO2A	361(13)	153(5)	507(4)
HO3A	254(13)	394(6)	346(4)
HN1A	403(11)	265(6)	93(4)
HN2A	228(12)	316(6)	101(3)
HN3A	263(11)	170(5)	91(3)
H2B	743(12)	249(6)	291(3)
H5B	849(11)	−34(5)	441(3)
H71B	927(12)	324(5)	443(3)
H72B	692(12)	318(5)	446(3)
H81B	600(11)	404(6)	352(3)
H82B	862(11)	409(5)	335(3)
HO2B	794(13)	−118(6)	339(4)
HN1B	937(12)	507(6)	424(3)
HN2B	677(12)	499(6)	438(3)
HN3B	765(12)	554(6)	388(4)

$3\sigma(I)$ were considered to be observed, the remaining reflections were excluded from the further calculations.

The intensity data were corrected for Lorentz and polarization effects, the computer programs employed during this as well as the subsequent calculations are described in Ref. 9. Atomic form factors used were those of Doyle and Turner [10] for the chlorine ion, oxygen, nitrogen, and carbon and of Stewart *et al.*[11] for hydrogen.

CRYSTAL DATA

$C_8H_{12}O_3NCl.C_8H_{10}O_3NCl$
Space group $P2_1/n$, monoclinic
$a = 6.815(1)$ Å; $b = 13.108(3)$ Å; $c = 20.365(6)$ Å;
$\beta = 95.43(2)°$
$V = 1811.0$ Å^3; F.W. = 409.27; $F(000) = 856$;
$Z = 4$
D_{obs} (flotation) = 1.50 g cm^{-3};
$D_{calc} = 1.502$ g cm^{-3}

STRUCTURE DETERMINATION

The coordinates of the two chlorine ions were determined from a sharpened Patterson func-

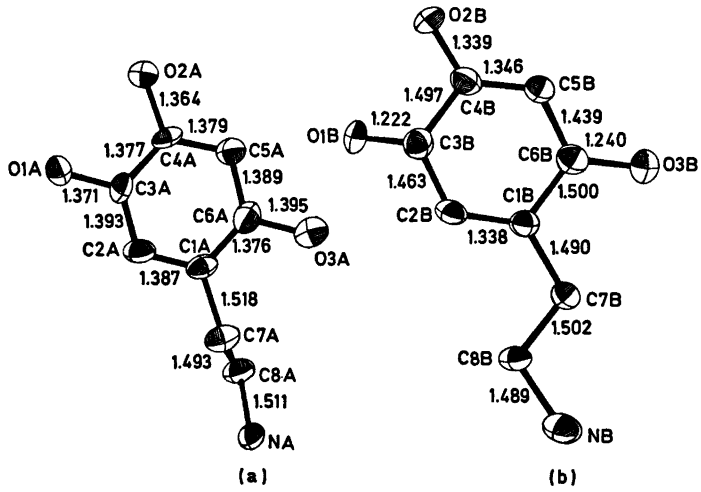


Fig. 1. Bond lengths and 50 % probability ellipsoids. (*a*) The 6-hydroxydopammonium ion. (*b*) The *p*-quinonoid ion. (The drawings were prepared using the computer program ORTEP.[18])

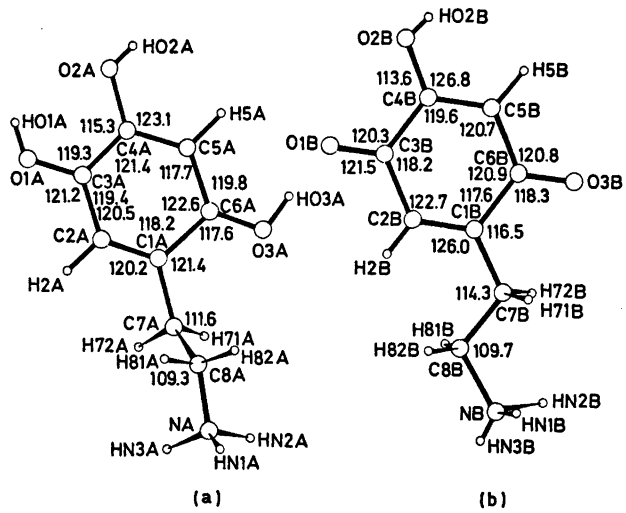


Fig. 2. Bond angles. (*a*) The 6-hydroxydopammonium ion. (*b*) The *p*-quinonoid ion.

tion and subsequent weighted Fourier maps [12] indicated the positions of the remaining non-hydrogen atoms. After a few cycles of least-squares refinement, anisotropic thermal parameters for the heavy atoms were introduced. Calculations of coordinates of the hydrogen atoms were based on stereochemical considerations. Further least-squares refinements with a common isotropic thermal parameter for the hydrogen atoms yielded a final conventional *R*-factor of 0.065 and a weighted *R*-factor of 0.056. The final parameters for non-hydrogen atoms are listed in Table 1 and for hydrogen atoms in Table 2.

An analysis of the molecular motions in terms of rigid body vibrations indicated that the cations could not be regarded as rigid units and the bond lengths were not corrected for thermal

Table 3. Deviations in Å of individual atoms from least squares planes.

(1) Through the benzene ring atoms.

C1A	0.002	O1A	−0.009
C2A	−0.005	O2A	−0.001
C3A	0.014	O3A	−0.010
C4A	−0.020	C7A	−0.073
C5A	0.017		
C6A	0.008		

(2) Through the quinone ring atoms.

C1B	0.020	O1B	0.054
C2B	0.009	O2B	−0.101
C3B	0.019	O3B	−0.020
C4B	−0.037	C7B	−0.048
C5B	0.025	C8B	−0.162
C6B	0.004	NB	−0.117

vibration effects. The ellipsoids of thermal motion of the two organic cations are illustrated in Fig. 1. Bond lengths and angles are presented in Fig. 1 and 2, respectively.

The structure factor list may be obtained from the authors upon request.

DESCRIPTION AND DISCUSSION OF THE STRUCTURE

The crystals were found to consist of a 1:1 molecular complex of 6-hydroxydopamine and its oxidized, *p*-quinonoid, form.

The 6-hydroxydopammonium ion. The structure of the 6-hydroxydopammonium ion corresponds closely to that of the pure compound [8] in bond lengths, angles and conformation. The hydroxyhydroquinone part of the molecule is nearly planar (*cf.* Table 3) and the ethylamine side chain is maximally extended. The dihedral angle C1A−C7A−C8A−NA is −175.7° and the angle between the benzene plane and the plane of the ethyl part of the side chain is 80.0°. This is the conformation found in most crystal structures of phenylethylamines.[13]

The p-*quinonoid ion.* The oxidized ion has a *p*-quinonoid structure with C=C and C=O bond lengths corresponding to those in *p*-benzoquinone.[14] The C−C single bond lengths in the ring are in the range from 1.439 Å to 1.500 Å but such variations are also found in other unsymmetrical substituted derivatives of *p*-benzoquinone.[14] The ethylamine side chain of the oxidized ion is fully extended but in contrast to the situation in the 6-hydroxydopammonium ion the side chain and the ring are almost coplanar. The deviation of the individual atoms from the least-squares plane through the quinone ring atoms are given in Table 3. The dihedral angle C1B−C7B−C8B−NB is 177.4° and C2B−C1B−C7B−C8B is −5.1°. Among the crystal structures of the phenylethylamines a similar coplanarity of the benzene ring and side chain is only found in (−)-adrenaline hydrogen (+)-tartrate.[13] In the latter study this exceptional arrangement of the ring and side chain may be explained by the dense packing of the structure and the unusually strong and elaborate hydrogen bond system in the crystals. A similar explanation for the unexpected planarity of the oxidized ion in the present investigation has not been found. The molecular packing and hydrogen bond system correspond closely to that found in the crystal structure of 5-hydroxydopamine hydrochloride,[15] which has the conformation usually encountered among related compounds. The bond lengths in the side chain of the *p*-

Table 4.
a. Hydrogen bonded interactions X−H···Y.

X	Y	X···Y	H···Y	X−H···Y
NA	Cl1	3.372	2.49	153
NA	Cl2	3.204	2.13	172
NA	O1A(*a*)	2.904	1.95	170
O1A	O2A	2.633	2.28	114
O2A	Cl1(*c*)	3.014	2.28	164
O3A	Cl2(*a*)	3.181	2.34	165
NB	Cl1(*a*)	3.161	2.55	140
NB	Cl2(*a*)	3.332	2.15	167
NB	Cl2(*c*)	3.324	2.65	130
O2B	Cl1(*a*)	3.086	2.37	156

b. Other short contacts.

	(Å)	
NA···O3B(*c*)	2.902	
NB···O1B(*a*)	3.000	$a=-x+\frac{1}{2},y+\frac{1}{2},-z+\frac{1}{2}$
O1A···O2A(*b*)	2.953	$b=-x,-y,-z$
C8B···O1B(*a*)	3.103	$c=x+\frac{1}{2},-y+\frac{1}{2},z+\frac{1}{2}$
C4B···O1A	3.386	
C5B···O1A	3.362	
C6B···O2A	3.366	
C7A···O1B	3.402	
C1A···C2B	3.358	

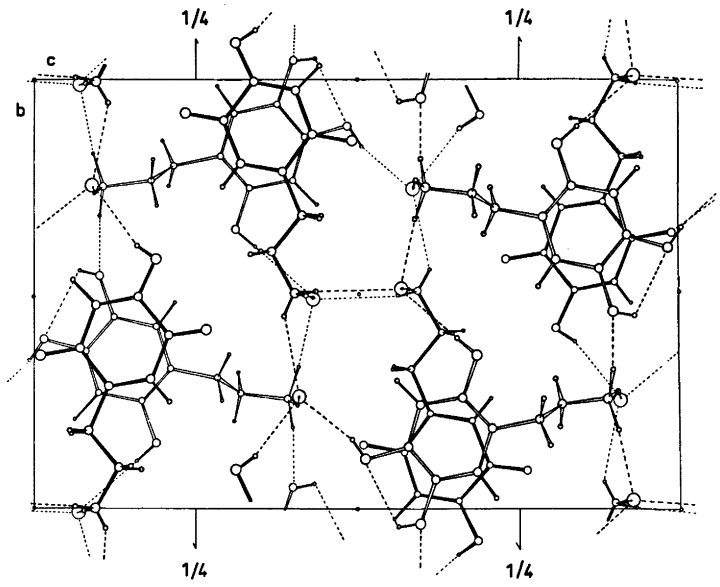


Fig. 3. The crystal structure as seen down the *a*-axis. The *p*-quinonoid ions are drawn with the marked bonds.

quinonoid ion are not significantly different from the normal values for these types of bonds.

Hydrogen bonding and molecular packing. The arrangement of the molecules in the crystals is illustrated in Fig. 3. The two components of the complex are stacked along the *a*-axis, the angle between the ring planes is 3.8°. The compound is thus possessing the characteristic geometry of crystalline π-molecular complexes with mixed stacks of almost parallel molecules of electron donating and accepting ability.[16] The distances from the center of the phenyl ring to the center of the two adjacent quinone rings within the stack are 3.49 and 3.42 Å, respectively. This separation of the component ring systems is larger than usually found for π-molecular complexes.[16] However, the component molecules are closer together than found in related aromatic one-component systems with a similar molecular arrangement. The distance between the centers of the benzene rings in 5-hydroxydopamine hydrochloride, also stacked along a crystallographic axis, is 3.94 Å.[15]

Crystalline 6-hydroxydopamine hydrochloride is colourless and the colour of the *p*-quinonoid form of 6-hydroxydopamine is reported to be yellow.[6,17] Thus, the interactions between almost parallel phenol and quinone rings in the present structure may possibly be the reason for the red colour of the crystals.

Short contacts assumed to represent hydrogen bonds in the crystals are presented in Table 4*a* and also in Fig. 3. In addition to these interactions there are N···O, O···O and C···O contacts in the range 2.9 – 3.1 Å (*cf.* Table 4*b*). These are not believed to represent hydrogen bonds because of unfavourable X – H···Y arrangements. Each 6-hydroxydopammonium ion is thus considered to be linked to four chlorine ions and two other 6-hydroxydopammonium ions through hydrogen bonds of the types N – H···Cl, N – H···O and O – H···Cl. Each oxidized ion appears to be connected to four chlorine ions by N – H···Cl and O – H···Cl hydrogen bonds. In the 6-hydroxydopammonium ion one phenolic hydrogen atom is situated less than 2.3 Å from the oxygen atom in *o*-position. This arrangement, which is not found in the pure compound, may indicate the presence of an intramolecular O – H···O hydrogen bond. The two component cations of the complex are not connected by hydrogen bonds and the strongest interactions between these cations are those between the rings stacked along the *a*-axis. The cations and

chlorine ions within a stack are linked by hydrogen bonds of the N–H···Cl type.

CHEMICAL IMPLICATIONS

The present investigation shows that the first oxidation product of 6-hydroxydopamine hydrochloride is the uncyclized *p*-quinone. This conclusion has been reached by other authors [1,3,5] on the basis of electrochemical and spectroscopic evidence. On standing in solution the *p*-quinone undergoes further reaction to yield melanin-like polymers by reaction paths that have been much discussed.[1–4] The formation of a complex of low solubility between the hydrochlorides of 6-hydroxydopamine and the *p*-quinone probably stabilizes the *p*-quinone and thus delays a further reaction.

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Tobacco Chemistry. 28. Structure and Synthesis of Drim-8-en-7-one, a New Tobacco Constituent

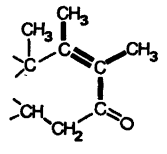
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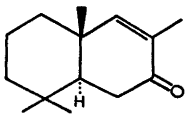
A new sesquiterpene ketone of the drimane series was isolated from Greek *Nicotiana tabacum* L. and shown to be drim-8-en-7-one** (*10*). This structure was subsequently confirmed synthetically using drimenol (*3*) as starting material.

Research on tobacco flavour has revealed that the material released as head-space volatiles or produced during the smoking-process comprises numerous constituents [1–4] of which a large number has been characterized as degradation products of terpenoids of either the carotenoid or the thunbergane-type.[5–10] Tobacco is also known to produce diterpenoids of the labdane-class [11–15] and earlier this year we reported on the structures and syntheses of five new natural products [16–18] which, together with norambreinolide [19] and six other very recently published tobacco constituents,[20] can be viewed as either being genuine sesquiterpenoids of the drimane group, or products derived from labdanes. We now wish to discuss the identification and confirmative synthesis of another bicyclic, terpenoid tobacco constituent which can be allotted to the drimane/labdane-derived category.

The compound was isolated in minute quantities from a medium-polar, neutral fraction (B4) [10] previously obtained from an ether-extract of sun-cured Greek *Nicotiana tabacum* L. and the structure gleaned exclusively from spectral properties. The presence of a conjugated, disubstituted, endocyclic enone system was indicated from UV [21] and IR absorption (248.5 nm, 1612 and 1661 cm^{-1}). Two mutually spin-spin coupled three-proton resonances [22] (homoallylic coupling [23] *ca.* 1 Hz) at δ 1.74 (α-CH_3) and δ 1.84 (β-CH_3) verified this and showed the two substituents to be methyls. With no evidence in the NMR spectrum of oxygen substituents or unsaturations apart from the enone moiety, and the elemental composition $C_{15}H_{24}O$ for the ion at highest mass, a bicyclic, conjugated ketone was implied which is in accord with the polarity of the fraction under investigation. The appearance of a two-proton multiplet in the δ 2.17 – 2.66 region as the AB-part of an ABC-system revealed that a methylene group, in turn linked to a methine group, was attached to the carbonyl group. These data and the presence of three methyl singlets at δ 0.88, 0.92 and 1.09, the last one assigned to a methyl group on a vinylic, quaternary carbon atom, implied the partial structure *1*. Since this partial structure contains the tail-end of three head-to-tail-linked isoprene units, implied by the C_{15}-structure comprising five methyl groups, the complete structure, *10*, disregarding stereochemistry, may be advanced for the new compound.



1



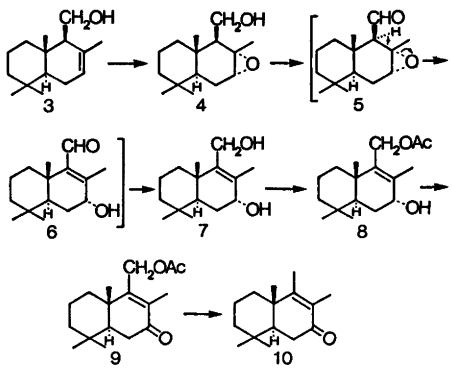
2

This was verified by comparison of its spectral data with those of (4a*R*,8a*S*)-4a,5,6,7,8,8a-hexahydro-3,4a,8,8-tetramethylnaphthalen-2(1H)-one (*2*, 11-nordrim-8-en-7-one) which recently

** Nomenclature and stereochemistry as defined in Ref. 25.

has been identified by us as a constituent of the same tobacco.[16] Thus the NMR and mass spectra of the two compounds were quite similar except for features expected on the basis of structural differences, *i.e.* the new tobacco isolate exhibited the molecular and base peaks fourteen mass units above those of *2*, and displayed an additional vinylic methyl signal (δ 1.84), but lacked the olefinic resonance of the 11-nor derivative *2*.

The assigned structure, drim-8-en-7-one (*10*), was unequivocally established by synthesis using drimenol [24,25] as starting material, see Scheme 1. 7α,8-Epoxydriman-11-ol (*4*),[25] prepared by epoxidation of drimenyl acetate, was converted in good yield to drim-8-en-7α-11-diol (*7*) in three steps. These were performed *in situ* and involved oxidation to the epoxyaldehyde *5* followed by base-catalyzed isomerization to the conjugated aldehyde *6*, and hydride-reduction of this aldehyde *7*. On attempted characterization the intermediates *5* and *6* proved unstable. Acetylation of the diol *7* at reduced temperature furnished 11-acetoxydrim-8-en-7α-ol (*8*) as the major product which on oxidation with Jones' reagent [26] yielded the keto-ester *9*. Hydrogenolysis of the conjugated keto-ester *9* applying zinc in acetic acid [27] gave drim-8-en-7-one (*10*) in excellent yield and this product was found identical to the tobacco constituent. Since the absolute configuration of drimenol (*3*) has previously been established [25] by correlation to oleanolic [28] and abietic acid [29] and, since synthetic and natural *10* exhibited rotatory powers of similar magnitude and the same sign, it follows that the absolute configuration of the natural ketone *10* at C-5 and C-10 are as shown in formula *10* (both centres have *S*-configuration).



Scheme 1.

The final product and the intermediates *3* – *9* are structurally related to a large number of oxygenated *trans*-decalins which exhibit a typical ambra odour.[30,31] However, the present *trans*-decalins are devoid of any significant smell thus implying that they do not meet the spacial requirements of the receptor sites of the human olfactory system.

We have previously isolated five structurally related compounds, (4a*R*,8a*S*)-4a,5,6,7,8,8a-hexahydro-3,4a,8,8-tetramethylnaphthalen-2(1*H*)-one (*2*),[16] (1'*S*,6'*R*)-4-(2',2',6'-trimethyl-6'-vinylcyclohexyl)-2-butanone (*13*),[16]* 14,15-bisnor-8-hydroxylabd-11*E*-en-13-one (*14*),[17] driman-8-ol (*15*),[18] and driman-8,11-diol (*16*),[18] from the same tobacco. A question of considerable interest is if these and the new compound (*10*), whose structures have been confirmed by syntheses from drimenol (*3*), all are degradation products of labdane diterpenoids or if only one (*14*) is of this origin and the others are true drimane sesquiterpenoids (*10*, *15*, *16*) and derivatives thereof (*2*, *13*). While the number and amounts of labdane diterpenoids predominate over those of the C_{14} and C_{15} compounds, as might be expected if they were precursors, no conclusion can be drawn from the steric relationships and valid arguments in favour of this alternative has to await tracer-studies and/or degradation studies such as photooxygenations of labdane diterpenoids.

EXPERIMENTAL

NMR, IR, UV, rotations and mass spectra were recorded on Varian XL-100, Digilab FTS-14, Perkin-Elmer 257, Beckmann DK-2A, Perkin-Elmer 141, and LKB 9000 instruments, respectively. Melting points were obtained using a Leitz Wetzlar instrument and are uncorrected. Accurate mass determinations were carried out at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm. Analytical and preparative gas chromatography was performed on a Varian 1700 instrument using steel

* In the original publication (Hlubucek *et al. Acta Chem. Scand. B 28* (1974) 18) the configuration and formula of this compound are correct but the Cahn, Ingold, Prelog-convention was incorrectly appiied with respect to the designation of the configuration at C(6').

capillary columns (50 m × 0.25 mm) coated with Ucon Oil HB 2000,[32] and a 3 m × 3.2 mm glass column packed with 5 % Carbowax 20 M on Chromosorb G, respectively. The extraction of 295 kg sun-cured Greek tobacco, *Nicotiana tabacum* L., grown in Serres 1968, and the fractionation of the extract have been described in a previous communication.[10] The present compound was isolated subfraction B4,[10] the further components of which will be published later.[33]

Drim-8-en-7-one (*10*) was isolated (4 mg) from subfraction No. 2 of B4[10] by liquid chromatography on alumina followed by preparative gas chromatography. For spectral data other than those listed here, *cf.* synthetic drim-8-en-7-one (*10*). $[\alpha]^{20}$ +58.7° (589 nm), +60.0° (578), +70.7° (546), +166.7° (436), +736.7° (365) (*c* 0.2; $CHCl_3$); λ_{max} ($CHCl_3$): 248.5 nm (ε 7060); accurate mass measurements: C_6H_9O, found 97.0652, calc. 97.0653; $C_{15}H_{24}O$, found 220.1829, calc. 220.1827.

Synthetic products

7α,8-Epoxydriman-11-ol (*4*). Drimenol (*3*, 196 mg), $[\alpha]^{20}$ −22° (589 nm), −22° (578), −25.2° (546), −46° (436), −82° (365) (*c* 0.5; benzene), lit.:[24] $[\alpha]_D^{25}$ −19.1° (benzene), was dissolved in dry pyridine (10 ml) and acetic anhydride (3 ml) and kept at room temperature for 24 h. The acetate (230 mg) isolated in the usual manner, was dissolved in CH_2Cl_2 (35 ml) and cooled in a dry-ice/acetone bath. A cooled solution of *m*-chloroperbenzoic acid (320 mg) in CH_2Cl_2 was added dropwise and the reaction mixture kept at −50 °C for 48 h. Water was added to the mixture which was extracted with CH_2Cl_2. The extract was washed with $NaHCO_3$, brine, and evaporated to dryness. The acetate was hydrolyzed with 5 % KOH/CH_3OH (10 ml) at room temperature within 1 h. Dilution with water and extraction with ether furnished after removal of the solvent, a crystalline residue which was recrystallized from pentane/ether. The crystals (154 mg) consisted solely of the 7α,8-epoxide, while the mother liquor (75 mg) contained a mixture of the 7α,8-epoxide and the 7β,8β-epoxide. The 7α,8-epoxide possessed the following physical properties: MS: 238 (M^+, 1.1), 109 (100), 123 (73), 69 (71), 41 (67), 124 (53), 95 (49), 81 (47), 55 (47), 177 (47), 43 (42), 138 (36), 191 (36); $\delta(CDCl_3)$: 0.79 (3 H, s), 0.87 (6 H, s), 1.49 (3 H, s), 3.02 (1 H, m, W_H = 5 Hz), 3.66 (1 H, q, *J* 9.5 and 11.5 Hz), 3.98 (1 H, q, *J* 3.5 and 11.5 Hz); ν_{max} (KBr): 3475 (s), 2966 (s), 2931 (s), 2920 (s), 1441 (s), 1392 (s), 1378 (m), 1368 (m), 1240 (m), 1048 (s), 1038 (m), 991 (m), 983 (m), 949 (s), 925 (m), 851 (s), 760 (s), 700 (m); $[\alpha]^{20}$ +14° (589 nm), +17° (578), +18° (546), +29° (436), +38° (365) (*c* 0.2; $CHCl_3$), lit.:[25] $[\alpha]_D$ +20° (*c* 1.56; $CHCl_3$); m.p. 98–99 °C, lit.:[25] 96–97 °C. The epoxyalcohol *4* could also be prepared directly from drimenol by epoxidation analogous to the procedure of Appel *et al.*[25] but the yields of the 7α,8-epoxide appeared to be lower.

Drim-8-en-7α,11-diol (*7*). Brown's [34] reagent (1 ml) was added to a solution of 7α,8-epoxydriman-11-ol (*4*, 100 mg) in ether (30 ml) and the mixture stirred at room temperature for 20 min. When the conversion to the aldehyde, 7α,8-epoxydriman-11-al (*5*), was complete (TLC), excess CrO_3 was destroyed by the addition of methanol (1.5 ml) and the stirring continued for 10 min. Methanolic potassium hydroxide (2 ml, 5 %) was added to the mixture and the isomerization to the conjugated aldehyde, 7α-hydroxydrim-8-en-11-al (*6*), was followed by TLC. The isomerization was complete after 30 min at room temperature as shown by the appearance of a single, UV-visible, more polar spot. Excess $NaBH_4$ (200 mg) was added and the mixture stirred for another 10 min. When the UV-visible TLC-spot had dissappeared, water (20 ml) was added and the mixture extracted four times with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and the solvent distilled. The white, solid residue (80 mg) was recrystallized from ether/CH_3OH. MS: M^+ at *m/e* 238 not visible, 207 (100), 109 (91), 119 (91), 123 (65), 69 (52), 41 (52), 43 (48), 55 (44), 220 (39), 95 (39); $\delta(CD_3OD)$: 0.88 (3 H, s), 0.93 (3 H, s), 0.97 (3 H, s), 1.83 (3 H, s), 3.92 (1 H, t with further splittings), 4.07 (1 H, d, *J* 12 Hz), 4.18 (1 H, d, *J* 12 Hz), AB-system; ν_{max} (KBr): 3250 (s), 2945 (s), 1460 (m), 1390 (m), 1378 (m), 1214 (m), 1060 (s), 1026 (m), 1019 (m), 991 (s), 976 (m), 880 (m); $[\alpha]^{20}$ +113° (589 nm), +118° (578), +136° (546), +246° (436), +408° (365) (*c* 0.1; methanol); m.p. 196–197 °C. The epoxyaldehyde *5* which also could be prepared from the epoxyalcohol *4* employing Sarrett's reagent (CrO_3-pyridine complex),[35] was attempted isolated and purified by liquid chromatography, but was found very unstable.

Selective acetylation of drim-8-en-7α,11-diol (*7*). The diol *7* (38 mg) dissolved in dry pyridine (2 ml) and two equivalents acetic anhydride (33 mg) was kept at 0 °C for 48 h. The reaction mixture was diluted with water and extracted four times with ether. The extract was washed with diluted H_2SO_4, $NaHCO_3$, water, and evaporated. The residue was carefully chromatographed on silica gel and the diacetate, 7α,11-diacetoxy-8-drimene (*11*, 7 mg) was eluted with 10 % ether in pentane, the monoacetates, 11-acetoxydrim-8-en-7α-ol (*8*, 23 mg) and 7α-acetoxydrim-8-en-11-ol (*12*, 9 mg) was eluted with 25 % ether in pentane, the latter monoacetate was the more polar, and unreacted diol (*7*, 9 mg) was eluted with pure ether.

7α,11-Diacetoxy-8-drimene (*11*). MS: M^+ at *m/e* 322 not visible, 262 (13, M^+ − AcOH), 220 (100), 43 (88), 119 (80), 133 (47), 187 (38), 41 (36), 205 (33), 189 (31); $\delta(CDCl_3)$: 0.84 (3 H, s), 0.86 (3 H, s), 0.96 (3 H, s), 1.66 (3 H, s), 2.06 (3 H, s), 2.08 (3 H, s), 4.10 (2 H, broad s), 5.20

(1 H, t with further splittings); ν_{max} (film): 2933 (s), 1740 (s), 1446 (m), 1375 (s), 1236 (s), 1159 (w), 1022 (s), 981 (m), 967 (m), 939 (m), 919 (w), 848 (w); $[\alpha]^{20}$ +96.3° (589 nm), +100.9° (578), +116.1° (546), +214.3° (436), +373.9° (365) (*c* 0.46; $CHCl_3$).

11-Acetoxydrim-8-en-7α-ol (8). MS: M+ at *m/e* 280 not visible, 262 (5, M+ $-H_2O$), 220 (100), 43 (69), 123 (64), 119 (44), 109 (44), 97 (43), 41 (43), 69 (40), 135 (34), 55 (34), 205 (31), 133 (31); $\delta(CDCl_3)$: 0.86 (3 H, s), 0.93 (3 H, s), 0.94 (3 H, s), 1.81 (3 H, s), 2.05 (3 H, s), 4.0 (1 H, t with further splittings), 4.50 (2 H, s); ν_{max} (film): 3300 (m), 2930 (s), 1740 (s), 1461 (m), 1387 (m), 1378 (m), 1240 (s), 1158 (w), 1138 (w), 1058 (m), 1026 (m), 1005 (m), 985 (m), 961 (m), 885 (w); $[\alpha]^{20}$ +128.6° (589 nm), +132.9° (578), +148.6° (546), +270° (436), +453.6° (365) (*c* 0.14; $CHCl_3$).

7α-Acetoxydrim-8-en-11-ol (12). MS: M+ at *m/e* 280 not visible, 220 (93, M+ −AcOH), 109 (100), 119 (58), 43 (56), 95 (44), 41 (44), 124 (40), 97 (40); $\delta(CDCl_3)$: 0.85 (3 H, s), 0.86 (3 H, s), 0.97 (3 H, s), 1.75 (3 H, s), 2.08 (3 H, s), 4.12 (1 H, d, *J* 12 Hz), 4.24 (1 H, d, *J* 12 Hz), 5.21 (1 H, t with further splittings); ν_{max}(KBr): 3440 (m, broad), 2960 (s), 2938 (s), 1732 (s), 1461 (m), 1379 (m), 1245 (s), 1211 (m), 1018 (m), 989 (m), 965 (m), 951 (m), 938 (m), 881 (m), 849 (m); $[\alpha]^{20}$ +102.5° (589 nm), +110° (578), +122.5° (546), +240° (436), +432.5° (365) (*c* 0.1; $CHCl_3$); m.p. 144–145 °C.

11-Acetoxydrim-8-en-7-one (9). To a solution of 11-acetoxydrim-8-en-7α-ol (*8*, 13 mg) in acetone (3 ml) was added a few drops of Jones' reagent [26] and the mixture stirred at ambient temperature for 30 min and the oxidation followed by TLC. The mixture was diluted with water and extracted with ether twice. The extract was washed with $NaHCO_3$, water and evaporated to yield pure (TLC) ketone *9* (10 mg, 78 %). The ketone *9* could also be prepared from the alcohol *8* in excellent yield employing Brown's two-phase system.[34] MS: 278 (M+, 17), 205 (100), 43 (89), 135 (74), 236 (42), 41 (41), 113 (38), 123 (36), 69 (36), 55 (31); $\delta(CDCl_3)$: 0.90 (3 H, s), 0.94 (3 H, s), 1.13 (3 H, s), 1.80 (3 H, s), 2.09 (3 H, s), *ca.* 2.4 (H_A, 'q', J_{AC} *ca.* 12 Hz, J_{AB} *ca.* 18 Hz), *ca.* 2.53 (H_B, 'q', J_{BC} *ca.* 6 Hz, J_{AB} *ca.* 18 Hz), 4.73 (1 H, d, *J* 12 Hz), 4.79 (1 H, d, *J* 12 Hz); ν_{max} (film): 2932 (s), 1742 (s), 1671 (s), 1622 (w), 1465 (m), 1446 (m), 1379 (s), 1321 (m), 1232 (s), 1153 (m), 1140 (m), 1029 (s), 970 (m), 919 (w), 781 (w), 758 (w); $[\alpha]^{20}$ +60.6° (589 nm), +64.5° (578), +76.4° (546), +181.2° (436), +587.9° (365) (*c* 0.3; $CHCl_3$); λ_{max} ($CHCl_3$): 245 nm (ε 10 530).

Drim-8-en-7-one (10). 11-Acetoxydrim-8-en-7-one (*9*, 10 mg) dissolved in acetic acid (2.5 ml) was stirred for 30 min at room temperature in the presence of activated Zn (400 mg).[36] The reduction [27] to *10* was monitored by TLC and when complete the mixture was poured into a saturated solution of $NaHCO_3$ and extracted with ether twice. Evaporation of the solvent left the ketone (8 mg, 100 %), essentially pure by TLC, as a colourless oil which crystallized on storage at 0 °C. MS: 220 (M+, 40), 97 (100), 135 (40), 69 (30), 41 (30), 123 (27), 55 (23), 109 (19), 137 (17); $\delta(CDCl_3)$: 0.88 (3 H, s), 0.92 (3 H, s), 1.09 (3 H, s), 1.74 (3 H, q, *J* *ca.* 1 Hz), 1.84 (3 H, q, *J* *ca.* 1 Hz), 2.17–2.66 AB-part of an ABC-system: *ca.* 2.34 (H_B, 'q', J_{BC} *ca.* 12 Hz, J_{AB} *ca.* 17 Hz), *ca.* 2.52 (H_A, 'q', J_{AC} *ca.* 6 Hz, J_{AB} *ca.* 17 Hz); ν_{max} (film): 2929 (s), 2869 (m), 2851 (m), 1661 (s), 1612 (m), 1460 (m), 1377 (m), 1324 (m), 1256 (w), 1216 (w), 1152 (w), 1140 (w), 1090 (w), 1076 (m), 1059 (w), 980 (w), 962 (w), 935 (w), 914 (w), 780 (w), 685 (w), 621 (w); $[\alpha]^{20}$ +49.6° (589 nm), +51.9° (578), +62.8° (546), +154.4° (436), +720° (365) (*c* 0.8; $CHCl_3$); λ_{max} ($CHCl_3$): 248 nm (ε 7100); m.p. 52–53 °C. The NMR, IR, and mass spectra of synthetic drim-8-en-7-one (*10*) were superimposable on those of the new tobacco isolate. Furthermore, the two compounds were indistinguishable when coinjected on a capillary GC-column.

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Purification and Properties of *Phaseolus vulgaris* Leghemoglobin (PhLb)

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Leghemoglobin components *a* and *b* (PhLb*a* and PhLb*b*) were purified from root nodules of *Phaseolus vulgaris*. Homogeneity of PhLb*a* was demonstrated by polyacrylamide gel disc electrophoresis. Calibrated gel filtration gave a molecular weight of 16 900 for PhLb*a*. The total amino acid compositions of PhLb*a* and PhLb*b* suggest that PhLb*b* is a deamidation product of PhLb*a*. The amino acid composition of PhLb*a* is: Lys_{12}, His_2, Arg_2, Asx_{14}, Thr_7, Ser_{11}, Glx_{15}, Pro_4, Gly_8, Ala_{25}, Val_{11}, Ile_4, Leu_{17}, Tyr_4, Phe_7, Trp_2. These 145 amino acid residues correspond to a molecular weight of 16 115 for the hemoprotein. The *N*-terminal sequence of PhLb*a* is *Gly-Ala-Phe-Thr-Glx-* and the *C*-terminal residue is alanine. The isoelectric point of PhLb*a* is 4.70 and that of PhLb*b* 4.55.

Leguminous root nodule leghemoglobin may make an important contribution to the knowledge of the structure—function relationship of heme proteins. Leghemoglobin is structurally and functionally related to animal hemoglobins and myoglobins. So far only soybean leghemoglobin component *a* has been extensively studied.[1] The present work describes the purification and some molecular properties of kidney bean (*Phaseolus vulgaris*) leghemoglobin.

MATERIALS AND METHODS

Planting of kidney beans. Seeds of kidney bean *Phaseolus vulgaris*, var. Kaiser Wilhelm, were surface sterilized, inoculated with *Rhizobium phaseoli* (Lantbrukshögskolan, Ultuna, Sweden) and grown in sea sand in a greenhouse essentially as described previously.[2] EDTA-Fe(III)-Na salt was used instead of ferric citrate. The harvested root nodules were stored at −16 °C.

Purification of leghemoglobin. The nodules were extracted in a mortar with ice-cold 20 mM phosphate buffer, pH 6.8. All other operations were performed at 4 °C. Leghemoglobin was precipitated from the centrifuged nodule extract by adding crystalline ammonium sulfate to 55 − 80 % saturation. The pH was maintained at 6.5 − 7.0 with ammonium hydroxide. The centrifuged precipitate was dissolved in the extraction buffer, dialyzed against distilled water and concentrated by pressure filtration on an Amicon UM-10 membrane. Fractionation of PhLb was performed on DEAE-Sephadex A-25 (Pharmacia) equilibrated with 15 mM acetate buffer pH 5.6. Convex gradient elution with respect to ionic strength was employed. A closed mixing chamber containing 250 ml of the equilibration buffer was connected to a 52 mM buffer reservoir, and, after PhLb*a* had emerged from the column, to a 100 mM buffer of the same pH.

Disc electrophoresis. Disc electrophoresis in 7.5 % polyacrylamide gels at pH 7.5 was conducted as described by Maurer,[3] except that the samples were laid directly onto the separation gels. The protein bands were visualized by staining with Coomassie blue.[4]

Analytical gel filtration. A column (1.5 × 92 cm) of Sephadex G-50 superfine (Pharmacia) equilibrated with pH 6.0 phosphate buffer of ionic strength 0.05 was used. 1.5 ml fractions were collected at a flow rate of 6 ml/h. The blue dextran void volume was detected by following A_{625}, and the elution volumes of the marker hemoproteins by A_{409}. The molecular weight of PhLb*a* was determined essentially as described by Andrews.[5]

Isoelectric focusing. The leghemoglobin fraction precipitating at 55 − 80 % ammonium sulfate saturation from the root nodule extract was dialyzed, and a sample of 6 − 10 mg was used for the electrofocusing performed according to Vesterberg and Svensson[6] in a 110 ml LKB 8101 column. A 1 % concentration of carrier ampholytes in the pH range 4.5 − 5.0 (Ampholine, LKB) was used. The column was cooled with 2 °C water. After focusing for 24 h

at 700 V, fractions of 2 or 1 ml were collected and the pH values were measured immediately at 2 °C (Radiometer PHM 4c). The pH meter was calibrated with 0.05 M potassium diphthalate and pH 7 standard buffer (Beckman No. 3581). Leghemoglobin was detected by following the absorbance at 404 nm.

Dry weight determination. Dialyzed leghemoglobin samples were dried to constant weight at 105 °C and weighed on a Cahn electrobalance.

Amino acid composition. About 2.5 mg of PhLb*a* or PhLb*b* was hydrolyzed in 2.0 ml of 6 N HCl containing 10 µl of 0.1 M phenol for 20 or 72 h in evacuated, sealed tubes at 110 °C. The amino acid composition was determined on a Beckman Spinco Model 120 B amino acid analyzer according to Spackman *et al.*[7] Values for serine, threonine, tyrosine, and phenylalanine were extrapolated to zero hydrolysis time, and for valine, isoleucine, and leucine the 72 h values were taken to be the final ones. The spectrophotometric method of Spies and Chambers [8] was used for the estimation of tryptophan from unhydrolyzed samples.

Apoleghemoglobin. ApoPhLb*a* and apoPhLb*b* were prepared as described previously.[9] Centrifugation of the apoprotein from acid acetone was performed at −13 °C.

N-*Terminal sequence determination.* The *N*-terminal amino acid sequence of apoPhLb*a* was determined by the dansyl-Edman procedure.[10] The dansylated amino acids were identified by thin layer chromatography on 3 × 3 cm polyamide sheets (BDH).[11]

Hydrazinolysis. Hydrazinolysis was performed according to Braun and Schroeder.[12] Hydrazine

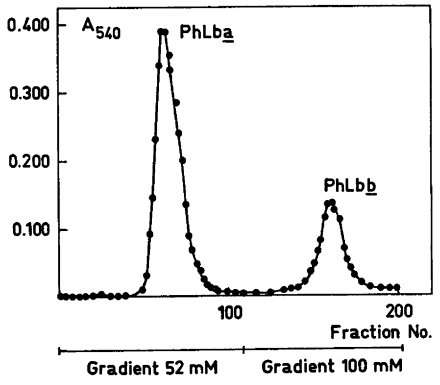


Fig. 1. Fractionation of *Phaseolus vulgaris* leghemoglobin on a DEAE-Sephadex A-25 column (2.5 × 36 cm) equilibrated with 15 mM acetate buffer pH 5.6. Gradient elution was performed with 52 mM and 100 mM acetate buffer pH 5.6. The sample has been purified from 200 g of wet nodules after 1 − 1.5 year's storage at −16 °C.

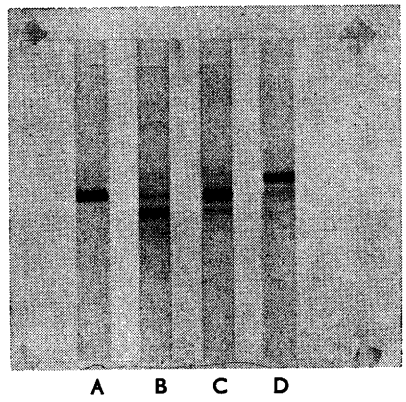


Fig. 2. Polyacrylamide disc electrophoresis (pH 7.5, 7.5 % polyacrylamide) of purified PhLb*a* (A), PhLb*b* (B), purified PhLb*a* after prolonged storage at −16 °C with occasional melting and refreezing (C) and apoPhLb*a* (D). Migration is from top to bottom.

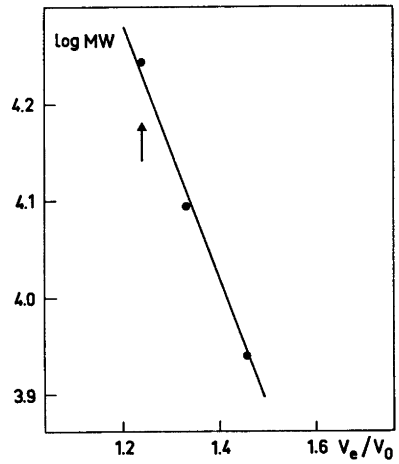


Fig. 3. Estimation of the molecular weight of PhLb*a* by gel chromatography on Sephadex G-50 superfine. The logarithms of the molecular weights are plotted against V_e/V_0. The reference proteins of known molecular weight are: (1) *Pseudomonas aeruginosa* cytochrome *c*-551, 8700;[13] (2) horse heart cytochrome *c*, 12 400;[25] (3) horse myoglobin, 17 600.[26] The position of PhLb*a* is marked by an arrow.

(95+ %, Eastman) was distilled and stored at 4 °C. Thoroughly dialyzed apoPhLb*a* or apoPhLb*b* (0.25 µmol) was lyophilized and dried in an Abderhalden dryer at 64 °C for 24 h, with 50 mg of Amberlite CG-50 resin in the hydrogen form added to the sample. After 72 h hydrazinolysis at 80 °C the liberated amino

acids were separated from hydrazides on a 1 x 25 cm Whatman cellulose phosphate P-70 column equilibrated with 0.4 M pyridine-formate buffer pH 3.2. Small aliquots (about 1/100) of the effluent fractions were analyzed by high voltage paper electrophoresis at pH 1.9.[13] The paper was stained with ninhydrin-cadmium acetate.[14] The fractions containing the neutral or basic amino acids were pooled, lyophilized and analyzed in the amino acid analyzer.

Pyridine hemochrome was determined according to Paul *et al.*[15]

Spectra. Absorption spectra were recorded with a Cary 15 spectrophotometer.

Abbreviations. EDTA = ethylenediaminetetraacetic acid, dansyl = 1-dimethylaminonaphthalene-5-sulfonyl.

RESULTS AND DISCUSSION

Purification. Fig. 1 represents the elution diagram of the PhLb components *a* and *b* from the anion exchanger. The yield of PhLb*a* is about 0.6 mg from 1 g of wet nodules. The amount of PhLb*b* varied from 5 % to 30 % of the amount of PhLb*a*, the smallest value for PhLb*b* being obtained from the brightest red nodules.

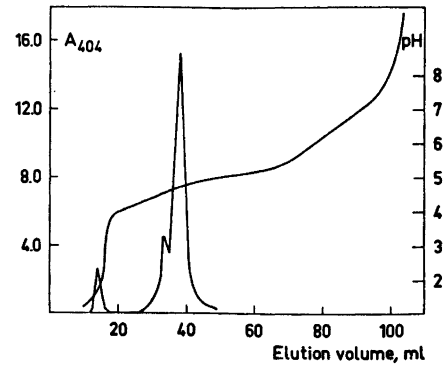


Fig. 4. Isoelectric focusing of the PhLb components in a sucrose density gradient. The experimental conditions are described in Methods section.

Criteria for homogeneity. In the isoelectric focusing column very faint red bands with somewhat higher pI than PhLb*a* can be seen

Table 1. The amino acid compositions of PhLb*a* and PhLb*b*.

Amino acid residue	PhLb*a* Hydrolysis time 20 h	70 h	Extrapolated or average value	Nearest integer	PhLb*b* Hydrolysis time 20 h	70 h	Extrapolated or average value	Nearest integer
Lys	11.98	11.47	11.73	12	11.76	11.98	11.87	12
His	1.82	1.85	1.84	2	1.95	1.84	1.90	2
Arg	1.86	1.60	1.73	2	1.94	2.06	2.00	2
Asp	14.37	14.32	14.35	14	14.67	14.43	14.55	15
Thr	6.68	6.00	6.95	7	6.85	6.14	7.12	7
Ser	9.82	7.95	10.60	11	9.80	8.09	10.60	11
Glu	15.02	15.10	15.06	15	15.10	15.12	15.11	15
Pro	4.24	4.37	4.31	4	4.28	4.39	4.34	4
Gly	7.96	7.71	7.84	8	8.22	7.97	8.10	8
Ala	25.03	24.80	24.92	25	24.62	24.69	24.66	25
Cys	0	0	0	0	0	0	0	0
Val	9.04	10.45	10.45	11	8.73	10.51	10.51	11
Met	0	0	0	0	0	0	0	0
Ile	3.70	3.70	3.70	4	3.81	3.93	3.93	4
Leu	17.02	17.44	17.44	17	16.62	17.07	17.07	17
Tyr	3.41	2.83	3.72	4	3.22	2.93	3.50	4
Phe	6.86	6.64	6.98	7	6.55	6.34	6.70	7
Trp	2.18 [a]		2.18	2	2.21 [a]		2.21	2
Total number of residues				145				
Recovery				97.2 %				93.2 %

[a] Determined spectrophotometrically (see Methods).

close above the major bands. This microheterogeneity possibly represents minor genetic variants of leghemoglobin sequence in a large population. Point mutations have been detected in the soybean leghemoglobin sequence.[16] The purified PhLb*a* migrated as a single band in acrylamide gel electrophoresis (Fig. 2), although in many extensively dialyzed PhLb*a* samples some apoprotein was also detected.

Prosthetic group. The pyridine hemochrome of PhLb*a* has a spectrum typical of protoheme IX compounds, with absorption maxima at 557 and 526 nm. The iron content of PhLb*a* is 0.313 % and that of PhLb*b* 0.312 % as determined by the pyridine hemochrome method. The theoretical value from the total amino acid analysis, assuming one heme group per molecule, is 0.347 %. The heme of PhLb seems to have a slight tendency to detach from the apoprotein, which can also be seen in disc electrophoresis.

Molecular weight and amino acid composition. The molecular weight of PhLb*a* obtained by analytical gel filtration is 16 900 (Fig. 3). Total amino acid analysis (Table 1) gives a molecular weight of 15 500 for the apoprotein and 16 115 for the hemoprotein. The value for the molecular weight of kidney bean leghemoglobin is close to that of soybean Lb*a* and Lb*c* (15 775 [16] and 16 695,[9] respectively). Values of 15 260 [17] for lupin and 16 400–16 700 [18] for snake bean leghemoglobin have been reported. Molecular weights of 17 500–20 600 for lupin and serradella leghemoglobins [19,20] are considerably higher. Variations in leghemoglobin molecular weights over such wide limits (15 300–20 600) would be somewhat unexpected.

No sulfur containing amino acids are present in kidney bean leghemoglobin. To the nearest integer, the amino acid compositions of PhLb*a* and PhLb*b* are identical except for aspartic acid (Table 1). The average number of aspartic acid residues per molecule is 14.35 in PhLb*a* and 14.55 in PhLb*b*. This difference is within experimental error. Accordingly, it may be concluded that PhLb*a* and PhLb*b* most probably have identical amino acid compositions.

Isoelectric point. The isoelectric point of PhLb*a* is 4.70 and that of PhLb*b* 4.55 at a temperature of 2 °C (Fig. 4). Component *b* is most probably a deamidation product of component *a*. The fraction with an isoelectric point of 1.6–2.0 is a free porphyrin. A low isoelectric point has been a general feature of all leghemoglobins so far studied.[18,19,21]

N- *and* C-*Terminal characterization of PhLba.* The dansyl-Edman procedure recommended for peptides was employed for PhLb*a* apoprotein. According to this method the by-products were removed only after the appropriate number of Edman steps had been performed. The unambiguous results obtained show that the method can also be applied to some small proteins. The *N*-terminal sequence of PhLb*a* is *NH_2-Gly-Ala-Phe-Thr-Glx-*. This sequence has a striking similarity with the first five *N*-terminal residues of human Hb*γ*, *NH_2-Gly-His-Phe-Thr-Glu-*.[22]

The *N*-terminal residue is also glycine in both the main leghemoglobin components of lupin [19] and of serradella [19] and in soybean Lb*c*,[23] while in soybean Lb*a* it is valine.[23] Glycine and valine are the typical *N*-terminal residues in animal globins.[24]

At first, hydrazinolysis of PhLb*a* and PhLb*b* hemoproteins met with some difficulties due to the bulk of the free amino acids bound to the protein. It was found necessary to remove the heme group and dialyze the apoprotein extensively against 50 mg/ml sodium bicarbonate solution and distilled water at 4 °C. After this treatment a 44 % recovery of alanine was obtained in the amino acid analysis, as calculated from the weight of the lyophilized sample before more thorough drying in the drying pistol. The yields of all other amino acids were 0–5 %, the most prominent impurities being serine and glycine. The carboxyterminal residue of PhLb*a* was thus concluded to be *alanine*.

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Mass Spectra of Organoarsenic Compounds. IV. Electron Impact-induced Fragmentation of 1,3,2-Dioxarsolanes, 1,3,2-Dithiarsolanes and 1,3,2-Oxathiarsolanes

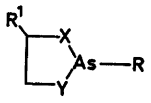
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The electron impact-induced fragmentations of a series or 2-substituted 1,3,2-dioxarsolanes, 1,3,2-oxathiarsolanes, and 1,3,2-dithiarsolanes have been studied. The five-membered cyclic arsenium ion formed by loss of the 2-substituent from the dioxarsolanes appears to be less stable than the corresponding six-membered cyclic arsenium ion formed in the fragmentation of 1,3,2-dioxarsenanes. The stability of the five-membered ring system increases when one or two sulfur atoms are introduced in the ring. The formation of the $[C_7H_7]^+$ ion is discussed.

As a part of our investigation of organic arsenic compounds [1-3] we now report the mass spectra (Figs. 1 to 4) of the following 1,3,2-dioxarsolanes, 1,3,2-dithiarsolanes, and 1,3,2-oxathiarsolanes.

In a brief communication [4] the electron impact-induced fragmentation of IV, VI, and VIII has been reported to give rise to the formation of very stable five-membered cyclic arsenium ions by the ready loss of the 2-substituent. Furthermore a rearrangement leading to the formation of the tropylium ion was observed.



	R	X	Y	R^1
I	CH_3	O	S	H
II	CH_3	O	S	CH_3
III	CH_3	S	S	H
IV	C_6H_5	O	O	H
V	C_6H_5	O	O	CH_3
VI	C_6H_5	O	S	H
VII	C_6H_5	O	S	CH_3
VIII	C_6H_5	S	S	H
IX	C_6H_5	S	S	CH_3

In accordance with these results the $[M-R]^+$ ion gives rise to the base peak in the mass spectra of I, II, and III (Fig. 1) where $R=CH_3$. However, when R is phenyl (IV to IX) the As—R bond scission is less likely, probably due to $p_\pi - p_\pi$ interaction of the lone pair electrons on arsenic with the benzene nucleus and consistent with our previous findings.[2] In the mass spectrum (Fig. 2) of 2-phenyl-1,3,2-dioxarsolane (IV) the $[C_6H_5As]^{+\cdot}$ ion thus gives rise to the base peak whereas the five-membered cyclic $[M-C_6H_5]^+$ ion has an abundance of only 12 %. The corresponding six-membered arsenium ion forms in the fragmentation of 2-phenyl-1,3,2-dioxarsenane the base peak. In this case the $[C_6H_5As]^{+\cdot}$ ion amounts to 42 %. For both types of compounds introduction of a methyl group in the heterocyclic ring tends to decrease the abundance of the $[M-C_6H_5]^+$ ion.

Introduction of one or two sulfur atoms in the five-membered ring causes a considerable increase in the stability of the molecular ions and of the $[M-C_6H_5]^+$ ions (Figs. 3 and 4). The difference between the As—O and As—S bond is clearly reflected in the fragmentation of the $[M-C_6H_5]^+$ ion. Thus the oxathiarsolanes (VI and VII) are forming the abundant $[AsS]^+$ ion (*m/e* 107) by elimination of $R^1C_2H_3O$ ($R^1=H$, CH_3). The corresponding loss of $R^1C_2H_3S$ (to yield the $[AsO]^+$ ion at *m/e* 91) is here almost

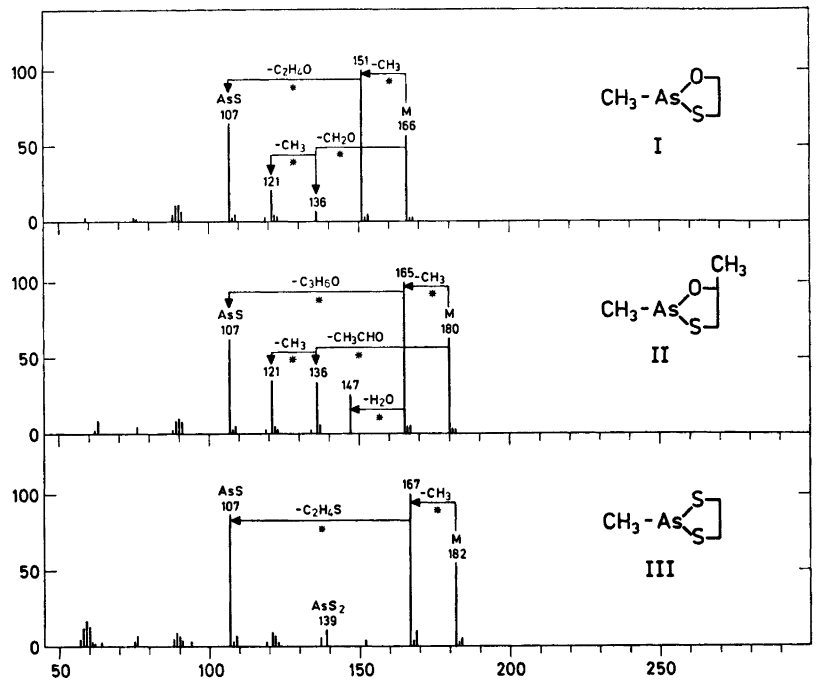


Fig. 1. Mass spectra of I, II, and III.

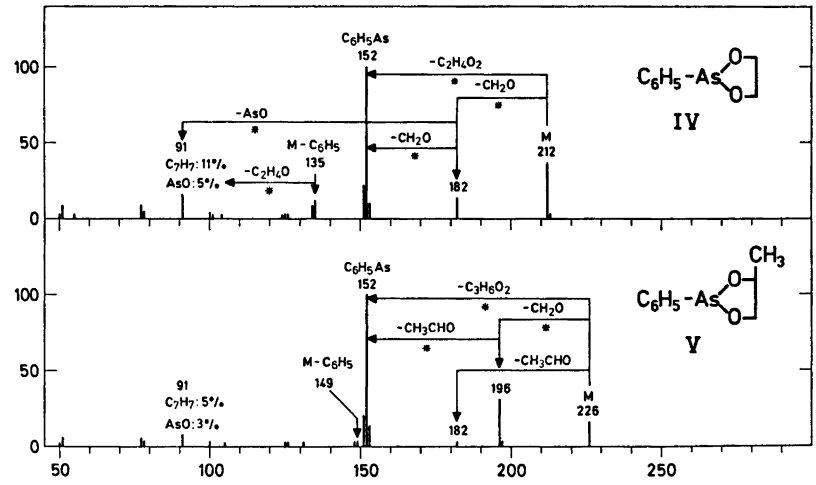


Fig. 2. Mass spectra of IV and V.

negligible. However, in the spectra of the dithiarsolanes elimination of this fragment leads to the formation of the prominent $[AsS]^+$ ion.

Formation of $[C_7H_7]^+$. Although $[C_7H_7]^+$ (most probably the tropylium ion) is formed in all cases when $R = C_6H_5$ it gives rise to prominent peaks in the spectra of VI and VII only. In these cases it was found to be formed both directly from the molecular ion and indirectly *via* the $[M - R^1CHO]^{+\cdot}$ ion. This suggests that

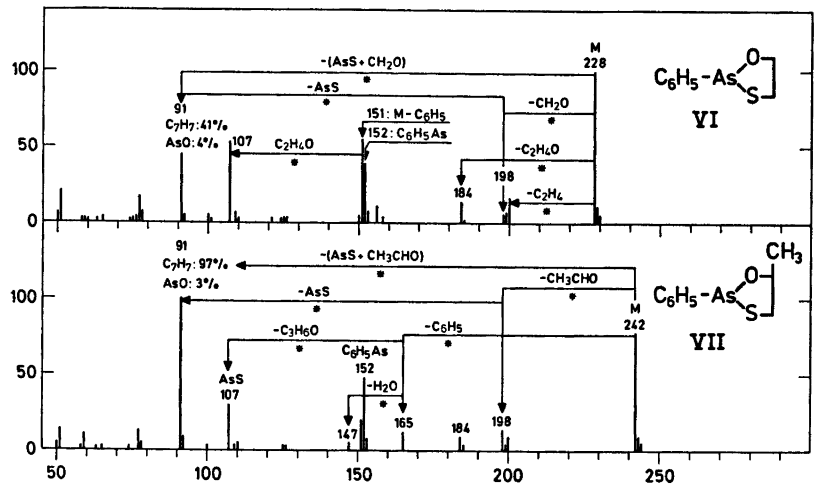


Fig. 3. Mass spectra of VI and VII.

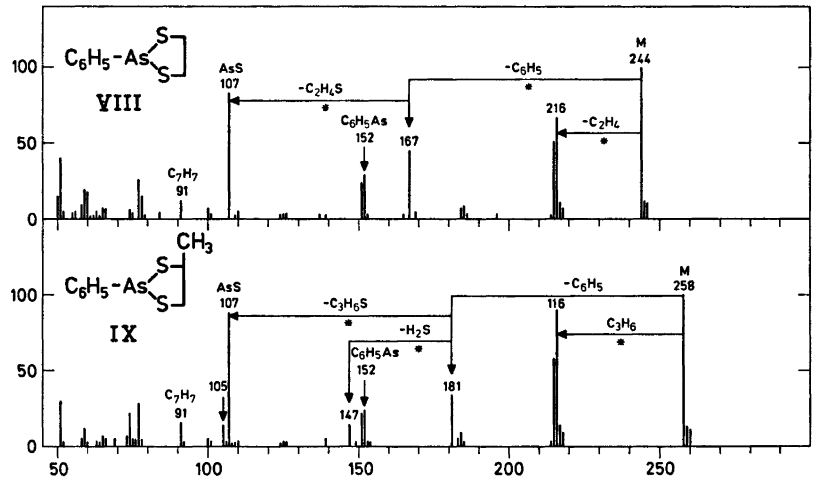
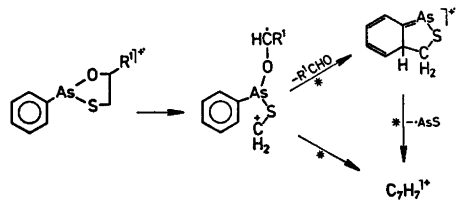


Fig. 4. Mass spectra of VIII and IX.

the primary bond scission occours at the C–C bond in the heterocyclic ring (Scheme 1). Accordingly, the high abundance (100 %) of the $[C_7H_7]^+$ ion in the fragmentation of VII may be due to preferred fragmentation at the branching point.



Scheme 1.

The mass spectra of the dioxarsolanes also exhibit ions corresponding to the elimination of RCHO from the molecular ions. The further loss of ·AsO yields the $[C_7H_7]^+$ ion. However, in case of V the ion corresponding to the loss of CH_2O is much more pronounced than that corresponding to the elimination of CH_3CHO, and a direct formation of the $[C_7H_7]^+$ ion from the molecular ions of IV and V was not ob-

served. This may suggest that in these cases the primary scission takes place at one of the As – O bonds.

It is notable that ions corresponding to the initial loss of CH_2S are absent in the spectra of the oxathiarsolanes. This was found also to be the case in the spectra of the dithiarsolanes (nor was there observed any loss of CH_3CHS in IX). The spectrum of IX exhibit in addition to the $[C_7H_7]^+$ ion a possible methyltropylium ion (*m/e* 105).

The different ability of the three types of 2-phenyl substituted arsolanes to form the $[C_7H_7]^+$ ion may be explained in terms of differences in the As – O and As – S bond energies. (No thermochemical data are available for As – S bonds, but the present mass spectral results strongly suggest that the As – S bond energy is greater than that of the As – O bond).

The higher stability of CH_2O and CH_3CHO as compared to CH_2S* is probably also reflected in the activation energies for the various fragmentation pathways.

It is interesting that the corresponding 2-phenylborolanes [7] give rise to the formation of $[C_7H_7]^+$ in analogous processes. However, in these cases CH_2S is lost preferentially to CH_2O. As pointed out by the authors,[7] this behaviour may be attributed to the very large diffference in the B – O and B – S bond energies.

Primary ejection of aldehyde was found also to be a characteristic feature upon ionisation of the corresponding phenyl substituted six-membered heterocyclic dioxarsenanes.[2] Subsequent ejection of ·AsO leads in these cases to the formation of an $[C_8H_9]^+$ ion. The methyltropylium structure has been suggested for this fragment.

An alternative to the loss of thioaldehyde is the elimination of alkene from the molecular ions of VI to IX. In the spectra of VIII and IX this process leads to the important $[C_6H_5AsS_2]^{+\cdot}$ ions. The corresponding $[C_6H_5AsOS]^{+\cdot}$ ion is formed in less abundance in the spectra of VI and VII, whereas the $[C_6H_5AsO_2]^{+\cdot}$ ion could not be detected (Fig. 2).

The $[C_6H_4AsS_2]^+$ ion (*m/e* 215, Fig. 4) is formed directly from the molecular ion as well as from *m/e* 216. It is a precursor for the $[AsS]^+$ ion (*m/e* 107).

EXPERIMENTAL

The mass spectra were all recorded with an AEI MS-902 mass spectrometer operating at 70 eV. Samples were introduced through the heated glass inlet system, and the source and sample temperatures were kept as low as possible. The exact mass measurements were made relative to heptacosafluorotributylamine. The metastable defocusing technique has been applied to establish evidence for some of the important transitions not accompanied by metastable ions in the spectra.

The purity of the compounds under investigation was established by elemental analysis. The various arsolanes were prepared according to well known methods,[8] and were purified by fractional distillation.

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* The heat of formation of CH_2S and CH_2O are 24 [5] and −27 [6] kcal/mol, respectively.

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Organic Hydroxylamine Derivatives. XIII.* The Configurations of a Series of Stereoisomeric 3-Methoxy-5-acylisoxazole Ketoximes

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As part of the synthesis of a series of compounds structurally related to muscimol (3-hydroxy-5-aminomethylisoxazole zwitterion) the 3-methoxy-5-acylisoxazoles (Ia—c) were converted into the corresponding ketoximes. In each case a mixture of two compounds were formed, as shown by thin layer chromatography. The ^{1}H NMR spectra of the isolated products were consistent with mixtures of the stereoisomeric oximes (II) and (III).[1] The corresponding oximes were separated by column chromatography, and the constitutions were confirmed by UV, IR, and ^{1}H NMR spectroscopy, and elemental analyses.

A number of acylisoxazole ketoximes have previously been synthesized,[2,3] but the problems relating to the configurations of the products have in no case received any attention. Attempts to establish unambiguously the structures of (IIa—c) and (IIIa—c) by ^{1}H NMR spectroscopic methods,[4,5] including lanthanide induced [6] and aromatic solvent induced shift experiments,[5,7] were unsuccessful.

The configurations of the oximes were determined by Beckmann rearrangements (Scheme 1) using phosphorus pentachloride in ether, which is reported to favour stereospecific rearrangements.[8] The oximes (IIa-c) having the greater R_F values were rearranged to give the amides (IVa—c) indicating *Z*-configurations of these oximes. The amides (IVa—c) were identified by comparison with authentic amides prepared from the acid chloride (VI) and the amines (VIIa—c), respectively. Correspondingly the oximes (IIIa—c) having the smaller R_F values were rearranged to give the amides (Va—c). Thus the oximes (IIIa—c) possess *E*-configurations. The structure determinations of (Va—c) are based on UV, IR, and ^{1}H NMR spectroscopy and supported by elemental analyses. The UV spectroscopic data originating from the 3-methoxy-5-aminoisoxazole moieties are in agreement with those published for 3-hydroxy-5-aminoisoxazoles.[9,10] Thin layer chromatography indicated that no isomerizations of the oximes took place prior to the rearrangements, as only the expected amides could be detected in the reaction products.

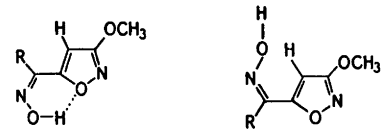


Fig. 1. Two possible conformations of *Z*-3-methoxy-5-(1-hydroxyiminoalkyl)isoxazoles.

Some spectroscopic data of the ketoximes (IIa—c) and (IIIa—c) and the amides (IVa—c) and (Va—c) are listed in Table 1.

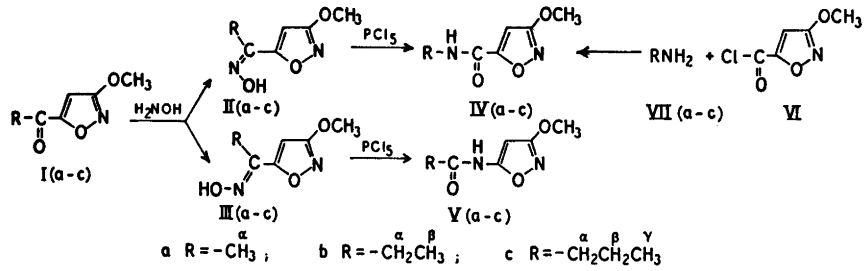
The *Z*-isomers of some 2-acylfurane ketoximes have been proposed to form *intra*molecular hydrogen bonds as revealed by IR spectroscopic investigations of diluted solutions.[11] However, similar investigations seem to indicate that (IIb) as well as (IIIb) form hydrogen bonds in 0.04 % chloroform solutions and furthermore absorption bands at 3550 cm^{-1} appeared in the IR

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Table 1. UV, IR, and ^{1}H NMR data of the oximes (IIa–c) and (IIIa–c) and the amides (IVa–c) and (Va–c). The UV spectra were recorded in 99.9 % ethanol solutions. The IR spectra were recorded in the solid state (KBr). Unless otherwise stated the ^{1}H NMR spectra were recorded in deuteriochloroform solutions using TMS as an internal reference. The singlet, doublet, triplet, quartet, and multiplet patterns of the ^{1}H NMR spectra are designated s, d, t, q, and m, respectively.

Compound	UV data λ_{max} (nm)	$\varepsilon \times 10^{-4}$	IR data (cm^{-1})	^{1}H NMR data (δ-values) Alkyl protons α	β	γ	C=C*H*-C	C*H*$_3$-O	O*H*	N*H*
IIa[a]	248	1.23	3400–3100(s), 1605(s), 1540–1500(s)	2.25s			6.75s	3.95s		
IIIa[a]	250	1.29	3400–3120(s), 1640(m), 1595(s), 1540–1500(s)	2.20s			6.07s	3.96s		
IVa[b]	226	1.21	3260(s), 3140(m), 1660(s), 1610(s)	2.76d			6.74s	3.93s		9.1–8.6
Va[c]	241	1.25	3020(s), 1680(s), 1640(m), 1580(s)	2.22s			5.92s	3.90s		10.3–10.0
IIb	249	1.18	3400–3100(s), 1605(s), 1540–1500(s)	2.70q	1.23t		6.81s	4.00s	9.8	
IIIb	251	1.29	3400–3100(s), 1640(m), 1595(s), 1540–1500(s)	2.70q	1.18t		6.08s	3.98s	8.7	
IVb	226	1.24	3320(s), 3140(m), 1660(s), 1600(m)	3.7–3.2m	1.25t		6.48s	3.98s		6.9–6.3
Vb	241	1.31	3260(s), 1690(s), 1620(s), 1540–1500(s)	2.43q	1.21t		5.98s	3.91s		8.6
IIc[a,d]	250	1.15	3400–3100(s), 1640(w), 1605(s), 1540–1500(s)	2.55t	1.9–1.4m	0.95t	6.60s	3.95s		
IIIc	252	1.41	3400–3100(s), 1640(m), 1595(s), 1540–1500(s)	2.67t	1.9–1.3m	0.98t	6.07s	3.97s	9.3	
IVc	228	1.11	3270(s), 3120(m), 1660(s), 1600(s)	3.6–3.1m	1.9–1.4m	1.1–0.8t	6.45s	3.96s		6.9–6.4
Vc	244	1.37	3210(s), 1680(s), 1620(s), 1580–1500(s)	2.6–2.2m	2.0–1.5m	1.2–0.8t	6.05s	3.98s		9.1–8.8

[a] The OH signal could not be detected in the ^{1}H NMR spectrum. [b] The ^{1}H NMR spectrum was recorded in hexadeuteriodimethyl sulfoxide. [c] The ^{1}H NMR spectrum was recorded in deuteriochloroform-hexadeuterioacetone (4:1). [d] The ^{1}H NMR spectrum was recorded in tetrachloromethane.



Scheme 1.

spectrum of (IIb) as a result of increasing dilution.

The distinctly greater R_F values of the *Z*-forms compared with those of the corresponding *E*-forms, and the pronounced deshielding of the isoxazole protons in the ^{1}H NMR spectra of the *Z*-isomers (see Table 1) may be explained by assuming an *intra*molecular hydrogen bonded *s-trans* conformation of the bond system C=C–C=N (Fig. 1, A). The latter effect, however, is not inconsistent with an *s-cis* conformation of the *Z*-isomers (Fig. 1, B). In this conformation the hydroxyimino group has approached the isoxazole proton, and thus the observed deshielding might arise from the NOH anisotropy.[4,5] Similar deshieldings of the corresponding furane protons in a series of *Z*-2-furanaldoximes are explained by assuming the *s-cis* conformations.[12,13] Also the very small difference between the chemical shift of the isoxazole proton of (IIb) recorded in deuteriochloroform and that recorded in hexadeuteriobenzene (0.08 ppm) indicates, that the hydroxyl oxygen is situated near this proton.[12]

EXPERIMENTAL

Unless otherwise stated the determinations of melting points, the recording of IR, UV, and ^{1}H NMR spectra, and the performance of microanalyses were accomplished as described in a previous paper.[14] Thin layer and column chromatographic procedures were accomplished using silica gel GF_{254} plates and silica gel, 0.05–0.20 mm (Merck), respectively.

Z-3-Methoxy-5-(1-hydroxyiminoethyl)isoxazole (IIa) and E-3-methoxy-5-(1-hydroxyiminoethyl)-isoxazole (IIIa). A mixture of (IIa) and (IIIa) was prepared as previously described,[1] using 1.41 g (10 mmol) of (Ia), 1.50 g (11 mmol) of sodium acetate trihydrate, and 0.77 g (11 mmol) of hydroxylammonium chloride. The mixture was concentrated to 10 ml *in vacuo*, and extracted with two 20 ml portions of ether. The combined ether phases were dried, and the ether was evaporated *in vacuo* to give 1.25 g of a mixture of the two oximes as colourless crystals. Column chromatography of the mixture of isomers (120 g of silica gel; eluent: methylene chloride to which increasing amounts of ethyl acetate were added), and rechromatography of appropriate fractions afforded 0.2 g of the apolar isomer (IIa) and 0.5 g of the polar isomer (IIIa). Recrystallization (water) gave (IIa) as colourless crystals, m.p. 132–133 °C. (Found: C 46.35; H 5.26; N 18.20. Calc. for $C_6H_8N_2O_3$: C 46.15; H 5.16; N 17.94). Recrystallization (water) afforded (IIIa) as colourless crystals, m.p. 134–135 °C. (Found: C 45.95; H 5.33; N 17.90. Calc. for $C_6H_8N_2O_3$: C 46.15; H 5.16; N 17.94).

Beckman rearrangement of Z-3-methoxy-5-(1-hydroxyiminoethyl)isoxazole (IIa). A suspension of 52 mg (0.24 mmol) of phosphorus pentachloride in ether (1 ml) was added at 0 °C to a solution of 25 mg (0.16 mmol) of (IIa) in ether (2 ml). The stirred mixture was allowed to warm to room temperature, stirred for further 1.5 h, and then poured over crushed ice. The organic phase was isolated, washed with 2 ml of an aqueous solution of sodium carbonate (1 M), dried and concentrated *in vacuo* to give 20 mg of a crystalline product, which was submitted to column chromatography (3.9 g of silica gel; eluent: methylene chloride to which increasing amounts of ethyl acetate were added) to give 4.5 mg (18 %) of colourless crystals. The IR spectrum was identical with that of (IVa), prepared from (VI) and (VIIa).

Beckmann rearrangement of E-3-methoxy-5-(1-hydroxyiminoethyl)isoxazole (IIIa). (IIIa) (139 mg; 0.89 mmol) was rearranged and the reaction product isolated as described above for (IIa). *N*-(3-Methoxyisoxazol-5-yl)acetamide (Va) (13 mg; 10 %) was obtained as colourless crystals, m.p. 156–157.5 °C (benzene). (Found: C 46.05; H 5.21; N 17.83. Calc. for $C_6H_8N_2O_3$: C 46.15; H 5.16; N 17.94).

N-Methyl-3-methoxyisoxazole-5-carboxamide (IVa). To 1 ml of a 40 % aqueous solution of methylamine was added at 0 °C a solution of

322 mg (2.0 mmol) of (VI) in ether (1 ml). After stirring for 15 min the precipitate was isolated and recrystallized (water) to give (IVa) (161 mg; 50 %) as colourless crystals, m.p. 154.5—155.5 °C. (Found: C 46.05; H 5.27; N 18.10. Calc. for $C_6H_8N_2O_3$: C 46.15; H 5.16; N 17.94).

Z-3-Methoxy-5-(1-hydroxyiminopropyl)isoxazole (IIb) and E-3-methoxy-5-(1-hydroxyiminopropyl)isoxazole (IIIb). (Ib) (1.55 g; 10 mmol) was converted into a mixture of the isomeric oximes (IIb) and (IIIb) as previously described.[1] The reaction mixture was worked up as described above for (IIa) and (IIIa) to give (IIb) (0.46 g; 27 %) as colourless needles, m.p. 96—98 °C (water-ethanol), (Found: C 49.85; H 6.18; N 16.17. Calc. for $C_7H_{10}N_2O_3$: C 49.40; H 5.92; N 16.46) and (IIIb) (0.50 g; 28 %) as colourless crystals, m.p. 114.5—118.5 °C (water-ethanol). (Found: C 49.45; H 5.99; N 16.48. Calc. for $C_7H_{10}N_2O_3$: C 49.40; H 5.92; N 16.46).

Beckmann rearrangement of Z-3-methoxy-5-(1-hydroxyiminopropyl)isoxazole (IIb). (IIb) (300 mg; 1.77 mmol) was rearranged and the reaction product isolated as described above for (IIa) to give colourless crystals (20 mg; 7 %), m.p. 75—77 °C (benzene-cyclohexane), the IR spectrum of which was identical with that of (IVb), prepared from (VI) and (VIIb).

Beckmann rearrangement of E-3-methoxy-5-(1-hydroxyiminopropyl)isoxazole (IIIb). (IIIb) (113 mg; 0.59 mmol) was rearranged and the reaction product isolated as described above for (IIa) to give (Vb) (20 mg; 18 %) as colourless crystals, m.p. 95.5—97.5 °C (benzene-cyclohexane). (Found: C 49.45; H 5.97; N 16.55. Calc. for $C_7H_{10}N_2O_3$: C 49.40; H 5.92; N 16.46).

N-Ethyl-3-methoxyisoxazole-5-carboxamide (IVb). A solution of 322 mg (2 mmol) of 3-methoxy-5-chloroformylisoxazole in ether (2 ml) was slowly added to a solution of 225 mg (5 mmol) of ethylamine in ether (3 ml) at 0 °C. The mixture was allowed to warm to room temperature, washed with two 5 ml portions of water, dried, and concentrated *in vacuo* to give 151 mg of crystals. (IVb) (99 mg; 29 %) was obtained as colourless crystals, m.p. 74.5—76.5 °C (benzene-cyclohexane). (Found: C 49.15; H 5.91; N 16.55. Calc. for $C_7H_{10}N_2O_3$: C 49.40; H 5.92; N 16.46).

Z-3-Methoxy-5-(1-hydroxyiminobutyl)isoxazole (IIc) and E-3-methoxy-5-(1-hydroxyiminobutyl)isoxazole (IIIc). (Ic) (1.69 g; 10 mmol) was converted into a mixture of the isomeric oximes (IIc) and (IIIc) as previously described.[1] The reaction mixture was worked up as described above for (IIa) and (IIIa) to give (IIc) as colourless needles, m.p. 119—121 °C (benzene), (Found: C 52.25; H 6.67; N 15.17. Calc. for $C_8H_{12}N_2O_3$: C 52.16; H 6.57; N 15.21) and (IIIc) (1.0 g; 54 %) as colourless needles, m.p. 97—98 °C (cyclohexane). (Found: C 52.10; H 6.32; N 15.23. Calc. for $C_8H_{12}N_2O_3$: C 52.16; H 6.57; N 15.21).

Beckman rearrangements of Z-3-methoxy-5-(1-hydroxyiminobutyl)isoxazole (IIc). (IIc) (300 mg; 1.63 mmol) was rearranged and the reaction product solated as described above for (IIa) to give *N*-propyl-3-methoxyisoxazole-5-carboxamide (IVc) (25 mg; 8 %) as colourless crystals, m.p. 74.5—75 °C (cyclohexane). The IR spectrum was identical with that of (IVc) prepared from (VI) and (VIIc).

Beckmann rearrangement of E-3-methoxy-5-(1-hydroxyiminobutyl)isoxazole (IIIc). (IIIc) (200 mg; 1.09 mmol) was rearranged as described above for (IIa) to give (Vc) (32 mg; 16 %) as colourless crystals, m.p. 113—114 °C (benzene-cyclohexane). (Found: C 52.20; H 6.55; N 15.32. Calc. for $C_8H_{12}N_2O_3$: C 52.16; H 6.57; N 15.21).

N-Propyl-3-methoxyisoxazole-5-carboxamide (IVc). (IVc) was synthesized as described above for (IVb). As starting materials were used 242 mg (1.50 mmol) of (VI) and 300 mg (5.0 mmol) of propylamine (VIIb). Obtained was 197 mg (71 %) of (IVc) as colourless crystals. An analytical sample was recrystallized (cyclohexane) to give (IVc) as colourless crystals, m.p. 75—77 °C. (Found: C 52.35; H 6.62; N 15.30. Calc. for $C_8H_{12}N_2O_3$: C 52.16; H 6.57; N 15.21).

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Chlorination of Tetra-*O*-benzoyl-1-deoxy-D-*arabino*-hex-1-enopyranose. Formation of an Orthoacid Chloride*

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Chlorination of tetra-*O*-benzoyl-1-deoxy-D-*arabino*-hex-1-enopyranose (*4*) gave tetra-*O*-benzoyl-2-chloro-α-D-mannopyranosyl chloride (*8*) and tetra-*O*-benzoyl-2-chloro-β-D-glucopyranosyl chloride (*6*). Similar results were obtained by chlorination of 2,4,6-tri-*O*-benzoyl-3-*O*-methyl-1-deoxy-D-*arabino*-hex-1-enopyranose (*16a*) and tetra-*O*-benzoyl-1-deoxy-D-*lyxo*-hex-1-enopyranose (*16b*). The structures of the dichlorosugars *6* and *8* are discussed using ^{1}H NMR and ^{13}C NMR spectroscopy.

Chlorination of *4*, at low temperature gave the orthoacid chloride *5*, which could be observed by NMR spectroscopy at −20 °C and which could be converted to the corresponding orthoester (*10*), orthoacid fluoride (*11*), and a benzylidene compound (*12*). Hydrolysis of *5* gave tetra-*O*-benzoyl-α-D-*arabino*-hexapyranosulose (*2*). At room temperature the orthoacid chloride (*5*) rearranges to the dichlorosugar (*6*).

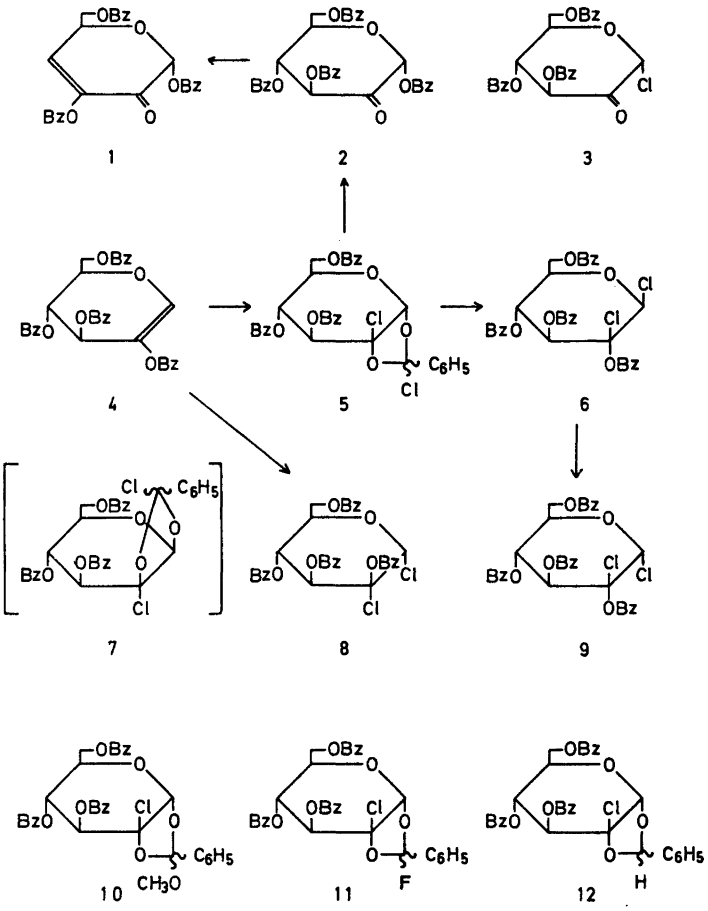
In connection with previous work on the reactions of 1-deoxy-hex-1-eno-pyranoses with hydrogen halides [1,2] we became interested in the behaviour of these compounds towards halogen. In a series of papers Maurer *et al.*[3] described the reaction of acylated 1-deoxy-hex-1-enopyranoses with chlorine. When tetra-*O*-benzoyl-1-deoxy-D-*arabino*-hex-1-enopyranose (*4*) was treated with chlorine in benzene a crystalline dichloro-compound was obtained in 22 % yield.[3] No other compounds were isolated. When Maurer and Petsch [3] hydrolyzed the crude chlorination product they obtained a compound which they assumed was tetra-*O*-benzoyl-D-*arabino*-hexopyranosulose (*2*). We were, however, not able to obtain *2* by this procedure [4] and we therefore decided to reinvestigate the chlorination of *4*.

When *4* was treated with chlorine in carbon tetrachloride at room temperature a crystalline dichloro-compound (*8*), identical with that described by Maurer *et al.*, was isolated in *ca.* 25 % yield. The material in the mother liquor was an almost pure compound and chromatography gave a dichlorosugar (*6*), isomeric with *8*. Comparison of the ^{1}H NMR spectra of *6* and *8* (Table 1) shows that H1 and H5 of *8* are found 0.4 − 0.5 ppm downfield from those of *6*. This indicates that *8* is an α-chloride and *6* a β-chloride. The axial chlorine at C1 in *8*, which causes a downfield shift of H5, should also deshield H3. However, this proton is found at higher field than H3 of *6*. This may indicate that *6* and *8* have different configurations at C2.

Since *6* was assumed to be a β-chloride an anomerization with titanium tetrachloride was attempted. This gave a new, isomeric dichlorosugar (*9*). A ^{1}H NMR spectrum of *9* showed that the signals of H1 and H5 had moved downfield relative to those of *6*, thus confirming that an α-chloride had been formed. The ^{1}H NMR spectrum of *9* is quite similar to that of *8*, but the optical rotations and the ^{13}C NMR spectra (Table 2) are different. From this it is concluded that *8* and *9* are α-chlorides with different configuration at C2 and that *6* and *9* are a pair of anomers. Treatment of *8* with titanium tetrachloride gave no reaction. This confirms that *8* is an α-chloride and shows that isomerization does not take place at C2 by this treatment.

In order to get more information about the structures of *6*, *8*, and *9* their ^{13}C NMR spectra

* Part of this work was presented at the Chemical Society Carbohydrate Group Meeting in Brighton, April 1972.



were measured (Table 2) and for comparison ^{13}C NMR spectra of the anomeric tri-*O*-acetyl-2-chloro-2-deoxy-D-gluco- (*13*) and -D-manno-pyranosyl chlorides (*14*) were also measured. These compounds were prepared according to Igarashi *et al.*[5] Besides, the anomeric tri-*O*-acetyl-2,2-dichloro-2-deoxy-D-*arabino*-hexo-pyranosyl chlorides (*15*) were prepared and their spectra were measured. Bradley and Buncel [5] obtained a crystalline product by addition of chlorine to tri-*O*-acetyl-1,5-anhydro-2-chloro-2-deoxy-D-*arabino*-hex-1-enopyranose and described it as the α-anomer (α-15). We isolated both a crystalline and a syrupy product from this reaction and found from ^{1}H NMR and ^{13}C NMR spectra that the crystalline product is the β-anomer (β-15) whereas the syrup is (α-15).

The ^{13}C chemical shifts of all the chloro-sugars shown in Table 2 were assigned by selective proton decoupling.[7] It is seen that the signal of C1 in an α-chloride is found at lower field than that of a β-chloride. This was also observed previously in case of the anomeric tetra-*O*-acetyl-D-glucopyranosyl chlorides [8] whereas in most other cases the signal of C1 is at lowest field in a β-pyranose derivative.[9] The signals of C3 and C5 in a β-chloride are shifted downfield compared to those of the corresponding α-chloride, in agreement with the γ-effect.[10] The chemical shifts of C4 and C6 are nearly independent of the anomeric configuration while C2 in compounds with an axial substituent at C1 (α-anomers) is at higher field than in β-anomers.

The ^{13}C chemical shifts of *6*, *8*, and *9* are in accordance with the structural assignments made from the proton spectra. Thus the β-

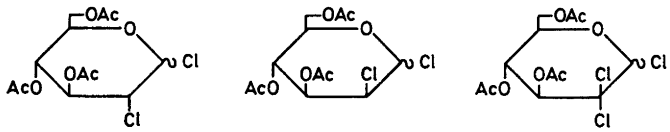
Table 1. Proton chemical shifts (δ) in deuteriochloroform and coupling constants (Hz) of 1,2-dichloro-sugars and some related compounds.

Compound	H1	H3	H4	H5	H6	H6′	J_{34}	J_{45}	J_{56}	$J_{66'}$	$J_{56'}$
8	7.50*a*	6.48	6.17	4.82	4.59	4.43	9.4	10.2	2.8	12.6	3.8
6	7.0	6.69	6.02	4.42	4.76	4.51	9.2	9.6	3.4	12.1	4.6
9	7.36*a*	6.55	6.17	4.78	4.7	4.54	9.6	10.0	2.8	12.6	4.6
17a	7.33*a*	4.34	5.92	4.64	4.53	4.34	9.4	10.2	2.6	12.4	4.2
18a	6.68	4.94	5.77	4.30	4.63	4.40	9.2	9.2	2.4	12.0	4.6
17b	7.52*a*	6.27	6.20	5.19	4.68	4.47	4.2	1.8	6.8	11.7	6.3
18b	6.93	6.81	6.14	4.67	4.35—4.9		4.5	1.8			
α15	6.24	5.88	5.37	4.46	4.2 —4.3		9.4	10.4	3.4		3.4
β15	5.58	5.52	5.25	3.95	4.2 —4.3		9.5	9.5	3.0		4.3
5	6.41	5.95	5.45	3.8	4.3 —4.6		1.2	7.8			
10	6.20	5.94	5.49	3.94	4.2 —4.6		1.2	7.8			
11	6.30	5.92	5.50	3.97	4.55—4.4		1.0	7.4	3.4	12.2	4.8
12[b]	6.50	6.05	5.61	4.37	4.60—4.43		1.0	7.6	4.0	13.0	4.6

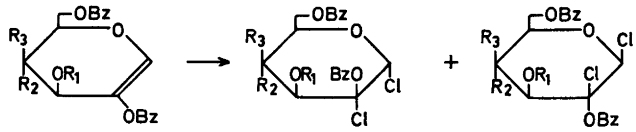
[a] The signal was hidden by the signals of the aromatic protons. Its position was therefore determined through the ^{13}C NMR spectrum by selective proton decoupling, finding the proton frequency at which Cl collapses to a singlet.[7]
[b] Benzylidene proton δ 6.15.

chloride (*6*) has Cl at higher field than the α-chloride (*9*); C2, C3, and C5 of *6* are at lower field than those of *9* in agreement with the results described above. The α-chlorides *8* and *9* have rather similar spectra. The largest differences are in the shifts of Cl and C3, in agreement with the proposed structures in which only the configuration of C2 is different.

Undecoupled ^{13}C NMR spectra of a large number of carbohydrates have shown that hexopyranoses with an axial H1 have a $^1J[^{13}C-H(1)]$ value which is *ca.* 10 Hz lower than that of the corresponding compound with an equatorial H1.[8,11]



13 14 15



16 17 18

a: = R_1 = CH_3, R_2 = OBz, R_3 = H

b: = R_1 = Bz, R_2 = H, R_3 = OBz

Table 2. ^{13}C Chemical shifts (ppm relative to Me_4Si) and $^1J[^{13}C\text{-}H(1)]$ values (Hz) of 1,2-dichlorosugars and some related compounds.

Compound	C1	C2	C3	C4	C5	C6	$^1J[^{13}C\text{-}H(1)]$
8	90.7	96.2	73.0	67.6	70.8	61.6	190
6	88.8	102.0	72.3	68.0	76.6	62.0	174
9	92.0	96.7	71.0	67.0	71.3	61.8	193
17a	91.2	96.8	82.3	69.2	70.7	61.8	190
18a	87.2	103.9	81.2	70.5	76.5	62.7	171
17b	91.1	95.0	70.2	67.5	69.6	61.3	190
18b	88.5	100.5	69.5	67.6	76.3	61.7	172
α13	92.2	57.4	70.9	67.8	70.5	60.7	185
β13	89.5	61.0	73.8	67.6	75.0	61.0	171
α14	90.6	59.6	68.1	64.2	71.5	61.0	187
β14	86.8	61.8	71.7	64.2	76.5	61.8	166
α15	94.6	86.4	72.4	66.8	71.2	60.8	189
β15	91.9	87.8	76.4	66.7	76.4	61.4	173
5	106.4	97.4					≃190
10	105.2	98.0	71.0[a]	67.2	68.9[a]	63.3	188
11	105.2	97.2					190
12	104.8	100.7	71.4[a]	67.6	69.3[a]	63.9	185

[a] May be reversed.

This difference between $^1J[^{13}C-H(1)]$ values of a pair of anomers is also found in the dichlorides (*13*) with the *gluco*-configuration, although it is slightly bigger (14 Hz), while in the dichloro compounds with *manno*-configuration (*14*) the difference is unusually large (21 Hz) (Table 2). With two electronegative substituents at C2 such as in the trichlorides (*15*) the difference is 16 Hz. A similar large difference (19 Hz) is found between the anomeric pair *9* and *6*. Between *8* and *6* the difference is 16 Hz indicating that *8* is also an α-chloride.

Thus the ^1H NMR and ^{13}C NMR spectra indicate that *6* is tetra-*O*-benzoyl-2-chloro-β-D-glucopyranosyl chloride, *9* is the corresponding α-anomer, and *8* is tetra-*O*-benzoyl-2-chloro-α-D-mannopyranosyl chloride.

Despite the fact that the pure chlorides *6* and *8* are very resistant towards hydrolysis Maurer *et al.*[3] were able to obtain a hydrolysis product when they treated the crude chlorination mixture with aqueous sodium hydrogencarbonate. This seems to indicate that products other than *6* and *8* are present in the crude mixture.

To investigate this *4* was treated with chlorine in tetrachloro methane at −20 °C and a ^1H NMR spectrum was measured within a few minutes at −20 °C. This showed that a small amount (*ca.* 25 %) of *8* was present whereas the major product was a compound different from *6*. Spectra run at intervals showed that only small changes took place during 2 h at −20 °C; but at room temperature the major

product disappeared rapidly and *6* was formed instead. The amount of *8* remained unchanged. Thus chlorination of *4* yields *8* and an unstable intermediate which rearranges to *6* at room temperature. The intermediate is assumed to be an orthoacid chloride *5* on the basis of its ^{1}H NMR spectrum and reactions. Its spectrum (Table 1) showed a small coupling (1.2 Hz) between H3 and H4, indicating that it has a distorted chair conformation similar to that of 1,2-*O*-α-alkylidene derivatives.[12–14] Orthoacid chlorides have not been isolated, but they have been observed in solution at −60 °C.[15]

When a solution of *5*, prepared as described above, was treated with water it reacted immediately to give tetra-*O*-benzoyl-α-D-*arabino*-hexopyranosulose (*2*). Maurer and Petsch [3] hydrolyzed the crude chlorination product with aqueous sodium hydrogencarbonate in boiling benzene, and under these conditions *2* is unstable.[4] When *2* was subjected to this treatment it lost benzoic acid and gave *1* which is probably the product isolated by Maurer and Petsch. Treatment of crude *5* with methanol in pyridine gave a stable orthoester *10*. Reaction with silver fluoride yielded a rather unstable fluoride, which is probably *11*. Reduction of *5* with lithium borohydride gave a benzylidene derivative *12*. Proton and ^{13}C NMR spectra of *10*, *11*, and *12* are quite similar to those of *5*. The rather large $^1J[^{13}$C-H(1)] values (185 – 190) may indicate that all four compounds are α-anomers (Table 2).

The dichlorocompound *8* could be formed from *4* by direct *cis*-addition of chlorine or, possibly, *via* a very unstable orthoacid chloride *7*. The latter has not been observed.

The chlorination of two other compounds was also investigated. Treatment of tri-*O*-benzoyl-3-*O*-methyl-1-deoxy-D-*arabino*-hex-1-enopyranose (*16a*) with chlorine gave *17a* and *18a* in 67 % and 16 % yield, respectively. From the spectra (Tables 1 and 2) the structures were shown to be analogous to those of *8* and *6*. No intermediates analogous to *5* could be observed by chlorination at low temperature. Similar results were obtained when tetra-*O*-benzoyl-1-deoxy-D-*lyxo*-hex-1-enopyranose (*16b*) was chlorinated. No intermediates were observed at low temperature. Work up gave the compounds *17b* and *18b* the structures of which were derived from their spectra.

The dichlorosugars *6* and *8* are very unreactive. Hydrolysis or methanolysis gave only unchanged starting materials. Catalytic reduction using palladium on carbon did not affect any of the chlorine atoms. Treatment with pyridine converted both *6* and *8* into di-*O*-benzoyl-kojic acid.[3] With anhydrous hydrogen fluoride *6* was destroyed, but *8* gave tri-*O*-benzoyl-α-D-*arabino*-hexopyranosulosyl chloride (*3*).

EXPERIMENTAL

Melting points are uncorrected. For thin-layer chromatography (TLC) silica gel PF_{254} (Merck) was used; preparative TLC was conducted with 1 mm layers on 20 × 40 cm plates. ^{1}H NMR spectra were recorded with a Varian HA-100 and a Bruker HXE-90 instrument using tetramethylsilane as the internal reference. ^{13}C NMR spectra were measured as previously recorded.[11] Optical rotations were measured in chloroform solution.

*Chlorination of tetra-*O*-benzoyl-1-deoxy-*D*-arabino-hex-1-enopyranose (4).* 3.17 g of *4* was dissolved at 0 °C in carbon tetrachloride (*ca.* 10 ml) which was previously saturated with chlorine. The solution was kept for 15 min at 0 °C and for 15 min at room temperature. The solvent was evaporated and carbon tetrachloride was added and again evaporated. The residue (*ca.* 4 g) was crystallized from ethanol (40 ml) to give 1.03 g (29 %) of *8*, m.p. 153 – 155 °C. Recrystallization gave a product with m.p. 155.5 – 157 °C, $[\alpha]_D^{25}$ +10.1° (*c* 1) (reported [3] m.p. 156 °C, $[\alpha]_D$ +10.3°).

Evaporation of the mother liquor gave a syrup (2.5 g, 71 %) which was almost pure as seen from TLC and ^{1}H NMR spectra. A sample was purified by preparative TLC eluting twice with benzene. The fast moving fraction gave a trace of *8*. The main fraction gave *6* as a syrup, $[\alpha]_D^{25}$ +28.0° (*c* 3). (Found: C 63.07; H 4.06; Cl 10.87. Calc. for $C_{34}H_{26}Cl_2O_9$: C 62.87; H 4.04; Cl 10.92). The product could be crystallized with some difficulty from cyclohexane, m.p. 84 – 88 °C, $[\alpha]_D^{25}$ +24.4°. A ^{1}H NMR spectrum showed that this product contained *ca.* one equivalent of cyclohexane.

Treatment of 6 *with titanium tetrachloride.* Pure *6* (200 mg) was dissolved in dry chloroform (20 ml). 10 ml of the solvent was evaporated to remove moisture. To the solution was added titanium tetrachloride (0.1 ml). The mixture was heated to 85 °C and the reaction was monitored by TLC (benzene). After 2 h *6* was no longer present. The dark mixture was poured on ice and the product was extracted with chloroform. The extract was washed with aqueous sodium hydrogencarbonate, dried and evaporated to give a syrup (140 mg) which was

almost pure as seen from TLC and an NMR spectrum. The product was purified by preparative TLC (benzene) to give 100 mg (50 %) of *9* as a syrup, $[\alpha]_D^{25}$ +25.2° (*c* 3.4). (Found: C 63.16; H 4.23; Cl 10.94). No other products could be isolated.

A similar treatment of *8* with $TiCl_4$ led to recovery of 70 % unchanged *8*. No other products could be isolated.

Tetra-O-*benzoyl*-α-D-arabino-*hexopyranosulose* (*2*). The glycal *4* (160 mg) was treated with chlorine in carbon tetrachloride at 0 °C as described above. The solution was stirred at 0 °C for 5 min with water (0.5 ml) and sodium hydrogencarbonate (0.5 g). It was then filtered, dried ($MgSO_4$) and evaporated. The residue (200 mg) was separated into two fractions by preparative TLC (ether-pentane 2:1). The fast moving fraction gave 43 mg (24 %) of *8*. The next fraction gave 116 mg (71 %) of partly hydrated *2*, which was dried *in vacuo* over P_2O_5, $[\alpha]_D^{25}$ +38.6° (*c* 4.6). A ^1H NMR spectrum was identical with that of the product described previously.[4]

1,3,6-Tri-O-*benzoyl*-*4-deoxy*-α-D-glycero-*hex*-*3*-*enopyranosulose* (*1*). Chlorination of *4* (4.6 g) followed by hydrolysis as described above gave a crude product which was crystallized from ethanol (10 ml) to give 1.4 g (27 %) of *8*, m.p. 153–155 °C. Evaporation of the mother liquor gave 3.7 g of residue which was boiled for 1 h in benzene (20 ml) in the presence of water (0.5 ml) and sodium hydrogencarbonate (5 g). The mixture was then diluted with dichloromethane, dried (Na_2SO_4), filtered and evaporated. The residue (2.8 g) was crystallized from ethanol (15 ml) to give 1.2 g (32 %) of *1*, m.p. 122–124 °C (recorded [4] m.p. 126.5–127 °C). A ^1H NMR spectrum [4] further confirmed the structure.

Preparation of the orthoester (*10*). 2.0 g of *4* was treated with chlorine in carbon tetrachloride at 0 °C. A mixture of dry methanol (6 ml) and pyridin (6 ml) was cooled to 0 °C and added in one portion to the chlorination mixture. After 30 min at 0 °C the mixture was diluted with carbon tetrachloride and washed five times with ice-water. The organic phase was dried by pouring through filter paper and evaporated at room temperature. The residue was crystallized from ethanol to remove some *8*. The ethanol was evaporated and the residue was chromatographed on a column of silica gel (100 g) eluting with ether-pentane (1:1). The main fraction gave 1.06 g (47 %) of *10* as a syrup, $[\alpha]_D^{25}$ −12.4° (*c* 3). (Found: C 65.84; H 4.66. Calc. for $C_{35}H_{29}ClO_{10}$: C 65.15; H 4.53).

Preparation of the orthoacid fluoride (*11*). 2.0 g of *4* was chlorinated as described above. Silver fluoride (5 g) was suspended in dry acetonitrile (5 ml) and stirred with Siccon for 15 min at room temperature and then cooled to 0 °C. The carbon tetrachloride solution, containing the chlorinated sugar, was poured into the silver fluoride suspension at 0 °C and the mixture was stirred for 30 min. It was then filtered under dry air and the solid was washed with carbon tetrachloride. The combined filtrate was evaporated *in vacuo* at room temperature to give a syrupy product which contained some silver salts. Spectra were measured on a carbon tetrachloride solution. A ^{19}F NMR spectrum gave a singlet 50 ppm upfield from $CFCl_3$. The product was too unstable to be purified further.

Preparation of the benzylidene-derivative (*12*). A solution of chlorinated *4* (1.04 g) was added slowly with stirring to an excess of lithium borohydride suspended in ether, which was cooled in an ice-salt bath. The stirring was continued for 10 min at room temperature and the mixture was then diluted with dichloromethane. Water was then added with caution and the organic phase was washed twice with water, dried ($MgSO_4$) and evaporated to give a syrup (1.2 g). The product was purified by preparative TLC eluting twice with benzene. The fast moving fraction gave 266 mg (23 %) of *8*. The next fraction gave 546 mg (50 %) of the benzylidene-derivative *12*. A sample was rechromatographed (ether-pentane 1:1), $[\alpha]_D^{25}$ −52.7° (*c* 1.1). (Found: C 66.52; H 4.40; Cl 5.70. Calc. for $C_{34}H_{27}ClO_9$: C 66.40; H 4.42; Cl 5.76).

Treatment of 8 *with anhydrous hydrogen fluoride*. The dichlorocompound *8* (1.36 g) was dissolved in anhydrous hydrogen fluoride (3 ml) at −10 °C and the solution was kept at +5 °C for 24 h. It was then diluted with dichloromethane and poured on ice; the organic phase was washed with aqueous sodium hydrogencarbonate, dried and evaporated. The syrupy residue (1.03 g) was crystallized from ether to give 407 mg (38 %) of *3*, m.p. 161–163 °C. Recrystallization gave the pure product, m.p. 165–167 °C, $[\alpha]_D^{25}$ +137° (*c* 2). (Found: C 63.81; H 4.14; Cl 6.84. Calc. for $C_{27}H_{21}ClO_8$: C 63.71; H 4.16; Cl 6.97).

Tri-O-*acetyl*-*2,2-dichloro*-*2*-*deoxy*-α- *and* β-D-arabino-*hexopyranosyl chlorides* (*15*). Tri-*O*-acetyl-2-chloro-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (5.0 g) was chlorinated as described by Brandley and Buncel [6] and worked up to give 667 mg (11 %) of a product with m.p. 161–163 °C, $[\alpha]_D^{25}$ −4.5° (*c* 6). A ^1H NMR spectrum was identical with that reported,[6] but showed that the product was the β-anomer (β-15).

The material in the mother liquor (4.2 g) was chromatographed on a column of silica gel (400 g) using benzene-ether (2:1) as eluent. The main fraction gave 1.76 g (29 %) of pure (α-15) as a syrup, $[\alpha]_D^{25}$ +113° (*c* 9). (Found: C 38.18; H 4.06; Cl 28.08. Calc. for $C_{13}H_{15}Cl_3O_7$: C 38.18; H 4.01; Cl 28.17).

Tri-O-*benzoyl*-*1,2-dideoxy*-*3*-O-*methyl*-D-arabino-*hex*-*1*-*enopyranose* (*16a*). Elimination of hydrogen bromide from tri-*O*-benzoyl-3-*O*-methyl-α-D-glucopyranosyl bromide (13 g) according to Ferrier [16] gave 11 g of crude *16a* as a syrup. Chromatography on a column of silica gel (500 g) using ether-pentane: (11) as eluent gave 7.8

g (70 %) of almost pure *16a*. Rechromatography with benzene-ether (19:1) as eluent gave 4 g (36 %) of pure *16a*, $[\alpha]_D^{25}$ −7.7° (*c* 6.8). (Found: C 68.53; H 4.96. Calc. for $C_{28}H_{24}O_8$: C 68.85; H 4.95).

Reaction of 16a *with chlorine.* Chlorination of *16a* (1.4 g) in carbon tetrachloride was performed as described above. Evaporation gave 1.34 g of a product which was crystallized from ethanol (10 ml) to give 931 mg (58 %) of tri-*O*-benzoyl-2-chloro-3-*O*-methyl-α-D-mannopyranosyl chloride (*17a*), m.p. 129 – 130 °C. Recrystallization gave the pure product, m.p. 131 – 132 °C, $[\alpha]_D^{25}$ +109.5° (*c* 4). (Found: C 60.05; H 4.36; Cl 12.34. Calc. for $C_{28}H_{24}Cl_2O_8$: C 60.12; H 4.33; Cl 12.68). The material in the mother liquor was separated into two fractions by preparative TLC (ethyl acetate-pentane 1:4). The first fraction gave 131 mg (8 %) of *17a*. The next fraction gave 259 mg (16 %) of tri-*O*-benzoyl-2-chloro-3-*O*-methyl-β-D-glucopyranosyl chloride (*18a*), which was crystallized from ether-pentane, m.p. 69 – 71 °C, $[\alpha]_D^{25}$ +16.8° (*c* 1.5). (Found: C 60.25; H 4.48; Cl 12.52).

*Tetra-O-benzoyl-1-deoxy-*D-lyxo-*hex-1-enopyranose* (16b) was prepared by the method of Ferrier [16] from tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (9.5 g). The crude product (8.4 g) was purified by chromatography on a column of silica gel (500 g) eluting with ether-pentane (2:1). This gave a fraction containing 1.6 g (20 %) of pure *16b* as a syrup, $[\alpha]_D^{25}$ +16.9° (*c* 3). (Found: C 70.42; H 4.67. Calc. for $C_{34}H_{26}O_9$: C 70.58; H 4.53). Further elution of the column gave *16b* which was not quite pure.

Treatment of 16b *with chlorine.* Chlorination of *16b* (700 mg) in carbon tetrachloride as described above gave a product (771 mg) which was separated into two fractions by preparative TLC (benzene). The fast moving fraction gave 341 mg (44 %) of tetra-*O*-benzoyl-2-chloro-α-D-talopyranosyl chloride (*17b*) as a syrup, $[\alpha]_D^{25}$ +85.5° (*c* 8.5). (Found: C 63.04; H 4.21; Cl 10.66. Calc. for $C_{34}H_{26}Cl_2O_9$: C 62.87; H 4.04; Cl 10.92).

The next fraction gave 158 mg (20 %) of tetra-*O*-benzoyl-2-chloro-β-D-galactopyranosyl chloride (*18b*) as a syrup, $[\alpha]_D^{25}$ +93.6° (*c* 2.7). (Found: C 62.69; H 4.23; Cl 10.68).

Microanalyses were performed by Dr. A. Bernhardt, Mikroanalytisches Laboratorium, or by Novo Microanalytical Laboratory.

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Resolution of (±)-2,2′-Spirobi[indan]-1,1′-dione

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Optically active 2,2′-spirobi[indan]-1,1′-dione has been prepared in good yield and high optical purity by a procedure including the following four steps: 1. stereoselective reduction of (±)-2,2′-spirobi[indan]-1,1′-dione to (±)-1′-hydroxy-2,2′-spirobi[indan]-1-one; 2. acylation of this compound with (−)-ω-camphanic acid chloride to give two diastereomeric camphanic esters; 3. reduction of the higher melting diastereomer to optically active 2,2′-spirobi-[indan]-1,1′-diol, and 4. oxidation of the latter compound to (+)-2,2′-spirobi[indan]-1,1′-dione. The optical purity of this compound was higher than 95 %, according to differential thermal analysis.

In continuing the studies of some aromatic spiro-compounds, *e.g.* (±)-2,2′-spirobi[indan]-1,1′-dione,[1] I, we have also recently resolved I. Two recent publications, [2,3] prompt us to publish our results at the present stage of studies.

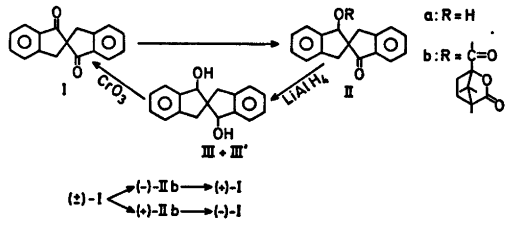
The primary aim was to elaborate a resolution, which could yield the optically active compound I in quantity and in high optical purity. Two methods were examined, resolution by chromatography and *via* diastereomer formation. No resolution was, however, obtained by chromatography of racemic I on partially acetylated cellulose with benzene as eluent.

The method applying diastereomer formation involves reduction of the racemic ketone to the corresponding diastereomeric secondary alcohols' followed by resolution of one of these and finally reoxidation of the enantiomeric alcohols to the enantiomeric ketones.

Reduction of I with excess of lithium aluminium hydride in tetrahydrofuran gave predominantly one of the three possible diastereomeric dihydroxy derivatives. The yield of this isomer (III, Scheme 1) was 65 %.

The NMR spectrum of III in DMSO-d_6 demonstrates, that the secondary alcohol groups at the 1 and 1′ positions have opposite configurations, and that the methylene groups at the 3 and 3′ positions are not equivalent. It is thus concluded that III is identical with (±)-2,2′-spirobi[indan]-(1*R*,1′*S*)-diol.

The monoreduction of I could be achieved by using lithium tri-*t*-butoxyaluminium hydride as reducing agent in tetrahydrofuran. The reduction shows high stereoselectivity since the isomer (IIa, Scheme 1) could be isolated in a yield higher than 70 %.



Scheme 1.

Chemical reactions (Scheme 1) and spectroscopic properties (NMR, IR) of the monoreduction product are consistent with structure IIa.

Experiments to resolve III were not successful. Resolution of IIa according to Scheme 1 did, however, succeed. This method follows the reaction sequence used in resolution of spiro[4.5] decan-1,6-dione [4] (Scheme 1).

Acylation of (±)-IIa with (−)-ω-camphanic acid chloride in pyridine afforded the camphanic ester IIb, (−)-IIb + (+)-IIb, in about 95 % yield. The separation of the two diastereomers (−)-IIb and (+)-IIb, respectively, could be achieved by fractional crystallization from

benzene or acetone. This separation gave the higher melting diastereomer (−)-IIb in almost pure form in 25−30 % yield.

The NMR spectra of the two diastereomers (+)-IIb and (−)-IIb are identical except for the camphanic acid residues, whose proton signals appear at higher field than those of the rest of the molecule. The signals from the three methyl groups in (−)-IIb and (+)-IIb, respectively, appear as three singlets. The separation of the methyl signals of the two diastereomers allows evaluation of the ratio between (−)-IIb and (+)-IIb.

Reduction of (−)-IIb or (+)-IIb, respectively, with excess of lithium aluminium hydride in tetrahydrofuran afforded a mixture of the two possible epimeric dihydroxy derivatives (III+III′), which was optically active.

In the dihydroxy derivative III′, the configuration at C_1 and C_1' must be the same; this is proved by NMR, which shows only one AB system for the secondary alcohol groups at C_1 and C_1' and only one AB system for the two methylene groups at the 3 and 3′ positions.

An alternative route to that outlined in Scheme 1 for conversion of IIb to I is hydrolysis of the camphanic ester (IIb) to give IIa, followed by oxidation of IIa to I.

Hydrolysis of (−)-IIb in alkaline solution gave a nearly quantitative yield of IIa, but the product was optically inactive; it is concluded that this racemization must involve cleavage of one of the bonds to the spiro atom C_2.

Oxidation of optically active 2,2′-spirobi-[indan]-1,1′-diol (III+III′) with Jones reagent gave (+)-I or (−)-I, respectively, according to the correlation outlined in Scheme 1. The yield of optically active 2,2′-spirobi[indan]-1,1′-dione was about 70 %.

Differential thermal analysis performed on (+)-I indicated that the optical purity was high, since the corresponding thermogram showed only one sharp endotherm on heating; (+)-I thus gives a thermogram typical for a pure compound.

Differential thermal analysis performed on a mixture of (±)-I and (+)-I, prepared with an optical purity of 93 %, displayed a thermogram on which an endotherm corresponding to (±)-I easily could be detected; from the peak height of the endotherm it is judged that the optical purity of (+)-I is higher than 95 % If one accepts the melting point curve in Ref. 3 (Fig. 3), then the optically purity of our product would be ~90 %.

The CD spectrum of (+)-I in methanol shows two positive and two negative absorption maxima in the $n-\pi^*$ range at 354, 340, 329, and 318 nm, respectively, while two positive and one negative CD maxima are observed in the $\pi-\pi^*$ range at 297, 244, and 230 nm.

The CD spectrum of (+)-I is thus in gross accordance with that recently published.[2]

The ORD spectrum of (+)-I in methanol shows three cotton effects in the wavelength range 250−400 nm, positive cotton effects at 355 and 295 nm, respectively, and a negative one at 331 nm.

The ORD spectrum of (+)-I could thus be correlated to the CD spectrum in the $n-\pi^*$ and the first $\pi-\pi^*$ band.

EXPERIMENTAL

NMR spectra were recorded at 60 MHz on a Varian A-60 spectrometer, using TMS as internal standard. The chemical shifts are expressed in τ values and the coupling constants numerically in Hz. IR spectra were recorded on a Perkin-Elmer model 521 spectrophotometer. The mass spectra were determined on a CEC 21/104 instrument. Optical rotations were measured with a Zeiss polarimeter, using a 2 dm cell to determine specific rotations, c being expressed as g/100 ml of solution. The circular dichroism was measured on a Rousset-Jouan Dichrographe. The ORD spectrum was performed on a F.J.C.A. spectropol instrument. Differential thermal analyses were performed with a Du Pont 900 Differential Thermal Analyser Instrument. Elemental analyses were determined in the laboratories of Dornis und Kolbe, Mülheim.

(±)-2,2′-Spirobi[indan]-1,1′-dione (I) was prepared by cyclization of (±)-2-benzyl-3-(*o*-carboxyphenyl)propionic acid.[1] Instead of purification by sublimation, the dried raw product was recrystallized directly (without pretreatment with Na_2CO_3) from benzene (charcoal). The yield was 75 %.

(±)-2,2′-Spirobi[indan]-(1R,1′S)-diol (III). To a stirred mixture of $LiAlH_4$ (1.52 g, 0.04 mol) and tetrahydrofuran (35 ml), distilled over $LiAlH_4$ was added a solution of (±)-2,2′-spirobi[indan]-1,1′-dione (I) (2.48 g, 0.01 mol) in tetrahydrofuran (100 ml) at 20 °C. The temperature of the mixture rose to 36 °C. Stirring was continued at 30 °C for 1 h. The reaction mixture was decomposed by successive addition of ethyl acetate (20 ml) and H_2O (80 ml). The inor-

ganic precipitate was filtered with suction and washed with warm $CHCl_3$. The $CHCl_3$ phase in the filtrate was separated and washed with H_2O and saturated NaCl solution. After drying ($CaSO_4$), the filtered solution was evaporated, and the crystalline residue (2.19 g) recrystallized from benzene (30 ml) to give III (1.61 g, 64 %), m.p. 155–157.5 °C. (Found: C 80.44; H 6.29. Calc. for $C_{17}H_{16}O_2$; C 80.92; H 6.39). MS (70 eV): *m/e* 234 ($M^+ - H_2O$, 100 %), 118 (64 %), 91 (79 %), 69 (67 %). NMR [$(CD_3)_2SO$]: τ 2.63–2.86 (m, 8 H, arom. protons), 4.72 (d, 1 H, $J = 6.5$ Hz), 5.40 (d, 1 H, HO–C–H), 4.87 (d, 1 H, $J = 6.5$ Hz), 5.21 (d, 1 H, HO–C–H), 6.54 (d, 1 H, $J = 15.5$ Hz), 7.45 (d, 1 H, CH_2), 7.20–7.26 (m, 2 H, CH_2).

(±)-1′-Hydroxy-2,2′-spirobi[indan]-1-one (IIa). To a stirred mixture of $LiAlH(OC_4H_9)_3$ (3.81 g) (≥ 90 %, Fluka) and tetrahydrofuran (50 ml), distilled over $LiAlH_4$, was added a solution of (±)-2,2′-spirobi[indan]-1,1′-dione (I) (2.48 g, 0.01 mol) in tetrahydrofuran (100 ml) at room temperature. The mixture was refluxed with stirring for 2 h. To the warm reaction mixture was added ethyl acetate (5 ml). After the exothermic reaction had subsided, the mixture was cooled and H_2O (10 ml) added; stirring was continued for further 15 min. The reaction mixture was filtered with suction, and the inorganic precipitate washed with warm $CHCl_3$. The combined filtrate was evaporated to dryness and the residue recrystallized from benzene (140 ml) to give IIa (1.81 g, 72 %), m.p. 198.5–199.5 °C. (Found: C 81.28; H 5.99. Calc. for $C_{17}H_{14}O_2$; C 81.58; H 5.64). MS (70 eV): *m/e* 250 (M^+, 24 %), 232 ($M^+ - H_2O$, 100 %), 131 (68 %). ν_{max}($CHCl_3$): 1719 cm^{-1} (C=O). NMR [$(CD_3)_2SO$]: τ 2.17–2.78 (m, 8 H, arom. protons), 4.21 (d, 1 H, $J = 6.2$ Hz), 4.65 (d, 1 H, HO–C–H), 6.31 (d, 1 H, $J = 17.5$ Hz), 7.23 (d, 1 H, CH_2), 6.78 (d, 1 H, $J = 15.5$ Hz), 7.11 (d, 1 H, CH_2).

(−)-ω-Camphanic acid was prepared by hydrolysis of (−)-α-bromocamphoric anhydride as previously described.[5,6]

Acylation of (±)-IIa with (−)-ω-camphanic acid chloride. The sodium salt of camphanic acid was prepared from camphanic acid (2.97 g, 0.015 mol), $NaHCO_3$ (1,32 g, 0.016 mol) and H_2O (10 ml). The resulting solution was evaporated. Benzene (5 ml), was added to the residue, and the mixture was again evaporated; this operation was repeated twice followed by drying at 50–60 °C. Benzene (10 ml) and pyridine (0.2 ml) were added to the dry sodium salt. The mixture was cooled in ice and a solution of oxalyl chloride (6.43 ml, 0.075 mol) in benzene (10 ml) was added during 5–10 min, while the reaction flask was shaken. After cooling for further 10 min the mixture was placed at room temperature for 19 h. After evaporation *in vacuo*, benzene (5 ml) was added to the residue followed by evaporation *in vacuo*, this operation was repeated twice. To the flask with the prepared camphanic acid chloride was added (±)-1′-hydroxy-2,2′-spirobi-[indan]-1-one (IIa) (3.0 g, 0.012 mol). The reaction flask was cooled in ice and pyridine (20 ml) added. After cooling for 0.5 h the reaction flask was placed at room temperature for 92 h. To the mixture was added $CHCl_3$ (30 ml). The $CHCl_3$ solution was washed successively with H_2O, HCl (2 N) and Na_2CO_3 (5 %). After drying ($CaSO_4$) the $CHCl_3$ solution was evaporated *in vacuo* to give crystalline IIb (4.85 g, 94 %). One recrystallization of the camphanic ester (IIb) from acetone (40 ml) gave (−)-IIb (1.52 g, 29.4 %), m.p. 198–200 °C. (Found: C 75.23; H 6.24. Calc. for $C_{27}H_{26}O_5$; C 75.33; H 6.09). MS (70 eV): *m/e* 249 ($M^+ - 181$, 24 %), 232 ($M^+ - 198$, 100 %). ν_{max} ($CHCl_3$): 1734, 1758, 1808 cm^{-1} (C=O). NMR ($CDCl_3$): τ 2.12–2.72 (m, 8 H, arom. protons), 3.44 (s, 1 H, H–C–OR), 6.22–7.22 (m, 4 H, CH_2–C–CH_2), 7.60–8.40 (m, 4 H, CH_2–CH_2), 8.97 (9.12, 9.41, 3 s, 3 CH_3). $[\alpha]_D^{26}$ −89.75° (*c* 2.0, $CHCl_3$). Evaporation of the filtrate from the isolation of (−)-IIb afforded a mixture of (+)-IIb and (−)-IIb (2.96 g, 57.3 %). NMR ($CDCl_3$): τ 2.12–2.72 (m, 8 H, arom. protons), 3.44 (s, 1 H, H–C–OR), 6.20–7.20 (m, 4 H, CH_2–C–CH_2), 7,5–8.6 (m, 4 H, CH_2–CH_2), 8.97 (9.12, 9.41, 3 s, 3 CH_3), 8.95 (9.12, 9.17, 3 s, 3 CH_3). The ratio between (−)-IIb and (+)-IIb in the two isolated fractions were 97:3 and 30:70, respectively, as determined from the NMR spectra.

2,2′-Spirobi[indan]-1,1′-diol (III + III′). To a stirred mixture of $LiAlH_4$ (1.73 g, 45.5 m mol) in tetrahydrofuran (12 ml), distilled over $LiAlH_4$, was added a solution of the camphanic ester (−)-IIb (1.31 g, 3.03 m mol) in tetrahydrofuran (30 ml) at room temperature. The mixture was refluxed with stirring for 5 h. Excess $LiAlH_4$ was destroyed by dropwise addition of ethyl acetate (10 ml). After the exothermic reaction had subsided, the reaction mixture was cooled and H_2O (40 ml) added dropwise. After work up as described above for III, the $CHCl_3$ solution was evaporated and the crystalline residue treated with warm benzene (5 ml) to give preferentially III (0.51 g). $[\alpha]_D^{25}$ +10.9° (*c* 4.0, tetrahydrofuran). The filtrate from the isolation of III was evaporated, and the residue (0.25 g) subjected to further reduction with $LiAlH_4$ (0.33 g) in tetrahydrofuran (10 ml). The mixture was stirred and refluxed for 2 h. After work up as described for the isolation of III, 0.1 g crystalline dihydroxy derivative (III + III′) was isolated. NMR (of III′) [$(CD_3)_2SO$]: τ 2.63–2.85 (m, 8 H, arom. protons), 4.52 (d, 2 H, $J = 6.4$ Hz), 4.99 (d, 2 H, HO–C–H), 7.03 (d, 2 H, $J = 15.5$ Hz), 7.35 (d, 2 H, CH_2). The total yield of dihydroxy derivative was 80 %. From the integrated intensities of the (HO–C–H) signals in the two isolated fractions, the ratio of III to III′ was evaluated to be 9:1 and 4:6, respectively.

Alkaline hydrolysis of (−)-IIb. A mixture of (−)-IIb (0.22 g, 0.5 mol), ethanol (5 ml, 96 %),

H_2O (3 ml) and aqueous KOH (2.0 ml, 0.5 N) was refluxed for 8 min. After cooling in ice-water the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ phase was extracted successively with H_2O and saturated NaCl solution. After drying ($CaSO_4$), the $CHCl_3$ solution was evaporated *in vacuo* to give crystalline IIa (0.12 g, 96 %). The product, which was optically inactive could be oxidized to (±)-I by means of Jones reagent.

(+)-2,2'-Spirobi[indan]-1,1'-dione [(+)-I]. To a mixture of III + III' (0.32 g, 1.27 m mol), prepared by reduction of (−)-IIb, and acetone (10 ml), distilled from $KMnO_4$, was added rapidly at room temperature with stirring 0.80 ml of 8 N CrO_3 reagent (10 ml, 8 N reagent was prepared from CrO_3 (2.67 g), conc. H_2SO_4 (2.3 ml) and H_2O). After addition of CrO_3 reagent the temperature in the reaction mixture rose to 39 °C. The reaction flask was placed in a water-bath of room temperature and stirring was continued for further 20 min. To the reaction mixture, which contained a dark coloured precipitate, was added H_2O (10 − 15 ml). The dark coloured precipitate disappeared, and a white precipitate was formed. After filtration and drying the product (0.26 g) was recrystallized from ethanol (18 ml, 96 %) to give (+)-I (0.215 g, 68.3 %) m.p. 210.5 − 212.5 °C. $[\alpha]_D^{24.5}$ +151.86° (*c* 3.22, $CHCl_3$). The NMR spectrum was identical with that of (±)-I previously reported.[1] ν_{max}(KBr): 1705, 1735 cm^{-1} (C=O). CD spectrum (methanol), (ν_{max} nm,$\Delta\varepsilon$): 354 (+0.34), 340 (+0.27), 329 (−0.58), 318 (-1.1_6), 297 ($+10._0$), 244 ($+5.2_3$), 230 (-8.5_4).

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Initial Velocity and Product Inhibition Studies on L-Iditol:NAD Oxidoreductase

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The mechanism of action of the enzyme L-iditol:NAD oxidoreductase (EC 1.1.1.14; sorbitol dehydrogenase) from sheep liver has been investigated. The catalyzed reaction:

D-sorbitol + $NAD^+ \rightleftharpoons$ D-fructose + NADH + H^+

was followed from both sides in series of spectrophotometric measurements of initial velocity and product inhibition. All measurements were carried out at pH 7.0.

The results indicates that this two-substrate reaction follows a Rapid Equilibrium Random mechanism in which one of the possible ternary complexes — the combination of enzyme, D-sorbitol, and NADH — is a dead-end complex.

The kinetic constants of the reaction are given.

L-Iditol:NAD oxidoreductase (EC 1.1.1.14; sorbitol dehydrogenase) catalyzes tke reversible reaction shown in eqn. 1.

D-sorbitol + $NAD^+ \rightleftharpoons$ D-fructose + NADH + H^+ (1)

The enzyme is found in the livers of a variety of mammalian species and it has been purified from several sources. [1–6] Interest in it has hitherto been concentrated upon its clinical use in the diagnosis of liver diseases,[7,8] although the substrate specificity,[5,6,9–11] the effects of NAD analogs,[5] the pH and temperature optima,[4,6,10] and the effects of various inhibitors [6,9,11,12] have been investigated in earlier studies.

This paper reports the results of kinetic studies that were designed to determine the mechanism by which sheep liver sorbitol dehydrogenase exerts its catalytic action in reaction (1). The attempt to distinguish between various possible mechanisms has involved measurements of initial velocities, obtained in the absence of products, and a determination of the product inhibition pattern.[13,14] The results indicate that sorbitol dehydrogenase acts with a Rapid Equilibrium Random mechanism with one dead-end complex, the enzyme—sorbitol—NADH complex.

EXPERIMENTAL

Materials

L-Iditol:NAD oxidoreductase (sorbitol dehydrogenase) from sheep liver was obtained from Boehringer, Mannheim, 60 mg lyophilisate contained 10 mg enzyme and 50 mg maltose. When stored at −18 °C, the lyophilisate lost no activity over several months. Agarose gel electrophoresis in barbitone buffer 0.075 M, calcium lactate 0.002 M, pH 8.6 showed one protein band.

The specific activity was not enhanced by gel filtration on a Sephadex G-150 column (1.5 × 35 cm). The commercial product was therefore used directly. Enzyme stock solutions (0.1 or 1 mg lyophilisate per ml phosphate buffer) were prepared each day and were kept at 0—5 °C. Under these conditions the activity decreased approximately 2 % per hour.

NAD and Na_2-NADH, both grade III, were purchased from Sigma Chemical Company. Stock solutions (1 or 10 mM) were prepared on the day they were to be used.

D-Sorbitol and D-fructose were analytical grades from BDH Chemicals Ltd.

All solutions were made in 0.05 M phosphate buffer, pH 7.0, containing 10 μM EDTA to prevent influence from heavy metal ions.

Methods

The equilibrium constant and the initial velocities were determined spectrophotometri-

cally. The change in the concentration of NADH was followed by measuring the light absorbance at $\lambda = 340$ nm as a function of time. Quartz cuvettes with a light path length of 10 mm were used. A Zeiss PM QII spectrophotometer was connected through a linear amplifier and a log-converter, to a recorder with adjustable sensitivity and multiple-speed chart drive. Full scale sensitivities of the recorder ranged from 0.04 to 0.2 absorbance units. Background absorbances could be eliminated.

The equilibrium constant was determined from experiments in which all four substrates were present in known concentrations at zero time. The reaction was measured until equilibrium was reached. Substrate equilibrium concentrations were calculated and the equilibrium constant, K_{eq}, was determined from eqn. 2.

$$K_{eq} = [\text{D-fructose}]_{eq}[\text{NADH}]_{eq}[\text{H}^+]/([\text{D-sorbitol}]_{eq}[\text{NAD}]_{eq}) \quad (2)$$

In each initial velocity experiment one substrate, the product inhibitor (if any) and a suitable amount of buffer were mixed in a 1×8 cm pyrex test tube. The tubes for a set of experiments (16—48 experiments with 4—8 concentrations of the varied substrate and 4—6 concentrations of inhibitor or fixed substrate) were placed in a thermostat at 23.5 °C. For each experiment, enzyme was added to one tube and exactly 10 min after this the reaction was initiated by adding the other substrate. After vigorous mixing the content was transferred to a cuvette and the absorbance was measured for 5 min. The slope of the tangent to the progress curve at zero time was taken as the initial velocity. A standard assay of enzyme activity was performed at least three times within each set of experiments and the results of these assays were used to correct the initial velocities for the loss of enzyme activity in the stock solution. Values of initial velocities were scaled up to the enzyme concentration 1 mg/l, the unit thus being μmol mg^{-1} min^{-1}. All experiments were run twice; mean values of the initial velocities were used in data processing.

Reciprocal velocities were plotted graphically against the reciprocals of the substrate concentrations. The data, which gave linear primary plots, were fitted to eqn. 3 using a least squares method assuming a normal distribution and equal variances of initial velocities.

$$s/v = s/V + K/V \quad (3)$$

Slopes ($S = K/V$) and intercepts ($I = 1/V$) (Lineweaver-Burk plots) were replotted against either inhibitor concentration or the reciprocal of the fixed substrate concentration to determine the type of inhibition.[14] When the intercept values for all inhibitor concentrations in a product inhibition experiment were equal to within the 67 % confidence limit, the inhibition was considered to be competitive. Data from the apparently linear replots were fitted to a linear equation, using a least squares method in which the weighting factors were the reciprocals of the squares of the standard errors. Values of the kinetic constants and estimates

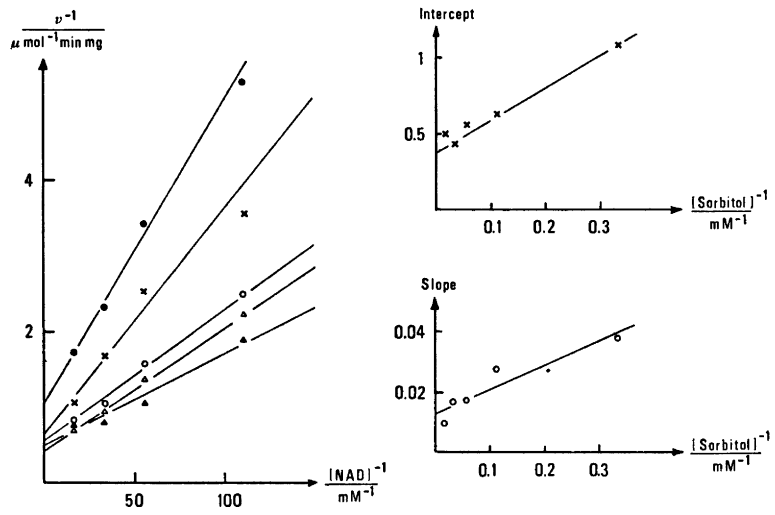


Fig. 1. Double reciprocal plot of the results of the initial velocity experiments with D-sorbitol and NAD as substrates. Intercept and slope replots are also shown. Concentrations were: sorbitol dehydrogenase 1 mg/l; NAD 9 μM, 18 μM, 30 μM and 60 μM; and D-sorbitol (●) 3 mM, (×) 9 mM, (○) 18 mM, (△) 30 mM and (▲) 60 mM.

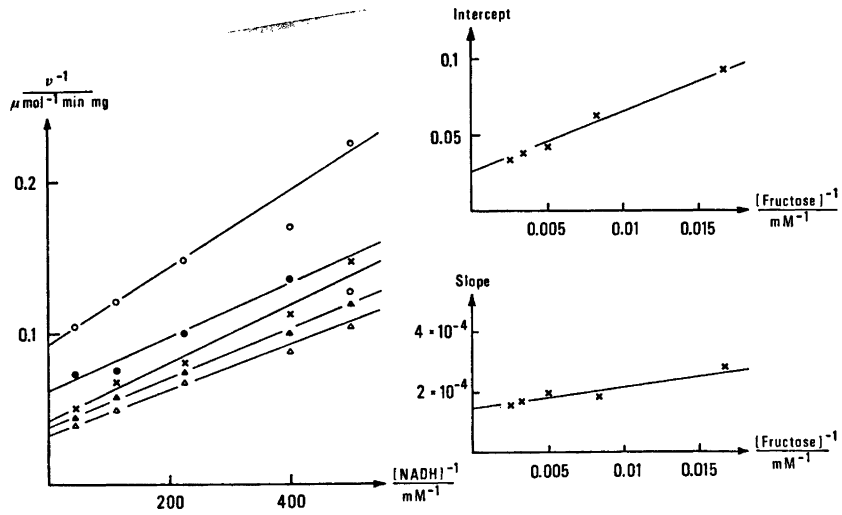


Fig. 2. Double reciprocal plot of the results of the initial velocity experiments with D-fructose and NADH as substrates. Intercept and slope replots are also shown. Concentrations were: sorbitol dehydrogenase 1 mg/l; NADH 2 μM, 2.5 μM, 4.5 μM, 9 μM and 22.5 μM; and D-fructose (○) 60 mM, (●) 120 mM, (×) 200 mM, (▲) 300 mM and (△) 400 mM.

of their accuracy were calculated from the slopes (IS = the slope of the intercept replot; SS = the slope of the slope replot) and the intercepts (II = the intercept of the intercept replot; SI = the intercept of the slope replot) and their estimated standard errors. The results given in Tables 3 and 4 are the best such estimates and their estimated standard errors, $y \pm S_y$.

RESULTS AND DISCUSSION

The results of a series of initial velocity experiments, in which sorbitol dehydrogenase was reacted with D-sorbitol and NAD and in which no products were present at zero time, are shown in Fig. 1. The results of a series of similar experiments on the reaction with D-fructose and NADH is illustrated in Fig. 2. The results are consistent with the initial rate equation (eqn. 4).

$$v = V/(1 + K_A/A + K_B/B + K_{iA}K_B/AB) \quad (4)$$

Eqn. 4 is consistent with most sequential mechanisms, but not with a Ping Pong mechanism.[14]

Table 1 shows the qualitative results of the product inhibition experiments; the results of one of these is shown in Fig. 3. The only simple sequential mechanism which fits the product inhibition pattern (Table 1) is the Rapid Equilibrium Random mechanism with one dead-end complex, Fig. 4.[14] The steady state rate equation for this mechanism is

Table 1. The product inhibition pattern of sorbitol dehydrogenase.

Fixed substrate	Varied substrate	Inhibitor	Resulting inhibition
D-Sorbitol	NAD	NADH	Competitive
D-Sorbitol	NAD	D-fructose	Competitive
NAD	D-sorbitol	D-fructose	Competitive
NAD	D-sorbitol	NADH	Noncompetitive
D-Fructose	NADH	D-sorbitol	Noncompetitive
D-Fructose	NADH	NAD	Competitive

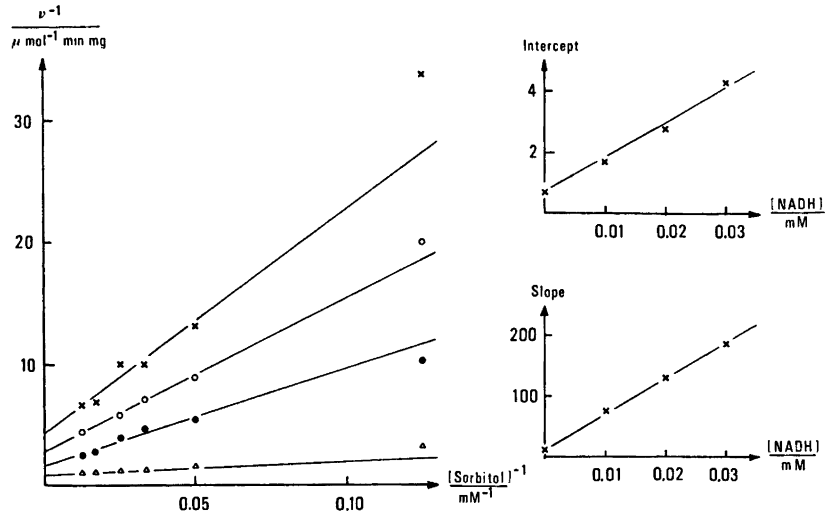


Fig. 3. Double reciprocal plot, and intercept and slope replots of the product inhibition experiments on sorbitol dehydrogenase with NAD as fixed substrate, D-sorbitol as varied substrate and NADH as inhibitor. Concentrations were: sorbitol dehydrogenase 1 mg/l; NAD 20 μM; D-sorbitol 8 mM, 20 mM, 30 mM, 40 mM, 60 mM, and 80 mM, and NADH (△) 0 μM, (●) 10 μM, (○) 20 μM and (×) 30 μM.

$$v = \frac{V_1 - V_2\ \mathrm{PQ}\ K_{iA}K_B/(\mathrm{AB}K_{iP}K_Q)}{1 + \frac{K_A}{A} + \frac{K_B}{B} + \frac{Q}{B}\frac{K_B}{K_{\alpha Q}} + \frac{K_{iA}}{AB}\frac{K_B}{}\left(1 + \frac{P}{K_{iP}} + \frac{Q}{K_{iQ}} + \frac{PQ}{K_{iP}K_Q}\right)} \qquad (5)$$

K_A, K_{iA} and $K_{\alpha A}$ are the equilibrium constants of the reactions:

$$\mathrm{EAB} \overset{K_A}{\rightleftharpoons} \mathrm{EA} + \mathrm{B};\ \mathrm{EA} \overset{K_{iA}}{\rightleftharpoons} \mathrm{E} + \mathrm{A}$$

and $$\mathrm{EQA} \overset{K_{\alpha A}}{\rightleftharpoons} \mathrm{EQ} + \mathrm{A};$$

$$K_{iA}K_B = K_{iB}K_A\ ,\ K_{iP}\ K_Q = K_{iQ}\,K_P$$

and $$K_{iA}K_{\alpha Q} = K_{\alpha A}\,K_{iQ} \qquad (6)$$

Eqn. 5 reduces to eqn. 4 when no product is present, P=Q=0.

The steady state initial rate equations for the product inhibitions can be obtained from eqn. 4 by setting either P or Q equal to zero.

If Q inhibits, P=0

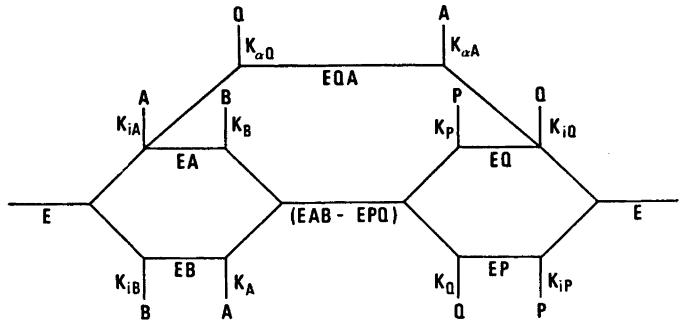


Fig. 4. Rapid Equilibrium Random mechanism with one dead-end complex. The conversion of the central complexes, EAB⇌EPQ, is the only rate-determining step in both reaction directions. All other steps are fast and therefore reach equilibrium rapidly after initiation of the reaction. Here E=sorbitol dehydrogenase, A=D-sorbitol, B=NAD, P=D-fructose, and Q=NADH.

$$v=\frac{V_1}{1+\frac{K_A}{A}+\frac{K_B}{B}\left(1+\frac{Q}{K_{\alpha Q}}\right)+\frac{K_{iA}K_B}{AB}\left(1+\frac{Q}{K_{iQ}}\right)} \quad (7)$$

If P inhibits, Q=0

$$v=\frac{V_1}{1+\frac{K_A}{A}+\frac{K_B}{B}+\frac{K_{iA}K_B}{AB}\left(1+\frac{P}{K_{iP}}\right)} \quad (8)$$

Eqn. 7 predicts linear noncompetitive inhibition when A is varied and linear competitive inhibition when B is varied. Eqn. 8 predicts linear competitive inhibition in both cases. The kinetic expressions of the linear coefficients derived from eqns. 7 and 8 are shown in Table 2. The results of the experiments (Table 1) exhibit the product inhibition pattern predicted by eqn. 7 and 8. The noncompetitive inhibition is between D-sorbitol and NADH, and this identifies A as D-sorbitol, B as NAD, P as D-fructose and Q as NADH (Fig. 4, eqns. 7 and 8). Table 3 gives the values of the kinetic constants calculated from the results shown in Figs. 1, 2 and 3, the kinetic expressions (Table 2) and eqns. 6. Table 4 shows the quantitative results of the experiments and, where an experimental value was not used in a determination, the corresponding values calculated from the kinetic constants. Most pairs of experimental and theoretical values are identical within the estimated standard errors.

The value of the equilibrium constant of reaction (1) is $K_{eq}=3.71\times10^{-9}\pm8\times10^{-11}$ M (mean from five experiments). The Haldane relation for the Rapid Equilibrium Random mechanism with one dead-end complex is

$$K_{eq}=\frac{V_1K_{iF}K_{NADH}\,[H^+]}{V_2\,K_{iS}\,K_{NAD}} \quad (9)$$

This gives $K_{eq}=3.8\times10^{-9}\pm3.7\times10^{-9}$ M, identical with the experimental and in agreement with the value $K_{eq}=2.4\times10^{-9}\pm1.3\times10^{-10}$ M reported by Blakley.[1]

No Ordered BiBi or Ping Pong mechanism fits the results. The product inhibition pattern, the identity between the calculated and experimental value of the equilibrium constant and the agreement of experimental and theoretical linear coefficients of the initial velocity and product inhibition data show that sorbitol

Table 3. The kinetic constants of sorbitol dehydrogenase.

V_1	=	2.7 ± 0.14 μmol min^{-1} mg^{-1}
K_S	=	5.9 ± 0.26 mM
K_{NAD}	=	19.0 ± 0.92 μM
K_{iS}	=	7.7 ± 0.55 mM
K_{iNAD}	=	25 ± 1.8 μM
$K_{\alpha S}$	=	45 ± 1.6 mM
V_2	=	38.5 ± 0.85 μmol min^{-1} mg^{-1}
K_F	=	150 ± 6.4 mM
K_{NADH}	=	11.1 ± 0.30 mM
K_{iF}	=	7.0 ± 0.39 mM
K_{iNAD}	=	0.53 ± 0.023 μM
$K_{\alpha NADH}$	=	3.1 ± 0.11 μM

Table 2. The kinetic expressions of the linear coefficient derived from the rate equation.

Fixed substrate	Varied substrate	Inhibitor	II	IS	SI	SS
A	B	—	$1/V_1$	K_A/V_1	K_B/V_1	$K_{iA}\ K_B/V_1$
A	B	Q	$(1+K_A/A)/V_1$	0	$K_B(1+K_{iA}/A)/V_1$	$K_B(1/K_{\alpha Q}+K_{iA}/A\ K_{iQ})/V_1$
A	B	P	$(1+K_A/A)/V_1$	0	$K_B(1+K_{iA}/A)/V_1$	$K_{iA}K_B/A\ K_{iP}\ V_1$
B	A	P	$(1+K_B/B)/V_1$	0	$K_A(1+K_{iB}/B)/V_1$	$K_{iA}K_B/B\ K_{iP}\ V_1$
B	A	Q	$(1+K_B/B)/V_1$	$K_B/B\ K_{\alpha Q}\ V_1$	$K_A(1+K_{iB}/B)/V_1$	$K_{iA}K_B/B\ K_{iQ}\ V_1$

II=intercept of intercept replot; IS=slope of intercept replot; SI=intercept of slope replot and SS=slope of slope replot.

Table 4. The experimental (E) and theoretical (T) values of the linear coefficients. The theoretical values were calculated from the kinetic expressions in Table 2 using the kinetic constants in Table 3. The unit of initial velocity is μmol min^{-1} mg^{-1} and the units of concentration and the kinetic constants are mM.

Fixed substrate	Varied substrate	Inhibitor		II	IS	SI	SS
D-Sorbitol	NAD	—	E	$3.7\times10^{-1}\pm1.2\times10^{-2}$	$2.2\pm2\ 10^{-1}$	$1.2\times10^{-2}\pm2.5\times10^{-3}$	$8\times10^{-2}\pm2\times10^{-2}$
			T		$2.16\pm6.5\ 10^{-2}$	$7.0\times10^{-3}\pm5\times10^{-4}$	$5.4\times10^{-2}\pm5.9\times10^{-3}$
D-Sorbitol 30 mM	NAD	NADH	E	$4.3\times10^{-1}\pm4\times10^{-2}$	0	$8.1\times10^{-3}\pm5\times10^{-4}$	$3.2^{-}\ \pm8\times10^{-1}$
			T	$4.4\times10^{-1}\pm1\times10^{-2}$	0	$8.8\times10^{-3}\pm6\times10^{-4}$	$5.6\ \pm6.2\times10^{-1}$
D-Sorbitol 40 mM	NAD	D-fructose	E	$4.2\times10^{-1}\pm1.3\times10^{-2}$	0	$7.7\times10^{-3}\pm3\times10^{-4}$	$1.9\times10^{-3}\pm4\times10^{-4}$
			T		0	$8.4\times10^{-3}\pm6\times10^{-4}$	$2\times10^{-4}\pm2.2\times10^{-5}$
NAD 0.02 mM	D-sorbitol	D-fructose	E	$6.4\times10^{-1}\pm5\times10^{-2}$	0	$6.6\ \pm6.4\times10^{-1}$	$7\times10^{-1}\pm1\times10^{-1}$
			T	$7.2\times10^{-1}\pm1.4\times10^{-2}$	0	$4.9\ \pm3\times10^{-1}$	$3.9\times10^{-1}\pm4.3\times10^{-2}$
NAD 0.02 mM	D-sorbitol	NADH	E	$7.2\times10^{-1}\pm1.4\times10^{-2}$	$114\ \pm2.3$	$4.9\ \pm3\times10^{-1}$	$5.6\ \pm4.5\times10^{-1}$
			T				$5.1\ \pm5.6\times10^{-1}$
D-Fructose	NADH	—	E	$2.6\times10^{-2}\pm6\times10^{-4}$	$3.9\ \pm1.4\times10^{-1}$	$1.5\times10^{-1}\pm2\times10^{-2}$	$6.6\times10^{-3}\pm3.5\times10^{-3}$
			T			$2.88\times10^{-1}\pm6\times10^{-3}$	$2.1\times10^{-3}\pm2.2\times10^{-4}$
D-Fructose 400 mM	NADH	NAD	E	$3.9\times10^{-2}\pm8\times10^{-4}$	$2.18\times10^{-4}\pm1.5\times10^{-6}$	$3.11\times10^{-1}\pm5\times10^{-3}$	$6.7\times10^{-7}\pm4\times10^{-8}$
			T	$3.6\times10^{-2}\pm1.3\times10^{-4}$			$6.7\times10^{-7}\pm7.4\times10^{-8}$
D-Fructose 400 mM	NADH	NAD	E	$3.8\times10^{-2}\pm3.6\times10^{-3}$	0	$3.1\times10^{-1}\pm1.8\times10^{-2}$	$6\times10^{-4}\pm1.4\times10^{-4}$
			T	$3.6\times10^{-2}\pm1.3\times10^{-3}$	0	$3.11\times10^{-1}\pm5\times10^{-3}$	$2\times10^{-4}\pm2.2\times10^{-5}$

dehydrogenase most probably follows a Rapid Equilibrium Random mechanism with one dead-end complex, the enzyme-sorbitol-NADH complex.

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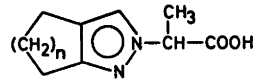
^{1}H NMR Spectroscopy of Some α-(Cyclopolymethylenepyrazolyl)-propionic Acids. Resolution and Configurational Assignment of α-(Cyclopentapyrazolyl-2)propionic Acid

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α-(4,5,6,7-Tetrahydroindazolyl-1)propionic acid (V) has been synthesized by catalytic hydrogenation of α-(indazolyl-1)propionic acid (IX) with 10 % palladium-on-charcoal as catalyst. The difference in the chemical shifts for the 3-proton in 1- and 2-substituted pyrazoles was used for determination of the structure of α-(cyclopentapyrazolyl-2)propionic acid (I), α-(cycloheptapyrazolyl-2)propionic acid (III) and α-(cyclooctapyrazolyl-2)propionic acid (IV). I was resolved *via* the brucine salt in ethyl acetate giving the acid with $[\alpha]_D^{25} = +26.7°$ in chloroform. The absolute configuration of I was determined by circular dichroism measurements. The rubidium salt of (−)-III was prepared for an unequivocal X-ray determination of the structure of I−V, IX and α-(indazolyl-2)-propionic acid (X) and the absolute configuration of active I−III.

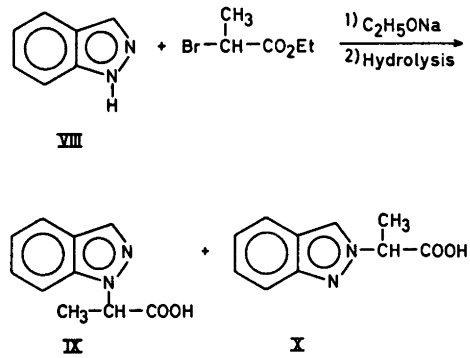
A series of α-(cyclomethylenepyrazolyl-2)propionic acids (I−IV) has been synthesized earlier.[1,2] The present investigation deals with the structure determination of these compounds.



I: n=1, II: n=2, III: n=3, IV: n=4.

The substitution of II was decided by hydrogenation of α-(indazolyl-2)propionic acid (X),[1] but its position in I, III, and IV, however, was not settled. To elucidate this question α-(4,5,6,7-tetrahydroindazolyl-1)propionic acid (V) was prepared.

Indazole (VIII) was reacted under reflux with ethyl α-bromopropionate in a solution of sodium ethoxide in absolute ethanol according to von Auwers and Allardt [3] giving a mixture of 1- and 2-esters (Scheme 1).



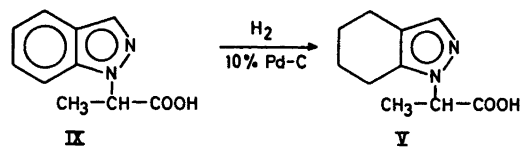
Scheme 1.

IX and X were successfully separated without prior picrate formation by column chromatography on silica gel. IX and X was also separated by recrystallization from hydrochloric acid of suitable concentration in which X, the more basic, is more soluble than IX.

The ^{1}H NMR data of IX and X and their ethyl esters in Table 1 show that the 3-proton shift in DMSO-d_6 appears at a higher field for the 1-isomers than for the 2-isomers.

Table 1. Chemical shifts (δ; ppm rel. TMS) of some 1- and 2-substituted indazoles and indazole in DMSO-d_6 and trifluoroacetic acid.

Compound	DMSO-d_6		Trifluoro-acetic acid	
	3-H	α-H	3-H	α-H
IX	8.01	5.57	8.84	6.02
IX, ethyl ester	8.02	5.64	8.75	5.93
X	8.34	5.50	8.87	5.91
X, ethyl ester	8.34	5.54	8.75	5.82
VIII	8.13		8.68	



IX V

Scheme 2.

STRUCTURAL DETERMINATION

von Auwers and Düesberg [4] found that the lower boiling isomers obtained by alkylation of indazole with alkylhalides in the presence of alkali were identical with those obtained according to the method of Fischer and Tafel [5] which gives exclusively the 1-isomer. Due to this and the mode of synthesis the 2-structure was assigned to the higher boiling isomer. The correctness of this structural assignment is supported by work of Grandberg *et al.*[9] and Schwan and Davis.[10] The structural determination of 1- and 2-alkylated indazoles is now easily done by means of their characteristic UV absorption properties.[6–8]

The structure of IX and X were determined by comparison of their UV spectra with those of 1- and 2-methylindazoles. IX and X were hydrogenated to V and II, respectively (Scheme 2 and Ref. 1). The 1-substitution of V and 2-substitution of II was further confirmed by ^{1}H NMR spectroscopy using the difference in chemical shifts of the vinylproton on 1,3,4- and 1,4,5-trialkylated pyrazoles.[12] III was used as a reference substance. The 2-substitution of III was determined by an X-ray crystallographic investigation [11] of its rubidium salt. The latter determination of the structure of II independently proves the correctness of the structure assignment of IX and X and that one of von Auwers and Düesberg of other indazoles. The chemical shifts of the vinyl protons of the compounds listed in Table 2 show that also I and IV are 2-substituted.

IR data on I–V were not useful for the structural determination, and the difference in UV maxima between I–IV and V was too small for a safe structural assignment.

PRELIMINARY TESTS ON RESOLUTION

Preliminary tests on resolution of racemic I were done with the common alkaloids and also with some synthetic bases in different solvents and solvent mixtures. In most cases only oils were formed, but salts were obtained with cinchonidine, (−)-ephedrine, brucine, and dehydroabietyl amine in ethyl acetate. The cinchonidine and (−)-ephedrine salts gave acid with $[\alpha]_D$ −2°, brucine +4°, and dehydroabietyl amine +1 and −1° in two different experiments. Brucine and cinchonidine were chosen for a full scale resolution. After four crystallizations of the brucine salt, an $[\alpha]_D$ value of +25° was obtained which did not change upon further recrystal-

Table 2. Chemical shifts of some pyrazoles in $CDCl_3$, DMSO-d_6 and CF_3COOH.

Compound	$CDCl_3$		DMSO-d_6		CF_3COOH	
	3-H	α-H	3-H	α-H	3-H	α-H
I	7.00	4.92	7.21	4.88	7.82	5.56
II	7.11	5.00	7.35	4.93	7.87	5.53
II ethyl ester			7.37	5.05		
III	7.11	4.99	7.28	4.82	7.87	5.48
IV	7.12	5.04	7.36	4.93	7.92	5.54
V	7.35	4.93	7.13	4.93	7.92	5.53
V ethyl ester			7.15	5.04		

lization. The cinchonidine salt of the acid obtained from the mother liquor after resolution with brucine gave after the first crystallization acid with $[\alpha]_D$ −11.7°. For some reason the specific rotation diminished upon further recrystallization.

DETERMINATION OF THE ABSOLUTE CONFIGURATION

The absolute configuration of (+)-I was determined from the CD data of (+)-I and(*R*)-(−)-II.[14] Fig. 1 clearly shows that (+)-I has the *S*-configuration.

The rubidium salt of (−)-III was synthesized for a crystallographic X-ray investigation in order to confirm unequivocally the 2-substitution of III and the absolute configuration of I−III. The X-ray investigation is under progress.[11]

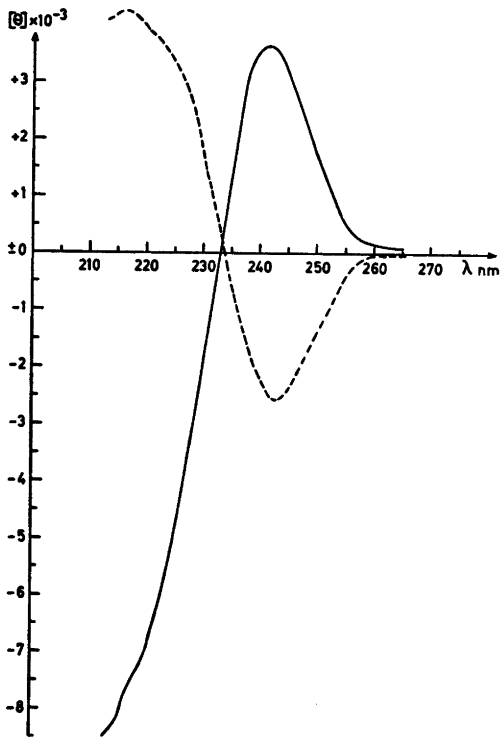


Fig. 1. CD spectra of (+)-I (- - -, $c = 3.90 \times 10^{-2}$) and (*R*)-(−)-II (———, c 3.56×10^{-2}) in methanol.

EXPERIMENTAL

The CD curves were recorded in 1 mm cells at 27 °C with a Cary 60 spectropolarimeter equipped with a circular dichroism accessory. The optical activity was measured with a Perkin-Elmer 141 spectropolarimeter in 1 ml micro cells of 10 cm length with chloroform as solvent. The IR spectra were recorded on a Perkin-Elmer 157 spectrophotometer. The ^{1}H NMR spectra were recorded on a Varian A-60 spectrometer with solutions of about 15 % concentration, when possible, using TMS as internal standard. The mass spectra were recorded at 70 eV with an LKB 9000 instrument.

GLC was performed on a Perkin-Elmer 900 gas chromatograph fitted with a flame ionization detector. Column temperature 200 °C, injection port temperature 230 °C, detector temp. 250 °C. Nitrogen carrier gas, flow rate 70 ml/min. Column: Varaport 30, AW, DMCS, 100−120 mesh, coated with 5 % Carbowax 20 M AW, DMCS-treated, packed in a 2.8 m glass column AW, DMCS-treated, I.D. 3.5 mm. The column was conditioned at 230 °C before use. Column chromatography was performed on silica gel (0.05−0.2 mm, 70−325 mesh ASTM, E. Merck). The thin layer chromatograms were run in glacial acetic acid/benzene (4/1) on non-activated 5 × 20 cm plates coated with silica gel F 254 (E. Merck) with a nominal thickness of 0.25 mm and analyzed with UV light or developed with iodine vapor.

All melting points were taken with a hot-stage microscope and are corrected. The microanalyses were carried out at the Analytical Department, Institute of Chemistry, Uppsala University.

(+)-Ephedrine was isolated from its hydrochloride (Fluka AG $[\alpha]_{546}^{20}$ +39±2° (*c* 11.5; water) by addition of 2 M sodium hydroxide and subsequent extraction with ether. The ether solution was dried over magnesium sulfate, the ether evaporated and the residue distilled *in vacuo*. M.p. 39 °C (lit[13] m.p. 40 °C).

rac-*Ethyl α-(indazolyl-1 and -2)propionate.* A mixture of racemic ethyl α-(indazolyl-1 and -2)-propionate was synthesized according to von Auwers and Allardt.[3]

I. Without sodium ethoxide. 1.0 g (8.5 mmol) indazole and 2.9 g (16.0 mmol) ethyl α-bromopropionate were warmed at 120 °C for 3 h. A slight excess of 2 M sodium hydroxide was then added. The mixture was extracted with three 6 ml portions of ether, the ether solution dried over magnesium sulfate and the ether distilled off. A GLC and a NMR analysis of the residue showed that it contained the 1-ester and 2-ester in the ratio 6:94 together with a minor amount of unreacted indazole. The retention times were 42, 85, and 55 min, respectively.

II. With sodium ethoxide. 1.50 g (13 mmol) indazole was dissolved in a solution of 0.437 g (19 mmol) sodium in 10 ml absolute ethanol, and 4.00 g (22 mmol) ethyl α-bromopropionate was

added. The temperature rose to 55 °C within a few minutes. The mixture was then refluxed until it was neutral and worked up as described earlier. A GLC analysis showed that 1- and 2-esters were formed in the ratio 66:34. 13.6 g of product was obtained after distillation from a run with 10 times the amounts given above. 4 g indazole was recovered upon distillation. The isomeric composition was not changed significantly after the distillation.

rac α-*(Indazolyl-1)propionic acid.* A. The isomeric esters were separated via the picrate of the 2-ester.[3] The acid obtained after hydrolysis was not quite free from X. Therefore the product (0.150 g) was chromatographed on a 28 × 1 cm column of 17.0 g silica gel. The column was eluted at a flow rate of 1 ml min^{-1} with 220 ml benzene, 50 ml 3 % (v/v) acetic acid/benzene, and 450 ml 5 % acetic acid/benzene. 5 ml fractions were collected and analyzed by TLC. Fractions 77 – 100 were pooled and evaporated to dryness yielding 0.134 g pure IX. M.p. 113 – 114.5 °C. IR (KBr) λ_{CO} 5.80 μ: UV (99 % EtOH) λ_{max} 254.9 nm (log ε 3.68) and 290.8 nm (log ε 3.76). R_F 0.52. (Found: C 63.11; H 5.30; N 14.73. Calc. for $C_{10}H_{10}N_2O_2$ (190.2): C 63.14; H 5.30; N 14.73).

B. 15.0 g ester mixture was hydrolyzed in 60 ml 2 M sodium hydroxide at 110 °C for 2 h. The alkaline solution was extracted with ether and concentrated hydrochloric acid was added to pH 2. After one night in a refrigerator, 11.5 g acid mixture was obtained. A ^{1}H NMR investigation showed that the product contained 59 % IX and 41 % X.

1.5 g of the acid mixture was dissolved in a mixture of 7.5 ml 2 M and 1.2 ml concentrated hydrochloric acid. The pH of the solution was 1.5 – 2.0. After cooling 0.55 g IX nearly free from X was isolated. After another recrystallization and thorough washing with water 0.42 g pure IX was obtained.

rac α-*(4,5,6,7-Tetrahydroindazolyl-1)propionic acid (V).* The conditions for the hydrogenation of IX were worked out on the 5 mg scale. Thin layer chromatography followed by mass spectrometry was used to test the hydrogenation product for the presence of V. Hydrogenation of IX in a Parr apparatus at 5 atm for one day at room temperature with 10 % palladium-on-charcoal as catalyst and glacial acetic acid as solvent yielded at least four products. No IX remained. Hydrogenation in 96 % ethanol at a higher pressure and temperature gave a more uniform product and was therefore chosen for hydrogenation on a larger scale. Only two spots were seen in the TLC test, a large one from V and a small one which probably was the ethyl ester of V. No IX remained.

0.40 g (2.1 mmol) IX was dissolved in 4 ml of 96 % ethanol and hydrogenated by shaking with hydrogen gas at 110 atm and 70 °C with 0.80 g 10 % palladium-on-charcoal as catalyst. After hydrogenation for 15 h a TLC test showed that only a small amount of IX remained. A further 0.50 g catalyst was then added and the hydrogenation continued for another 9 h. No or very little IX remained, but more by-product, probably the ethyl ester of V, was formed. The filtrate was then evaporated to dryness and the residue dissolved in 3 ml of chloroform, filtered and added to 10 ml of ligroin (b.p. 60 – 71 °C). An oil was first obtained, which crystallized on scratching. The product was made alkaline with 2 M sodium hydroxide and extracted with chloroform. The alkaline solution was filtered and then acidified by addition of hydrochloric acid to pH 3.5. The solid product was washed with ice water and dried. 0.20 g acid was obtained with m.p. 140 – 141 °C and R_F 0.43. UV (99 % EtOH) λ_{max} 229.8 nm (log ε 2.67). IR (KBr) λ_{CO} 5.75 μ. (Found: C 61.79; H 7.30; N 14.28. Calc. for $C_{10}H_{14}N_2O_2$ (194.2): C 61.81; H 7.27; N 14.44).

(S)-(+)-α-(Cyclopentapyrazolyl-2)propionic acid ((+)-I). Racemic α-(cyclopentapyrazolyl-2)propionic acid (I) was synthesized according to Gustafsson *et al.*[2] M.p. 170 – 71 °C. 13.5 g (0.075 mol) I and 32.2 g (0.075 mol) brucine were dissolved in 620 ml of boiling ethyl acetate and allowed to crystallize overnight in a refrigerator. The salt obtained was then recrystallized. After each crystallization, acid was liberated from 0.10 g salt by addition of 2 M sodium hydroxide, extraction with chloroform several times and subsequent acidification with hydrochloric acid to pH 5. The acid was filtered off and dried, and the optical activity was measured in chloroform. After five recrystallizations the acid was liberated from its salt by addition of 2 M sodium hydroxide. The brucine was filtered off and the filtrate acidified to pH 3.5 with concentrated hydrochloric acid. The course of the resolution is given in Table 3. After recrystallization from 5 ml ethanol/water (2/1) 0.50 g acid was obtained with m.p. 180 – 87 °C. $[\alpha]_D^{25}$ +26.7°, $[\alpha]_{365}^{25}$ +95.8° (*c* 0.524). CD: $[\alpha]_{234}$ ±0, $[\theta]_{242}$ −2590. The somewhat low $[\theta]$ value at λ_{max} 242 and the broad melting point interval make it probable that the acid was not quite optically

Table 3. Resolution of α-(cyclopentapyrazolyl-2)propionic acid (I).

Crystallization	1	2	3	4	5	6
Solvent, ml	620	100	75	80	40	40
Salt, g	16.0	13.3	10.9	7.8	5.3	4.1
$[\alpha]_D^{25}$, deg	13.6	18.8	19.3	25.5	25.5	25.7

pure. (Found: C 59.98; H 6.74; N 15.44. Calc. for $C_9H_{12}N_2O_2$ (180.2): C 59.99; H 6.71; N 15.54).

The rubidium salt of (−)-α-(cycloheptapyrazolyl-2)propionic acid. A concentrated solution of 0.0924 g (−)-III in acetone was mixed with an equimolar amount of rubidium hydroxide in a minimal amount of water. The combined solution was slightly alkaline. More (−)-III was added to neutral reaction. The mixture was evaporated to dryness and 8 ml warm acetone was added. The salt was dissolved by adding a minimal amount of water (~0.25 ml) to the warm mixture. After cooling, 0.060 g (41 %) rubidium salt monohydrate of (−)-III was obtained. The salt was recrystallized from acetone with a small amount of water. The rubidium and potassium salts of II were made in the same way. (Found: C 42.58; H 5.48; N 8.79. Calc. for $C_{11}H_{17}N_2O_3Rb$ (310.74): C 42.52; H 5.51; N 9.02).

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A Facile Route to α-Hydrazinopropionic Acid of High Optical Purity. The Absolute Configuration of α-(4,5,6,7-Tetrahydroindazolyl-1 and -2)propionic Acid

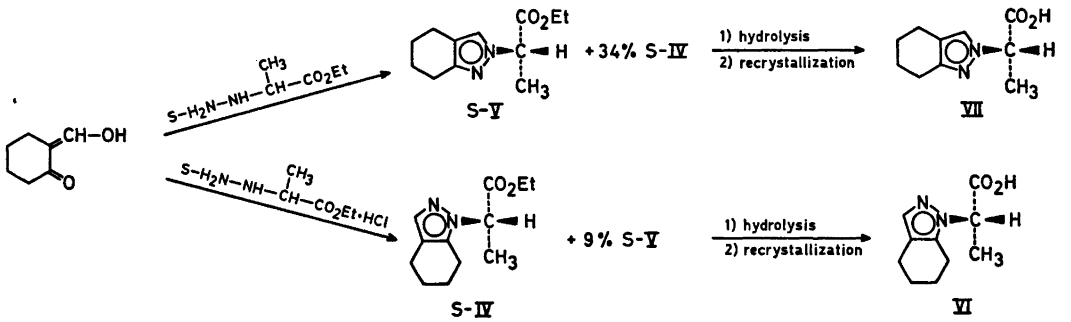
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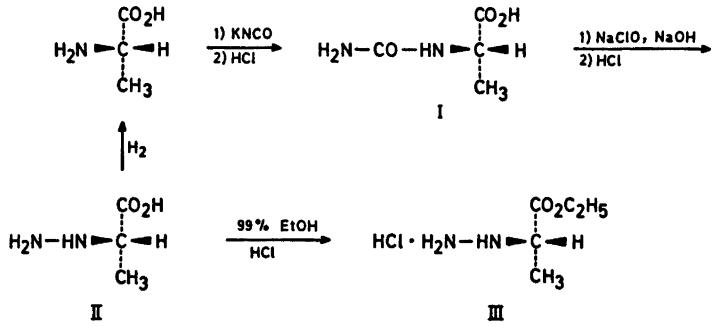
(*S*)(−)-α-Hydrazinopropionic acid (II) with $[\alpha]_D^{25} = -30°$ (6 M HCl) and an enantiomeric purity of 0.995 ± 0.002 has been synthesized *via* Hofmann rearrangement of (*S*)(−)-α-ureidopropionic acid (I). Ethyl α-hydrazinopropionate hydrochloride (III) was reacted with α-hydroxymethylenecyclohexanone to give a mixture of ethyl α-(4,5,6,7-tetrahydroindazolyl-1)propionate (IV) and ethyl α-(4,5,6,7-tetrahydroindazolyl-2)propionate (V). The ratio was influenced by the degree of protonation. (*S*)(+)-α-(4,5,6,7-tetrahydroindazolyl-1)propionic acid (VI) and (*S*)(+)-α-(4,5,6,7-tetrahydroindazolyl-2)propionic acid (VII) were synthesized for configurational assignment of some α-azolylpropionic acids.

The aim of the present work was to determine the absolute configuration of α-(indazolyl-2)-propionic acid,[1] α-(benzotriazolyl-2)-propionic acid [2] and a series of α-(cyclomethylenepyrazolyl-2)-propionic acids.[3,4] In the present case the quasi-racemate method [5,6] failed. The absolute configurations of the acids mentioned have now been determined by synthesis of (*S*)(+)-α-(4,5,6,7-tetrahydroindazolyl-2)propionic acid (VII) (Scheme 1) and CD measurements. Some of the results are given in Ref. 2—4. (−)-α-(Indazolyl-2)propionic acid was shown to have (*S*)-configuration by hydrogenation to VII. (*S*)(+)-α-(4,5,6,7-tetrahydroindazolyl-1)-propionic acid (VI) (Scheme 1) was also synthesized for the determination of the absolute configuration of α-(benzotriazolyl-1)-propionic acid.[7]

(*S*)(−)-α-Hydrazinopropionic acid (II) was obtained by Hofmann rearrangement of (*S*)(−)-α-ureidopropionic acid (I) with sodium hypochlorite in sodium hydroxide (Scheme 2). The mother liquor after separation of II gave a crystalline residue which was not further characterized. No II was obtained when using sodium hypobromite under different conditions instead of sodium hypochlorite. Separation of



Scheme 1.



Scheme 2.

II from the salt by means of ion exchange chromatography according to Carmi *et al.*[8] is not convenient for a separation on a large scale due to the large amount of salt formed. Purification via the ethyl ester of II and subsequent saponification with barium hydroxide according to Darapsky [9] was successful, but the method was tedious. In the present work most of the sodium chloride was separated off by treatment with acetic acid and a mixture of acetic acid and chloroform.

The enantiomeric purity of II defined according to Horeau [10] was determined by degradation to alanine, which was analyzed without purification according to Manning and Moore.[11] Using a method of Dakin [12] for preparation of I and performing the Hofmann reaction of I at room temperature, II was obtained with an enantiomeric purity of 0.995 ± 0.002. When the Hofmann reaction was done at 80 °C, II was obtained with enantiomeric purity of $0.97 - 0.98$.

A slight racemization also during the preparation of I with the Dakin method cannot be excluded. Therefore a milder method was worked out for its preparation. Alanine was reacted with potassium cyanate at room temperature for one day in a triethylamine-acetic acid buffer of pH 8. The preparation of II according to this method has not yet been done.

Examination of the IR spectrum of II reveals that it largely exists as a zwitterion. Absorption at 6.35 and 7.12 μm, typical for a carboxylate anion,[13] is found but absorption at 5.8 μm, normal for the carbonyl of an unionized carboxylic acid, is lacking. The IR spectrum of II is given in Fig. 1. The solubility properties of II, which are similar to those of alanine, and its high melting point support the zwitterionic character.

The cyclization of 1,3-dicarbonyl compounds or their sodium salts with hydrazines is a standard method for synthesis of pyrazoles.[14,15] β-Chlorovinylketones, β-alkoxyvinylketones, 1,3-diacetals, and β-aminovinylketones can be used instead of the 1,3-dicarbonyl compounds. 2-Chlorocyclohex-1-ene-1-carboxaldehyde and

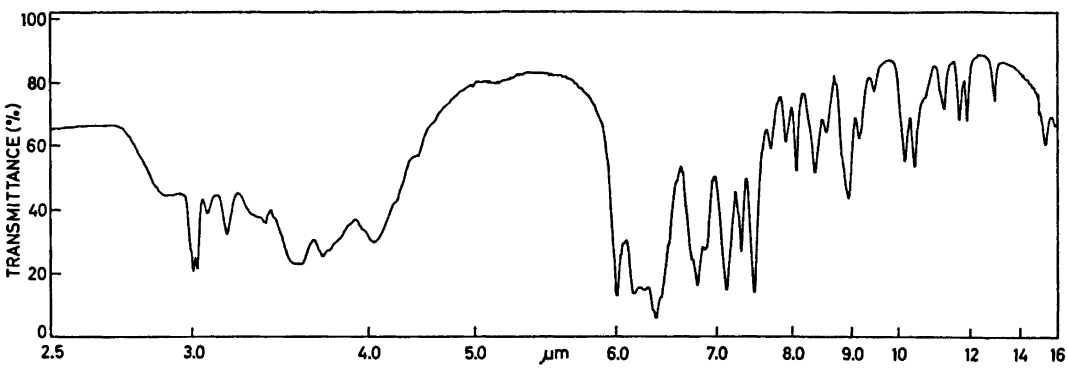


Fig. 1. IR spectrum of (S)(−)-α-hydrazinopropionic acid. Solid in KBr.

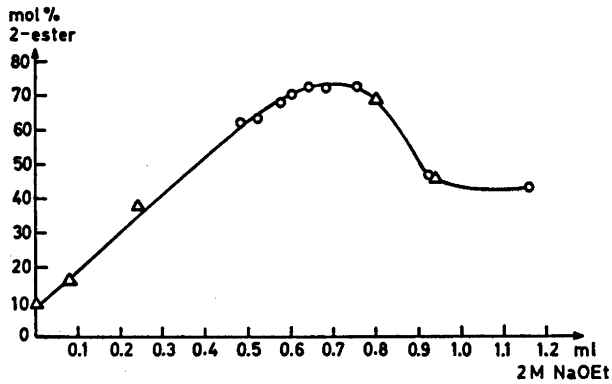


Fig. 2. Reaction of III with α-hydroxymethylenecyclohexanone upon addition of different amounts of sodium ethoxide. △ and ○ represent data obtained from ^{1}H NMR and GLC analysis, respectively.

benzylhydrazine in ethanol and hydrochloric acid are reported [16] to give exclusively 1-isomer, but no proof was given. The corresponding reaction with ethyl α-hydrazinopropionate hydrochloride (III), however, gives 1- and 2-isomer in the ratio 87:13.

A series of reactions was done with III and α-hydroxymethylenecyclohexanone in 99 % ethanol by adding sodium ethoxide in various amounts. The result is given in Fig. 2.

At strongly acidic or strongly basic conditions much of the 1-ester was formed in comparison to the 2-ester. The total yield of pyrazoles was high in the first case but rather low in the second case. At intermediary conditions more 2-ester than 1-ester was formed. These results are probably the consequence of the different degree of protonation in the different experiments. Protonation of the β-ketoaldehyde and the hydrazine compound as well as deprotonation of the β-ketoaldehyde ought to influence the course of reaction.

The reaction between α-hydroxymethylenecyclohexanone and β-hydroxyethylhydrazine in 99 % ethanol with or without addition of sodium ethoxide at −100 °C was followed by ^{1}H NMR spectroscopy. The reaction was quite fast and no intermediate was observed with certainty. Although the reaction between a β-ketoaldehyde and a hydrazine is a commonly used reaction, its mechanism is not known. Coispeau and Elguero [17] point out that the keto-enol equilibria and the two nucleophilic centra of monoalkyl-substituted hydrazines could give rise to twelve possible reaction mechanism and two isomers. Six of the mechanisms were ruled out. They do not discuss the influence of the degree of protonation on the reaction.

The reaction of II with α-hydroxymethylenecyclohexanone in absolute ethanol gave ~66 % VI and ~34 % VII. The same reaction but with triethylamine added (a drop of the reaction mixture on a moisted indicator paper gave pH 5.5) gave ~45 % VI and ~55 % VII. The reaction of α-hydroxymethylenecyclohexanone with ethyl (*S*)(−)-α-hydrazinopropionate and its hydrochloride was chosen for synthesis of VII and VI, respectively (Scheme 1), both of which thus have (*S*)-configuration.

The ester (*S*)-IV was easily racemized by hydrolysis with 2 M sodium hydroxide. A complete deuterium exchange of the α-proton by hydrolysis in 5 M sodium deuterium oxide at 100 °C was found by an ^{1}H NMR investigation. The acid isolated after acidification showed no or very low optical activity. Hydrolysis of IV with 2 M deuteriochloric acid at 100 °C did not give deuterium exchange and the acid isolated was optically active. The ester (*S*)-V, on the contrary, did not racemize on alkaline hydrolysis and the isolated acid had the maximal optical activity.

EXPERIMENTAL

The optical activity was measured with a Perkin-Elmer 141 spectropolarimeter in micro cells of 10 cm length. The CD curves were recorded in 1 ml cells in methanol solutions with

a Cary 60 spectropolarimeter equipped with a CD accessory. The ^{1}H NMR spectra were recorded on a Varian A-60 instrument with solutions of about 10 % using TMS as internal standard. The mass spectra were recorded at 70 eV with an LKB 9000 instrument and the IR spectra with a Perkin-Elmer 257 spectrophotometer in KBr pellets. The GLC analyses were performed with a Perkin-Elmer 990 instrument fitted with a flame ionization detector. Column temperature 160 °C, injection port temperature 240 °C and detector temperature 240 °C. Nitrogen carrier gas with flow rate 38 ml/min. The column was of 3.0 m length and made of glass, AW, DMCS-treated. I.D. 3.5 mm. It was packed with chromosorb W, AW, DMCS-treated, 100 – 200 mesh, coated with 7.5 % UCON 50LB-550 X. The column was conditioned at 200 °C for 24 h before use.

The thin layer chromatograms were run in butanol-glacial acetic acid-water (60:30:10) on non-activated plates (E. Merck) coated with silica gel F 254 with a nominal thickness of 0.25 mm. The chromatograms of α-hydrazinopropionic acid were developed with ninhydrin in 96 % ethanol (0.25 %, w/v) or molybdophosphoric acid (5 % in 96 % ethanol) according to Stahl.[18]

The melting points were determined with a hot stage microscope and are uncorrected. The microanalyses were carried out at the analytical department of the Institute.

The amino acid analyses were done with a Beckman Spinco automatic amino acid analyzer. The (*S*)-alanine used was purchased from Ajinomoto Co., Tokyo, Japan and contained less than 0.1 % (*R*)-alanine. The ligroin used had a specified b.p. of 60 – 71 °C.

(S)(−)-α-*Ureidopropionic acid (I).* 4.5 ml triethylamine and 4.5 ml water were mixed and glacial acetic acid was added to give a buffer of pH 8. 1.0 g (12.3 mmol) potassium cyanate was added to this buffer and then 1.0 g (11.2 mmol) of (*S*)-alanine in 3 ml of water. After one day at room temperature 2 M hydrochloric acid was added and I isolated, washed with ice-water and dried. 0.40 g (27 %) α-ureidopropionic acid was obtained with $[\alpha]_D^{25}$ −7.9°, $[\alpha]_{365}^{25}$ −17.9° (α_D^{52} −0.070°, α_{365}^{25} −0.159°, *c* 1.779; water) (lit.[12] $[\alpha]_D^{25}$ −9.4°). The product was not recrystallized to avoid eventual racemization.

(S)(−)-α-*Hydrazinopropionic acid (II).* An ice-cooled solution of 2.20 g (0.016 mol) of I was dissolved in 10 ml cooled 2 M sodium hydroxide, and 65 ml of 0.5 M sodium hypochlorite [20] in 10 % sodium hydroxide was added. The solution was kept at room temperature for 1.5 h. Then 1.6 ml of 99 % hydrazine hydrate was added (in another experiment sodium sulfite solution). The pH was adjusted to 3 with hydrochloric acid and the water distilled off in vacuo at 80 °C. The residue was suspended in acetic acid and most of the sodium chloride was filtered off. Chloroform was added in portions to the filtrate for selective precipitation of sodium chloride, I, and II in that order. II was dissolved in a small amount of water and precipitated by addition of 99 % ethanol. Yield 0.47 g (27 %). Recrystallization twice in the same way yielded II with m.p. 211 – 213 °C and $[\alpha]_D^{25}$ −30° (α_D^{25} −0.288°; *c* 0.964; 6 M HCl), R_F 0.38 (lit.[19] $[\alpha]_D^{25}$ −30.3°, m.p. 206 – 208 °C). The product contained a minor amount of sodium chloride; in another experiment 0.5 %.

Found: C 34.43; H 7.38; N 25.80. Calc. for $C_3H_8N_2O_2$ (104.11): C 34.61; H 7.75; N 26.91.

Deamination of (S)(−)-α-hydrazinopropionic acid. 0.2 mmol II was dissolved in 2 ml of water and 0.10 g 10 % palladium-on-charcoal was added. Hydrogenation was performed at a hydrogen pressure of 5 atm. in a Parr catalytic hydrogenation apparatus at room temperature for 24 h with continuous shaking. The catalyst was then filtered off, and the residue evaporated to dryness. The enantiomeric purity of the alanine formed was determined without purification. After the reaction, no II was detected in a TLC test. Neither absorption at 6.76 μm nor the double peak absorption at 3.0 μm typical for II was found.

Ethyl (S)(−)-α-hydrazinopropionate hydrochloride (III). (S)(−)-α-Hydrazinopropionic acid was esterfied before purification according to Darapsky.[9] The ester product was a viscous oil with $[\alpha]_D^{25}$ −23.3° (α_D^{25} −1.210°; *l* = 1; *c* 5.19; 99 % ethanol). The ester undergoes self-condensation upon standing (*cf.* Ref. 21). $[\alpha]_D^{25}$ of the ester hydrochloride was −2.8° (α_D^{25} −0.11°, *l* = 1, *c* 3.92; 99 % ethanol). ^{1}H NMR spectra showed that the ethyl α-hydrazinopropionate and its hydrochloride were not quite pure.

(S)(+)-α-(4,5,6,7-Tetrahydroindazolyl-1-)-propionic acid (VI). 0.40 g (3.2 mmol) α-hydroxymethylenecyclohexanone [22] was dissolved in 1.5 ml of 99 % ethanol. The solution was added dropwise at room temperature to 0.78 g (5.2 mmol) ethyl (*S*)(−)-α-hydrazinopropionate hydrochloride in 6 ml of 99 % ethanol. The temperature rose to 27 °C. The course of reaction was followed by ^{1}H NMR spectroscopy. After 10 min no further formation of IV and V was observed. After standing for half an hour the solvent was evaporated off at reduced pressure. The residue was warmed with 4 ml of 1 M hydrochloric acid on a boiling water bath for 90 min. 2 M sodium hydroxide was then added to pH 3.5 and the mixture was extracted with ether. Excess of sodium hydroxide should be avoided since the ester easily racemizes under alkaline conditions. The ether phase was dried with magnesium sulfate and treated with charcoal. The nearly colorless ether solution was evaporated to dryness. The residue (0.3 g) was dissolved in 1 ml of chloroform and precipitated by adding 6 ml of ligroin. After one further recrystallization in the same way and one from ethanol-water, a colorless product was obtained free from 2-isomer according to an IR and ^{1}H NMR analysis. 0. 06 g (10 %) of VI

was obtained with m.p. 139–140° C. $[\alpha]_D^{25}$ +16.8°, $[\alpha]_{365}^{25}$ +60.3° (α_D^{25} +0.219°, α_{365}^{25} +0.784°, *l* 1, *c* 1.30; $CHCl_3$). The specific rotation is strongly dependent on concentration. With *c* 3.518 an $[\alpha]_D^{25}$ value of +4.0° was obtained. IR (KBr), λ_{CO} 5.8 μm. UV λ_{max} 229.8 nm (log ε 3.67). CD, $[\theta]_{225}$ ±0, $[\theta]_{235}$ +1150 (in methanol). ^{1}H NMR ($CDCl_3$) $-CH_2-$ and $-CH_3$ (multiplet with the highest peak at δ 2.48), α-H (quartet at δ 4.90) and >CH= singlet at δ 7.30).

(S)(+)-α-(4,5,6,7-Tetrahydroindazolyl-2)-propionic acid (VII). Ethyl (S)(−)-α-hydrazinopropionate was isolated by suspending its hydrochloride in a mixture of chloroform and an excess of dry sodium carbonate. After stirring for some hours the chloroform solution was filtered and the chloroform evaporated off. It is advisable to prepare ethyl α-hydrazinopropionate just prior to use since it is not stable at room temperature.

0.5 g (4 mmol) of α-hydroxymethylenecyclohexanone was mixed with a solution of 0.5 g (3.8 mmol) of ethyl (*S*)(−)-α-hydrazinopropionate in 3 ml 99 % ethanol. The mixture was refluxed for 45 min. The solvent was distilled off and the residue was warmed for 2 h in a boiling water-bath. The hydrolyzate was extracted with ether and the water phase acidified to pH 3 with concentrated hydrochloric acid. The product was dissolved in 5 ml chloroform and 5 ml of ligroin was added. 0.05 g VII was obtained free from 1-acid according to IR and ^{1}H NMR data. M.p. 199–200 °C. $[\alpha]_D^{25}$ +41° (α_D^{25} +0.190°; *c* 0.464; $CHCl_3$).

The ratio of IV:V as a function of the amount of sodium ethoxide solution added. 0.200 g portions of racemic ethyl α-hydrazinopropionate hydrochloride were dissolved in 1.2 ml 99 % ethanol and various amounts of a 2 M solution of sodium ethoxide in 99 % ethanol were added. Then 0.40 ml of a solution of α-hydroxymethylenecyclohexanone in 99 % ethanol with a concentration of 0.5 g/ml was added to each portion under stirring and the mixture was warmed at 85 °C for 45 min. The solvent was distilled off and water was added to the residue. 2 M sodium hydroxide was added to slightly alkaline reaction if the water phase was acidic. The mixture was extracted with two portions of 5 ml ether and the ether phase was washed with a small amount of water and dried with magnesium sulfate. The ratio of IV:V in the ether solution was determined by GLC analysis. In some cases the ratios were determined by ^{1}H NMR analysis in DMSO solution of the residue after distilling off the solvent. The results are given in Fig. 2. Addition of more than 0.76 ml 2 M sodium ethoxide solution resulted in a marked decrease in the ester yield.

IV and V from 2-chlorocyclohexenecarboxaldehyde. 2-Chlorocyclohexenecarboxaldehyde was prepared according to Ziegenbein and Lang.[23] A solution of 1.0 g (5.4 mmol) rac. ethyl α-hydrazinopropionate hydrochloride in 1 ml 6 M hydrochloric acid and 2 ml 96 % ethanol was added at room temperature to 1.0 g (6.9 mmol) 2-chlorocyclohexenecarboxaldehyde dissolved in 3 ml 99 % ethanol. The course of reaction was followed by ^{1}H NMR spectroscopy using the difference in the vinyl proton shifts of the starting material, the intermediate and the products. A small amount of 2-chlorocyclohexenecarboxaldehyde remained after 30 min. After subsequent boiling for 30 min nearly no intermediate hydrazone was visible but much of the pyrazoles was observed. Boiling was continued for 30 min more. 2 M sodium hydroxide was then added to neutral reaction and the mixture was extracted with ether. The ether solution was dried, the ether distilled off and the residue analyzed by GLC. The compounds IV and V were formed in the ratio 87:13.

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NMR-Studies of the Interaction of Metal Ions with Poly(1,4-hexuronates). IV. Proton Magnetic Resonance Study of Lanthanide Binding to Sodium Methyl α-D-Galactopyranosiduronate in aqueous Solutions

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The interaction of Eu^{3+} and Pr^{3+} with sodium methyl α-D-galactopyranosiduronate in aqueous solutions has been studied by ^1H NMR. The results indicate a large formation constant for 1:3 adducts and that only negligible amounts of 1:2 and 1:1 adducts are present at low molar ratios of lanthanide to substrate. From the LIS-curves at very low ratios of lanthanide to substrate, evidence was obtained for a remarkably high degree of axial symmetry in the 1:3 complex. A lanthanide-binding site has been determined by a computer search for the best fit of calculated and observed LIS assuming only dipolar (pseudocontact) mechanism and an axially symmetrical complex in which the three ligands are magnetically equivalent.

The binding of metal ions to active sites in anionic biopolymers plays an important role in determining their physical and biological properties. Information on binding sites and complex-formation constants is thus of great importance, and we have applied ^1H NMR spectrometry to obtain such information by using paramagnetic lanthanide ions as probes. The ability of these ions to induce chemical shifts in NMR spectra is well established,[1] and spectra which are normally too complex for complete interpretation, such as those of carbohydrates, may become amenable to analysis. A valid analysis of the lanthanide induced shifts (LIS) to provide information on the structure of the complex requires knowledge of the stoichiometry of the complex, the complex-formation constants and, very importantly, also, the symmetry of the complex.[2]

The present ^1H NMR study of sodium methyl α-D-galactopyranosiduronate with $Eu(NO_3)_3$ and $Pr(NO_3)_3$ as shift reagents in D_2O leads to structural assignment for the complexes formed, which predominantly consist of Ln(Me α-D-galactopyranosiduronate)$_3$ (Ln = Eu or Pr). It is possible to visualize the conformation of this complex by molecular models, but more than one conformation seems to be acceptable. A preliminary account has been published previously.[3]

The theoretical background for the structural calculations is given by Reuben [4] and Bleaney.[3,5]

EXPERIMENTAL

Methyl α-D-galactopyranosiduronic acid methyl ester (*1*) was synthesized according to Jones and Stacey.[15] The yield of the crude product was 20 %. After recrystallization from methanol-acetone (1:1, v/v) the product had a melting point of 148 °C and a specific rotation $[\alpha]_D^{20} = +127°$ (*c* 1.0; water).

Sodium methyl α-D-galactopyranosiduronate was prepared by hydrolysis of the methyl ester (*1*). The methyl ester (*1*) (50 mg) was dissolved in sodium hydroxide (0.02 N, 25 ml) at room temperature. Aliquots (1 ml) were removed at intervals and titrated with HCl (0.01 N) to follow the progress of the reaction. When the reaction was complete (*ca.* 20 min), excess alkali was removed by passing the sample through a cation-exchange column (Dowex 50W × 8, H^+-form). The eluate was neutralized with dilute sodium hydroxide and the sodium methyl α-D-

galactopyranosiduronate was isolated by freeze-drying. The yield was virtually quantitative. The product was used for the NMR studies without further characterization.

NMR spectrometry. Sodium methyl α-D-galactopyranosiduronate (monohydrate) was dissolved in D_2O to give the desired concentration, and *ca.* 0.3 ml of the solution was accurately weighed into the NMR tube. The pH of the solution was checked by means of a Radiometer C-297 capillary glass electrode and adjusted to 5–6 by addition of HNO_3 (1.5 N in D_2O). For measuring the initial slopes of the LIS curves, the appropriate lanthanide nitrate was added as a D_2O solution (125 mg/ml) in amounts ranging from 2 to 20 μl. The volume corrections due to these additions were neglected. For higher ratios of lanthanide to substrate, $[Ln_0]/[S_0]$, the nitrates were weighed directly into the NMR tube and dissolved *in situ*. The spectra were recorded on a Varian-A-60A NMR spectrometer at 39 °C using dioxan or sodium 3-(trimethylsilyl)propane sulfonate as an internal reference. Downfield shifts were assigned as positive. All assignments were confirmed by double resonance experiments using a Varian HA-100 NMR spectrometer.

Analysis of data. A computer program was written to carry out an automatic search for the position of the lanthanide ion relative to the substrate which gave the best fit of the observed and the calculated LIS, assuming an axially symmetrical complex and only dipolar mechanism for the LIS. Space coordinates were taken from an X-ray study of methyl α-D-galactopyranosiduronic acid methyl ester.[7] A reasonable starting position for the lanthanide ion was chosen and space coordinates for an auxiliary atom introduced to define the orientation of the principal magnetic axis as the straight line through this atom and the lanthanide. The distances from the lanthanide to the various protons, r_i, and the angles between the vectors $\vec{r_i}$ and the symmetry axis, θ_i, could be calculated. In the case of rapid-ligand exchange, averaged induced dipolar shifts, $\Delta\nu_i$, for the actual substrate protons, H_i, caused by a certain species LnS_n can be calculated according to the formula [4,5]

$$\Delta\nu = \frac{n[LnS_n]}{[S_0]} C \frac{(3\cos^2\theta_i - 1)}{r_i^3} = \frac{n[LnS_n]}{[S_0]} \Delta_{ni} \quad (1)$$

The bound shift, Δ_{ni} is the induced shift for proton i when n substrate molecules are bound to the lanthanide ion. The stoichiometry parameter n, the complex/substrate molar ratio $[LnS_n]/[S_0]$, and the constant C are universal for all protons. The value of C depends on the magnetic properties of the lanthanide ion and the crystal-field coefficients, and is not known. The theory gives only relative, not absolute signs, *i.e.* C can be either positive or negative. Both possibilities have been taken into account here. To avoid introduction of the factors $[LnS_n]$ and C, initial shift ratios relative to those of proton H_1 was used. An agreement factor,[4] ε, was expressed by

$$\varepsilon = \left[\frac{1}{N-1}\sum_{i=2}^{N}\left(\frac{\Delta\nu_{calc}^{(i)}}{\Delta\nu_{calc}^{(1)}} - \frac{\Delta\nu_{obs}^{(i)}}{\Delta\nu_{obs}^{(1)}}\right)^2\right]^{\frac{1}{2}} \quad (2)$$

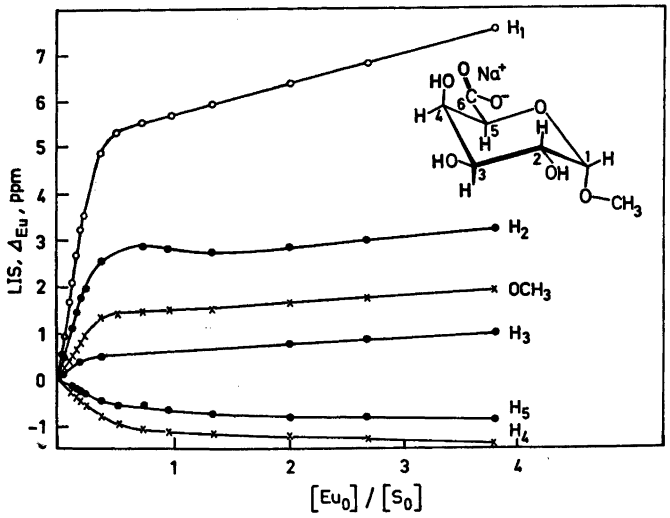


Fig. 1. The induced shifts for the carbon-bound protons of sodium methyl α-D-galactopyranosiduronate in D_2O solution with $Eu(NO_3)_3$ as shift reagent. (Substrate concentration $[S_0] = 0.336$ mol/l).

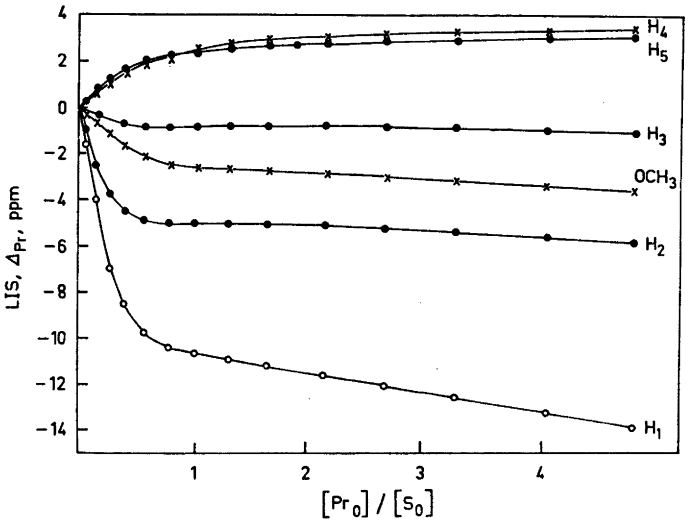


Fig. 2. The induced shifts for the carbon-bound protons of sodium methyl α-D-galactopyranosiduronate in D_2O solution with $Pr(NO_3)_3$ as shift reagent. (Substrate concentration $[S_0] = 0.216$ mol/l.

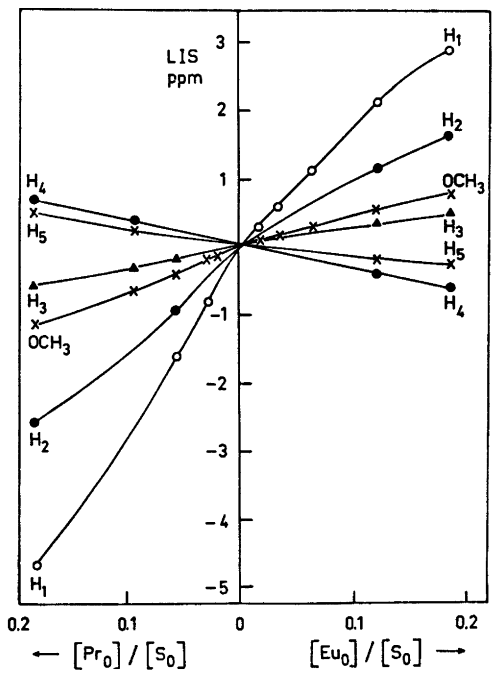


Fig. 3. Same as Figs. 1 and 2, expanded scale. (Substrate concentrations: $[S_0]_{Pr} = 0.216$ mol/l, $[S_0]_{Eu} = 0.336$ mol/l).

where N is the number of protons involved. By changing the x, y, and z coordinates of the lanthanide and the auxiliary atom, respectively, in a stepwise manner with a pre-selected step-size, and selecting the position with the lower ε value, the program searched for a best-fit position defined by a minimum value of ε.

RESULTS

a. Presentation of data. The observed lanthanide-induced shifts (LIS) in ppm for five protons ($H_1 - H_5$) and the CH_3-group in sodium methyl α-D-galactopyranosiduronate in D_2O solutions are shown in Figs. 1, 2, and 3 as a function of the $[Ln]/[S_0]$ ratio with $Eu(NO_3)_3$ and $Pr(NO_3)_3$ as shift reagents. Almost identical curves were obtained with $Pr(ClO_4)_3$ instead of $Pr(NO_3)_3$.

The fact that both upfield and downfield shifts occur clearly demonstrates the angular dependence in the dipolar shift predicted by eqn. (1). This is markedly different from observations with the complexes $Ln(dpm)_3$ and $Ln(fod)_3$ in organic solvents where LIS are almost exclusively in the same direction for all protons in the substrate. Due to overlapping of the resonance peaks, the LIS curves for H_3 and H_2 were difficult to obtain at very low $[Eu_0]/[S_0]$ ratios.

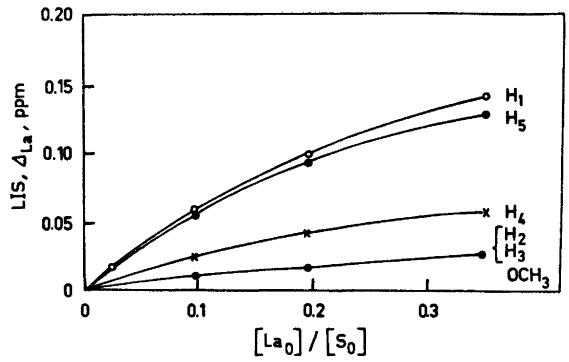


Fig. 4. The induced shifts for the carbon-bound protons of sodium methyl α-D-galactopyranosiduronate in D_2O solution due to complex formation with $La(NO_3)_3$. (Substrate concentration $[S_0] = 0.21$ mol/l).

As was to be expected from theory [5] and earlier experiments,[2] the shifts induced by Pr^{3+} are larger than those induced by Eu^{3+} and of opposite sign as can be seen from Fig. 3. In order to compare the LIS which are due to the paramagnetic nature of the Eu^{3+} and Pr^{3+} ions the LIS must be corrected for the shifts due to electrostatic shielding of the ions.[3] Experiment with the diamagnetic ion La^{3+} therefore was carried out and the results are shown in Fig. 4. From Fig. 4 it is seen that the shift contribution due to complex formation of La^{3+} is largest for H_1. The induced paramagnetic shifts are, as may be seen by comparison with Figs. 1 to 3, completely overriding this effect and the correction is thus not important. For protons H_4 and H_5, on the other hand, this effect is significant and a correction has been applied to the initial slopes given in Table 1. Ratios of Eu^{3+} and Pr^{3+} induced shifts are nearly the same for all protons at low $[Ln_0]/[S_0]$ ratios as appears from the last column of Table 1. This is a characteristic feature for dipolar LIS when the anisotropy of the magnetic susceptibility has axial symmetry provided the structure of the complex is the same for the two lanthanides.[2]

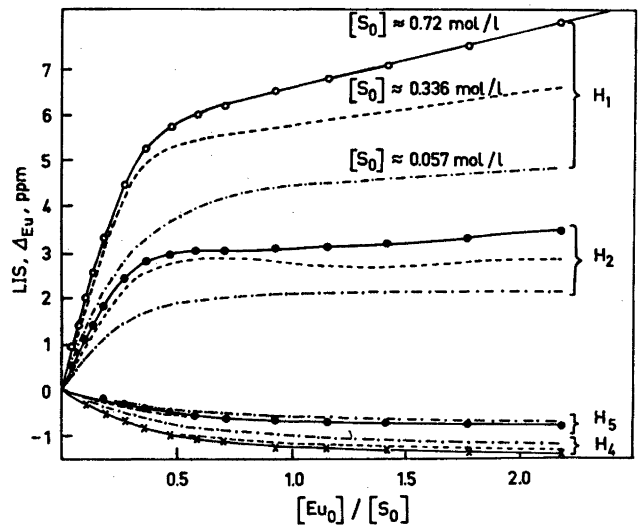


Fig. 5. The induced shifts for the carbon-bound protons of sodium methyl α-D-galactopyranosiduronate in D_2O solution as a function of $[Eu_0]/[S_0]$ for three different substrate concentrations.

b. Stoichiometry. Our first aim was to estimate the formation constants K_1, K_2, and K_3 for the complexes 1:1, 2:1 and 3:1 defined by:

$$\begin{aligned} Ln + S &\rightleftharpoons LnS & K_1 \\ LnS + S &\rightleftharpoons LnS_2 & K_2 \\ LnS_2 + S &\rightleftharpoons LnS_3 & K_3 \end{aligned}$$

and the corresponding bound shifts for proton H_1, Δ_{11}, Δ_{21}, and Δ_{31}.

Even at the highest $[Ln_0]/[S_0]$ ratio the LIS are still increasing remarkably linearly (see Figs. 1 and 2) and it is not possible to estimate any asymptotic value for the observed shifts. For a pure 1:1 complex this would give the bound shift Δ_{1i} of the various substrate protons directly. Information on the stoichiometry of the complexes and their bound shifts for the different protons must therefore be sought from the shapes of the curves in Fig. 5, which give the LIS as a function of $[Eu_0]/[S_0]$ ratio for three different substrate concentrations, $[S_0] =$ 0.72, 0.336, and 0.057 mol/l, respectively. The curves have a characteristic knee at molar ratios $[Eu_0]/[S_0] \approx 0.3$, and the initial LIS slopes $k = \delta(\Delta\nu_i)/\delta([Eu_0]/[S_0]) = n\Delta_{ni}$, increase very slowly at high $[S_0]$ values. This is characteristic for a system in which 3:1 complexes are formed when the formation constant $K_3 = [LnS_3]/[S][LnS_2]$ is fairly large.[8]

Since the presence of both LnS_3, LnS_2, and LnS has to be considered with their corresponding bound shifts, a theoretical fitting of the LIS-curves requires six-parameter (K_1, K_2, K_3, Δ_{3i}, Δ_{2i}, and Δ_{1i}). By extending the method of Shapiro and Johnston [9] to a six parameter fit almost complete fitting could be obtained for the LIS curve of proton H_1 for the intermediate substrate concentration $[S_0] = 0.336$ mol/l. The best fit values for the Eu^{3+}-induced bound shifts for 3:1, 2:1, and 1:1 complexes were found to be: $\Delta_{31} = 5.83$ ppm, $\Delta_{21} = -2.67$ ppm and $\Delta_{11} = 40.0$ ppm, respectively. The formation constants giving the best fit were: $K_3 = 350$ mol^{-1}, $K_2 = 160$ mol^{-1}, and $K_1 = 5.5$ mol^{-1} (standard deviation $\sigma \approx 4$). The ratio $K_1:K_2:K_3$ was 0.034:1:2.19, whereas binding to identical and independent sites requires 3:1:0.33.[10] This result thus suggests that there is some kind of cooperativity in the binding process.

By comparing the estimated bound shift for H_1 for the 3:1 complex with the one obtained from the initial slope of the LIS curve assuming that the initial shifts are due to the 3:1 complex only (see Table 1, $3\Delta_{31} = 17.4$, *i.e.* $\Delta_{31} = 5.8$ ppm), it is suggested that the 3:1 complex and thus the species LnS_3 is the dominating one in the initial stage of the binding process.

However, the agreement between calculated and observed Δ_{31} is not so good for other substrate concentrations $[S_0]$. This is probably due to a change in activity coefficients over the large range of salt concentrations used. A full, self consistent analysis, including the activity coefficients (γ) for the lanthanide solutions is necessary to obtain a reliable set of parameters which will fit the LIS curves for all substrate concentrations. It was only intended here to see if the introduction of activity coefficients would improve the fit and a crude correction was employed by letting $[Ln_0]corr = \gamma^{\pm}[Ln_0]$. Using published values [11] for $\gamma^{\pm}$ for $Ln(NO_3)_3$ a marked improvement was obtained, especially for high $[Ln_0]/[S_0]$ ratios. The remarkably good fit obtained without taking activity coefficients into account for the substrate concentration 0.336 mol/l is probably partly due to the fact that in the range of Ln-concentrations used in that experiment, the activity coefficients are relatively independent on the concentration.[11] For the lowest substrate concentration the activity coefficients decrease markedly with increasing Ln-concentration, whereas the opposite is the case for the Ln-concentrations used in the experiments with the highest substrate concentrations. The formation constants given above should therefore not be regarded as true equilibrium constants. The given values for the bound shifts should also be regarded as rough estimates.

Due to the uncertainties introduced by the difficulties in taking activity coefficients properly into account an additional way was sought to find out if initial slopes and slope ratios are purely representative for the 3:1 complex.

Because of rapid ligand exchange between the three species and the solution, the ratios of the LIS ($\Delta\nu_i$) for protons $H_2 - H_5$ (H_i) relative to the LIS of proton H_1 ($\Delta\nu_1$) will in the general case be dependent on the concentration of the three different complexes according to the formula:

$$\frac{\Delta\nu_i}{\Delta\nu_1} = \frac{3\Delta_{3i}[LnS_3] + 2\Delta_{2i}[LnS_2] + \Delta_{1i}[LnS]}{3\Delta_{31}[LnS_3] + 2\Delta_{21}[LnS_2] + \Delta_{11}[LnS]} \qquad (3)$$

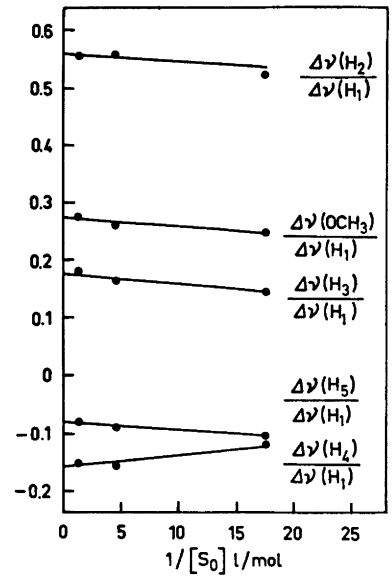


Fig. 6. The induced shift ratios relative to H_1 for the carbon-bound protons of sodium α-D-galactopyranosiduronate in D_2O solution as a function of the reciprocal substrate concentration ($1/[S_0]$).

With the approximate values of the formation constants given above, it is easy to realize that the 3:1 complex must dominate completely over the two other species at infinite concentration of substrate. The initial LIS ratios at different concentration of substrate obtained from Fig. 5 were therefore, plotted against $1/[S_0]$ in Fig. 6. The negligible small variation with substrate concentration shown in Fig. 6 strongly indicates, in agreement with the calculations above, that the initial LIS ratio to a very good approximation can be taken as representative for the 3:1 complex.

c. Structural assignment of the 3:1 complex. It now seems established with a high degree of certainty that the initial slopes of the LIS curves are representative for the 3:1 complex, but a test for the magnetic symmetry of the complex must be carried out before the initial slopes may be used for structural assignment of the 3:1 complex. As mentioned under *a.* the LIS ratio relative to H_1 obtained with $Eu(NO_3)_3$ and $Pr(NO_3)_3$ listed in Table 1, is in fair agreement with each other except for a slight deviation in the values for proton H_3. As mentioned before, initial LIS values are difficult to measure for H_3, and since this shift is rather small its relative value is less accurate. According to Bleaney [2] the data in Table 1, therefore, strongly suggest that the magnetic susceptibility has axial symmetry in the 3:1 complex.

The two sets of relative LIS ratios in Table 1 were used in a computer search for the conformational parameters assuming axial symmetry. Owing to the presumed free rotation of the methyl group around the C_1-O_1 and O_1-CH_3 linkages (*cf.* Fig. 1), a definite set of coordinates can not be assigned to $O-CH_3$ protons. The LIS for the $O-CH_3$ protons were therefore omitted during the search procedure. For both sets of shifts a best-fit position for the lanthanide ion and a best-fit orientation of the principal magnetic axis were obtained, and the data are summarized in Table 2. A step-size of 0.02 Å was used in the computer search procedure. The distance between the Eu^{3+} and Pr^{3+} position is ~0.35 Å, which is in the order

Table 1. Initial slopes in ppm for the LIS of sodium methyl α-D-galactopyranosiduronate with $Eu(NO_3)_3$ and $Pr(NO_3)_3$ as shift reagents. Substrate concentrations: $[S_0]_{Eu}=0.336$ mol/l, $[S_0]_{Pr}=0.216$ mol/l. All initial slopes are corrected for shifts due to electrostatic shielding (see Fig. 4).

Proton	Initial LIS slopes $\delta(\Delta\nu)/\delta([Ln_0]/[S_0])=k$		Relative LIS slopes				k_{Pr}/k_{Eu}
	Eu^{3+}	Pr^{3+}	Eu^{3+} exp.	calc.	Pr^{3+} exp.	calc.	
H_1	17.4	−29.8	1	1	1	1	−1.72
H_2	9.4	−17.2	0.540	0.547	0.578	0.577	−1.82
H_3	2.9	−3.67	0.166	0.155	0.123	0.133	−1.28
H_4	−2.7	4.08	−0.155	−0.160	−0.137	−0.139	−1.49
H_5	−1.47	2.67	−0.084	−0.083	−0.090	−0.090	−1.82
$O-CH_3$	4.47	−7.25	0.257		0.243		−1.62

Table 2. Conformational parameters for the binding of Ln^{3+} in the Ln(methyl α-D-galactopyranosiduronate)$_3$ complexes calculated from the LIS.

Ln^{3+}	Real-space coordinates (Å)						Ln^{3+}–{oxygen / carbon distances (Å)			θ_{H_1} (°)	ε
	Ln^{3+}			Auxiliary atom							
	x	y	z	x	y	z	O_4	O_5	C_6		
Eu^{3+}	−2.41	3.75	18.1	−0.57	1.33	16.47	3.01	1.93	2.08	12.47	0.0033
Pr^{3+}	−2.43	3.81	17.7	−0.67	1.49	16.40	2.99	2.00	2.39	12.91	0.0018

of the expected accuracy in positioning the lanthanide ion.[1] The main difference between the positions is their distances from C_6. They are both reasonable from a chemical point of view. The fit to the experimental data is remarkably good in both cases as judged by the root mean square deviations, ε. To illustrate this, calculated initial slopes are given in Table 1. The data are also compatible with a reasonable average position for the $O-CH_3$ protons. Sodium methyl α-D-galactopyranosiduronate

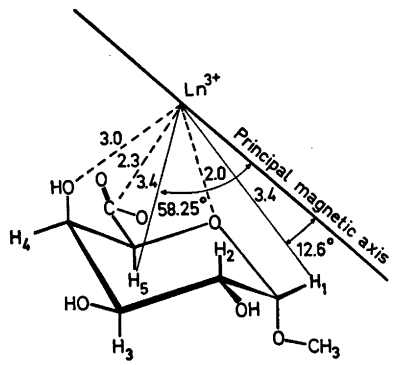


Fig. 7. Proposed binding site for the lanthanide ion and the orientation of the principle magnetic symmetry axis relative to methyl α-D-galactopyranosiduronate.

with the position of the Ln^{3+} ion and the orientation of the symmetry axis is shown in Fig. 7.

By using LIS ratios instead of LIS in the search, an ambiquity is introduced since both negative and positive LIS will give a positive ratio with a reference LIS of the same sign. The search for the positions reported above were carried out assuming downfield shifts to be positive with a positive C value [see eqn. (1)] for Eu^{3+} and a negative C value for Pr^{3+}. According to this sign convention the angle factor should be positive for the H_1 proton which shows a large downfield Eu^{3+}-induced shift. Similar calculations were carried out by assuming reversal of the signs of C, and the results are compared with the mean value of the previous ones in Table 3. A best-fit position was obtained also for this sign convention. However, the position is different, especially with reference to the orientation of the principal magnetic axis which is reoriented by approximately 90°. This is expected since the angular factor ($3\cos^2\theta - 1$) has to change sign. However, with a lanthanide $-O_5$ distance of only 1.57 Å, this position is physically less reasonable. The root means square deviation, ε, is also an order of magnitude larger.

Table 3. Comparison of conformational parameters obtained for the Pr(methyl α-D-galactopyranosiduronate)$_3$ complex according to the sign conventions applied; downfield shifts positive.

Sign conv.	Ln^{3+}			Auxiliary atom			Ln^{3+}–{oxygen / carbon distances (Å)			θ_{H_1} (°)	ε
	x	y	z	x	y	z	O_4	O_5	C_6		
$C(Eu) < 0$	−2.57	2.45	17.2	−1.03	5.02	19.44	3.74	1.57	3.15	115.8	0.012
$C(Eu) > 0$	−2.41	3.83	17.8	−0.63	1.43	16.41	2.97	1.99	2.26	12.6	0.0016

Table 4. Ln^{3+}-proton distances and θ values for the two alternative sites.

Sign convention		H_1	H_2	H_3	H_4	H_5
C(Eu) < 0	θ_i^0	115.8	80.14	66.14	38.19	51.63
	r_i (Å)	2.13	3.27	4.71	4.68	3.45
C(Eu) > 0	θ_i^0	12.93	27.76	39.00	66.13	58.25
	r_i (Å)	3.37	3.64	4.89	4.14	3.39

As a check on the binding sites obtained, small additions of $Gd(NO_3)_3$ were used to induce line broadening. To obtain data for all protons, $Eu(NO_3)_3$ was first added ($[Ln_0]/[S_0] = 0.18$) to spread out the signals and then $Gd(NO_3)_3$ was added. A line broadening proportional to r^{-6} is predicted by the theory,[12] and should reflect the differences in lanthanide – proton distances r_i. The observed broadening effects were of the order: $H_1 \approx H_2 \approx H_5 > H_3 > O-CH_3$. The broadening was difficult to estimate for H_4, but it was definitely less than for H_1 and larger than for $O-CH_3$. The most pronounced difference between the two sites, for which lanthanide – proton distances, r_i, and angles, θ_i, are given in Table 4, is the ratio $(r_2/r_1)^6$. The ratio $(r_2/r_1)^6$ was found to be 13 for the negative and 1.6 for the positive sign convention. The broadening experiments predict this ratio close to unity and thus favour the latter site to be the most likely.

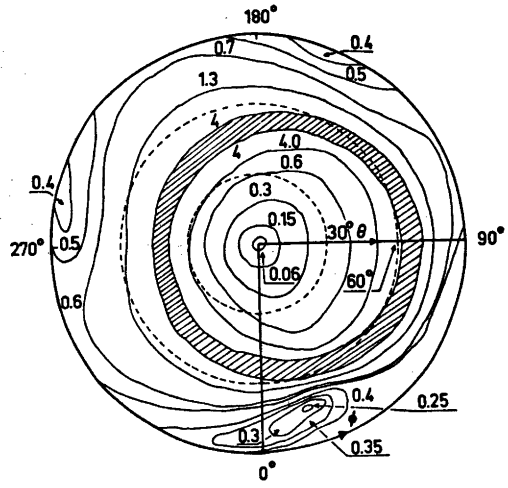


Fig. 8. Contours of the agreement factor ε as a function of the orientation of the principal magnetic axis given by the polar angles θ and ϕ. The centre in the diagram corresponds to the best fit with the axis pointing out of the paper plane.

To investigate whether there exist other orientations of the magnetic axis leading to a minimum value of the agreement factor ε, we have rotated the axis in all orientations, keeping the lanthanide ion position. The result is shown in Fig. 8 as a contour plot of the agreement factor ε. The contours are paths of constant ε values as a function of polar angles θ and ψ defining the axis orientation. The centre in the diagram corresponds to the best fit position with the axis pointing out of the paper plane. In addition to the minimum for the most reasonable site, only one more less defined minimum appeared.

DISCUSSION

The nitrates and perchlorates of Eu^{3+} and Pr^{3+} appear to be well suited as shift reagents for carbohydrates in aqueous solutions. However, Angyal's observations on methyl β-D-hammamelopyranoside [13] suggest that the requirements for structural analysis may not always be fulfilled.

It should be emphasized that a strict pH control is necessary to obtain reliable induced shifts for acidic carbohydrates. The pH curves for the induced shifts of protons H_1 and H_2 of D-galactopyranosiduronic acid published previously [3] clearly demonstrate this point. Owing to the low solubility of the lanthanide hydroxides the safe pH working range is limited to between 5 and 6.

As pointed out by Bleaney *et al.*[2] a number of conditions have to be fulfilled to allow any valid conclusion to be drawn from LIS concerning the conformation of the complexes. Among these, three primary requirements may be formulated: (i) The conformation of the

substrate must be retained in the complexed state, (ii) LIS of all protons must be of dipolar origin only, and (iii) it must be possible to measure or evaluate a quantity proportional to the bound shift for one particular stoichiometry of the complex.

(i) The ^{1}H NMR spectrum of sodium methyl α-D-galactopyranosiduronate after addition of $Eu(NO_3)_3$ to a molar ratio of 3.6 has been published previously.[3] The coupling constants were all in reasonable agreement with those observed for the free substrate, thus suggesting that the $^4C_1$ conformation is retained in the lanthanide complexed state.

(ii) Contact shifts have their origin in movement of unpaired electron spin density from the metal cation to the ligand protons by covalent bond formation and are independent of the factor $(3\cos^2\theta - 1)$. This interaction rapidly decreases with increasing Ln-proton distance through bonds and may possibly be significant only for protons H_4 and H_5 according to the lanthanide position shown in Fig. 7. However, since contact shift contributions by Eu^{3+} and Pr^{3+} differ markedly [4,14] in magnitude, and since the LIS for H_4 and H_5 fit the symmetrical scheme for the dipolar mechanism, a significant contact shift contribution seems [5] very unlikely.

(iii) For systems in which complexes of higher than 1:1 stoichiometry is formed, the observed LIS will in the general case be an average over all species according to eqn. (3). Bearing in mind that only the formation constants K_n are universal for all protons in the substrate, while the bound shift Δ_n generally are different, it turns out that even a system where $n=2$ may be undetermined if no additional information is available. In our case the LIS data strongly indicate a large formation constant for 3:1 complex, and that only small and negligible amounts of 2:1 and 1:1 complexes are present at low $[Ln_0]/[S_0]$ ratios.

In the computer search a set of space coordinates was calculated giving the best fit to the observed shift data without considering the question of more than one substrate molecule participating in the complex formation. The question therefore remains, whether a complex conformation with three magnetically equivalent carbohydrate molecules is physically possible. This is necessary for correlating the results to any physically reasonable complex.

The proposal of complex structures based on the present data is of course hypothetical. However, it is obviously possible to accomodate two sugar rings with the lanthanide ion as an inversion centre, as shown previously.[3] In order to be equivalent to the others, the third sugar moiety must be in a position that can be reached by a hypothetical rotation of the first or the second unit around the axial symmetry axis until no intermolecular interactions occur. Another possibility seems to be that the third unit is located in a position approaching the one corresponding to the negative sign convention in such a way that the shift contributions from two of the ligands cancel. Scaled molecular models indicate that these positions may be possible. If this is not the case it seems unlikely that the analysis should lead to a chemically reasonable site with an almost perfect fit to the observed data.

The induced shifts by lanthanide salts are smaller than those of the β-diketones (*e.g.* $Ln(dpm)_3$ and $Ln(fod)_3$) [15] for which the constant C is about 1000 ppm Å^3. In our case the value of C is only 120 ppm Å^3 or 360 ppm Å^3 if two ligands counteract. This may suggest that the position corresponding to the negative sign convention is more probable for the third ligand, but the dependence of C on solvents and ligands is complex, and more data are needed to settle this question.

The 3:1 stoichiometry leads to a solvation number 9 for the Ln^{3+} ions which is expected [16] in aqueous solutions. Since the LIS curves are similar with perchlorate and nitrate, and the HDO signal is unaffected by both shift- and broadening reagents, it is not likely that any NO_3-anions or water molecules are present in the first solvation sphere.

The interaction between the Ln^{3+} ions and the ligands is mainly of electrostatic nature. It is difficult to realize any amount of LnS_3 species exceeding the statistical value for identical and independent sites, without any formation of interligand hydrogen bonds which may stabilize the 3:1 complex.

Preliminary studies on the β-anomer, at present under way in our laboratories, show different results. The LIS data can be completely fitted by assuming only 1:1 and 2:1 complexes and the formation constants were found to be

$K_1 = 26$ M^{-1}, $K_2 = 35$ M^{-1}, and $K_3 \approx 0$, thus indicating a much weaker coordinating power in the case of the 3:1 complex. The induced shifts for the substrate protons are also entirely different in accordance with different stoichiometry and structure of the complex. Provided that the lanthanide position relative to the substrate is the same as in the α-anomer molecular model studies show that due to steric hindrance caused by the equatorial methoxy group, 3:1 stoichiometry is impossible or difficult for the β-anomer.

We hope that a full analysis of the LIS data for the β-anomer will shed some more light on the structural problems.

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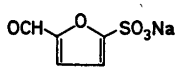
Reactions of 5-Formyl-2-furansulfonic Acid

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Twentynine new furan compounds have been prepared from 5-formyl-2-furansulfonic acid sodium salt.

5-Formyl-2-furansulfonic acid sodium salt (I) has recently become easily accessible.[1] It has been used to prepare 29 new furan compounds (II—XXX). Since all reactions employed are well-



I

known standard reactions, only structures, starting materials, and yields for these new compounds are given (Table 1).

The diversity of the reactions and the generally high yields obtained illustrate that furans with a sulfonyl group in α-position behave as one would expect from the structures in question. No rearrangements or openings of the furan ring, phenomena always to be counted with in furan chemistry, were observed.

The structures of the new furans follow from their syntheses and from elemental analyses. The structure of XI was further proved by the fact that this compound gave no color reaction with iron(III) chloride in aqueous solution.

EXPERIMENTAL

Microanalyses by G. Cornali and W. Egger, Leo Pharmaceutical Products.

5-Formyl-2-furansulfonic acid thiosemicarbazone sodium salt dihydrate (II). I (49.5 g, 0.250 mol) and thiosemicarbazide (22.7 g, 0.250 mol) were dissolved in water (3.6 l). The solution was evaporated from a water bath (80 °C) under reduced pressure. When almost all water had been removed, the residue crystallized. It was dissolved in water (150 ml), and propanol (1 l) was added. The crystalline precipitate formed was isolated by filtration; washing with ether, and drying (70 °C, 15 h). 58.0 g (76 %) of II was obtained as almost white crystals. When heated 2 °C/min in a capillary, the product decomposed with darkening and evolution of gases. When heated rapidly, a clear, colorless gel was formed with evolution of gases around 150 °C. [Found: C 23.6; H 3.5; N 13.6; S 20.6; sulfated ash 23.1 Calc. for $C_6H_6N_3NaO_4S_2.2H_2O$ (307.3): C 23.4; H 3.3; N 13.7; S 20.8; ash if Na_2SO_4 23.1]. The compound is sensitive to light.

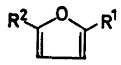
The corresponding anhydrous compound has previously been prepared by Ivanov and Yankov.[2]

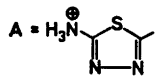
5-Dichloromethyl-2-furansulfonyl chloride(III). To a stirred suspension of I (19.8 g, 0.100 mol) in phosphorus oxychloride (20 ml) was added phosphorus pentachloride (50.0 g, 0.240 mol, Analar BDH) in portions, over a period of about 20 min at 20 °C (cooling). 20 min after the last portion of phosphorus pentachloride had been added, another 19.8 g of I was added in one portion, and again 50.0 g of phosphorus pentachloride was added portionwise at 20 °C over 20 min. These additions were repeated two times more. Thus totals of 79.2 g (0.400 mol) of I and of 200 g (0.960 mol) of phosphorus pentachloride were used. The suspension was stirred at 20 °C for another 90 min. The sodium chloride formed was filtered off and washed with methylene chloride, and the filtrate and washings distilled to give 91.0 g (91 %) of III as a slightly yellow oil, b.p.$_{10}$ 143—146 °C, n_D^{25} 1.5442. A sample was redistilled and analysed, b.p. $_{0.2}$ 84—85 °C, n_D^{25} 1.5431. [Found: C 24,2; H 1.3; Cl 42.8; S 12.6. Calc. for $C_5H_3Cl_3O_3S$ (249.5): C 24.1; H 1.2; Cl 42.7; S 12.8]. III is a strong vesicant. The product turns dark green on standing.

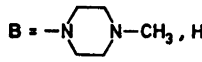
5-Hydroxymethyl-2-furansulfonic acid sodium salt (IV). I (19.8 g, 0.100 mol), water (50 ml), and wet Raney nickel (4.0 g, BASF H 1—50)

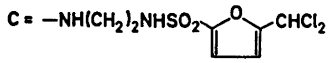
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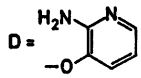
Table 1. New furan compounds from 5-formyl-2-furansulfonic acid sodium salt.

No.			Prepared from (yield %)	
II	SO_3Na	$CH{=}NNHCSNH_2.2H_2O$	I	(76)
III	SO_2Cl	$CHCl_2$	I	(91)
IV	SO_3Na	CH_2OH	I	(92)
V	SO_3Na	CH_2NH_2	I	(75)
VI	SO_3^-	A	II	(62)
VII	SO_2NH_2	$CHCl_2$	III	(80)
VIII	$SO_2N(C_2H_5)_2$	$CHCl_2$	III	(63)
IX	SO_2B	$CHCl_2$	III	(72)
X	SO_2C	$CHCl_2$	III	(94)
XI	SO_2D	$CHCl_2$	III	(76)
XII	SO_2Cl	CH_2Cl	IV	(80)
XIII	SO_3^-	$CH_2N{+}H_3$	V	(62)
XIV	SO_3Na	$CH_2NHCOCH_3$	V	(50)
XV	SO_2NH_2	$CH{=}NNHCONH_2$	VII	(66)
XVI	SO_2NH_2	$CH{=}NNHCSNH_2$	VII	(19)
XVII	SO_2B	$CH{=}NNHCONH_2$	IX	(40)
XVIII	SO_2NH_2	CH_2Cl	XII	(80)
XIX	$SO_2N(CH_3)_2$	CH_2Cl	XII	(90)
XX	$SO_2N(C_2H_5)_2$	CH_2Cl	XII	(58)
XXI	SO_2NH_2	$CH_2NH_2.HCl$	XVIII	(77)
XXII	SO_2NH_2	$CH_2N(CH_3)_2.HCl$	XVIII	(51)
XXIII	$SO_2N(CH_3)_2$	$CH_2NH_2.HCl$	XIX	(71)
XXIV	$SO_2N(CH_3)_2$	$CH_2N(CH_3)_2.HCl$	XIX	(74)
XXV	$SO_2N(NH_2)_2$	CH_2OH	XIX	(78)
XXVI	$SO_2N(C_2H_5)_2$	$CH_2N(C_2H_5)_2$	XX	(62)
XXVII	SO_2NH_2	$CH_2NHCONHCH_3$	XXI	(73)
XXVIII	$SO_2N(CH_3)_2$	$CH_2NHCONHCH_3$	XXIII	(92)
XXIX	$SO_2N(CH_3)_2$	$CH_2OCONHCH_3$	XXV	(80)
XXX	$SO_2N(C_2H_5)_2$	$CH_2N(C_2H_5)_2.HCl$	XXVI	(46)









were shaken in a glasslined autoclave under hydrogen (50 atm) at about 80 °C. After 2 h of shaking absorption of hydrogen ceased. The catalyst was filtered off, and the filtrate evaporated to dryness (100 °C, 10 mmHg). 18.3 g (92 %) of IV remained as faintly yellow crystals, m.p. about 195 °C (decomp.). [Found: C 29.9; H 2.6; S 15.5; sulfated ash 35.8. Calc. for $C_5H_5NaO_5S$ (200.2): C 30.0; H 2.5; S 16.0; ash if Na_2SO_4 35.5].

5-Aminomethyl-2-furansulfonic acid sodium salt (V). I (19.8 g, 0.100 mol), water (35 ml), ammonia (40 ml), and wet Raney nickel (2.4 g, BASF H 1–50) were shaken in a glasslined autoclave under hydrogen (50 atm) at 65 °C for 1 h. The catalyst was filtered off, and the filtrate evaporated to dryness from a water bath (100 °C, 10 mmHg). 20.6 g of crude V remained as white, hygroscopic crystals. The content of V in the crude product was at least 15 g (corresponding to a 75 % yield) as judged by the below transformation of V into XIII. A sample was crystallized from ethanol-water (4:1) and dried (100 °C, 0.1 mmHg). [Found: C 29.0; H 3.5; N 6.1; S 15.1. Calc. for $C_5H_6NNaO_4S$ (199.2): C 30.1; H 3.0; N 7.0; S 16.1. Calc. for $C_5H_6NNaO_4S + 3.8$ % H_2O: C 29.0; H 3.3.]

2-Amino-5-(2-sulfo-5-furyl)-1,3,4-thiadiazole (VI). II (20.0 g, 0.0650 mol) was dissolved in water (200 ml), and the solution mixed with a solution of iron(III) chloride hexahydrate (47.2 g, 0.172 mol) in water (400 ml). The solu-

tion was heated to 80 – 90 °C for 30 min and then cooled to 0 °C. The brown precipitate formed was filtered off and dried, whereby 10.0 g (62 %) of crude VI, m.p. above 300 °C, was obtained. Boiling with water (800 ml) and carbon black (1.5 g) followed by hot filtration, and partial evaporation of the filtrate gave 7.70 g of VI as slightly yellow needles, m.p. above 300 °C [Found: C 29.1; H 2.2; N 17.1; S 25.8; equivalent weight by acidimetric titration: 251 Calc. for $C_6H_5N_3O_4S_2$ (247.3): C 29.2; H 2.0; N 17.0; S 25.9].

5-Dichloromethyl-2-furansufonamide (VII). Gaseous ammonia was passed into a stirred solution of III (25.0 g, 0.100 mol) in ether (100 ml) at – 30 °C, until evolution of heat had ceased. Stirring was continued for another 10 min at 0 °C. The ammonium chloride formed was filtered off and washed with ether. The combined filtrate and washings were evaporated to dryness (25 °C, 10 mmHg) and the residue crystallized from chloroform (80 ml). 18.5 g (80 %) of VII was obtained as white crystals, m.p. 88 – 90 °C. A sample was crystallized to constant melting point, 90 – 92 °C. [Found: C 25.9; H 2.3; Cl 31.0; N 6.1; S 13.9. Calc. for $C_5H_5Cl_2NO_3S$ (230.1): C 26.1; H 2.2; Cl 30.8; N 6.1; S 13.9].

5-Dichloromethyl-N,N-diethyl-2-furansulfonamide (VIII). VIII was made from diethylamine (2.92 g, 0.0400 mol) and III (5.00 g, 0.0200 mol) in the same way as VII. The resulting, oily residue (5.30 g) was distilled to give 3.61 g (63 %) of VIII as a slightly yellow oil, b.p.$_{0.3}$ 140 – 141 °C, n_D^{25} 1.5147. A colorless sample was obtained by another distillation, b.p.$_{0.05}$ 122 – 123 °C, n_D^{25} 1.5140. [Found: C 37.9; H 4.4; Cl 24.9; N 4.9; S 11.3. Calc. for $C_9H_{13}Cl_2NO_3S$ (286.2): C 37.8; H 4.6; Cl 24.8; N 4.9; S 11.2].

4-(5-Dichloromethyl-2-furansulfonyl)-1-methylpiperazine (IX) hydrochloride. 1-Methylpiperazine (4.00 g, 0.0400 mol)in dry methanol (10 ml) was added dropwise to a stirred solution of III (10.00 g, 0.0400 mol) in dry methanol (60 ml) at 0 to – 10 °C. The resulting, thick slurry of IX was heated to 40 °C, whereby a yellow solution was obtained. This was allowed to stand for 15 min without further heating. Cooling to – 20 °C gave a white precipitate of IX, which was filtered off and washed with methanol of – 15 °C (10 ml) and with ether. 10.0 g (72 %) of IX, m.p. 206 °C (decomp.), was obtained. A sample was crystallized from methanol, m.p. 206 °C (decomp.). [Found: C 34.5; H 4.3; Cl 30.3; Cl^- 10.2; N 7.8; S 9.3. Calc. for $C_{10}H_{15}Cl_3N_2O_3S$ (349.7): C 34.4; H 4.3; Cl 30.5; Cl^- 10.2; N 8.0; S 9.2].

N,N'-Bis(5-dichloromethyl-2-furansulfonyl)-ethylenediamine (X). A solution of ethylenediamine (0.900 g, 0.0150 mol) and triethylamine (3.03 g, 0.0300 mol) in dry methanol (10 ml) was added dropwise to a stirred solution of III (7.50 g, 0.0300 mol) in dry methanol (25 ml) at – 10 °C. The resulting, yellow solution was kept for 15 min without cooling. Dropwise addition of water (75 ml) with stirring gave a yellow crystalline precipitate of X, which was filtered off and washed twice with water. 6.82 g (94 %) of X, m.p. 146 – 150 °C, was obtained. A sample was crystallized from methanol, m.p. 149 – 150 °C. [Found: C 29.9; H 2.5; Cl 29.0; N 5.5; S 13.4. Calc. for $C_{12}H_{12}Cl_4N_2O_6S$ (486.2): C 29.7; H 2.5; Cl 29.2; N 5.8; S 13.2].

2-Amino-3-(5-dichloromethyl-2-furansulfonyloxy)pyridine (XI). III (15.0 g, 0.0600 mol) in pyridine (15 ml) was added dropwise to a stirred solution of 2-amino-3-pyridinol (5.64 g, 0.0600 mol) in pyridine (60 ml) at 25 – 30 °C. The resulting, brown solution was left standing for 10 min at room temperature, then water (300 ml) was added slowly to give a precipitate of XI. Filtration and washing with water gave 14.7 g (76 %) of XI as yellow crystals, m.p. 107 – 109 °C. A sample was crystallized with carbon black from ethanol-water (1:2) to give white crystals, m.p. 107 – 109 °C. [Found: C 37.2; H 2.7; Cl 22.1; N 8.6; S 10.0. Calc. for $C_{10}H_8Cl_2N_2O_4S$ (323.2): C 37.2; H 2.5; Cl 22.0; N 8.7; S 9.9].

5-Chloromethyl-2-furansulfonylchloride (XII). Finely ground IV (100 g, 0.500 mol) was suspended in phosphorus oxychloride (230 g, 1.50 mol) and methylene chloride (250 ml). Pyridine (59.3 g, 0.750 mol) was added dropwise with stirring over a period of about 60 min. The temperature rose to that of the boiling point of the mixture (49 °C) and was kept here for another 30 min by external heating. The reaction mixture was cooled to 20 °C and poured slowly into water (500 ml) with stirring. The organic phase was separated, dried over sodium sulfate, treated with carbon black (5.0 g), and evaporated from a water bath (60 °C, 10 mmHg). The residual black liquid was taken up in boiling methylcyclohexane and the solution treated twice with carbon black. Toluene (65 ml) was added, and the solution was cooled to 0°C, seeded, and left standing for 2 h at – 10 °C. Filtration and washing with petroleum ether gave 74.0 g (69 %) of XII, m.p. 38 – 40 °C, yellow crystals turning grey overnight, but otherwise stable. [Found: C 28.0; H 1.9; Cl 32.7; S 14.9. Calc. for $C_5H_4Cl_2O_3S$ (215.1): C 27.9; H 1.9; Cl 32.9; S 14.9]. Another 12.0 g (11 %) may be obtained by distillation of the mother liquor (b.p. $_{0.1}$ 83 °C).

5-Aminomethyl-2-furansulfonic acid (XIII). Crude V (20.6 g) was suspended in water (20 ml) and the pH of the suspension brought to 3 by addition of concentrated hydrochloric acid (about 8 g). The suspension was heated to 100 °C and slowly cooled to 0 °C. Filtration, washing with water of 0 °C (5 + 3 ml) and ethanol, and drying at 100 °C gave 10.9 g (62 %) of XIII as white, slightly hygroscopic crystals. A sample was crystallized from water and dried (100 °C, 0.1 mmHg). [Found: C 33.6; H 4.2; N 7.2; S 17.8; equivalent weight by electrometric titration: 178. Calc. for $C_5H_7NO_3S$

(177.2): C 34.9; H 4.0; N 7.9; S 18.1. Calc. for $C_5H_7NO_4S + 3.7$ % H_2O: C 33.6; H 4.3].

5-Acetamidomethyl-2-furansulfonic acid sodium salt (XIV). V (1.99 g, 0.0100 mol) and acetic anhydride (10 ml) were mixed and heated in a water bath (100 °C) for 20 min. The resulting resin was dissolved in 99 % ethanol (15 ml) and the solution filtered hot. Evaporation of the filtrate to dryness from a water bath (100 °C, 10 mmHg) followed by 3 crystallizations from 99 % ethanol (each from 10 ml), and drying (50 °C, 0.1 mmHg) gave 1.20 g (50 %) of XIV as white, strongly hygroscopic crystals. [Found: C 31.0; H 3.9; N 4.9; S 11.4; sulfated ash 27.1. Calc. for $C_7H_8NNaO_5S$ (241.2): C 34.9; H 3.3 N 5.8; S 13.3; ash if Na_2SO_4 29.4. Calc. for $C_7H_8NNaO_5S + 10.6$ % H_2O: C 31.2; H 4.1].

5-Formyl-2-furansulfonamide semicarbazone (XV). VII (6.90 g, 0.0300 mol), semicarbazide hydrochloride (3.35 g, 0.0300 mol) and sodium acetate (7.38 g, 0.0900 mol) were stirred with water (50 ml) at 35 °C for 20 min. Suspended VII was slowly dissolved, and a new precipitate of XV was formed. Cooling to 0 °C, filtration, and washing with water, ethanol, and ether gave 4.60 g (66 %) of XV as slightly yellow crystals, m.p. 233 °C (decomp.). Crystallization from water (150 ml) with carbon black gave 2.91 g of white crystals, m.p. 235 °C (decomp.). [Found: C 30.8; H 3.5; N 23.7; S 13.9. Calc. for $C_6H_8N_4O_4S$ (232.2): C 31.0; H 3.5; N 24.1; S 13.8].

5-Formyl-2-furansulfonamide thiosemicarbazone (XVI). VII (69.0 g, 0.300 mol), thiosemicarbazide (27.3 g, 0.300 mol), and sodium acetate (49.2 g, 0.600 mol) were stirred with water (2 liter) at 50 °C for 3.5 h. Cooling to 20 °C and filtration gave a semisolid precipitate, which was crystallized from water (4 l) with carbon black. 14.2 g (19 %) of XVI was hereby obtained as yellow crystals, m.p. about 240 °C. A sample was recrystallized, m.p. 242 °C (decomp.). [Found: C 28.9; H 3.3; N 22.2; S 25.6. Calc. for $C_6H_8N_4O_3S_2$ (248.3): C 29.0; H 3.3; N 22.6; S 25.8].

4-(5-Formyl-2-furansulfonyl)-1-methylpiperazine semicarbazone hydrochloride (XVII). IX (0.525 g, 0.00150 mol) and semicarbazide hydrochloride (0.167 g, 0.00150 mol) were dissolved in water (5 ml). The solution was left standing (1 h) at room temperature and then evaporated to dryness from a water bath (100 °C, 10 mmHg). The white, crystalline residue was crystallized from methanol (10.0 ml, cooling to −15 °C); yield 0.210 g of XVII (40 %), m.p. 240 °C (decomp.). [Found: C 37.3; H 5.3; Cl^- 10.2; N 19.6; S 9.1. Calc. for $C_{11}H_{18}ClN_5O_4S$ (351.8): C 37.6; H 5.2; Cl^- 10.1; N 19.9; S 9.1].

5-Chloromethyl-2-furansulfonamide (XVIII). Gaseous ammonia (17.0 g, 1.00 mol) was passed into a stirred solution of XII (97.0 g, 0.450 mol) in ether (600 ml) at 10 °C over a period of about 100 min. Stirring was continued for another 60 min at 20 °C. The ammonium chloride formed was filtered off. The filtrate was evaporated to dryness (60 °C, 10 mmHg) and the residue crystallized from chloroform. 70.0 g (80 %) of XVIII was obtained as white crystals, m.p. 90 – 92 °C. A sample was recrystallized, m.p. 90 – 92 °C. [Found: C 30.5; H 3.1; Cl 18.5; N 7.0; S 16.3. Calc. for $C_5H_6ClNO_3S$ (195.6): C 30.7; H 3.1; Cl 18.1; N 7.2; S 16.4].

5-Chloromethyl-N,N-dimethyl-2-furansulfonamide (XIX). Dimethylamine (36.0 g, 0.800 mol) in ether (400 ml) was added to a stirred solution of XII (86.0 g, 0.400 mol) in ether (400 ml) at 20 °C over a period of about 30 min. The resulting suspension was stirred for another 60 min and filtered. The filtrate was extracted with 0.5 % hydrochloric acid (100 ml), dried over sodium sulfate, and evaporated to dryness (60 °C, 10 mm). Crystallization of the residual oil from carbon tetrachloride (150 ml) gave 80.0 g (90 %) of XIX as white crystals, m.p. 55 – 57 °C. A sample was recrystallized, m.p. 56 – 57 °C. [Found: C 37.4; H 4.5; Cl 16.1; N 6.1; S 14.6. Calc. for $C_7H_{10}ClNO_3S$ (223.7): C 37.6; H 4.5; Cl 15.9; N 6.3; S 14.3].

5-Chloromethyl-N,N-diethyl-2-furansulfonamide (XX). XX was made from diethylamine (16.4 g, 0.225 mol) and XII (24.0 g, 0.112 mol) in the same way as XIX. The crude product was distilled twice, whereby 16.2 g (58 %) of XX was obtained as a slightly yellow oil, b.p.$_{0.1}$ 120 – 123 °C, n_D^{25} 1.5080. [Found: C 43.0; H 5.7; Cl 14.3; N 5.5; S 12.6. Calc. for $C_9H_{14}ClNO_3S$ (251.7): C 43.0; H 5.6; Cl 14.1; N 5.6; S 12.7].

5-Aminomethyl-2-furansulfonamide hydrochloride (XXI). Sodium azide (3.25 g, 0.0500 mol) was added to a stirred solution of XVIII (9.78 g, 0.0500 mol) in dimethylformamide (100 ml) at 20 °C. Stirring was continued for 2 h and the sodium chloride formed filtered off. 5 % palladium on carbon catalyst (1.00 g) was suspended in the filtrate, and a solution of hydrazine hydrate (1.37 g, 0.0275 mol) in dimethylformamide (10 ml) was added dropwise with stirring at 20 °C over a period of 100 min. When the evolution of nitrogen had ceased [yield 1800 ml (100 %)], the catalyst was filtered off and the filtrate evaporated from a water bath (90 °C, 10 mmHg). The residue was taken up in water (10 ml), and the solution was adjusted to pH 1 with 3 N hydrochloric acid (17 ml). Removal of a small amount of impurity by filtration, evaporation of the solvent, and crystallization of the residue from ethanol (75 ml) and ether (50 ml) gave 8.20 g (77 %) of XXI as greyish crystals, m.p. 170 – 172 °C. A sample was recrystallized, m.p. 173 – 174 °C. [Found: C 28.2; H 4.3; Cl 16.6; N 13.1; S 15.0. Calc. for $C_5H_9ClN_2O_3S$ (212.7): C 28.4; H 4.2; Cl 16.6; N 3.1; S 15.0].

5-Dimethylaminomethyl-2-furansulfonamide hydrochloride (XXII). XVIII (9.80 g, 0.0500 mol) was dissolved in ether (50 ml) and the solution added to a stirred solution of dimethylamine (6.80 g, 0.150 mol) in ether (50 ml) at

0 – 10 °C over a period of about 90 min. Stirring was continued for another 15 min. Water (10 ml) was added to the resulting, thick suspension. The ethereal phase was separated and the aqueous phase extracted with chloroform (six 100 ml portions). The combined ether and chloroform phases were evaporated to dryness and the residue dissolved in 3 N hydrochloric acid (15 ml). Evaporation to dryness and crystallization of the residue from ethanol (100 ml) gave 6.20 g (51 %) of XXII as greyish crystals, m.p. 190 – 193 °C. A sample was recrystallized, m.p. 192 – 194 °C. [Found: C 34.8; H 5.5; Cl 14.9; N 11.4; S 13.4. Calc. for $C_7H_{13}ClN_2O_3S$ (240.7): C 34.9; H 5.4; Cl 14.7; N 11.6; S 13.3]. From the above aqueous phase 0.50 g (5 %) of dimethyl di(5-sulfamoylfurfuryl)ammonium chloride precipitated by scratching, m.p. 209 – 211 °C. [Found: C 36.1; H 4.7; Cl 8.9; N 10.4; S 15.9. Calc. for $C_{12}H_{18}ClN_3O_6S_2$ (399.9): C 36.1; H 4.5; Cl 8.9; N 10.5; S 16.0].

5-Aminomethyl-2-furansulfonic acid dimethylamide hydrochloride (XXIII). XXIII was prepared from XIX (22.4 g, 0.100 mol) in the same way as XXI. The crude compound was dissolved in ethanol (100 ml) containing hydrogen chloride (3.65 g, 0.100 mol), and a solid impurity was removed by filtration. Addition of ether (200 ml) and filtration gave 17.0 g (71 %) of XXIII, m.p. 145 – 146 °C. Another crystallization gave white, strongly hygroscopic crystals, m.p. 146 – 148 °C. [Found: C 34.7; H 5.9; Cl 14.5; N 11.2; S 13.4. Calc. for $C_7H_{13}ClN_2O_3S$ (240.7): C 34.9; H 5.4; Cl 14.7; N 11.6; S 13.3].

5-Dimethylaminomethyl-2-furansulfonic acid dimethylamide hydrochloride (XXIV). Dimethylamine (6.80 g, 0.150 mol) and XIX (11.20 g, 0.0500 mol) in ether (100 ml) were left standing at room temperature for 4 days. Water (15 ml) was added in order to dissolve the precipitate of dimethylamine hydrochloride formed. The ethereal phase was separated, washed with water (three 10 ml portions), and evaporated to dryness. The remaining oil was dissolved in 3 N hydrochloric acid (20 ml) and the solution extracted with ether (50 ml). Evaporation of the aqueous phase to dryness and crystallization of the residue from ethanol (50 ml) gave 10.0 g (74 %) of XXIV, m.p. 174 – 176 °C. A sample was recrystallized, m.p. 174 – 176 °. [Found: C 40.3; H 6.4; Cl 13.4; N 10.2; S 12.0. Calc. for $C_9H_{17}ClN_2O_3S$ (268.8): C 40.2; H 6.4; Cl 13.2; N 10.4; S 11.9].

5-Hydroxymethyl-2-furansulfonic acid dimethylamide (XXV). XIX (22.4 g, 0.100 mol) and potassium acetate (9.80 g, 0.100 mol) were heated under reflux in ethanol (100 ml) for 20 h. The potassium chloride formed was filtered off, and the filtrate was heated under reflux with water (50 ml) and sodium hydrogen carbonate (8.40 g, 0.100 mol), until the evolution of carbon dioxide ceased (4 h). Evaporation of the reaction mixture, addition of water (20 ml), extraction with chloroform (two 50 ml portions), and distillation of the chloroform solution gave 16.0 g (78 %) of XXV as a yellow oil, b.p.$_{0.05}$ 147 – 150 °C, n_D^{25} 1.5105. [Found: C 41.0; H 5.5; N 6.8; S 13.7. Calc. for $C_7H_{11}NO_4S$ (205.2): C 41.0; H 5.4; N 6.8; S 15.6].

5-Diethylaminomethyl-2-furansulfonic acid diethylamide (XXVI). XX (3.78 g, 0.0150 mol) was dissolved in diethylamine (15 ml) and the solution left standing at room temperature for 18 h. The diethylamine hydrochloride formed was filtered off and the filtrate evaporated to dryness. The residue was taken up in ether, and the solution was filtered in order to remove a small amount of solid material. Distillation of the filtrate gave 2.70 g (62 %) of XXVI as a yellow oil, b.p.$_{0.1}$ 123 – 124 °C, n_D^{25} 1.4906. [Found: C 54.4; H 8.2; N 9.3; S 11.1. Calc. for $C_{13}H_{24}N_2O_3S$ (288.4): C 54.2; H 8.3; N 9.7; S 11.1].

1-(5-Sulfamoylfurfuryl)-3-methylurea (XXVII). To a stirred suspension of XXI (21.3 g, 0.100 mol) and methyl isocyanate (5.70 g, 0.100 mol) in chloroform (200 ml) was added triethylamine (11.0 g, 0.110 mol) in chloroform (100 ml) over a period of 10 min. Stirring was continued for another 60 min and the precipitate formed filtered off and dried. Triethylamine hydrochloride was removed from the precipitate by trituration with water (50 ml) and filtration. The filter cake was dissolved in 1 N sodium hydroxide and the solution treated twice with carbon black. Addition of 3 N hydrochloric acid (30 ml) and filtration gave 17.0 g (73 %) of XXVII as white crystals, m.p. 168 – 169 °C. A sample was crystallized from nitromethane, m.p. 168 – 169 °C. [Found: C 36.2; H 4.8; N 17.8; S 13.7. Calc. for $C_7H_{11}N_3O_4S$ (233.3): C 36.0; H 4.7; N 18.0; S 13.7].

1-(5-Dimethylsulfamoylfurfuryl)-3-methylurea (XXVIII). XXIII (14.0 g, 0.0685 mol) was dissolved in acetone (140 ml). Methyl isocyanate (3.90 g, 0.0685 mol) was added and the solution left standing at room temperature for 1 h. Filtration gave 16.5 g (92 %) of XXVIII as white crystals, m.p. 163 °C. A sample was crystallized from ethanol, m.p. 163 °C. [Found: C 41.5; H 5.8; N 15.9; S 12.3. Calc. for $C_9H_{15}N_3O_4S$ (262.3): C 41.4; H 5.8; N 16.1; S 12.3].

Methylcarbamic acid 5-(dimethylsulfamoyl)-furfuryl ester (XXIX). About 2 mg of potassium hydroxide was crushed with a spatula in XXV (10.3 g, 0.0500 mol). Methyl isocyanate (2.85 g, 0.0500 mol) was added at 20 °C with stirring and cooling. Heating of the mixture to 45 °C caused the reaction to start as indicated by an increase in temperature. The temperature was kept at 65 °C, at first by external cooling, for 15 min. Crystallization of the reaction product from toluene (50 ml) with carbon black gave 10.5 g (80 %) of XXIX, m.p. 50 – 51 °C. A sample was recrystallized from ether, m.p. 52 – 53 °C. [Found: C 41.1; H 5.4; N 10.5; S 12.3; Calc. for $C_9H_{14}N_2O_5S$ (262.3): C 41.1; H 5.4; N 10.7; S 12.2].

5-Diethylaminomethyl-2-furansulfonic acid di-

ethylamide hydrochloride (XXX). The compound was made from XXVI in the usual way and crystallized from ethanol. Yield 46 % of XXX, m.p. 148 °C. [Found: C 48.0; H 7.7; Cl 10.9; N 8.5; S 10.0. Calc. for $C_{13}H_{25}ClN_2O_3S$ (324.9): C 48.1; H 7.8; Cl 10.9; N 8.6; S 9.9].

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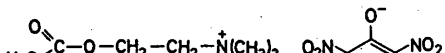
The Crystal Structure of Succinylcholine Picrate

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Succinylcholine picrate, $C_{26}H_{34}N_8O_{18}$ crystallizes in space group $P\bar{1}$ with a unit cell having dimensions $a = 11.147(7)$, $b = 7.091(8)$, $c = 11.332(8)$ Å, $\alpha = 101.59°(5)$, $\beta = 109.08°(5)$, and $\gamma = 94.68°(13)$. $Z = 1$. The structure was determined from a sharpened origin removed three-dimensional Patterson synthesis and refined by full matrix least squares technique to an R index of 0.063, using 1870 diffractometer-collected single crystal X-ray diffraction intensity data. The torsion angle $O-C-C-N^+$ is $\pm 87°$. A correlation of the three known crystal structures of succinylcholine salts provides a picture of the readiness with which changes from the preferred conformation take place in different parts of the succinylcholine ion.

The crystal structure of succinylcholine picrate has been determined as a part of an investigation [1-3] of the influence of different surroundings on the conformation of the succinylcholine ion.



EXPERIMENTAL

Succinylcholine picrate, $C_{26}H_{34}N_8O_{18}$, was prepared by mixing saturated aqueous solutions of succinylcholine chloride and of picric acid, and single crystals were grown by slow evaporation of an aqueous solution of the compound. M.p. 158.5 – 159 °C. The crystals are yellow, triclinic needles elongated in the b-direction. The unit cell parameters, obtained from precession photographs (Mo$K\alpha$ radiation, $\lambda = 0.7107$ Å) and refined by the least squares method using the program CELSIUS,[4] are: $a = 11.147(7)$, $b = 7.091(8)$, $c = 11.332(8)$ Å, $\alpha = 101.59°(5)$, $\beta = 109.08°(5)$, and $\gamma = 94.68°(13)$. The space group is $P\bar{1}$. The calculated density for one formula unit per unit cell is 1.515 g cm^{-3} and the density measured by flotation in a mixture of tetrachloromethane and chloroform is 1.51 g cm^{-3}. The linear absorption coefficient, μ(Mo$K\alpha$), is 1.4 cm^{-1}.

All X-ray data were measured from a crystal with the dimensions $0.35 \times 0.4 \times 0.5$ mm with quartz monochromatized Mo$K\alpha$-radiation using a NONIUS 3-circle automatic diffractometer. The ω-scanning mode was used with a scan speed of 0.6°/min, each reflection being scanned over a range of 1.4°. Of the about 4000 independent reflections within the range of $2.5° \leq \theta \leq 28.0°$, 1870 reflections with intensities greater than 2.5 standard deviations were considered as observed. The diffraction data were reduced to structure amplitudes by the use of an ALGOL-program OUTDIFF-5, written by A. M. Sørensen of this laboratory. No absorption corrections were applied.

DETERMINATION AND REFINEMENT OF THE STRUCTURE

An attempt to solve the structure applying symbolic addition procedures, using the Σ_2 relationship [5] was made, but without success. A program system written for centrosymmetric structures by Dewar and Stone [6] was used. The signs of 328 reflections were defined in terms of 7 symbols, 3 of which were origo defining. After the structure had been solved it became clear, that the correct combination of signs had a very low internal consistency.

The sharpened three-dimensional origin removed Patterson synthesis gave the orientation of the benzene ring of the picrate ion. Two picrate ions related by a center of symmetry give rise to a set of eight vectors equal to the imaginary vector between the centers of the two rings, and further 6 sets each consisting of eight practically equal vectors exist between the picrate ions. The Patterson synthesis was scan-

Table 1. Observed structure amplitudes and calculated structure factors for succinylcholine picrate. The column headings are the indices l, $|F_o|(\times 10)$, and $|F_c|(\times 10)$.

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Table 1. Continued.

-5 112 108	1 44 47	-6 50 46	4 284 288	2 119 102	-6,-6,L	-6 51 47	-4,-7,L
-6 113 107	3 66 62	-8 37 58	3 56 61	1 102 86	7 66 64		1 45 46
-8 65 73		-9 62 70	2 53 39	-1 196 185	6 173 165	5,-6,L	2 34 26
-9 74 79	9,-4,L		1 59 63	-2 177 168	5 46 51	-7 53 50	5 37 30
	4 74 72	0,-4,L	0 120 126	-5 114 103	1 85 87	-6 35 43	5 65 59
3,-3,L	1 45 42	-6 52 57	-1 134 132	-6 71 68	-1 40 41	-4 83 74	8 55 56
-11 58 54	0 53 43	-5 73 71				-3 38 39	
-10 69 69	-1 71 65	-4 246 243	-8,-4,L	1,-5,L	-5,-6,L	-2 40 34	-5,-7,L
-9 96 99	-3 81 66	-3 121 119	-2 45 47	-6 153 157	0 60 61	-1 99 103	8 82 88
-7 201 206	-4 58 53	-2 183 186	-1 100 113	-5 52 58	1 69 72	0 35 27	7 71 71
-6 30 20	-5 85 82	-1 185 166	0 54 54	-4 43 45	2 43 45	1 101 117	5 35 49
-5 49 55	-7 101 99	0 151 155	3 105 106	-3 43 36	3 166 176	2 44 53	1 63 39
-3 175 161		1 101 97	4 62 55	-2 64 55	4 50 50	4 91 104	
-2 212 211	8,-4,L	2 102 119	5 174 172	-1 162 157	5 107 110	5 32 29	-9,-9,L
-1 42 50	-8 43 41	3 176 171	6 153 153	0 39 38	6 85 88	7 39 44	4 74 68
0 38 51	-6 108 121	4 334 330	7 86 84	1 158 169	7 41 37		
1 108 108	-5 177 179	5 65 57		2 85 102		6,-6,L	-2,-8,L
2 161 179	-3 34 27	6 155 150	-9,-4,L	3 108 115	-4,-6,L	7 47 54	5 42 31
3 317 313	-2 79 77	7 38 30	5 86 94	4 199 201	10 37 32	6 59 59	4 44 27
4 27 29	-1 217 213	8 33 43	4 57 51	5 49 51	9 51 47	5 67 79	2 51 74
5 87 91	0 169 161	9 144 138	3 114 111	8 129 133	8 100 99	2 36 47	
6 70 70	1 59 53	10 127 133	2 41 37	9 73 71	5 36 27	1 52 60	-1,-8,L
7 59 50		11 44 48	0 55 47		4 39 40	-1 108 114	0 77 78
8 127 124	7,-4,L		-1 55 57	2,-5,L	3 80 80	-2 99 100	3 49 44
9 102 107	6 50 40	-1,-4,L		10 105 100	2 62 51	-6 137 138	4 96 92
	5 59 53	11 81 77	-10,-4,L	9 115 113	-1 33 34		5 64 76
4,-3,L	4 53 50	9 51 45	3 44 52	7 116 123	-2 41 44	7,-6,L	
8 126 124	0 79 84	7 165 162	5 86 92	6 65 67	-3 37 41	-5 52 60	0,-8,L
7 111 103	-1 156 153	6 128 127	6 36 29	4 51 59		-3 87 88	5 68 75
6 167 164	-2 53 58	5 153 141		2 73 73	3,-6,L	-2 39 42	4 47 47
5 56 58	-3 39 32	3 150 141	-11,-4,L	0 67 67	-3 56 63	-1 38 21	0 57 43
4 89 90	-4 34 28	2 219 189	5 41 29	-1 68 37	-2 41 55	0 38 49	
2 57 69	-5 62 61	1 100 96		-2 185 181	0 64 56	1 83 88	1,-8,L
1 86 76	-6 73 74	0 314 283	-9,-5,L	-3 59 56	2 39 31	2 49 51	-1 165 180
0 217 231		-1 149 155	2 79 85	-5 112 119	4 118 123		1 36 37
-1 155 170	5,-4,L	-2 28 7	5 39 39	-6 61 64	6 65 62	8,-6,L	3 74 75
-2 100 107	-10 49 45	-3 39 40		-7 38 40	7 49 35	2 59 51	6 36 41
-3 269 272	-9 65 65	-4 100 106	-8,-5,L		8 74 68	1 40 44	
-4 107 107	-8 56 52	-5 280 285	10 47 49	3,-5,L	9 64 63		2,-8,L
-7 43 45	-5 57 68	-6 78 67	2 69 80	-7 96 99	10 45 56	9,-6,L	5 42 51
-9 41 42	-5 136 128	-7 64 67	1 63 64	-3 36 58		0 44 44	3 35 55
	-3 73 73			-2 50 38	2,-6,L		1 51 47
5,-3,L	-1 36 38	-7,-5,L	-7,-5,L	-1 135 141	8 61 56	8,-6,L	0 63 74
-11 51 56	0 195 193	-2,-4,L	4 45 56	1 36 27	5 112 109	-3 59 53	
-9 147 147	1 136 132	-6 55 47	5 53 48	2 79 113	4 48 33	-4 75 66	4,-8,L
-8 100 95	2 92 90	-5 47 50	6 94 89	3 75 76	3 160 143	-5 44 47	1 73 88
-7 49 42	3 104 95	-4 198 199	7 65 68	4 146 156	2 48 46		
-6 135 135	4 45 40	-3 201 211	4 158 164	5 59 67	1 43 53	9,-6,L	5,-8,L
-4 35 24	6 120 117	-2 125 123	11 102 97	7 53 45	0 78 80	-4 121 122	1 41 57
-2 115 101		-1 39 26		8 54 54	9 46 40	-3 63 55	
-1 235 237	5,-4,L	0 83 74	-6,-5,L	9 63 62	10 101 95	-2 46 51	
1 161 163	6 117 121	1 352 326	9 53 59		11 42 43	-1 48 45	
2 173 176	5 148 148	2 35 41	8 74 78	4,-5,L		1 69 74	
5 47 59	3 35 27	3 125 114	7 77 79	7 41 35	1,-6,L		
6 96 86	2 133 139	4 227 208	6 126 138	6 66 61	1 39 39	7,-7,L	
7 85 93	1 45 41	5 204 197	5 176 181	5 100 112	-1 56 59	1 39 39	
8 50 46	0 52 57	6 35 17	4 158 164	4 137 146	-2 34 37	-1 56 59	
10 92 89	-2 132 115	9 50 56	3 72 85	3 49 57		-2 34 37	
	-3 103 132	10 50 46	-1 71 80	1 108 125	-3 112 114		
6,-3,L	-4 81 82	11 87 85		-1 39 49	-2 231 216	6,-7,L	
5 76 79	-6 69 64		-5,-5,L	-2 83 94	-1 98 102	-3 41 44	
4 66 63	-7 107 111	-3,-4,L	-2 83 94	-5 75 77	0 94 85	-1 48 40	
2 82 70	-8 45 55	11 141 139	0 43 41	-6 75 77	1 62 64	2 30 43	
1 100 92		10 44 52	2 28 20	-7 57 55	2 165 146		
0 60 59	4,-4,L	8 43 40	3 93 99	-8 41 45	8 111 108	5,-7,L	
-1 118 122	-8 102 108	7 287 284	5 91 91		9 46 40	5 39 43	
-2 199 198	-7 75 81	5 63 71	8 105 111	5,-5,L	10 101 95	-1 40 42	
-4 151 153	-4 51 53	4 65 56		-8 41 47	11 42 43	-4 73 79	
-6 177 179	-3 155 140	3 98 101	-4,-5,L	-7 40 39			
-7 183 184	-2 95 133	2 138 112	12 55 49	-4 66 69	0,-6,L	4,-7,L	
-8 91 91	-1 103 118	0 85 86	10 87 92	-2 36 40	8 89 90	-2 42 47	
	1 48 39	-1 136 136	8 182 179	0 32 22	7 112 107	0 119 125	
7,-3,L	3 245 259	-2 137 146	7 93 101	1 233 253	5 31 28	2 59 58	
-9 63 53	4 111 110	-4 183 188	6 34 46	2 90 102	4 62 78	4 39 47	
-7 216 218	5 88 83	-5 36 37	5 140 137	3 60 58	3 113 113	6 79 82	
-6 62 62	6 32 27	-6 68 68	2 158 159	7 46 57	2 96 86	7 89 106	
-4 81 83	7 46 35		0 59 54		1 81 86		
-1 84 84	10 61 61	-4,-4,L	-1 61 46	6,-5,L	-1 53 52	3,-7,L	
0 132 125		-6 86 97	-3 39 42	6 46 40	-2 158 160	7 47 55	
1 64 44	3,-4,L	-5 44 67		5 102 103	-3 152 146	2 69 120	
2 44 46	9 72 65	-2 32 31	-3,-5,L	1 76 81	-4 75 62	-1 70 75	
3 53 41	8 59 51	-1 36 29	-5 61 61	0 128 128		-3 44 42	
	7 56 63	0 75 75	-3 195 192	-2 39 35	1,-6,L	-5 75 69	
8,-3,L	5 43 44	2 189 186	-2 38 47	-3 35 34	-6 54 45		
6 69 50	3 222 226	3 215 215	1 176 184	-4 38 41	-5 130 129	2,-7,L	
4 165 159	2 342 371	4 180 176	-5 75 76	-5 80 76	-1 85 84	3 41 56	
3 52 46	1 115 139	5 124 118	4 55 54	-6 40 46	1 83 83		
1 58 57	0 77 89	6 135 122	6 32 30	-8 53 44	2 47 54	1,-7,L	
-1 131 125	-1 257 254	7 47 31	7 48 42		4 48 45	8 60 56	
-3 51 51	-2 307 284	8 50 37	9 36 37	7,-5,L	5 125 131	7 57 58	
-5 58 52	-3 43 24	10 31 13	10 35 25	-6 43 30	6 82 91	4 52 58	
-6 89 87	-4 90 97	12 73 68	12 45 41	-5 248 243	7 43 44	3 75 76	
-7 108 107	-5 33 18			-4 98 98	9 47 57	2 44 38	
	-7 122 125	-5,-4,L	-2,-5,L	-2 115 108		-1 80 83	
9,-3,L	-8 81 81	12 55 52	12 79 71	0 135 135	2,-6,L	-2 40 34	
-9 72 69		11 54 59	10 71 68	3 81 80	9 45 47	-3 105 94	
-8 62 50	2,-4,L	10 60 58	9 64 59	4 35 41	8 36 31		
-6 69 54	-8 122 129	9 132 141	7 85 75	5 45 31	7 58 58	0,-7,L	
-4 62 58	-7 153 147	8 71 73	6 122 120		6 51 49	-3 73 70	
-2 93 85	-6 137 140	7 63 63	5 30 29	8,-5,L	5 215 232	-2 38 35	
-1 98 98	-5 42 38	6 116 111	3 46 47	2 50 43	4 47 49	-1 42 37	
0 67 66	-4 104 97	4 66 69	2 121 118	1 110 117	2 32 44	0 103 137	
1 58 53	-3 98 92	3 53 54	1 47 54	0 36 42	1 35 59	1 43 54	
	-2 125 124	0 78 82	0 98 90	-2 94 95	0 140 164	3 69 53	
10,-3,L	-1 161 148	-1 29 25	-1 96 101	-3 69 66	-1 116 128	4 95 122	
0 128 122	1 143 150	-2 127 136	-2 87 95	-5 77 81	-6 74 82		
-1 91 93	3 317 315		-3 43 39	-6 84 70		-1,-7,L	
-3 86 75	4 305 293	-6,-4,L	-4 181 180	-7 48 43	3,-6,L	7 34 34	
-6 50 50	5 41 50	-4 39 44	-7 34 38	-8 68 57	-3 91 93	5 91 130	
-9 66 51	6 92 85	-3 64 70			-1 100 97	1 60 65	
	7 37 30	-2 60 67	-1,-5,L	9,-5,L	0 86 83	-2 115 108	
11,-3,L	8 147 138	-1 67 72	-6 70 63	-7 73 74	2 98 126	-3 42 38	
-4 74 75	9 101 94	0 61 60	-5 48 54	-6 83 74	3 50 71		
-3 55 46	10 85 80	1 69 73	-4 206 199	-4 43 46	4 102 116	-2,-7,L	
-2 81 72	11 55 52	2 97 96	-2 39 43	-3 38 45	5 193 203	-2 85 30	
0 68 56		3 69 73	-1 70 60	0 69 61	7 95 99	0 46 44	
3 50 41	1,-4,L	4 113 115	1 152 140		9 60 67	1 33 27	
	9 52 56	5 33 25	2 170 155	-8,-6,L		2 73 57	
11,-4,L	8 111 110	6 91 97	3 151 146	7 71 60	4,-6,L	5 53 54	
2 111 101	7 58 59	7 105 101	4 63 60	6 57 57	6 66 68	7 37 42	
-1 59 49	5 75 71	8 71 70	5 65 63	5 40 40	5 95 108	8 62 63	
-3 86 80	4 213 214	10 50 48	6 83 83	4 62 60	4 85 89		
	3 505 503	11 47 45	7 55 53	3 57 59	3 44 60	-3,-7,L	
10,-4,L	2 46 69	12 53 48	8 28 15		2 31 25	9 48 59	
-8 47 35	1 75 71		10 68 74	-7,-6,L	1 97 117	8 44 45	
-6 84 72	0 422 398	-7,-4,L		1 67 78	0 35 35	4 87 94	
-4 70 65	-1 47 29	11 69 74	0,-5,L	6 107 103	-1 97 96	2 39 45	
-3 63 60	-2 338 327	9 103 112	9 103 106	7 67 70	-2 99 103	-1 68 72	
2 84 71	-3 175 175	6 59 69	7 79 73		-3 78 86		
0 49 51	-5 36 37	5 63 63	6 99 84	-6,-6,L	-4 72 76		
			5 54 42	9 42 34			

Table 2. Final positional parameters and their estimated standard deviations. Parameters and e.s.d. of non-hydrogen atoms are $\times 10^4$, parameters and e.s.d. of hydrogen atoms are $\times 10^3$.

Atom	x/a	y/b	z/c	Atom	x/a	y/b	z/c
C(11)	4114(5)	0158(7)	2535(4)	C(5)	−1271(6)	4119(8)	1309(5)
C(12)	5313(4)	1482(6)	3389(4)	C(6)	−2531(5)	4772(7)	0732(5)
C(13)	5514(4)	2480(6)	4609(4)	N(7)	−2435(4)	6862(5)	0607(3)
C(14)	4519(5)	2427(6)	5083(4)	C(8)	−1731(5)	8258(8)	1889(5)
C(15)	3333(5)	1245(7)	4368(5)	C(9)	−3797(5)	7274(9)	0109(6)
C(16)	3163(4)	0183(7)	3153(4)	C(10)	−1778(6)	7142(8)	−0327(5)
O(11)	3988(4)	−0879(5)	1474(3)	H(11)	128 (6)	448 (8)	517 (6)
N(12)	6398(4)	1590(5)	2947(4)	H(12)	007 (5)	265 (9)	497 (5)
O(121)	6193(4)	1612(5)	1821(3)	H(51)	−054 (6)	417 (9)	102 (6)
O(122)	7474(4)	1666(6)	3721(4)	H(52)	−139 (6)	264 (9)	098 (6)
N(14)	4722(5)	3519(6)	6366(4)	H(61)	−299 (5)	383 (8)	−032 (5)
O(141)	3803(4)	3582(6)	6735(4)	H(62)	−301 (5)	464 (7)	129 (5)
O(142)	5815(4)	4373(6)	7034(3)	H(81)	−170 (5)	967 (9)	184 (5)
N(16)	1953(4)	−1187(6)	2484(5)	H(82)	−070 (6)	804 (8)	226 (5)
O(161)	1462(4)	−1918(6)	3141(4)	H(83)	−209 (6)	809 (8)	251 (6)
O(162)	1477(4)	−1493(7)	1324(4)	H(91)	−383 (5)	856 (8)	−018 (5)
H(13)	636 (5)	302 (7)	512 (5)	H(92)	−431 (5)	743 (8)	079 (5)
H(15)	259 (5)	136 (7)	468 (5)	H(93)	−426 (5)	628 (8)	−074 (5)
C(1)	0348(5)	4270(8)	4741(5)	H(101)	−187 (6)	845 (9)	−048 (6)
C(2)	0029(5)	3752(8)	3318(5)	H(102)	−211 (6)	595 (9)	−114 (6)
O(3)	0541(4)	2615(7)	2772(4)	H(103)	−069 (6)	698 (9)	010 (6)
O(4)	−0898(3)	4603(5)	2689(3)				

Table 3. Final thermal parameters and their estimated standard deviations $\times 10^3$. The anisotropic temperature factors are defined by the equation
T.F. $= \exp[-\frac{1}{4}(h^2a^{*2}B_{11} + k^2b^{*2}B_{22} + l^2c^{*2}B_{33} + 2hka^*b^*B_{12} + 2hla^*c^*B_{13} + 2klb^*c^*B_{23})]$

Atom	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C(11)	3.00(23)	1.94(19)	2.35(20)	0.01(17)	0.67(18)	0.55(16)
C(12)	2.84(22)	1.92(19)	2.47(20)	0.02(17)	1.08(17)	0.61(16)
C(13)	2.71(21)	1.65(19)	2.32(20)	0.08(16)	0.48(17)	0.36(15)
C(14)	3.33(23)	1.61(19)	2.04(19)	0.57(17)	0.88(17)	0.34(15)
C(15)	2.81(22)	2.59(22)	2.78(21)	0.75(18)	1.13(18)	0.97(17)
C(16)	2.40(21)	2.37(21)	2.50(21)	−0.05(17)	0.46(17)	0.68(17)
O(11)	4.94(20)	2.82(16)	2.79(16)	−1.05(14)	1.54(14)	−0.69(12)
N(12)	3.35(21)	1.60(16)	3.27(20)	−0.09(14)	1.26(17)	0.30(14)
O(121)	4.73(20)	3.46(17)	3.47(18)	−0.02(15)	2.08(15)	1.07(14)
O(122)	2.73(17)	4.82(21)	4.40(19)	0.17(14)	0.94(15)	0.74(15)
N(14)	5.30(26)	2.32(19)	2.74(20)	0.69(19)	1.92(15)	0.31(15)
O(141)	6.55(25)	4.72(21)	4.13(20)	0.64(18)	3.33(19)	−0.24(16)
O(142)	5.86(24)	3.48(18)	2.80(16)	−0.62(17)	0.91(16)	−0.66(14)
N(16)	3.04(20)	3.39(22)	3.81(23)	−0.42(16)	0.59(18)	0.63(18)
O(161)	4.38(21)	6.17(25)	5.47(22)	−2.15(18)	0.92(18)	2.35(19)
O(162)	4.16(21)	6.78(25)	3.03(19)	−1.43(18)	0.15(16)	−0.38(16)
C(1)	3.47(25)	4.11(27)	3.23(23)	1.15(20)	0.41(20)	0.52(20)
C(2)	2.95(24)	3.26(24)	3.55(25)	0.62(20)	0.42(20)	0.64(20)
O(3)	6.65(26)	7.29(28)	4.64(21)	4.41(23)	1.36(20)	0.73(19)
O(4)	3.50(16)	2.79(15)	2.76(15)	0.72(13)	0.61(13)	0.71(12)
C(5)	5.21(30)	3.16(24)	2.55(22)	1.28(22)	1.21(20)	0.40(18)
C(6)	3.85(26)	1.61(19)	2.96(22)	−0.41(18)	0.66(20)	0.28(16)
N(7)	2.89(18)	1.93(15)	2.31(15)	−0.00(13)	0.70(14)	0.46(12)
C(8)	3.96(28)	2.35(21)	2.92(22)	−0.39(19)	0.50(19)	−0.45(17)
C(9)	2.92(24)	3.80(26)	3.52(25)	0.91(21)	0.64(20)	0.52(21)
C(10)	4.72(29)	4.83(29)	3.72(25)	0.06(23)	2.15(23)	1.97(22)

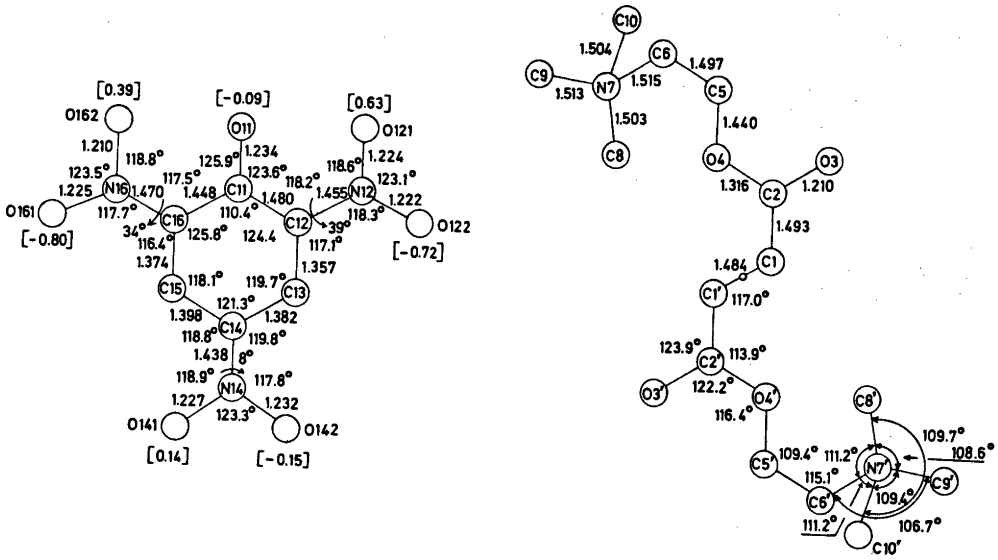


Fig. 1. Bond distances and angles. The e.s.d. on bonds are in the range 0.005—0.009 Å and on angles 0.4—0.5°. The numbers in angular parentheses are the distances in Ångström of the atoms from the least squares plane through the six carbon atoms in the ring.

ned for potential "center-center" peaks, and for each of them the fit of the additional 6 sets of vectors with the Patterson synthesis was examined. This, together with packing considerations, gave the clue to the solution. A three-dimensional electron density map calculated using the phases from the contributions of the atoms of the benzene ring and the three nitro groups (for the sake of simplicity arranged coplanar with the ring) indicated the positions of all ten non-hydrogen atoms in the succinylcholine ion and of the phenoxide oxygen atom.

The refinement was performed by the full-matrix least squares method. Positional parameters for all non-hydrogen atoms were varied together with their individual thermal parameters, initially isotropic but converted to anisotropic as the refinement neared convergence. When the R index had dropped to 0.1 a three-dimensional difference Fourier synthesis was calculated, from which the positions of all 17 hydrogen atoms in the asymmetric unit could be postulated. The positional parameters of the hydrogen atoms were added to the variables while their temperature factors were assumed to be equal to those of the atoms to which they are bonded. Some further cycles of least squares refinement were carried out, and after the last cycle the final shifts of all varied parameters were less than one third of their corresponding estimated standard deviations. All atoms were treated as uncharged, and the form factors were taken from *International Tables for X-ray Crystallography*.[7] The refinement was based on F, and the function minimized was $\sum w[|F_o| - |F_c|]^2$. Unit weight was given to each observed reflection with the exception of very weak reflections ($|F_o| < 10$), which were given weights less than 1.0. The final R value is 0.063. The observed structure amplitudes and the final calculated structures factors are given in Table 1. Nearly all calculations have been performed on the IBM 7094 computer at the NEUCC installation in Copenhagen with the use of the program systems *X-RAY 63* [8] and *WATSUP*.[9]

DESCRIPTION AND DISCUSSION OF THE STRUCTURE

The final positional and thermal parameters together with their estimated standard deviations are given in Tables 2 and 3. The interatomic distances and angles are shown in Fig. 1, in which the atomic numbering used in this paper is also given. A number of least squares planes through parts of the ions were calculated.

Table 4. Least squares planes and angles between them. The equations of the planes are in direct (unit cell) space. Distances (Å) to atoms defining the plane are asterisked.

A. $3.1826x-6.4014y+5.1107z-2.4967\text{Å}=0$
B. $-0.6196x+6.8770y+0.2238z-0.7650\text{Å}=0$
C. $-1.7944x+6.6204y-5.4084z+1.9550\text{Å}=0$
D. $7.1335x-5.7666y-1.2873z-1.7479\text{Å}=0$
E. $7.5084x+4.7930y-3.4255z-0.6837\text{Å}=0$

∠ Plane A Plane B 39°
∠ Plane A Plane C 8°
∠ Plane A Plane D 34°

Atom	A	B	C	D	E
C(11)	−0.006*				
C(12)	0.022*	−0.001*			
C(13)	−0.026*				
C(14)	0.014*		−0.002*		
C(15)	0.001*				
C(16)	−0.005*			0.003*	
O(11)	−0.088				
N(12)	−0.028	0.002*			
O(121)	0.627	−0.001*			
O(122)	−0.717	−0.001*			
N(14)	−0.007		0.006*		
O(141)	0.138		−0.002*		
O(142)	−0.150		−0.002*		
N(16)	−0.154			−0.010*	
O(161)	−0.801			0.004*	
O(162)	0.394			0.004*	
C(1)					0.000*
C(1′)					0.000*
C(2)					0.000*
C(2′)					0.000*
O(3)					−0.027
O(4)					0.073
C(5)					0.112
C(6)					0.547

The parameters of these planes, the deviations of the atoms from them and some angles between them are given in Table 4.

The dimensions of the picrate ion indicate a strong delocalisation of the negative charge of the phenoxide oxygen atom and are essentially as found by Maartmann-Moe [10] in the mutually isomorphous salts, potassium picrate and ammonium picrate, and by Thewalt and Bugg in the structure of serotonin picrate monohydrate.[11] The benzene ring is not completely planar (*cf.* Table 4) and atoms bonded directly to the ring show considerable deviations, (up to 0.15 Å), from the least squares plane through the six carbon atoms. The planes of the two nitrogroups in the *ortho*-positions to O(11) are twisted relative to the plane of the ring, the angles being 34 and 39°, respectively, and the distances O(121)−O(11) and O(162)−O(11) are 2.77 and 2.74 Å. The deviations of O(121) and O(162) from the ring plane have the same sign, while the deviation of O(11) from this plane has the opposite sign.

The bond lengths and angles of the succinylcholine ion show no deviations from the expected values. The central part of the ion, including the ten atoms C(1) to C(5) and C(1′) to C(5′), is in this crystal structure approximately planar (*cf.* Table 4). The alcohol moiety of the ester group is situated *s-cis* to the carbonyl group, an arrangement which agrees with the most commonly found ester con-

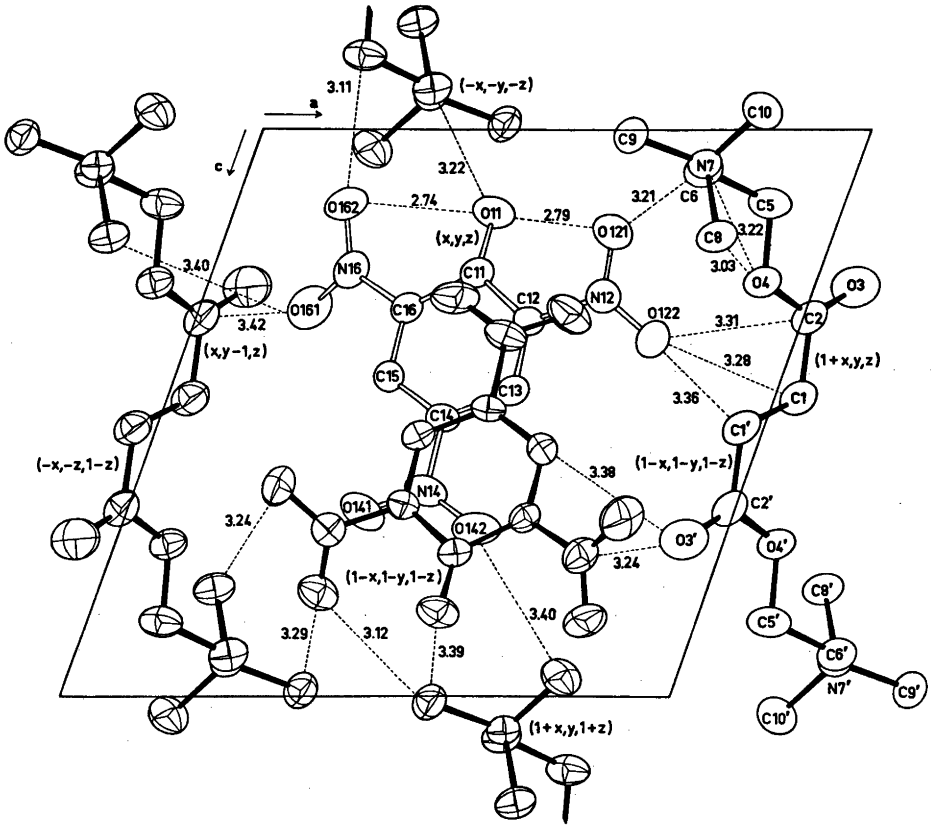


Fig. 2. Non-bonded distances less than or equal to the sum of the respective van der Waals radii of the atoms. The atoms are represented by their thermal ellipsoids scaled to enclose 50 % probability. The drawing was produced by ORTEP.[33]

formation,[12] but the β-carbon atom of the acid moiety [C(1')] is situated in a more unusual manner, namely *s-trans* to the carbonyl group. As in most of the examined solid state structures containing the atomic arrangement O–C–C–N$^+$, the system is in this structure found in an approximately *gauche* conformation, the torsion angle O(4)–C(5)–C(6)–N(7) being ±87° and the intramolecular distances N(7)–O(4) and C(8)–O(4) being 3.22 and 3.03 Å respectively. None of the hydrogen atoms bonded to C(8) are in a position favourable for a close contact to O(4). The distances H(82)–O(4) and H(83)–O(4) are 2.58 and 2.91 Å, respectively. This is in agreement with the situation found in *erythro*-α,β-dimethylacetylcholine iodide.[13] The conformation about all three N–C methyl bonds is staggered.

The structural packing on the whole is rather compact. Only few contacts (Fig. 2) between succinylcholine ions are found, whereas a number of close contacts between the picrate ions and between succinylcholine ions and picrate ions are observed. In agreement with the delocalisation of the negative charge of the phenoxide oxygen atom contacts to O(11) are not dominant and many contacts to the nitrogroups, especially the two in the *ortho*-positions to O(11), exist.

The crystal structures of three succinylcholine salts have been examined.[2-3] Some indications concerning the preferred conformation of the succinylcholine ion and the readiness with which alterations from this conformation

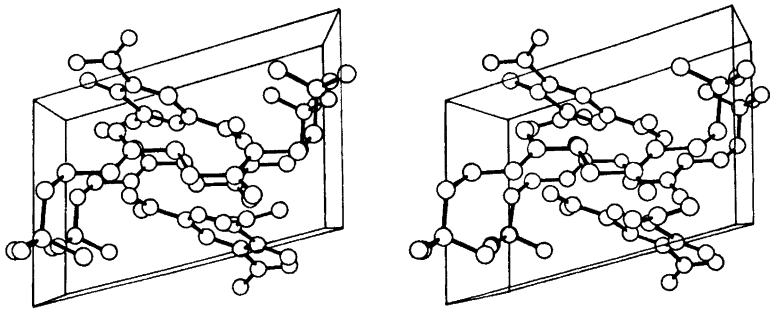


Fig. 3. Stereodiagram illustrating the packing. The *b* axis is ↑, the *c* axis is ←, and the *a* axis is directed upward. The drawing was produced by ORTEP.[33]

take place in different parts of the ion can be seen from a correlation of the three structures. The immediate impression is that the three succinylcholine ions are very dissimilar: in the not very compact succinylcholine perchlorate structure the ion has a rather extended shape, while it is more or less crumpled up in the more compact succinylcholine iodide and succinylcholine picrate structures. Nevertheless the differences are concentrated in certain parts of the chain, while other parts of it are unchanged from structure to structure (*cf.* Table 5). The two main differences are found in the torsion around C(1)–C(2), and around O(4)–C(5). More constant are the following: (I) The torsion angle C(2′)–C(1′)–C(1)–C(2), in succinylcholine perchlorate and picrate 180° owing to the symmetry and in the iodide about 170°. (II) The torsion angle C(1)–C(2)–O(4)–C(5), which deviates from 180° with less than 10° in all three structures. (III) The torsion angle O(4)–C(5)–C(6)–N(7), which is of the magnitude characteristic for the system O–C–C–N$^+$ in many solid state structures [14] (although deviations from this arrangement also have been reported.[15–17])

It can be concluded that the *gauche* conformation of the choline moiety as expected from the numerous known structures [18] is relatively stable and that alterations of the overall conformation of the succinylcholine ion preferably take place in other parts of the ion. This is in full agreement with Herdklotz and Sass,[19] who found that the conformation of the acetylcholine ions in acetylcholine chloride crystals differs from that found in acetylcholine bromide.[20] Also here the *gauche* choline conformation was a common feature, while different ester conformations were observed.

The very many crystal structures of compounds, which in some or another way intervene in the normal biological functions of acetylcholine have given good information about the preferred conformation of this sort of compounds, and it has been observed that alterations from this preferred state, which may be induced by the crystal packing, will only seldom take place in the choline moiety. These results

Table 5. The numerical value of torsional angles (°) of succinylcholine iodide, succinylcholine perchlorate, and succinylcholine picrate.

	Suc. iodide		Suc. perchlorate	Suc. picrate
C(2′)–C(1′)–C(1)–C(2)	170		180	180
C(1′)–C(1)–C(2)–O(4)	25	44	166	3
C(1)–C(2)–O(4)–C(5)	172	177	173	178
C(2)–O(4)–C(5)–C(6)	113	99	168	164
O(4)–C(5)–C(6)–N(7)	80	78	75	87

have been compared with measurements by IR [21] and NMR spectroscopy [22–24] in solution of compounds related to acetylcholine and with theoretical considerations,[25–27] and more or less detailed agreement has been found. Nevertheless we are still concerned only with preferred conformations, and in biological systems we meet the possibility that the molecules may change conformation under influence of enzymes or receptors [28–29] in a similar way as several enzymes [30–32] change conformation when acting on a substrate.

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Derivatives of Glutacondialdehyde. 2.* Ring Opening of 3-Methyl- and 3-Methoxypyridine. Preparation of 2- and 4-Substituted Glutacondialdehyde Derivatives. The Structure of the Glutacondialdehyde Bis-diacetal

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Ring opening of 3-methyl- and 3-methoxypyridinium-1-sulfonate and benzoylation of the salts obtained were found to occur *via* a specific reaction, yielding only 4-methyl- and 4-methoxy-5-hydroxy-*trans*-2,*trans*-4-pentadienal benzoate. A 1:1 mixture of the 2-methyl-5-hydroxy-*trans*-2,*trans*-4-pentadienal benzoate and the isomeric 4-methyl-benzoate was obtained by hydrolysis and benzoylation of 1,1,3,5,5-pentaethoxy-2-methylpentane. Bromination followed by benzoylation of the glutacondialdehyde sodium salt yielded the 2-bromo benzoate, which was also obtained from the glutacondialdehyde benzoate by bromination.

All double bonds in these derivatives were found to have the *trans* configuration, as well as the double bond in the bisdiacetal of glutacondialdehyde. This has been confirmed by microwave broad band spectroscopy in the vapour phase.

The preparation of 1,1,3,5,5-pentamethoxypentane directly from the sodium salt of glutacondialdehyde is reported.

In a previously paper [1] we described the final structure determination, mainly by ^{1}H NMR, of the sodium salt of glutacondialdehyde (IVa). Glutacondialdehyde in solution,[2] $\tau_{1/2}$ in ether < 10 min at room temperature, polymerises easily.

In the course of that work, we found the potentially quite useful ring opening of pyridines to glutacondialdehyde had never been subjected to much scrutiny,[3] except in the case of pyridine itself.[4] Furthermore, simple substituted glutacondialdehydes are not well examined, and since such dialdehydes offer many synthetic applications, we decided to examine further ring openings of substituted pyridines as well as the chemistry of the corresponding glutacondialdehydes.

RESULTS

Our first approach was to examine the ring opening reaction of a series of substituted pyridines, namely 3-methyl-, 4-methyl-, 3,5-dimethyl-, 4-*tert*-butyl-, and 3-methoxypyridine, under the reaction conditions where the two step reaction led to a high yield of ring opening product from pyridine itself.[4]

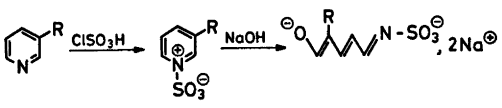
4-Methylpyridine yielded only tarry materials. 3,5-Dimethyl- and 4-*tert*-butylpyridine reverted to the parent amine, while 3-methyl- and 3-methoxypyridine nicely ring opened, to give the unstable yellow disodium salts IIIb and IIId, respectively, in excellent yield. However, attempts to transform compounds IIIb and IIId into the sodium salts of the corresponding glutacondialdehydes failed.*

Benzoylation of compounds IIIb and IIId led to the formation of the mono-benzoylated derivatives, VIb and VId, respectively. VIb in

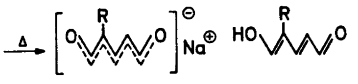
* Part 1, see Ref. 1.

** Section on microwave spectra only.

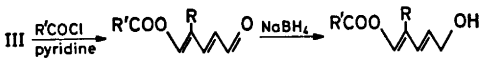
* See later in the discussion for the transformation of I to IV.



I II III



IV V

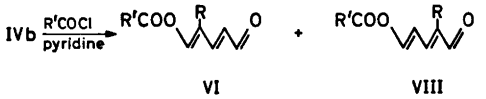


VI VII

a: R=H , R'=C_6H_5
b: R=CH_3 , R'=C_6H_5
c: R=CH_3 , R'=C_6D_5
d: R=OCH_3 , R'=C_6H_5
e: R=OCH_3 , R'=C_6D_5

turn was reduced to the expected [1] alcohol VIIb with sodium borohydride.

Yanovskaya and Kucherov [5] reported the preparation of a benzoyl derivative of Vb; but apparently were not sure of the position of the methyl group. Therefore we repeated their experiments, and found that a 1:1 mixture of the isomers VIb and VIIIb was obtained:

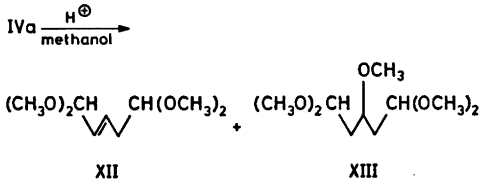


VI VIII

b: R=CH_3 , R'=C_6H_5
c: R=CH_3 , R'=C_6D_5

Furthermore, we reinvestigated the bromination of IVa and of the benzoyl derivative VIa. Upon bromination each yielded a mono bromo derivative, to which we assign structures IX and X, respectively. Baumgarten [4a] previously reported a lower melting point for X, for which he assumed the bromo atom to be substituted at the 4-position. It should be noted that IX was isolated as the benzoyl derivative X. Sodium borohydride reduction of X gave XI.

As a part of the structure determination we also reinvestigated the preparation of the tetramethyl acetal of IVa, previously described by Merländer.[6] We found that treatment of IVa with methanol led to the reported tetramethyl acetal XII, and as a higher boiling fraction the very stable 1,1,3,5,5-pentamethoxypentane (XIII).

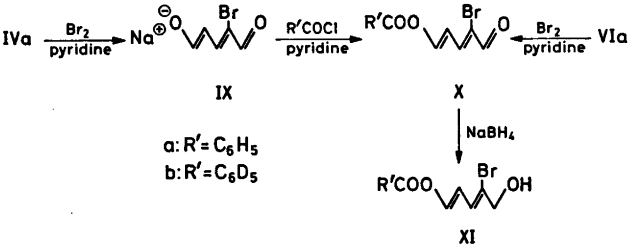


XII XIII

Structural assignments. All of the above described compounds showed the expected elemental composition. The ultraviolet and infrared spectra (Tab. 1) all are in good agreement with the assignments. However, the main evidence was found in the ^1H NMR spectra (Table 2).

The all-*trans* configuration is inferred from the IR-absorptions at 935 – 975 cm^{-1}; (see Ref. 1). The ^1H NMR spectra of all compounds,* as seen from the table, had the coupling constant $J_{2,3}=15$ Hz which is only compatible with a *trans* configuration. Similarly the coupling constant $J_{4,5}=11-12$ Hz is again consistent with a *trans* configuration. The location of the

* The numbering of the glutacondialdehyde derivatives has been defined in Ref. 1 as
RCOO–CH=CH–CH=CH–CHO.
5 4 3 2 1



IX X XI

a: R'=C_6H_5
b: R'=C_6D_5

Table 1. Spectral properties of glutacondialdehyde derivatives.

Compound	No.	UV absorption spectra in abs. ethanol		IR absorption spectra in KBr		
				OH	CHO	*trans* C=C
		λ_{max} mμ	log ε	cm^{-1}	cm^{-1}	cm^{-1}
5-Hydroxy-*trans*-2,*trans*-4-pentadienal benzoate						
2-methyl-	VIIIb	237 289	4.06 4.55		2580 2720 2815	936
4-methyl-	VIb	233 288	3.99 4.49		2700 2735 2810	970
2-bromo-	Xa	237 300	4.02 4.49		2842	948
5-Hydroxy-*trans*-2,*trans*-4-pentadienal hexadeuteriobenzoate						
2-methyl-	VIIIc	236 289	3.94 4.54		2690 2790 2890	945
4-methyl-	VIc	232 289	3.80 4.43		2700 2735 2815	970
2-bromo-	Xb	239 303	4.04 4.46		2845	948
trans-2,*trans*-4-Pentadiene-1,5-diol-5-benzoate						
4-methyl	VIIb	229 267	4.45 4.21	3340		965
2-bromo-	XIa	233 269	4.31 4.27	3250		938
trans-2,*trans*-4-Pentadiene-1,5-diol-5-hexadeuteriobenzoate						
2-bromo-	XIb	233 268	4.22 4.30	3400		937
1,1,5,5-Tetramethoxy-*trans*-2-pentene						
	XII					975
5-Hydroxy-*trans*-2,*trans*-4-pentadienal benzoate						
4-methoxy-	VId				2700 2735 2815	970
5-Hydroxy-*trans*-2,*trans*-4-pentadienal hexadeuteriobenzoate						
4-methoxy-	VIe				2700 2735 2815	970

substituents follows from the splitting of the individual signals, and from the chemical shifts.

The steric structure, *i.e. trans* or *cis*, of IIIa, IIIb or IIId was shown to be *trans* in the case of IIId, the ^1H NMR spectrum of which was found to be similar to the spectrum found for IVa.[1] The ^1H NMR spectra of IIIa and IIIb were not obtained due to the insolubility of these salts, but as their UV and IR spectra [12] closely resembled that of IIId, these salts must

also be assigned the *trans* structure.

All the assignments described above correspond to similar results for some amino derivatives of the glutacondialdehyde type reported by Dillon and Lewis,[7] where $J_{trans} = 11 - 15$ Hz, Schibe and Jutz [8] $J_{trans} = 11.8 - 12.7$ Hz and by Marvell, Li and Paik [9] $J_{trans} = 12$ Hz.

MICROWAVE SPECTRA

A low-resolution microwave-spectrum of 5-hydroxy-*trans*-2,*trans*-4-pentadienal acetate[1] has been recorded at a vapour pressure of 0.03 mmHg on a Hewlett-Packart MRR-spectrometer.

The spectrum (Fig. 1) consists of unresolved "bands" approximately 100 MHz wide and 703 MHz apart, with no appreciable fine structure. (30 000 MHz correspond to 1 cm^{-1} on the more

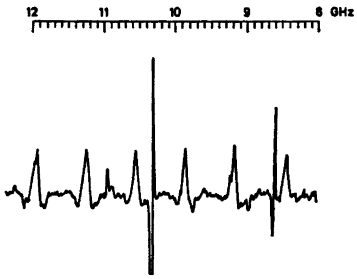


Fig. 1. Microwave spectrum of 5-hydroxy-*trans*-2,*trans*-4-pentadienal acetate.

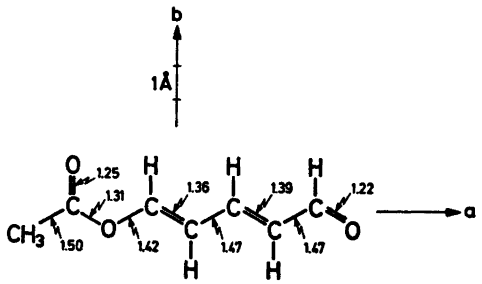


Fig. 2. 5-Hydroxy-*trans*-2,*trans*-4-pentadienal etacate.

familiar wave number scale). These findings indicate that the molecule is very close to the limit, where the two largest moments of intertia are equal (the prolate limit). They also indicate that the molecule possesses a dipole component in the direction of the axis of smallest moment of inertia. In Fig. 2 this axis is designated by *a*, while the axes of largest moments of inertia are designated by *b* and *c* (not shown in the figure). Also indicated on the figure are a set of reasonable geometrical parameters which give a good description of the spectrum in the following sense:

The spectrum reflects the free rotation of the molecule, and it is described to a good approximation by the formula:

$$\nu_{J \to J+1} = 2B^{+}(J+1)$$

with B^{+} expressed in terms of the moments of inertia as follows:

$$B^{+} = f(1/I_b + 1/I_c)$$

with ν in MHz and I_b, I_c in u$Å^2$, $f = 505\,376/2$. J is the rotational quantum number, which may be found from the spectrum by dividing the spacing between two peaks ($2B^{+}$) into the frequency of the lower of the two peaks.

The moments of inertia of the model of Fig. 2 are $I_b = 1449$ u$Å^2$ and $I_c = 1508$ u$Å^2$ giving $2B^{+} = 684$ MHz, in good agreement with the observed value of 703 MHz.

The conformation shown in Fig. 2, and the conformation obtained by rotating the acetyl group by 180°, must have almost the same moments of inertia, therefore the microwave spectra can say nothing conclusive in this regard. However, it is well known [10] that esters in general favour a conformation where C=O and the ester alkyl group are *cis*. We therefore presume that the conformation giving rise to the spectrum is the one shown in Fig. 2.

DISCUSSION

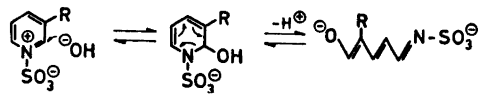
The benzoylation of III thus gives only the 4-substituted isomer VI, without a trace of the 2-substituted isomer. This highly specific reaction is mirrored in the bromination reactions of IVa and VIa, which reactions yielded only Xa. Thus the opening of the pyridine ring seems to occur as follows:

Table 2. Nuclear magnetic resonance parameters.[a]

Compound[b]	Protons at carbon number C_1	C_2	C_3	C_4	C_5	Other protons
VIIIb	0.59, s, 1 H	–	3.10, d,m, *J* 11.4, 1 H	3.45, t, *J* 11.4, 1 H	2.02, d, *J* 11.4, 1 H	8.11, s, 3 H, methyl H; 1.85–2.05, m, 2H and 2.44–2.68, m, 3H, aromatic H
VIb	0.36, d, *J* 7.5 1 H	3.74, d,d, *J* 7.5, J 15.0, 1H	2.74, d, *J* 15.0, 1 H	–	2.06, m, *J* 1.5, 1 H	1.75–1.92, m, 2H and 2.53–2.84, m, 3H aromatic H; 8.00, d *J* 1.5, 3 H methyl H
Xa	0.75, s, 1 H	–	2.45, d, *J* 10.5, 1 H	3.22, d,d, *J* 12.0, *J* 10.5, 1 H	1.80, d, *J* 12.0, 1 H	1.75–2.50, m 5 H, aromatic H
VIIIc	0.58, s, 1 H	–	3.10, d,m, *J* 11.4, 1 H	3.45, t, *J* 11.4, 1 H	2.02, d, *J* 11.4, 1 H	8.11, s, 3 H methyl H
VIc	0.36, d, *J* 7.5, 1 H	3.74, d,d, *J* 7.5 *J* 15.0, 1 H	2.75, d, *J* 15.0, 1 H	–	2.07, m, *J* 1.5 1 H	7.96, d, *J* 1.5, 3 H, methyl H
Xb	0.78, s, 1 H	–	2.48, d, *J* 10.9, 1 H	3.25, d,d, *J* 11.6, *J* 10.9, 1 H	1.80, d, *J* 11.6, 1 H	–
VIIb	5.80, d, *J* 5.3, 2 H	4.21, d,t, *J* 15.0 5.3, 1 H	3.72, d, *J* 15.0, 1 H	–	*ca.* 2.55, m, *J* 1.5, 1 H	8.05, d, *J* 1.5, 3 H, methyl H; s, 7.75, 1 H, OH; 1.91–2.08, m 2 H and 2.45–2.78, m, 3 H, aromatic H

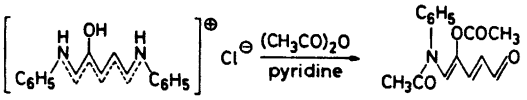
XIa	5.71, s, 2 H	–	3.31–3.80, m, *J* 12.0, *J* 10.5		2.24, d, *J* 10.5, 1 H	1.85–2.84, m, 5 H, aromatic H; 7.78, s, 1 H, OH
XIb	5.71, s, 2 H	–	*ca.* 3.53, m, *J* 10.5		2.24, d, *J* 10.5, 1 H	7.78, s, 1 H, OH
XII	5.24,d, *J* 5.4, 1 H	4.51, d,d, *J* 5.4, *J* 15.0 1 H	4.08, d,d, *J* 6.0, *J* 15.0 1 H	7.56, t, *J* 6.0, 2 H	5.56, t, *J* 6.0, 1 H	6.64, s,12 H, methyl H
XIII	5.50, t, *J* 6.0, 2 H	8.29, t, *J* 6.0, 4 H	6.78, t, *J* 6.0, 1 H	See C_2	See C_1	6.62, s, 3 H, 6.70, s, 12 H, methyl H
VId	0.40, d, *J* 7.5, 1 H	3.56, d,d, *J* 7.5, *J* 15.0, 1 H	3.13, d, *J* 15.0, 1 H	–	2.46, s, 1 H	1.80–1.98, m, 2 H and 2.33–2.55, m, 3 H, aromatic H, 5.98, s, 3 H, OCH_3
VIe	0.40, d, *J* 7.5, 1 H	3.56, d,d, *J* 7.5, *J* 15.0, 1 H	3.13, d, *J* 15.0, 1 H	–	2.46, s, 1 H	5.98, s, 3 H, OCH_3
IIId [c]	2.10, d, *J* 10.5, 1 H	4.72, d,d, *J* 10.5, *J* 13.5, 1 H	3.52, d, *J* 13.4 1 H	–	2.12, s, 1 H	6.53, s, 3 H, OCH_3

[a] All spectra were recorded at 60 Mc/s with TMS as internal reference. Chemical shifts are in τ-units, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, d,d = doublet of doublets, d,t = doublet of triplets, t,t = triplet of triplets, and the coupling constants are given in Hz.
[b] Deuteriochloroform was used as solvent, in the case of no remarks. [c] In hexadeuteriodimethyl sulfoxide.



The proposed reaction scheme explains the position for attack at the pyridine ring where R = CH_3 or OCH_3. The steps seem to be reversible, *e.g.* hydrolyses of the unstable salt III regenerates the parent pyridine.

A similar specific acylation reaction of a related glutacondialdehyde derivative has been reported by Dillon and Lewis.[7] They found that acetylation of the so-called Stenhouse salts in pyridine yielded exclusively one isomer, while the same reaction carried out in water gave a mixture of the two possible isomers.



a "Stenhouse salt"

These results suggest that acylation reactions performed in pyridine are specific for the glutacondialdehyde derivatives with an imide function such as in III or in the Stenhouse salts. However, in the case of the glutacondialdehyde sodium salts IVa and IVb acylation of the enol form resonance structure was non specific and the two possible isomers were obtained in a 1:1 ratio. This investigation will be continued with other pyridines as well as substituted glutacondialdehydes.

EXPERIMENTAL

Microanalyses were carried out in the Microanalytical Department of the University of Copenhagen by Mr. Preben Hansen. Melting points (uncorrected) were determined on a Büchi melting point apparatus.

Infrared spectra were recorded on a Perkin Elmer Model 457 grating infrared spectrophotometer. Ultraviolet spectra were recorded on a Beckman ACTA III spectrophotometer by Mrs. Bodil Kroneder. Proton magnetic resonance spectra were recorded on a Jeol C-60HL NMR spectrometer by Mrs. Eva Heyn Olsen, who also assisted with some of the preparations.

TLC, thin layer chromatography, and PTLC, preparative layer chromatography, were performed on silica gel with benzene as eluent.

Gas chromatography was carried out on a Varian Autoprep Gas Chromatograph.

5-Hydroxy-trans-*2*,trans-*4-pentadienal benzoate (VIa)*. The previously reported [1] benzoyl derivative VIa was prepared as described for VId. Thus 4.0 g of IIIa in 30 ml of pyridine and 2.0 ml of benzoyl chloride yielded 0.54 g (11 %) of VIa.

3-Methyl pyridinium-1-sulfonate (IIb).[11] 3-Methyl pyridine (12.4 g) dissolved in 40.0 ml of chloroform was cooled to −20 °C, whereupon chlorosulfonic acid (4.36 ml) was added slowly during 75 min while the temperature was maintained at −5 to 0 °C. Stirring was continued for 90 min at −20 °C. The product was filtered, washed with pentane (20 ml) and dried at 20 °C/1 mmHg. Yield of IIb as white crystals, m.p. 140−148 °C was 8.2 g (44 %). (Found: C 38.60; H 4.64; N 7.51; S 16.25. Calc. for $C_6H_7NO_3S.3/4H_2O$: C 38.60; H 4.59; N 7.50; S 17.17).

Ring opening of 3-methyl pyridinium-1-sulfonate (IIb) to give IIIb. Disodium 4-methyl-5-oxido-trans-*2*,trans-*4-pentadienylidenamin*-N-*sulfonate (IIIb)*. IIb (21 g) was cooled to −15 °C. To these crystals was added during 5 min with stirring a solution of sodium hydroxide (10 g) dissolved in 68 ml of water previously cooled to −20 °C. Stirring was continued at this temperature for 30 min. The suspension turned bright yellow and stirring was continued at room temperature for 2½ h. The product was filtered and washed with methanol to yield 21 g (74 %) disodium salt IIIb as bright yellow crystals (UV and IR nearly identical [12] with the corresponding salt [2] obtained from the unsubstituted pyridine). (Found: C 28.85; H 3.68; N 5.37; S 12.73. Calc. for $C_6H_6Na_2NSO_4.H_2O$: C 28.58; H. 3.20; N 5.56; S 12.71).

3-Methoxy pyridinium-1-sulfonate (IId). The above described method was used. Thus 3-methoxy pyridine (22 g) in 60 ml of chloroform and 13.4 ml of chlorosulfonic acid yielded 14.0 g (37 %) of IId as white crystals. A sample recrystallized from 1,2-dichloroethane had m.p. 118−120 °C. (Found: C 36.12; H 4.07; N 6.90; S 15.87. Calc. for $C_6H_7NSO_4.2/3H_2O$: C 35.82; H 4.18; N 6.96; S 15.94). 11.4 g of 2-methoxypyridinium hydrochloride may be isolated from the filtered reaction mixture, bringing the total yield to 76 %.

Ring opening of 3-methoxy pyridinium-1-sulfonate (IId) to give IIId. Disodium, 4-methoxy-5-oxido-trans-*2*,trans-*4-pentadienylidenamin*-N-*sulfonate (IIId)*. 4.0 g of IId was cooled to −20 °C whereupon 2.0 g of sodium hydroxide dissolved in 16 ml of water was added with stirring and cooling. Within 30 min the temperature was raised to 0 °C and 75 ml of 2-propanol was added. The precipitated very unstable yellow crystals were filtered, washed with pentane and dried (20 °C/1 mmHg), to yield 3.5 g (66 %) of IIId. (Found: S 11.28. Calc. for $C_6H_7Na_2NSO_4.2H_2O$: S 11.16).

4-Methyl-5-hydroxy-trans-*2*,trans-*4-pentadienal benzoate (VIb)*. To a suspension of III b (17.3 g) in 104 ml of pyridine was added

benzoyl chloride (8 ml) in 30 min at about −5 °C. Stirring was continued for 1 h and the temperature raised to 20 °C. The resulting yellow suspension was added to 1 litre of ice cold water. Isolation and drying of the precipitated pale yellow crystals yielded 9.12 g (61 %) of Vb, free from any trace of the isomer VIIIb, as seen from the NMR spectra and from TLC. Recrystallization from methanol yielded pale yellow needles m.p. 138−139 °C. (Found: C 72.20; H 5.68. Calc. for $C_{13}H_{12}O_3$: C 72.20; H 5.59).

4-Methyl-5-hydroxy-trans-*2*,trans-*4-pentadienal hexadeuteriobenzoate (VIc)*. The method described above was used. Thus 2.16 g of IIIb yielded 0.55 g (29 %) of VIc as pale yellow needles. A sample recrystallized from methanol had m.p. 137−138 °C. (Found: C 70.17; H+D 5.44. Calc. for $C_{13}H_7D_5O_3$: C 70.55; H+D 5.47).

4-Methoxy-5-hydroxy-trans-*2*,trans-*4-pentadienal benzoate (VId)* was prepared as described above. Thus 2.0 of IIId in 15 ml of pyridine and 1.0 ml of benzoyl chloride was stirred for 15 min at 15 °C, whereupon 300 ml of ice cold water was added, and the precipitated pale yellow needles were isolated yielding 0.46 g (27 %) of VId. Recrystallization from cyclohexane yielded white needles, m.p. 123−124 °C (Found: C 67.30; H 5.19. Calc. for $C_{13}H_{12}O_4$: C 67.23 H 5.21).

4-Methoxy-5-hydroxy-trans-*2*, trans-*4-pentadienal hexadeuteriobenzoate (VIe)* was prepared as above. Thus 2.0 g of IIId and hexadeuteriobenzoyl chloride yielded 0.48 g (27 %) of VIe. Recrystallization from cyclohexane yielded white needles, m.p. 122−123 °C. (Found: C 65.80; H+D 5.12. Calc. for $C_{13}H_7D_5O_4$: C 65.80; H+D 5.06).

2-Bromo-5-hydroxy-trans-*2*,trans-*4-pentadienal benzoate (Xa). Method a*. To IVa (6.0 g) stirred in 150 ml of pyridine at +5 °C was slowly added 2.0 ml of bromine. The clear solution was then cooled to 0 °C and 4.0 ml of benzoyl chloride added; stirring was continued for 5 min, whereupon the reaction mixture was poured into ice cold water (250 ml) and the product isolated. This gave 3.94 g (37 %) of Xa as pale yellow crystals. Recrystallization from methylcyclohexane yielded hygroscopic white needles, m.p. 128−129 °C (d). (Found: C 51.25; H 3.23; Br 28.17. Calc. for $C_{12}H_9BrO_3$: C 51.27; H 3.23; Br 28.43. Ref. 4a gives m.p. 108 °C for this mono brominated derivative.

Method b. To a suspension of 5-hydroxy-*trans*-2,*trans*-4-pentadienal benzoate (VIa) [1] (2.02 g) in 75 ml of ether at +5 °C was added 0.53 ml of bromine and stirring continued for 30 min. Pyridine (35 ml) was then added and after 5 min the reaction mixture was poured into ice cold water (100 ml) and the product was isolated. This gave 2.08 g (72 %) of Xa. Recrystallization from methylcyclohexane yielded analytically pure Xa, mixed m.p. and spectra identical with those of the product from *method a*.

2-bromo-5-hydroxy-trans-*2*-trans-*4-pentadienal hexadeuteriobenzoate (Xb). Method a* described above was used. Thus 2.0 g of I in pyridine (50 ml) was treated with bromine (0.67 ml) at +5 °C, whereupon hexadeuteriobenzoyl chloride (1 ml) was added. Working up as usual yielded 0.74 g (21 %) of Xb as pale yellow crystals. Recrystallization from methylcyclohexane gave colourless needles m.p. 135−136 °C (d). (Found: *J* 50.32; H+D 3.21; Br 27.87. Calc. for $C_{12}H_4D_5BrO_3$: C 50.35; H+D 3.16; Br 27.94.

4-Methyl-trans-*2*,trans-*4-pentadiene-1,5-diol-5-benzoate (VIIb)*. VIIb was prepared by borohydride reduction as described in Ref. 1. Thus VIb (0.50 g) dissolved in 20 ml of dioxane yielded 0.39 g (77 %) of VIIb as colourless unstable needles, m.p. 77−78 °C (benzene-pentane). (Found: C 71.50; H 6.56. calc. for $C_{13}H_{14}O_3$: C 71.54; H 6.47).

2-Bromo-trans-*2*,trans-*4-pentadiene-1,5-diol-5-benzoate (XIa)*. XIa was prepared as above. Thus Xa (0.70 g) in 25 ml of dioxane yielded 0.60 g (86 %) of XIa as white crystals, m.p. 127−128 ° (benzene). (Found: C 50.90; H 3.87; Br 28.00. Calc. for $C_{12}H_{11}BrO_3$: C 50.94; H 3.92; Br 28.22).

2-Bromo-trans-*2*,trans-*4-pentadiene-1,5-diol-5-hexadeuteriobenzoate (XIb)*. XIb was prepared as above. Thus Xb (0.185 g) in 10 ml of dioxane yielded 0.15 g (80 %) of XIb as white crystals, m.p. 138−140 °C (benzene-pentane). (Found: C 49.95; H+D 3.56; Br 27.58. Calc. for $C_{12}H_6D_5BrO_3$: C 50.00; H+D 3.85; Br 27.74).

Reaction of I with methanol−HCl, to give XII and XIII. IVa (25 g) was stirred in 190 ml of methanol and heated to reflux, whereupon 150 ml of methanol containing 0.068 g of HCl was added. Reflux was continued for 10 min and the reaction mixture left overnight at room temperature. Addition of water (600 ml) and potassium carbonate (100 g) to the reaction mixture followed by continuous extraction with ether yielded 12 g of a yellow oil. Distillation of this oil (12 g) gave three fractions: *A*, 3.89 g, b.p. 80−90 °C, n_D^{26} 1.4317; *B*, 4.19 g, b.p. 90−100 °C, n_D^{26} 1.4258; C, 3.48 g, b.p. 100−125 °C, n_D^{25} 1.4232.

Gas chromatography showed fraction *C* to be 95 % pure 1,1,3,5,5-pentamethoxypentane (XIII) corresponding to a yield of 11 % obtained directly by one batch distillation. Analytically pure XIII together with the glutacondialdehyde bis-dimethyl acetal (XII) [6] was obtained by preparative gas chromatography of the crude product. Thus XIII gave n_D^{26} 1.4210. (Found: C 54.00; H 10.02. Calc. for $C_{10}H_{22}O_5$: C 54.03; H 9.95).

Pure 1,1,5,5-tetramethoxy-*trans*-2-pentane (XII) gave n_D^{26} 1.4332. Merländer [6] reported n_D^{20} 1.4288 for XII. (Found: C 56.85; H 9.57. Calc. for $C_9H_{19}O_4$: C 56.82; H 9.54).

2-Methyl-5-hydroxy-trans-*2*,trans-*4-pentadi-*

enal benzoate (VIIIb). The known 2-methyl-1,1,3,5,5-pentamethoxy pentane [5] was hydrolysed to give the crude sodium salt IVb. This salt (0.6 g) in pyridine (6 ml) was treated with 0.5 ml of benzoyl chloride as described in Ref. 1 yielding 0.110 g of cream coloured crystals. Repeated TLC [13] (6 times) with benzene as eluent together with NMR showed these crystals to be a 1:1 mixture of IVb and VIIIb. Recrystallization of a sample from methanol gave colourless crystals m.p. 85 – 90 °C.

In another experiment 1.4 g of the crude product was separated by repeated PTLC to give 0.32 g of VIIIb as colourless needles, m.p. 103 – 105 °C (methanol). (Found: C 71.70; H 5.55). Furthermore this chromatogram yielded 0.35 g of IVb, mixed m.p. and spectra identical with those described for IVb above.

2-Methyl-5-hydroxy-trans-*2*,trans-*4-pentadienal hexadeuteriobenzoate (VIIIc).* VIIIc was prepared as described above. Thus crude IVb (0.8 g) and hexadeuteriobenzoyl chloride (0.6 ml) yielded 0.27 g of a 1:1 mixture of VIIIc and VIc as cream coloured crystals, m.p. 76 – 82 °C. Separation by repeated PTLC of 0.9 g of crude product from another experiment yielded 0.20 g of VIIIc as colourless needles, m.p. 105 – 106 °C (methanol). (Found: C 70.65; H + D 5.57). Furthermore this chromatogram yielded 0.19 g of VIc, mixed m.p. and spectra identical with those previously described for VIc.

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Short Communications

Studies on Coumarins. Pyrrolocoumarin

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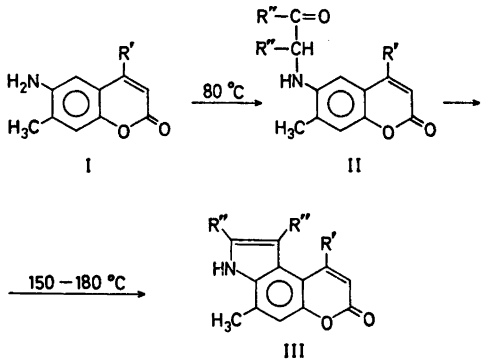
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Naturally occurring furocoumarins of the psoralen and angelicin type were isolated by Baerheim Svendsen *et al.*[1] from the roots of different *Heracleum* species of the family *Umbelliferae* which also occur in Scandinavia. They are all structurally related to umbelliferone (7-hydroxycoumarin). Martin *et al.*[2] have investigated the fungicidal effect of these same furocoumarins on some fungi, primarily *Gloesporium limetticola* and *Alternaria tenuis*.

Other naturally occurring coumarins with biocidal and physiological properties have been reviewed by Seshadri[3] and Soine.[4] On the other hand, the nitrogen analogues of furocoumarins, *viz.* pyrrolocoumarins, seem not to have been found in Nature and even not been prepared. Only a few papers on synthesis (by Skraup's method) of pyranoquinolines have been published.[5–7]

In order to investigate the biocidal properties of pyrrolocoumarins, one such substance was prepared starting from 6-amino-4,7-dimethylcoumarin. This was obtained by reduction of the corresponding nitrocoumarin with tin(II) chloride in conc. hydrochloric acid rather than iron and acetic acid,[8] since the tin method gave a better yield. The aminocoumarin was then treated with benzoin following Bischlers method, analogously to the preparation of 4- and 6-hydroxy-2,3-diphenylindole.[9–11]

The cyclization to a pyrrolocoumarin took place in two steps with *p*-toluenesulfonic acid or hydrochloric acid as a catalyst. The intermediate product, an α-anilidoketone, was obtained on refluxing the reactants in ethanol or benzene for 18 – 24 h. This intermediate was then treated in the presence of acid catalyst without any solvent at 150 – 180 °C for some hours. The overall yield was rather poor. In the reaction scheme the synthetic pathway is shown for the novel pyrrolocoumarin III.



Scheme 1. Synthesis of the pyrrolocoumarin. $R' = CH_3$, $R'' = C_6H_5$.

Experimental. All melting points are uncorrected. The ^1H NMR spectra were recorded on a Varian A 60 spectrometer and the mass spectra on a LKB 9000 mass spectrometer (temperature of the ion source 270 °C, electron energy 70 eV).

6-Amino-4,7-dimethylcoumarin (I, $R' = CH_3$). The nitrocoumarin (11.0 g, 50 mmol) and tin(II) chloride dihydrate (35.0 g, 155 mmol) were suspended in 60 ml of conc. hydrochloric acid at 20 °C. The mixture was heated with stirring on a boiling water bath for 20 h, cooled, and the undissolved amine hydrochloride removed by filtration with suction. The crude product was dissolved in hot water, filtered with suction, and the filtrate neutralized with sodium hydrogen carbonate. The precipitate, containing stannic salts, was filtered and dried. After Soxhlet extraction with dichloromethane the aminocoumarin was recrystallized once from dioxane (10 ml/g). Yield 5.1 g = 56.6 %. M.p. 210 – 211 °C (Lit.[8] 203 – 205 °C). The ^1H NMR spectrum in DMSO-d_6 exhibits the following δ-values (assignment, multiplicity, *J* Hz): 2.20 (7-CH_3, 1), 2.37 (4-CH_3, 2, 1.0), 5.0 (6-NH_2, 1), 6.30 (3-H, 2, 1.0), 7.0 and 7.12 (5- and 8-H or *vice versa*, 2, 0.5).

6-(1',2'-Diphenyl-2'-ketoethyl)amino-4,7-dimethylcoumarin. (II). Compound I (3.8 g), benzoin (4.3 g) and *p*-toluenesulfonic acid (*p*-TS, 0.1 g) were dissolved in 75 ml of absolute ethanol and refluxed for 24 h. The coumarin II, which had already precipitated from the hot solution, was collected after cooling and re-

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crystallized once from ethanol. Yield 3.8 g = 49.7 %. M.p. 192–194 °C. (Found: C 77.5; H 5.6; N 3.8. Calc. for $C_{25}H_{21}NO_3$: C 78.3; H 5.5; N 3.7). Mass spectrum (rel. intensity): M^+ 383, 279 (20.5), 278 (100), 105 (20.0), 91 (24.7), 77 (24.7).

4,5-Diphenyl-2,3-b-pyrrolo-4,7-dimethylcoumarin (III). Comarin I (1.9 g) and benzoin (2.2 g), or alternatively coumarin II (3.8 g), and *p*-TS (0.02 g) were mixed thoroughly and heated in an oil bath at 180 °C for 15 h. The crude product was treated with sodium hydrogen carbonate in water, dried and recrystallized twice from dimethylformamide (10 ml/g). Yield 0.7 g = 19.2 %. M.p. 323–325 °C. 0.2 g was chromatographed twice on preparative thin layer alumina with carbon tetrachloride-ethanol 25:1 for analysis. M.p. 330–331 °C. (Found C 82.3; 81.9; H 5.3; 5.0; N 3.7, 4.0. Calc. for $C_{25}H_{19}NO_2$: C 82.2; H 5.2; N 3.8). Mass spectrum M^+ 365 (100), 337 (42.6), 323 (23.0).

Acknowledgements. I am indebted to Miss Gurli Hammarberg for the ^1H NMR spectra and to Mr. Hans-Erik Högberg for the mass spectra. Dr. J. Thompson checked the English text. This work was financially supported by the Swedish Natural Science Research Council.

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Pseudomonas Cytochrome *c* Peroxidase. X. The Effect of *Pseudomonas* Neutral Proteinase on the Enzyme Molecule

RITVA SOININEN and NILS ELLFOLK

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Pseudomonas cytochrome *c* peroxidase (PsCCP, EC 1.11.1.5) has been isolated in a homogeneous form as revealed by disc electrophoresis and analytical ultracentrifugation.[1,2] It consists of a single polypeptide chain with an apparent molecular weight of 40 000 ± 1 500 according to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS).[3] In some preparations, however, two smaller components were observed in SDS-gel electrophoresis. *Pseudomonas aeruginosa* was found to produce several proteolytic enzymes,[4] and therefore the smaller components were suspected to derive from PsCCP by proteolysis by a proteinase still present in the preparation.

In this study the effect of a partly purified *Pseudomonas* neutral proteinase on PsCCP was investigated.

Experimental. PsCCP was prepared as described previously.[1,2] The absorbance ratio A_{407}/A_{280} of the preparations was 4.50–4.66. The purity of the preparations was checked by disc electrophoresis according to Maurer[5] (system No. 1a, pH 8.9, 7 % gel).

Pseudomonas neutral proteinase (elastase, "semialkaline proteinase") was prepared according to Morihara *et al.*[6] from a semisynthetic growth medium. After elution from DEAE-cellulose, the preparation was concentrated by ultrafiltration in a Diaflo apparatus (Amicon N.V., UM-10 membrane) and used without further treatment.

Incubation of PSCCP with the partly purified proteinase was carried out in 0.01 M Na-phosphate buffer pH 7.0 at 40 °C at an enzyme-substrate ratio appr. 1:100 (w/w). The reaction was stopped by inactivating proteinase by heating at 100 °C for 5 min.

Analytical SDS-polyacrylamide gel electrophoresis was performed according to Weber and Osborn[7] in 10 % gels. Standard proteins used for molecular weight estimation were: bovine serumin albumin (Fraction V, Armour), ovalbumin (Grade V, Sigma), pepsin (crysallized, Sigma), and horse heart cytochrome *c* (Type III, Sigma). Analytical SDS-polyacrylamide gel electrophoresis in 12.5 % gels in the presence of 8 M urea according to Swank and Munkres[8] was used to separate the peptides in the 1 500 to 10 000 molecular weight range. 10–30 μg of protein were applied per gel. After electrophoresis the gels were cut in the middle of the marker dye band, stained with

crystallized once from ethanol. Yield 3.8 g = 49.7 %. M.p. 192–194 °C. (Found: C 77.5; H 5.6; N 3.8. Calc. for $C_{25}H_{21}NO_3$: C 78.3; H 5.5; N 3.7). Mass spectrum (rel. intensity): M^+ 383, 279 (20.5), 278 (100), 105 (20.0), 91 (24.7), 77 (24.7).

4,5-Diphenyl-2,3-b-pyrrolo-4,7-dimethylcoumarin (III). Comarin I (1.9 g) and benzoin (2.2 g), or alternatively coumarin II (3.8 g), and *p*-TS (0.02 g) were mixed thoroughly and heated in an oil bath at 180 °C for 15 h. The crude product was treated with sodium hydrogen carbonate in water, dried and recrystallized twice from dimethylformamide (10 ml/g). Yield 0.7 g = 19.2 %. M.p. 323–325 °C. 0.2 g was chromatographed twice on preparative thin layer alumina with carbon tetrachloride-ethanol 25:1 for analysis. M.p. 330–331 °C. (Found C 82.3; 81.9; H 5.3; 5.0; N 3.7, 4.0. Calc. for $C_{25}H_{19}NO_2$: C 82.2; H 5.2; N 3.8). Mass spectrum M^+ 365 (100), 337 (42.6), 323 (23.0).

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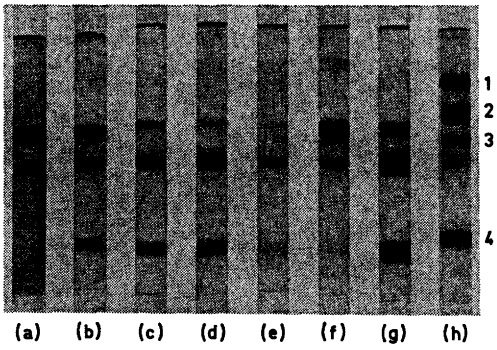


Fig. 1. SDS-polyacrylamide gel electrophoresis in 10 % gels according to Weber and Osborn [7] of untreated PsCCP (a); of PsCCP incubated with *Pseudomonas* neutral proteinase for 1 (b), 5 (c), and 30 (e) min in 0.01 M Na-phosphate buffer pH 7.0 at 40 °C; of PsCCP incubated without proteinase for 30 min under the same conditions as before (f); of a PsCCP preparation cleaved during the purification (g); and of standard proteins (h): 1, serum albumin; 2, ovalbumin; 3, pepsin; 4, horse heart cytochrome *c*.

Coomassie Brilliant Blue R 250 (Serva) and de-stained by soaking with anion exchange resin (Weber *et al.*).[9] The gels were photographed as described by Oliver and Chalkley.[10] SDS (pract., Fluka) and urea (pure, Merck) were recrystallized before use.

Preparative SDS-polyacrylamide gel electrophoresis in a 10 % gel was performed on a horizontal gel bed (100 × 150 × 3 mm). With slight modifications the procedure used was that of Weiner *et al.*[11] The gel was cast in a rectangular mold with a groove (2 × 85 × 2 mm) for sample application 2 cm from one end of the gel bed. After polymerisation the gel was pre-eluted by soaking for 4 days at 30 °C with gentle agitation in the electrophoresis buffer, which was changed every 8 – 16 h. β-Mercaptoethanol was omitted from the sample. 3 mg of protein was applied to the gel, and the electrophoresis was run in a Desaga thin-layer electrophoresis apparatus at 60 mA for 16 h at 25 °C. After this time a guide strip was cut from the edge of the gel and the rest of the gel was frozen. The guide strip was stained and de-stained in the same way as the analytical gels. Zones containing polypeptides were cut off, and eluted twice with 5 mM $NaCO_3$ containing 0.05 % SDS for 8 – 16 h at 30 °C. The eluate was centrifuged and lyophilised.

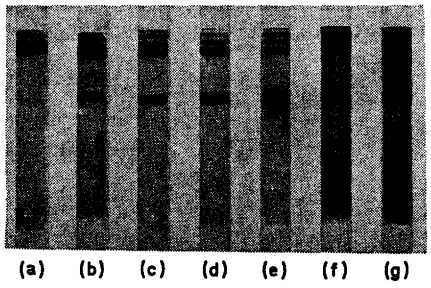


Fig. 2. SDS-polyacryalmide gel electrophoresis in 12.5 % gels in the presence of 8 M urea according to Swank and Munkres [8] of untreated PsCCP (a); of PsCCP incubated with and without *Pseudomonas* neutral proteinase as described in Fig. 1 (b-f); and of a PsCCP preparation cleaved during the purification (g).

N-Terminal amino acids and *N*-terminal amino acid sequences were determined by the dansyl-Edman procedure in the presence of SDS.[11]

The protein concentration of PsCCP was determined using the absorption coefficient at 280 nm $A(1\text{ cm}, 1\ \%) = 12.1$,[12] and that of the proteinase preparation by the Folin-Ciocalteau reaction.[13]

Chemicals were of analytical grade if not otherwise stated.

Results. The electrophoretic patterns of the samples from the incubation mixture of PsCCP and the partly purified *Pseudomonas* neutral proteinase are shown in Fig. 1 (b – e). Two polypeptide bands with apparent molecular weights of about 28 000 and 11 000, as calculated from the relative mobilities in SDS-gels, appear during the incubation. No peptides with lower molecular weights than 11 000 were observed in SDS-gel electrophoresis in the presence of 8 M urea within the first 30 min incubation time (Fig. 2). After prolonged incubation (60 min), smaller peptides appeared and larger peptides disappeared completely. Fig. 1 (f) and Fig. 2 (f) show the electrophoretic patterns of PsCCP incubated for 30 min without the addition of proteinase. Two faint bands appeared indicating the presence of some contaminating proteinase. Fig. 1 (g) and Fig. 2 (g) show the electrophoretic patterns of a PsCCP preparation suspected to be cleaved during the purification procedure. In addition to the band corresponding to the intact PsCCP, two faster moving bands were present. These had the same apparent molecular weights as those obtained by the cleavage of PsCCP by the added proteinase.

In order to study further the cleavage products, PsCCP was treated with proteinase for 10 min on a larger scale under the same conditions as before, the polypeptides being isolated by preparative SDS-gel electrophoresis (Fig. 3A, Preparations A-b and A-c). The polypeptides from the PsCCP preparation cleaved during the purification were isolated by the same method (Fig. 3B, Preparations B-b and B-c). The *N*-terminal amino acid sequence of Preparations A-c and B-c was the

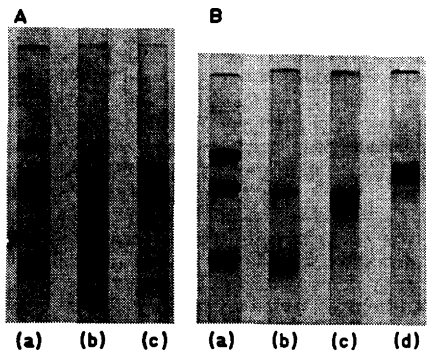


Fig. 3. Isolation of the polypeptide fragments obtained (A) by the limited proteolysis of PsCCP by *Pseudomonas* neutral proteinase (incubation of PsCCP with proteinase, appr. 100:1 w/w, in 0.01 M Na-phosphate buffer pH 7.0 for 10 min at 40 °C) and (B) from PsCCP cleaved during the purification. The preparations were analysed by analytical SDS-gel electrophoresis according to Weber and Osborn [7] in 10 % gels prior (a) and after (b-d) the preparative SDS-gel electrophoresis.

same as that of the intact protein: *Asp-Ala-Leu-*. Presumably these fractions arose from the *N*-terminal part of the protein molecule. More than one *N*-terminal amino acid was found in Preparations A-b and B-b that gave the apparently homogeneous bands of molecular weight 11 000 in the electrophoresis. The *N*-terminal amino acids in Preparation A-b were leucine, phenylalanine and valine, while those in Preparation B-b were leucine, phenylalanine and, obviously arising from the larger polypeptide fraction seen faintly in Fig. 3B, b, traces of aspartic acid. This indicates that the faster moving bands, and, correspondingly, the slower ones as well, consist of 2 – 3 fragment of about the same molecular weight. The *N*-terminal amino acids of the faster moving fragments were those expected to arise due to the hydrolysis catalysed by neutral proteinase, which cleaves the peptide bonds from the imino side of hydrophobic amino acids.[14]

Discussion. Pseudomonas neutral proteinase cleaves PsCCP into two large fragments similar to those arising in some purified preparations. The molecular weights of PsCCP and neutral proteinase, and the isoelectric points as well, are of the same magnitude.[1,6,12] Therefore it may be difficult to separate the two proteins completely by the methods used in the purification procedure for PsCCP (ion exchange and gel chromatography). PsCCP can, however, be prepared without noticeable proteolytic cleavage when all steps are carried out at a low temperature without unnecessary delay. The presence of the cleavage products in the final PsCCP preparation can easily be checked by SDS-gel electrophoresis as described in this paper.

The cleavage of the PsCCP molecule into a few, large, well-defined fragments, together with the *N*-terminal amino acid analyses of these, indicates that a few peptide bonds in the polypeptide chain are preferentially attacked by neutral proteinase.

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The Structure of Guaran

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The galactomannans from seeds of leguminous plants consist of chains of 1,4-linked β-D-mannose residues, part of which carry an α-D-galactopyranose residue linked to O-6. Studies by Courtois and Le Dizet,[1] using enzymatic methods, indicate that these polysaccharides are essentially block polymers, in which highly branched regions alternate with regions having a low degree of branching. Recent results from periodate-oxidation studies of guaran [2] have indicated the possibility of studying this problem by chemical methods, and we now report such studies.

In guaran the ratio of D-mannose to D-galactose residues is about 2:1. On periodate oxidation, however, the Malapradian oxidation limit of 1.33 mol of periodate per hexose residue was not observed. The consumption of periodate virtually stopped when 58 % of the mannose residues had been oxidized.[2] It has been demonstrated [3,4] that this inhibition of periodate oxidation is due to the formation of inter-residue hemiacetals between the aldehyde groups of oxidized residues and hydroxyl groups in unoxidized, adjacent residues. When there is a free hydroxyl at C-6, formation of intra-residue hemiacetals competes with the formation of inter-residue hemiacetals and the protection of adjacent residues against periodate oxidation is incomplete. Even if the rate is reduced, it is then practically possible to obtain the Malapradian oxidation limit. If guaran were a block polymer, only 6-substituted D-mannose residues should be completely protected. If the 6-substituents were randomly distributed, however, both unsubstituted and 6-substituted residues should be completely protected. This has now been investigated by methylation analysis of a number of samples, which have been subjected to periodate oxidation and borohydride reduction (Table 1). Hydrolysates of the methylated products were analysed, as the alditol acetates, by GLC-MS.[5] The two components with $T = 0.20$ and 0.35 are the acetates of 1,4-di-*O*-methyl-D-erythritol and 1-*O*-methyl-D-erythritol, respectively, derived from oxidized D-mannose residues without and with a substituent at O-6. These components are volatile and considerable parts of them have most probably been lost during concentration of solutions. The ratio between 2,3-di-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose is not very accurate but is consistently somewhat higher for the oxidized samples than for the original polysaccharide. If guaran were a block polymer, however, this ratio should have been much higher. The small increase observed may be due to a somewhat faster oxidation of unsubstituted than of 6-*O*-substituted D-mannose residues. This is also indicated by the higher ratio of di- to mono-*O*-methyl-D-erythritol obtained from the samples which had only been oxidized for a short time. In conclusion, therefore, the present results are consistent with a random distribution of the terminal α-D-galactopyranose residues in guaran.

Table 1. Methylation analysis of periodate oxidized-borohydride reduced guaran.

Time of oxidation	Components (area of peak, relative to 2,3,6-Man = 1.00) 1,4-Eryt	1-Eryt	2,3,4,6-Gal	2,3-Man
0 min	–	–	1.44	1.61
2 min	–	–	1.11	1.94
3 min	–	–	1.03	1.57
5 min	0.12	0.15	0.73	1.74
10 min	0.21	0.20	0.53	1.90
15 min	0.25	0.34	0.26	2.01
20 min	0.25	0.51	0.20	2.01
35 min	0.37	0.64	0.18	1.63
1.5 h	0.43	0.92	–	1.66
3.5 h	0.36	1.99	–	2.16
5 h	0.14	1.37	–	2.26
9 h	0.64	1.70	–	1.97
11 h	1.05	1.85	–	1.96
13.5 h	0.83	2.17	–	2.35
20 h	0.63	1.94	–	2.13
24 h	0.77	1.67	–	2.09
30 h	1.03	2.25	–	2.49

Experimental. The preparation of the modified guaran samples has been reported.[2] The samples were methylated according to Hakomori, hydrolysed, reduced, acetylated and analysed by GLC-MS as previously described.[5] A glass-column (200 × 0.3 cm) containing 3 % (w/w) of OV-225 on Chromosorb G (80 – 100 mesh) at 180 °C was used for GLC.

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A Simple Synthesis of [2.2.2.2]-Paracyclophane-1,9,17,25-tetraene by a Wittig Reaction

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Synthetic applications of the Wittig reaction are numerous, but only few deal with the synthesis of macrocyclic compounds, often by multistep sequences [1] or *via* low-yield reactions.[2,3] The major difficulty lies in the control of the *cis/trans* ratio at the double bonds formed in the Wittig reaction.

[2.2.2.2]Paracyclophanes can be prepared, together with smaller and larger paracyclophanes, from α,α′-dichloro-*p*-xylene and sodium.[4,5] A more general method enabling the introduction of a variety of substituents is desirable. Previous attempts to use a Wittig reaction gave only trace amounts of [2.2.2.2]-paracyclophanetetraenes.[3] We now wish to report a one-step synthesis of [2.2.2.2]paracyclophane-1,9,17,25-tetraene, or, systematically, pentacyclo[20.2.2.2$^{4-7}$.2$^{12-13}$.2$^{16-19}$]-dotriaconta-2,4,6,8,10,12,14,16,18,20,22,24,25,-27,29,31-hexadecaene, in a 15 % yield from terephthalaldehyde and the *bis*-triphenylphosphonium salt from α,α′-dibromo-*p*-xylene, Scheme 1.

Low reaction temperature favours ring formation, whereas high temperature favours polymerization. Addition of lithium iodide to the reaction mixture does not increase the yield.

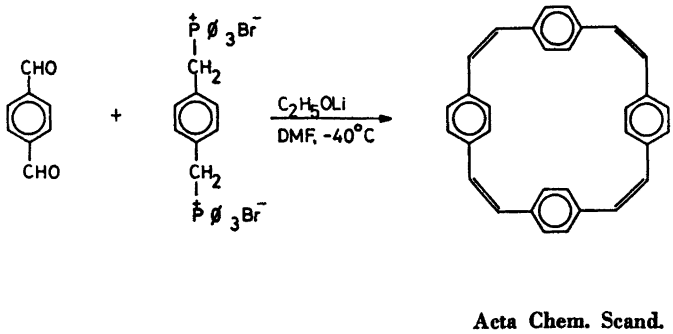
[2.2.2.2]Paracyclophane-1,9,17,25-tetraene is a yellow solid melting at 245 – 247 °C. Its NMR spectrum shows only two sharp singlets, demonstrating the equivalence of the aromatic hydrogens, as well as of the olefinic hydrogens. In contrast to [2.2.2.2]paracyclophane, the tetraene does not form any charge-transfer complex with tetracyanoethylene.

This new Wittig reaction makes it possible to study the chemistry of [2.2.2.2]paracyclophane-1,9,17,25-tetraene and substituted analogues, possible precursors for circulenes and new cage compounds.

Experimental. *[2.2.2.2]Paracyclophane-1,9,-17,25-tetraene.* To a solution of terephthalaldehyde (1.34 g, 10 mmol) and the *bis*-triphenylphosphonium salt from α,α′-dibromo-*p*-xylene (7.9 g, 10 mmol) [1] in dry dimethylformamide (200 ml) was added dropwise a solution of lithium (0.16 g, 22 mmol) in ethanol (50 ml). The mixture was vigorously stirred at −40 °C under nitrogen. The rate of addition was adjusted to maintain a faint red colour of the solution. The addition required 15 h. Water (200 ml) was added and the solution extracted with ether (3 × 150 ml). Evaporation of the solvent gave a yellowish oil which was purified by chromatography on silica gel with dichloromethane as solvent. The first fraction gave yellow needles (300 mg, 15 %), melting at 245 – 247 °C after recrystallization from carbon tetrachloride, identified as [2.2.2.2]paracyclophane-1,9,17,25-tetraene. (Found: C 93.7; H 5.90. Calc. for $C_{32}H_{24}$: C 94.1; H 5.92). MS: exact mass. found 408.1885, calc. 408.1878, *m/e*; 408 (M^+, 100 %), 204 (M^{++}, 5 %). NMR: sharp singlets at $\delta = 6.45$ (8 H) and $\delta = 7.32$ (16 H). UV: λ_{max} at 303 nm, log $\varepsilon = 4.76$. IR: 1603 m, 1507 m, 1424 m, 891 s, and 835 s cm^{-1}.

On gradient-sublimation of the crude product from the Wittig reaction, a small amount (total yield 1 %) of a less volatile product was obtained in addition to [2.2.2.2]paracyclophanetetraene. Its mass spectrum is consistent with a [2.2.2.2.2.2]paracyclophanehexaene; *m/e* 612 (M^+, 100 %) and 306 (M^{++}, 10 %). The IR spectrum indicates the presence of *trans*-double bonds, 970 cm^{-1} (s).

[2.2.2.2]Paracyclophane. [2.2.2.2]Paracyclophane-1,9,17,25-tetraene (408 mg, 1 mmol) was dissolved in benzene (100 ml) and hydrogenated at atmospheric pressure with palladium on charcoal as a catalyst. Four mmol of hydrogen was consumed in 120 min. Filtration and evaporation of the solvent gave, after recrystallization from acetic acid, [2.2.2.2]paracyclophane (366 mg, 90 %) melting at 180 – 181 °C (lit. 179 – 182 °C).[4,5] MS: exact mass,



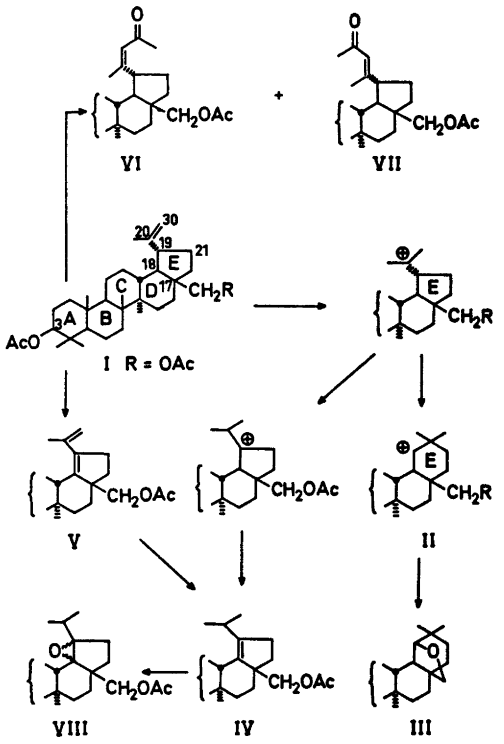
Scheme 1.

found 416.248; calc. for $C_{32}H_{32}$, 416.250; *m/e* 416 (M^+, 58 %), 311 (14 %), 207 (72 %) and 104 (100 %). NMR: singlets at $\delta = 6.72$ (16 H) and $\delta = 2.85$ (16 H).

Acknowledgements. We thank prof. Martin Nilsson for his kind interest and the Swedish Natural Science Research Council for financial support.

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Triterpenes. A Novel Acid Catalysed Double Bond Migration in 3β,28-Diacetoxy-lup-20(30)-ene (Betulin Diacetate)

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The acid catalysed rearrangement of $\Delta^{20(30)}$-derivatives in the lupane series has been reported [1-3] to proceed *via* the secondary carbocation (II). Subsequent reactions are highly dependent on the reaction conditions and on the nature of the function R at C-28. The common feature of the end products is the enlargement of the five-membered ring E to six-membered.

The acid catalysed rearrangement of betulin diacetate (I) has been performed by Vystrčil *et al.*[2] in H_2SO_4-AcOH-benzene mixture and by Pettit *et al.*[3] in HCl-EtOH-$CHCl_3$ mixture. Both reactions lead to the allobetulin ring system (III) with a six-membered ring E.

When we carried out the isomerisation of betulin diacetate (I) with HBr in AcOH-Ac_2O-benzene solution, another reaction pathway was observed. Instead of breaking the bond between C-19 and C-21, the hydrogen at C-19 was shifted, and the double bond migration product, Δ^{18}-isomer (IV), was obtained. The Δ-[18]structure (IV) was confirmed by its identity (TLC, IR, ^{1}H NMR, MS, m.p., mixed m.p., $[\alpha]_D$) with an authentic sample prepared by PtO_2-catalysed hydrogenation [4] of the mercuric acetate dehydrogenation product (V) of betulin diacetate (I). In addition, compounds (IV), obtained either by hydrogenation of diene (V) or by isomerisation of betulin diacetate (I), gave identical epoxides (VIII) with *m*-chloroperbenzoic acid.

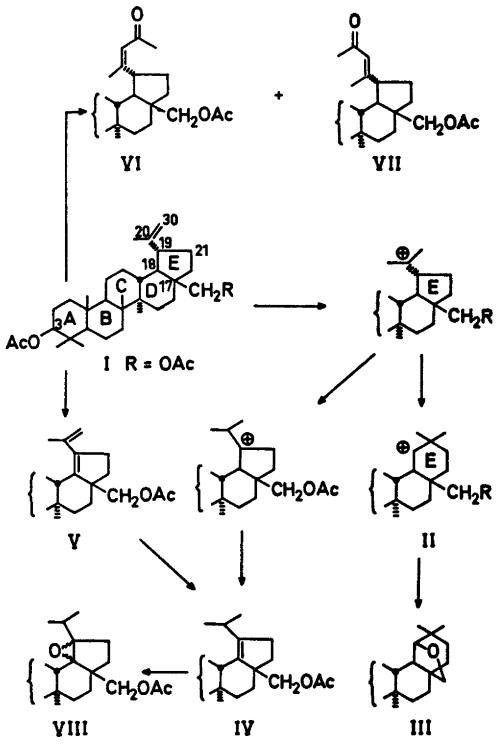
Increasing concentrations of both HBr and AcOH cause a more rapid reaction. Acetic anhydride addition gives a milder reaction but results in the formation of an *E/Z*-mixture of ketones (VI) and (VII), due to the addition of CH_3CO^+ to the double bond of betulin diacetate (I) followed by loss of a proton. Below 10 % HBr the reaction may demand months, while above 14 % HBr and 40 % AcOH all the starting material has reacted in a few hours or less and increasing amounts of less polar side products are formed. These side products were also obtained, when the Δ^{18}-isomer (IV) was treated with HBr in AcOH-Ac_2O-benzene solution. The ^{1}H NMR-spectrum of this mixture

found 416.248; calc. for $C_{32}H_{32}$, 416.250; *m/e* 416 (M^+, 58 %), 311 (14 %), 207 (72 %) and 104 (100 %). NMR: singlets at $\delta = 6.72$ (16 H) and $\delta = 2.85$ (16 H).

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Increasing concentrations of both HBr and AcOH cause a more rapid reaction. Acetic anhydride addition gives a milder reaction but results in the formation of an *E/Z*-mixture of ketones (VI) and (VII), due to the addition of CH_3CO^+ to the double bond of betulin diacetate (I) followed by loss of a proton. Below 10 % HBr the reaction may demand months, while above 14 % HBr and 40 % AcOH all the starting material has reacted in a few hours or less and increasing amounts of less polar side products are formed. These side products were also obtained, when the Δ^{18}-isomer (IV) was treated with HBr in AcOH-Ac_2O-benzene solution. The ^{1}H NMR-spectrum of this mixture

lacks the protons of the $-CH_2OAc$ side chain at C-17.

The isomerisation of betulin diacetate (I) with *p*-toluenesulfonic acid in AcOH-Ac_2O-benzene solution gave as main products the preparatively inseparable *E/Z*-mixture of ketones (VI) and (VII), and the Δ^{18}-compound (IV).

Experimental. Melting points are uncorrected PMR spectra were recorded on a Varian A-60 spectrometer in $CDCl_3$ and related to internal TMS, the IR spectra on a Perkin-Elmer 125 spectrometer using KBr pellets, mass spectra on a Perkin-Elmer 270B mass spectrometer, specific rotations in $CHCl_3$ solution on a Perkin-Elmer 141 polarimeter, and elemental analyses with a F & M 185 CHN-analyser.

Isomerisation of betulin diacetate (I) with HBr. (a) Betulin diacetate (1.0 g) in benzene (29.4 g) was added to a mixture of Ac_2O (15.0 g), AcOH (15.5 g), and 36.5 % HBr in AcOH (24.8 g) (resulting in 10.5 % HBr and 36.5 % AcOH) and allowed to stand for two weeks at room temperature. The reaction was followed on silica plates impregnated with 10 % $AgNO_3$. The mixture was washed with water and $NaHCO_3$-solution, dried and the solvent evaporated. Crystallisation from EtOH gave 3β,28-diacetoxy-lup-18-ene (IV) (0.87 g), m.p. 215 °C, $[\alpha]_D$ +16° (*c* 1.0) (Ref. 4, m.p. 211 °C, $[\alpha]_D$ +15°), mixed m.p. with authentic sample [4] 215 °C. M^+ 526; δ 4.45 (1 H, m), 4.0 (2 H, br.s), 3.15 (1 H, m), 2.1–2.7 (4 H, m), 0.83–1.07 (group of 7 Me).

(b) Betulin diacetate (1.0 g) in benzene (36.5 g), Ac_2O (15.0 g), AcOH (8.0 g), and 36.5 % HBr in AcOH (32.5 g) (resulting in 12.8 % HBr and 30.3 % AcOH) was treated as above for 18 h. Work up and chromatography [silica gel dry column and 1:3 $CHCl_3$-petroleum ether (b.p. 60–80 °C) eluent] gave a mixture of less polar side products (0.2 g) and 3β,28-diacetoxy-lup-18-ene (IV) (0.7 g).

(c) Betulin diacetate (1.0 g) in benzene (27 g), Ac_2O (25 g), 36.5 % HBr in AcOH (16.5 g) (resulting in 8.7 % HBr, 15.2 % AcOH, and 36 % Ac_2O) was treated as above for one month. According to TLC, the reaction mixture contained almost equal amounts of betulin diacetate (I), 3β,28-diacetoxy-lup-18-ene (IV), and 3β,28-diacetoxy-30-acetyl-lup-20(30)-ene *E/Z* isomers (VI + VII) characterised below.

Treatment of 3β,28-diacetoxy-lup-18-ene (IV) with HBr. 3β,28-Diacetoxy-lup-18-ene (IV) (0.2 g) in benzene (6.7 g) was added to a mixture of Ac_2O (1.0 g), AcOH (5.0 g), and 36.5 % HBr in AcOH (8.7 g) (resulting in 14.5 % HBr and 49 % AcOH) and kept for 7 h at room temperature and worked up. According to TLC, the complex mixture contained no starting material. The ^1H NMR spectrum of this mixture of unseparated compounds lacks the protons of the $-CH_2OAc$ side chain at C-17.

Treatment of betulin diacetate (I) with TsOH. Betulin diacetate (3.0 g) in benzene (30 g), AcOH (50 g), Ac_2O (10 g), and $TsOH.H_2O$ (3.0 g) was stirred for 4 days at 60 °C. The mixture was treated with water and $NaHCO_3$-solution, dried and the solvent evaporated. Chromatography [silica gel dry column and 1:2 $CHCl_3$-petroleum ether (b.p. 60–80 °C) eluent] gave 3β,28-diacetoxy-lup-18-ene (IV) (0.6 g) with physical constants identical to those given above, and 3β,28-diacetoxy-30-acetyl-lup-20(30)-ene (VI) and (VII) (1.2 g) as an inseparable mixture of (*E*)- and (*Z*)-isomers. M^+ 568; λ_{max} (EtOH) 244nm, ε 15 500; ν 1735, 1725, 1680, 1600; δ 6.15+6.0 (1 H), 4.7–3.6 (3 H, m), 2.5 (1 H, m), 2.13, 2.08, 2.04 [3 × (3 H, s)], 1.85 (3 H, br. s), 1.03, 0.96 [2 × (3 H, s)], 0.83 (9 H, s). (Found: C 75.61; H 9.96. Calc. for $C_{36}H_{56}O_5$: C 76.01: H 9.92).

Epoxidation of 3β,28-diacetoxy-lup-18-ene (IV). 3β,28-Diacetoxy-lup-18-ene (IV) (1.0 g), either from the isomerisation of betulin diacetate (I) or from the hydrogenation of diene (V), was stirred for 1 h in $CHCl_3$ (30 ml) with *m*-chloroperbenzoic acid (0.6 g) and Na_2CO_3 (1.0 g) The mixtures were treated with water, $NaHSO_3$-, and $NaHCO_3$-solutions, dried and the solvent evaporated. Crystallisations from EtOH gave 3β,28-diacetoxy-18ξ,19ξ-epoxy-lupane (VIII) (0.8 g), m.p. 222 °C, $[\alpha]_D$ +25.5° (*c* 1.7); M^+ 542; δ 4.53 (1 H, d 11 Hz), 4.42 (1 H, m), 3.88 (1 H, d 11 Hz), 1.12–1.08 (4 Me groups), 0.89–0.83 (3 Me groups). (Found: C 75.39; H 10.09. Calc. for $C_{34}H_{54}O_5$: C 75.23; H 10.03).

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Reactions between Azolium Anions and Electrophilic Reagents. I. Direct Thiation of 1,3-Disubstituted 1,2,3-Triazolium Anions with Sulfur

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Direct thiation of 1,3-disubstituted 1,2,3-triazolium tosylates *1* with elemental sulfur and sodium hydride in dimethylformamide proceeds selectively to give 1,3-disubstituted 4-(1,2,3-triazolio)sulfides *2* in high yields.

4-(1,2,3-Triazolio)sulfides *2* have been obtained previously by treatment of halogen-substituted 1,2,3-triazolium salts *3* with sulfide ions.[1]

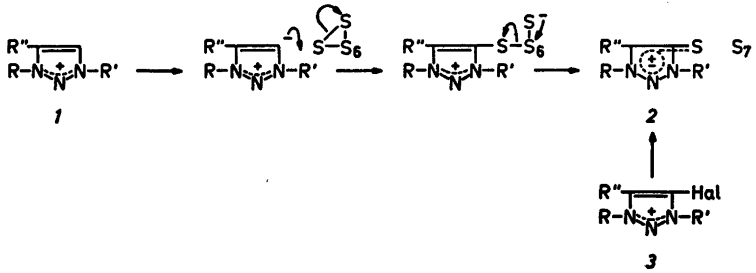
Recently, imidazolium-,[2] thiazolium-,[3] and 1,2,4-triazolium [4,5] salts have been thiated with elemental sulfur *via* their anions using pyridine or triethylamine as proton abstractors. A similar thiation of 1,3-disubstituted 1,2,3-triazolium salts *1* to give 1,3-disubstituted 4-(1,2,3-triazolo)sulfides *2** would be attractive since simple 1,2,3-triazolium salts *1* are more readily accessible than their halogen substituted derivatives *3*.

* The reaction is formulated as an ionic process analogous to the thiation of other carbanions.[6–9]

However, thiation of 1,2,3-triazolium salts *1* by treatment with sulfur, pyridine, and triethylamine proved to be inconvenient since separation of the products from the triethyl ammonium salts formed was tedious and caused loss in yield. Thiation of *1* with sulfur using other bases and media was therefore attempted. Among several systems sodium hydride in dimethylformamide was found to be the best, resulting in a simpler isolation procedure and in high yields of thiation products (Table 1). This modified thiation method is superior to the substitution process (*3*→*2*). In addition, the direct thiation method makes new types of 4-(1,2,3-triazolio)sulfides available.

Until now, halogen substituted 4-(1,2,3-triazolio)sulfides *2* (R″=Hal) have been inaccessible since treatment of dihalogen substituted 1,2,3-triazolium salts *3* (R″=Hal) with sulfide ions leads to decomposition.* The alternative halo-

* Begtrup, M. Unpublished results.



Scheme 1.

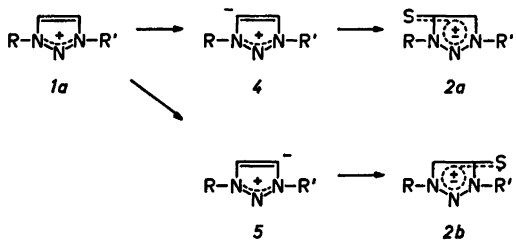
Table 1. Conversion of 1,3-disubstituted 1,2,3-triazolium tosylates into 1,3-disubstituted 4-(1,2,3-triazolio) sulfides.

Starting material *1* (or *1a*)			Ref.	Product(s) *2* (or *2a* or *2b*)			Crude product Yield %	Crude product M.p. °C	M.p. of pure compound °C	Reported m.p. °C	Analytical data Found / Calc. C %	H %	N %	S %	Hal %
R	R′	R″		R	R′	R″									
CH_3	CH_3	H	13	CH_3	CH_3	H	100	170–171	171–172[b]	172–173[1]					
$CH_2C_6H_5$	$CH_2C_6H_5$	H	10	$CH_2C_6H_5$	$CH_2C_6H_5$	H	97	95–96	122–123[c]		66.60	5.26	14.56	10.90	
											68.32	5.37	14.94	11.39	
CH_3	CH_3	Br	13	CH_3	CH_3	Br	56	158–160	162–163[b]		23.17	2.95	20.01	15.47	Br: 38.47
											23.09	2.91	20.19	15.41	38.40
$CH_2C_6H_5$	CH_3	Cl	14	$CH_2C_6H_5$	CH_3	Cl	71	134–139	141–144[b]		50.09	4.11	17.36		Cl: 14.70
											50.10	4.21	17.53		14.79
CH_3	$CH_2C_6H_5$	Cl	14	CH_3	$CH_2C_6H_5$	Cl	38	131–134	131–134[d]		49.95	4.20	17.46	13.39	Cl: 14.98
											50.10	4.21	17.53	13.38	14.79
CH_3	C_6H_5	CH_3	10	CH_3	C_6H_5	CH_3	87	148–149	148–149[d]		58.40	5.47	20.50	15.58	
											58.50	5.40	20.47	15.62	
$CH_2C_6H_5$	CH_3	H	10	$CH_2C_6H_5$	CH_3	H	32[a]	119–123	130[b]	127–129[1]					
				CH_3	$CH_2C_6H_5$	H	47[a]	132–133	132–134[d]	137–139[1]					
C_6H_5	CH_3	H	10	C_6H_5	CH_3	H	10[a]	188–191	202–204[b]	193–194[15]					
				CH_3	C_6H_5	H	73[a]	98–100	107–108[b]	112[1]					

[a] Yields isolated by column chromatography. The composition of the initial product mixture is given in Experimental. [b] Recrystallized from ethyl acetate. [c] Recrystallized from chloroform-ether. [d] Recrystallized from ethyl acetate-hexane.

genation of 4-(1,2,3-triazolio)sulfides leads to attack on the sulfur atom with formation of sulfenyl halides.[1] However, direct thiation of monochloro- or monobromo 1,2,3-triazolium salts *1* (R″=Hal) affords halogen substituted 4-(1,2,3-triazolio)sulfides *2* (R″=Hal) in good yields (Table 1).

Direct thiation of 1,2,3-triazolium salts *1a* with two different *N*-substituents gives rise to a mixture of two isomeric 4-(1,2,3-triazolio)-sulfides *2a* and *2b*.



Scheme 2.

A separate experiment indicated that *2a* (R=CH_3, R′=$CH_2C_6H_5$) does not isomerize to *2b* (R=CH_3, R′=$CH_2C_6H_5$) under the reaction conditions.

Provided that the deprotonation of *1a* is the rate limiting step, the ratio between *2a* and *2b* is determined by the relative stability of the anions *4* and *5*. This, in turn, is reflected by the ratio between the rates of base catalyzed deuterium exchange of the ring protons in the starting material *1a*. In *1a* (R=$CH_2C_6H_5$, R′=CH_3), H-4 is exchanged 1.2 times faster than H-5.[10] Thus, thiation of *1a* (R=$CH_2C_6H_5$, R′=CH_3) is expected to produce *2a* and *2b* (R=$CH_2C_6H_5$, R′=CH_3) in this ratio. The ratio found is 1.4. This agreement would be in accordance with the suggested mechanism assuming kinetic control. On the other hand, H-4 is exchanged 2.4 times faster than H-5 in *1a* (R=C_6H_5, R′=CH_3) [10] whereas thiation of *1a* (R=C_6H_5, R′=CH_3) yields *2a* and *2b* (R=C_6H_5, R′=CH_3) in the ratio 9.3. This discrepancy is incompatible with deprotonation as the rate limiting step. Hence precautions are advisable in predicting the distribution of thiation products, based on deuterium exchange measurements on the starting material.

EXPERIMENTAL

Column chromatography was carried out as described previously.[11] Melting points are uncorrected. NMR spectra were obtained on a Varian A-60 instrument. IR spectra were measured in potassium bromide pellets. The purity of all compounds were checked by TLC.

General procedure for the preparation of 1,3-disubstituted 4-(1,2,3-triazolio) sulfides *2*.

Equimolar amounts of 1,3-disubstituted 1,2,3-triazolium tosylate *1* and sulfur (S_8) were dried carefully over phosphorus pentaoxide at 0.1 mmHg. A 55 % suspension of sodium hydride in mineral oil (Fluka) was then added using 1.1 equiv. when the starting material was halogen substituted and 3 equiv. in all other cases.*

The air in the reaction flask was replaced with dry nitrogen and the mixture was cooled to −70 °C.

Dry dimethylformamide [12] (20 ml per g of sodium hydride suspension) was then added with magnetic stirring. Stirring was continued at −70 °C for ½ h. The cooling bath was then removed and the mixture was stirred overnight at room temperature. Moisture was carefully excluded in all operations. The dimethylformamide was then removed *in vacuo*, the temperature in the flask being kept below 100 °C. The residue was extracted with methanol (4 × 20 ml per 0.01 mol of starting material) and the solution was neutralized with conc. acetic acid. The methanol was evaporated and the residue was extracted with boiling ethyl acetate (4 × 40 ml per 0.01 mol of starting material). The ethyl acetate was evaporated and the residue was extracted three times with hexane (20 ml per 0.01 mol of starting material) leaving the crude product.

When an NMR-spectrum of the crude product proved that only one isomer was present purification was effected by recrystallization as described in Table 1.

When the NMR spectrum indicated that the crude product contained two isomers separation was achieved by column chromatography as described below.

1-Methyl-3-benzyl-1,2,3-triazolium tosylate 1a (R=$CH_2C_6H_5$, R′=CH_3) gave a mixture of the 4-(1,2,3-triazolio)sulfides *2a* and *2b* (R=$CH_2C_6H_5$, R′=CH_3), in the ratio 1.44:1 according to the NMR spectrum. Column chromatography (30 g of silica gel per 100 mg of mixture) eluting with ethyl acetate first gave [1-benzyl-3-methyl-4-(1,2,3-triazolio)]sulfide *2b* (R=$CH_2C_6H_5$, R′=CH_3). The second fraction contained [1-methyl-3-benzyl-4-(1,2,3-triazol-

* Use of only 1.1 equiv. of sodium hydride in the latter cases reduced the yield with up to 10 %, absolute.

Table 2. NMR spectroscopic data (δ) of 1,3-disubstituted 4-(1,2,3-triazolio)sulfides.[a]

Compound *2*	H-5	N-CH_2	N-CH_3	C-CH_3
[1,3-Dibenzyl-4-(1,2,3-triazolio)]sulfide	[b]	8.72 9.05		
[1,3-Dimethyl-5-bromo-4-(1,2,3-triazolio)]-sulfide			4.10 4.12	
[1-Methyl-3-benzyl-5-chloro-4-(1,2,3-triazolio)]sulfide		5.76	4.07	
[1-Benzyl-3-methyl-5-chloro-4-(1,2,3-triazolio)]sulfide		5.58	4.08	
[1,5-Dimethyl-3-phenyl-4-(1,2,3-triazolio)]sulfide			3.98	2.37

[a] The spectra were recorded in deuteriochloroform solution. Position of signals are given in ppm relative to tetramethylsilane (TMS) (δ-values).
[b] The signal is hidden by the phenyl group absorption.

io)]sulfide *2a* (R=$CH_2C_6H_5$, R′=CH_3). The compounds were purified further by recrystallization. Yields and details are given in Table 1.

1-Methyl-3-phenyl-1,2,3-triazolium tosylate 1a (R=C_6H_5, R′=CH_3) produced *2a* and *2b* in the ratio 9.27:1 as shown by the NMR spectrum of the crude product. Column chromatography (7.5 g of silica gel per 100 mg of mixture, ethyl acetate) afforded [1-phenyl-3-methyl-4-(1,2,3-triazolio)]sulfide *2b* (R=C_6H_5, R′=CH_3). The column was then eluted with ethyl acetate-methanol (1:1) to give [1-methyl-3-phenyl-4-(1,2,3-triazolio)]sulfide *2a* (R=C_6H_5, R′=CH_3). Yields and recrystallization media are given in Table 1.

All previously described compounds were identified through their melting point, IR, and NMR spectra. The new compounds were identified through their analyses (Table 1) and their NMR spectra (Table 2).

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Studies on the Chemistry of Lichens. XI. Chemical Investigations of Five Norwegian *Alectoria* Species

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Five Norwegian *Alectoria* species have been studied with regard to their chemical content. A mixture of higher tetrahydroxy fatty acids and 5,7-dihydroxy-6-methyl phthalide have been isolated from *A. nigricans*. Fumarprotocetraric acid was identified as the main component of *A. bicolor*. From *A. fremontii* was isolated a crystalline sterol, of the composition $C_{29}H_{48}O_2$, fremontol, for which a probable structure is proposed. A mixture of higher homologues of tetrahydroxy fatty acid alkyl esters was isolated from the same lichen. Atranorin and the aminosulfonic acid taurine, were detected in all the species investigated.

A number of species of the *Alectoria* have been chemically studied by different research workers. Recently a survey was given by Huneck and Follmann [1] concerning the secondary metabolites of 21 species of the genus *Alectoria*. Hawksworth [2–4] reported extensive investigation of the same genus. Alectorialic acid, thamnolic acid, and atranorin were reported to be the frequently occurring chemical constituents of these lichens. The excellent publications of Culberson [5–6] have been of great value to the present work.

In a previous communication from this laboratory [7] investigations on the occurrence of alectorialic and decarboxyalectorialic acid in *A. nigricans* and *Parmelia alpicola* have been reported. The structure of alectorialic acid has recently been established by Persson and Santesson.[8]

The present report describes a chemical examination of the species *Alectoria nigricans*, *A. capillaris*, *A. nadvornikiana*, *A. bicolor*, and *A. fremontii*. No attention has been given to the occurrence of sugar alcohols in the species studied.

Alectoria nigricans (Ach.) Nyl. *Investigation of the tetrahydroxy fatty acids.* The hydroxy acids were obtained as a white powder, m.p. 181–182 °C from the acetone extract of the lichen (fraction 1; found C 65.85; H 11.05; O 22.77). Analyses of hydroxy acids from five other lichen species (*Haematomma ventosum*, *Ochrolechia tartarea*, *Lecanora myrinii*, *Ramalina siliquosa*, and *Parmelia alpicola*) in our laboratory gave the average values C 65.97; H 11.01; O 22.82. On account of the close similarity that always has been found by our laboratory [9] with regard to the elemental composition and the infrared spectra of these hydroxy compounds, and on the basis of the publication of Susi,[10] it is expected that the four hydroxyl groups have the same positions (9,10,12,13) in the acid molecules in all the investigated cases.

The NMR spectra of the isolated tetrahydroxy fatty acids from *A. nigricans* in DMSO-d_6 at 73 °C, exhibited signals corresponding to methyl-protons (δ 0.87) and straight chain methylene-protons (δ 1.28).

It was found expedient to carry out investigations on the acid mixture and this sufficed to unravel the structures of the components. Four homologous species are discernible in the mass spectrum of the mixture. The interpretation of the low resolution mass spectrum has been made by comparing it with the spectra of authentic di- and tetrahydroxystearic acid. The spectrum showed series of strong peaks corresponding to ions of the empirical formula $C_nH_{2n+1}^+$, $C_nH_{2n}^+$, and $C_nH_{2n-1}^+$. Base peaks were found at masses 57(C_4H_9), 71(C_5H_{11}), and 97(C_7H_{13}), but no peak for a molecular ion.

Table 1. Masses (*m/e*) and relative abundances (%) of significant fragment-ions in the low resolution mass spectrum of tetrahydroxy fatty acid mixture isolated from *Alectoria nigricans*, $CH_3-(CH_2)_n-\underset{OH}{CH}-\underset{OH}{CH}-CH_2-\underset{OH}{CH}-\underset{OH}{CH}-(CH_2)_7-COOH$, and of 9,10,12,13-tetrahydroxystearic acid ($n=4$).

Fragment-ions	$n=4$		$n=6$		$n=7$		$n=8$		$n=9$	
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
I	101	14	129	65	143	13	157	9	171	13
I−H_2O	83	58	111	69	125	31	139	21	153	13
II	113	70	141	27	155	36	169	17	183	21
III	157	85	185	14	199	93	213	16	227	40
III−H_2O	139	43	167	12	181	21	195	9	209	13
III−$2H_2O$	121	40	149	38	163	39	177	7	191	12
IV	169	18	197	14	211	12	225	9	239	8

The most significant peaks in the spectrum of the acid mixture correspond to the fragment-ions

$CH_3(CH_2)_nCH=OH$ (I),

$CH_3(CH_2)_n-\underset{\diagdown\,/}{\overset{O}{CH-CH}}$ (II),

$CH_3(CH_2)_n-\underset{\diagdown\,/}{\overset{O}{CH-CH}}-CH_2-CH=OH$ (III),

and

$CH_3(CH_2)_n-\underset{\diagdown\,/}{\overset{O}{CH-CH}}-CH_2-\underset{\diagdown\,/}{\overset{O}{CH-CH}}$ (IV),

where n has the values of 6, 7, 8, and 9. The masses of all the different fragments and their relative abundances are listed in Table 1. The elemental composition of the fragments has been confirmed by high resolution mass spectrometry. The fragments given by 9,10,12,13-tetrahydroxystearic acid are also included in the same table.

It should be emphasized that the epoxi-structures depicted above are only formal representations.

Three important metastable ions are found in the spectrum corresponding to the following fragmentation processes:

		Metastable peak	
		Found	Calc.
(1) *m/e* 129 $\xrightarrow[-H_2O]{m^*}$ *m/e* 111		95.5	95.5
(2) *m/e* 199 $\xrightarrow[-2H_2O]{m^*}$ *m/e* 163		133.7	133.5
(3) *m/e* 181 $\xrightarrow[-H_2O]{m^*}$ *m/e* 163		146.7	146.8

The metastable ion at *m/e* 133.5 indicates that the ion *m/e* 163 is formed, in part, by a one-step loss of two molecules of water.

High resolution mass spectrometry has shown that the ion at *m/e* 99 (96 %) corresponds to the fragment

$\overset{O^+}{\underset{\diagdown\,/}{CH-CH}}-CH_2-\underset{\diagdown\,/}{\overset{O}{CH-CH_2}}$

As a result of the data reported above it must be concluded that the hydroxy fatty acids isolated from *A. nigricans* contained significant proportions of 9,10,12,13-tetrahydroxy fatty acids with chain length of C_{20}, C_{21}, C_{22}, and C_{23}. The presence of such hydroxy acids with chain length of C_{21}, C_{22}, and C_{23} in the lichens *Parmelia centrifuga* and *Haematomma ventosum* has been pointed out in an earlier publication.[9]

Alectoria capillaris (Ach.) Cromb. and *A. nadvornikiana* (Gyeln.) separately were extracted with acetone. TLC and IR of the different fractions which were obtained by working up the residues, demonstrated that the species both contained the components *atranorin*, *barbatolic acid*, *alectorialic acid*, and decomposition products of these compounds. Mass spectrometry confirms the conclusion obtained by TLC. Dominant and characteristic peaks are

found at *m/e* 194(36 %), corresponding to 5,7-dihydroxy-6-formyl phthalide (A), 176 (36 %, A−H_2O), 166 (79 %, A−CO), and 137 (34 %, A−CO−CHO).

In addition to the compounds mentioned above, *A. capillaris* is reported to contain norstictic, psoromic, and salazinic acid. These compounds have not been detected in the sample collected here.

Alectoria bicolor (Ehrh.) Nyl. A sparsely soluble acid obtained by extraction of this lichen was identified as *fumarprotocetraric acid* by comparison with an authentic sample using TLC. This conclusion was supported by IR and MS analyses of the acid. A dominant peak in the mass spectrum at *m/e* 116 corresponds to ionized fumaric acid. A metastable peak was observed at *m/e* 66.6 in the pattern of the isolated fumarprotocetraric acid. The same metastable peak by authentic acid was found at 66.6, and by authentic fumaric acid at *m/e* 66.7. The attached process is

$$m/e\ 116 \xrightarrow[-CO]{m^*(\text{calc.}=66.8)} m/e\ 88$$

Three products, A, B, and C, positive to *p*-phenylenediamine were isolated by PLC from the acetone-soluble part of the extract, Fraction 2. On the basis of colour reactions, TLC, UV, and MS analyses the products were found to consist of *fumarprotocetraric* and *decarboxythamnolic acid* (A), *decarboxyalectorialic acid* (B), and *atranorin* (C).

On further investigation of Fraction 2, an unsaturated sterol compound was isolated. TLC on silica gel with solvent C revealed a spot with R_F less than 0.20. Spraying with sulfuric acid and heating gave a gray-bluish coloured spot. Extraction of the sterol-zone from preparative plates, afforded a residue sufficient for TLC and MS analyses. The lower part of the mass spectrum revealed the characteristic sterol fragmentation known form the literature.[11] The molecular ion which corresponds to the formula $C_{28}H_{44}O_2$, was observed at *m/e* 412 (6 %). The most important observations from the mass spectrum are summarized in Table 2. Owing to the lack of sufficient material, a complete structural determination of this sterol could not be accomplished.

Alectoria fremontii (Tuck.) From a smaller sample of the lichen *vulpinic acid* was isolated by PLC on Woelm SiO_2 plates and solvent C. TLC of the isolated and authentic vulpinic acid on activated SiO_2 plates proved their identity. The solvents used were hexane-chloroform-acetone (2:3:1) and solvent C.

Table 2. Mass fragmentation of the sterol isolated from *Alectoria bicolor*. The side-chain is designated R.

m/e	%	Fragment-ion
397	3	M−CH_3
394	6	M−H_2O
383	5	M−29
379	7	M−CH_3−H_2O
376	6	M−$2H_2O$
369	3	M−C_3H_7
287	4	M−R (R=C_9H_{17})
285	5	M−(R+2H)[a]
269	8	M−R−H_2O
267	7	M−(R+2H)−H_2O
251	18	M−R−$2H_2O$
249	7	M−(R+2H)−$2H_2O$
228	100	M−R−C_3H_5−H_2O[b]
227	41	M−R−C_3H_6−H_2O[b]

[a] C_9-unsaturated side-chain. [b] Cleavage of ring D.

From the greater part of the lichen material three fractions were obtained according to solubility. TLC of the light petroleum extracted part of the residue (I) showed a complex mixture of components. Four of these components were isolated and investigated by TLC, IR, and MS.

Fremontii 1 gave a yellow colour with *p*-phenylenediamine and a faint yellow colour with sulfuric acid on the chromatograms. With solvent A the component has an R_F-value quite different from that of alectorialic, norstictic, or thamnolic acid.

Its mass spectrum showed a probable molecular peak at *m/e* 372. Peaks for M−H_2O (*m/e* 354), M−CO_2 (*m/e* 328), M−CO_2−CO (*m/e* 300), and M−CO_2−CHO (*m/e* 299) were present. The substance must be regarded as unidentified.

Fremontii 2 gave a strong yellow colour with *p*-phenylenediamine, gray-black or brown colour with sulfuric acid and heat on TLC plates. Yellow colour with KOH, and a yellow-brown colour with ferric chloride in ethanolic solution. A long series of known lichen substances have

been cochromatographed with Fremontii 2, but it is still unidentified.

Fremontii 3. By means of TLC with three different solvents and spraying with *p*-phenylenediamine, sulfuric acid and bisdiaz. benzidine, the component was identified as *atranorin.*

Fremontii 4 gave a dark blue colour with phosphomolybdic acid, and gray-green or green colour with sulfuric acid on TLC plates. Ergosterol and sitosterol, in comparison, gave brown and violet colour reactions with the same reagent, respectively. The compound was recrystallized from ethanol-water (4:1). Faint-yellow-coloured crystals, yield 3.8 mg, showed only one spot on the chromatograms and gave an unambiguous and easily interpretable mass spectrum.

The compound, for which the name *fremontol* is proposed, was soluble in conc. sulfuric acid with a strong red colour, and the solution fluoresced yellow-green or green in UV-light. An acetic anhydride solution gave a blue-bluegreen-green reaction with one drop of sulfuric acid; Liebermann-Burchard reaction. A chloroform solution gave a pale yellow colour reaction with tetranitromethane, indicating that fremontol was an unsaturated sterol.

The IR spectrum, Fig. 1, of the compound displays characteristic bands of a sterol, and exhibits marked similarity with the spectrum of ergosterol. The spectrum contains bands at 3390 and 3520 cm^{-1} corresponding to more than one hydroxyl group. The small band at 1735 cm^{-1} is a reflection of the strong hydrogen bonded hydroxyl group at 3390 cm^{-1}. The spectrum shows also olefinic unsaturation (1650, 965, and 935 cm^{-1}) and characteristic bands at 857 and 820 cm^{-1} associated with a trisubstituted double bond. It exhibits a split absorption band at 1380 cm^{-1} corresponding to δ_s $CH(CH_3)_2$.

Table 3. Mass fragmentation of *fremontol* isolated from *Alectoria fremontii.* The fragments A and B are explained in Fig. 2.

m/e	% Abundance	Fragment-ion
413	4	$M-CH_3$
411	5	$M-OH$
410	9	$M-H_2O$
397	11	$M-CH_2O$
396	31	$M-CH_3OH$
395	6	$M-CH_3-H_2O$
394	10	$M-2OH$
392	4	$M-2H_2O$
385	5	$M-CH(CH_3)_2$
378	5	$M-CH_3OH-H_2O$
377	7	$M-CH_3-2H_2O$
367	5	$M-CH(CH_3)_2-H_2O$
363	21	$M-CH_3OH-CH_3-H_2O$
287	4	M−B
271	8	$M-C_{10}H_{19}-H_2O$
269	8	$M-B-H_2O$
255	8	$M-B-CH_3OH$
253	19	$M-C_{10}H_{19}-2H_2O$
251	28	$M-B-2H_2O$[a]
239	11	$M-B-CH_3O-OH$
229	7	$M-A-H_2O$
215	9	$M-A-CH_3OH$
211	13	$M-A-2H_2O$
197	13	$M-A-CH_3OH-H_2O$

[a] Ionised nucleus.

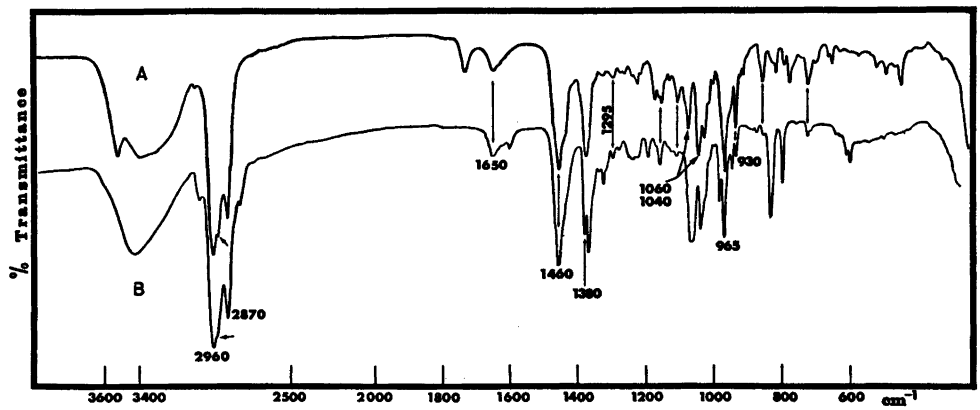


Fig. 1. Infrared spectra of fremontol (A) and ergosterol (B) isolated from *Alectoria fremontii.*

The bands at 1070 and 1040 cm^{-1} and the characteristic bands between 850 and 790 cm^{-1} may be attributed to a *3β-hydroxysteroid.*

The mass spectrum (Table 3) was typical for that of sterol, and the lower part of it was quite similar to that of cholesterol, ergosterol, and stigmasterol.[11] The lack of peak at M−72 indicated that the nucleus in the sterol molecule must have an unsaturated character; Ryhage and Stenhagen.[12] The spectrum exhibited a molecular peak at *m/e* 428 (7 %), and the sterol was assigned the formula $C_{29}H_{48}O_2$. Of value are the ions $(M-H_2O)$, $(M-2H_2O)$, $(M-H_2O-CH_3)$, and $(M-2H_2O-CH_3)$, which unambiguously indicate that fremontol must be *a diol.* Base peak in the spectrum was observed at *m/e* 69 corresponding to the fragment-ion $\overset{+}{C}H_2-CH=C(CH_3)_2$. The dominant peaks at *m/e* 396 and 363 due to the ions $(M-CH_3OH)$ and $(M-CH_3OH-CH_3-H_2O)$ should be noticed.

Probably a hydroxyl group is to be found in position C-18. Fragmentation due to the loss of CH_3OH might indicate the presence of a methyl ether. However, additional IR and MS data did not support this assumption. Among other things, neither a peak in the mass spectrum at *m/e* 381 (M−47) nor loss of methanol from the $(M-CH_3)$ fragment, was observed.

The usual peaks due to the loss of the side-chain are observed, and a peak at $M-CH(CH_3)_2$, supported interpretation of the IR band at 1380 cm^{-1}. The isopropyl group corresponds to the end of the side-chain. The peak at *m/e* 251, due to the *ionized nucleus* formed with loss of the side-chain and two molecules of water from the molecule-ion, indicates the presence of two hydroxyl groups. Other important processes caused by cleavage of the molecule as

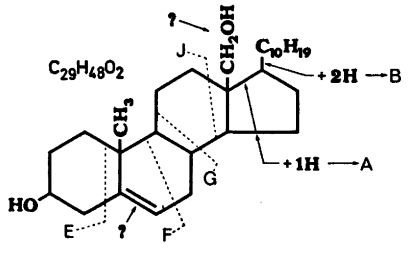


Fig. 2. Preliminary structure of fremontol isolated from *Alectoria fremontii.*

shown in Fig. 2 are found as welldefined peaks in the spectrum.

Owing to the remarkable similarity of IR and the lower part of MS of fremontol, and those of cholesterol, ergosterol, and stigmasterol, it can be assumed that fremontol has a molecular structure as drawn in Fig. 2, and belongs to the group of C_{29}-sterols. The band at 965 cm^{-1} in the IR spectrum showed the side-chain to have a double bond (*trans*) in 22−23 position; Spiteller-Friedmann and Spiteller.[11]

It is essential to underline that the fixation of the second hydroxyl group in C-18, and the nuclear double bond at 5,6-position are to some extent arbitrary, and is therefore marked with a questionmark in the figure. However, MS and IR support the postulated structure. The small yield of fremontol prevented a complete determination of the molecular structure.

The acetone-soluble product (II) gave a positive Liebermann-Burchard reaction identical with that of ergosterol. In the mass spectrum the molecular-ion was observed at *m/e* 396. The IR spectrum and the elemental analysis confirmed that the isolated compound was identical with *ergosterol.*

Water- and acetone-insoluble fraction (III). After purification of this fraction by recrystallization, the component sublimed in the temperature interval 182−185 °C. It did not give any of the usual colour reactions. The main IR absorption bands in the higher part of the spectrum were detected at 1735 (ester-carbonyl), 1468 (CH_3 and CH_2 hydrogen bending), 1175 (C−O stretch), 1062 (C−O stretch), 940 and 720 cm^{-1}. The last band was weak and unsplit. The lower part of the spectrum (Fig. 3) shows the intensity of the hydroxyl band as compared with the asymmetric carbon-hydrogen stretching band around 2900 cm^{-1}. The figure demonstrates that the chainlength of the esters in the mixture obviously must be appreciable larger than that of stearic acid.

The high sublimation interval, lack of IR signal between 1600 and 1650 cm^{-1}, and no hydrogenation in the system dioxane/PtO_2, proved that the esters were saturated.

The mass spectrum of the ester mixture has no molecular ion, which is in accordance with the observation of Ryhage and Stenhagen.[12] The fragmentation in the lower part of the

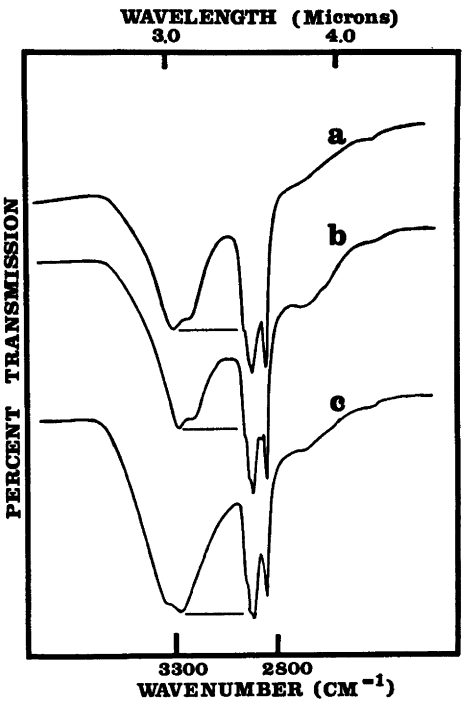
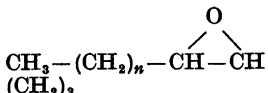
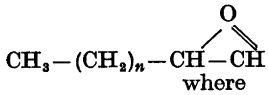


Fig. 3. Infrared spectrum of the tetrahydroxy fatty acid alkyl esters (a) isolated from *Alectoria fremontii*, compared with spectra of dihydroxystearic acid (b) and tetrahydroxystearic acid (c).

spectrum is nearly identical with the spectrum reported for the hydroxy acids of *A. nigricans*. Outstanding peaks in the spectrum of the ester mixture occur at *m/e* 213 (25 %), 199 (100 %), 185 (25 %), and 183 (50 %). Other important fragments were



where $n=6$ *m/e* 239 (26 %)
$n=7$ *m/e* 253 (63 %)
$n=8$ *m/e* 267 (18 %)



where $n=6$ *m/e* 141 (23 %)
$n=7$ *m/e* 155 (68 %)
$n=8$ *m/e* 169 (21 %)

$HO{=}CH{-}(CH_2)_x{-}COO(CH_2)_yCH_3$ $(x+y=18)$ *m/e* 341 (16 %)

The following processes were confirmed by the appropriate metastable peaks

$$m/e\ 199 \xrightarrow[-H_2O]{m^*} m/e\ 181\ (40\ \%)$$

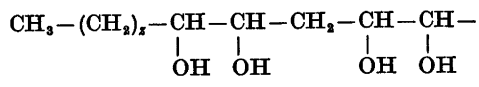
$$\xrightarrow[-H_2O]{m^*} m/e\ 163\ (65\ \%)$$

$$m/e\ 271\ (18\ \%) \xrightarrow[-H_2O]{m^*} m/e\ 253\ (63\ \%)$$

$$\xrightarrow[-H_2O]{m^*} m/e\ 235\ (38\ \%)$$

The whole series of the ions $(CH_2)_x{-}COO{-}(CH_2)_yCH_3$ $(x+y$ up to and including 12) with intensities 11 % and higher, were recognized. The peak corresponding to the case of $x+y=12$, was observed at *m/e* 227 (45 %).

Considering the IR-spectrum given in Fig. 3 and spectra reported earlier,[9] it seems probable that the esters are of relatively high molecular weights. The ratio between the contents of carbon and oxygen in the molecule itself is found to be about 4.0. This value agreed well with the molecular weight of 558, calculated for $C_{33}H_{66}O_6$. From the present results, the recrystallized fraction III most probably contains a mixture of *tetrahydroxy fatty* acid alkyl ester homologues of the general formula $C_nH_{2n}O_6$, where $n=32$, 33, and 34 are given as tentative values. The four hydroxyl groups may be located in positions as demonstrated for the molecule



$(CH_2)_x{-}COO(CH_2)_y{-}CH_3$

where z has the values of 6, 7, and 8, and $(x+y=18)$

The five *Alectoria* species were finally extracted with water and the extracts worked up as described by Solberg.[13] It is worth noting that 2-aminoethanesulfonic acid (taurine) was present in all of them and in amounts as follows: *Alectoria nigricans* 50 ppm; *A. capillaris* 196 ppm; *A. nadvornikiana* 212 ppm; *A. bicolor* 128 ppm; *A. fremontii* 171 ppm.

EXPERIMENTAL

Materials and methods. Elemental analyses were performed by Alfred Bernhardt, Mikroanalytisches Laboratorium, Elbach-BRD. The melting points have not been corrected. IR spectra were all taken in KBr pellets; NMR spectra were run in deuteriochloroform, deuterium oxide, and dimethyl sulfoxide-d_6. Chemical shifts were recorded in δ [ppm downfield from internal standard tetramethylsilane (TMS)].

The mass spectra recorded in this communication were obtained by direct sample insertion into the ion source of the mass spectrometer. Ionising energies of 70 eV were used, and the temperature of the solid heater and in the chamber was lying between 250 and 280 °C. The relative abundances are given in parenthesis.

All evaporation of extracts and solutions was performed with a rotating evaporator under reduced pressure.

Thin layer chromatography. Thin layer chromatography (TLC) and preparative layer chromatography (PLC) of the depside, depsidone, and sterol components were carried out on precoated plates (Woelm, Germany); Silica gel F 254/366 was used as adsorbant. The solvents (A, B, C) used were the same as reported by Culberson and Kristinsson.[14] The sterols were chromatographed with the solvent system chloroform-methanol (95+5).

Isolation of the lichen substances

Alectoria nigricans. The lichen (500 g) was collected on Reinøy, Troms, and extracted in a Soxhlet extractor with acetone for 12 h. A colourless solid which slowly separated from the acetone solution was collected, washed with ethanol, recrystallized twice from boiling glacial acetic acid and dried for several hours at about 80 °C; yield 0.36 g (Fraction 1). The fraction was identified as a mixture of tetrahydroxy fatty acids.

The acetone solution was concentrated to a smaller volume, and an almost colourless solid was deposited; yield 11.2 g (Fraction 2). After repeated crystallizations from diluted acetic acid and ethanol, colourless needles was obtained, m.p. 178.5 °C. The fraction was identified as alectorialic acid by TLC, IR, MS, and NMR.

The mother liquor from the acetone extract was evaporated and the residue subjected to a preliminary investigation by TLC, solvents A and C. In addition to several known components an unknown PD-negative compound, AN−X, with relatively low R_F-value was seen on the chromatograms. Preparative chromatography of the residue and repeated crystallizations from hot water and diluted acetic acid yielded 129 mg colourless needles, m.p. 224−225 °C (d) (Fraction 3). (Found: C 60.00; H 4.42; O 35.56. Calc. for $C_9H_8O_4$: C 60.00; H 4.47; O 35.52), M^+ 180. AN−X was fairly soluble in organic solvents, and one active hydrogen atom was detected by analysis.

Main IR bands: 3460, 3300, 1740, 1690, 1630, 1610 cm^{-1}. UV in ethanol gave: λ_{max} at 260 nm, λ_{min} at 237 nm.

The NMR (acetonitrile-d_3) of AN−X at 100 MHz showed a singlet at δ 2.04, due to an arylmethyl group, a two-proton singlet at δ 5.16 arising from a $C-CH_2-O$ group, and one aromatic ring proton singlet at δ 6.50. AN−X was identified as *5,7-dihydroxy-6-methyl phthalide.*

The 5,7-dimethoxy-6-methyl phthalide crystallized from methanol as colourless needles, m.p. 169.4 °C. The 4-bromo-5,7-dihydroxy-6-methyl phthalide was purified by recrystallization from dilute ethanol, giving colourless needles melting between 155 and 160 °C.

Alectoria capillaris. This lichen (37 g) was collected from spruce at Golå, Oppland and Brydal, Hedemark, 1970−1971, and extracted with acetone. The solvent was distilled off and the green coloured residue macerated on a filter with light petroleum and acetone. A sparsely soluble product was obtained (0.73 g). Colour reactions, IR, and MS were in agreement with those of barbatolic acid.

The acetone soluble part of the residue was investigated by TLC with solvents A, B, and C. The chromatograms were sprayed with *p*-phenylenediamine (PD), sulfuric acid (10 %) and bisdiazotized benzidine. 5,7-Dihydroxy-6-methyl and 5,7-dihydroxy-6-formyl phthalide both gave a dark brown colour with the benzidine reagent.

Alectoria nadvornikiana. The material used in this investigation was collected at Hauge in Dalane, Rogaland, 1969, and extracted with acetone. By concentration the solution a residue (147 mg) was obtained. The fraction was investigated by TLC with the same solvents and spraying reagents as mentioned for *A. capillaris.* Mass spectrometry confirmed the conclusions obtained by TLC.

Alectoria bicolor. The material (19.6 g) was collected at Hauge in Dalane, Rogaland, and refluxed with acetone for 2 h. The residue treated with cold acetone gave 54 mg of a sparsely soluble product which was relatively easily soluble in hot 80 % acetone. It gave a reddish brown colour with $FeCl_3$, a yellow colour with KOH, and an orange-red with PD. TLC with solvents A and C, and with butanol-acetone-water (5:1:2), followed by spraying with PD and sulfuric acid unambiguously demonstrated the presence of fumarprotocetraric acid.

The acetone-soluble part of the extract was at first investigated by TLC. Three dominant spots (A, B, C) were noticed when the chromatograms were sprayed with PD. Small amounts of these products were isolated subsequently by PLC.

Product A. The bright yellow colour with PD on TLC plates was persistent. This is an important sign as several PD-positive substances at first may produce a yellow colour which darkens more or less after a short period of time. Colour reactions with sulfuric acid was gray, dark gray or gray-black. The spot gave a bluish-white fluorescense in UV-light. Hawksworth [15] has reported decarboxythamnolic acid as having the same property.

The mass spectrum is rather complicated and in all probability the product A contained fumarprotocetraric acid and decarboxythamnolic acid.

Product B. On the basis of chromatographic behaviour, colour reactions and UV absorption in ethanol (λ_{max} 375 nm, λ_{min} 326 nm) this product was identified as decarboxyalectorialic acid. R_F-values with solvents A and C are given below:

Compound	$R_F \times 100$ Solv. A	Solv. C
Alectorialic acid	59	33
Decarboxyalectorialic acid	62	42
Substance isolated	62	42
Psoromic acid (standard)	67	43

Product C. Chromatographic properties, colour reactions with PD and sulfuric acid on TLC plates, and the UV absorption (compared with an authentic sample) established that product C is identical with atranorine.

Alectoria fremontii. This species was collected from *Picea exelsa* at Kvernmoen, Alvdal, Hedemark, 1971–1972. The material was divided into two portions, a smaller one (70 g) which was particularly rich in yellow soredia, and a greater part (1.8 kg) poor in soredia. The two portions were separately dealt with according to the usual procedure. The second and greater part of the material was refluxed with acetone for 22 h. Evaporation of the filtered extract left a dark green, amorphous, solid residue, which was divided into three fractions (I, II, III) according to solubility.

TLC of the light petroleum extracted part of the residue (I) showed a complex mixture of components. Some of these components were only present as traces and not studied. However, four components were isolated by PLC from the extract and named Fremontii 1, 2, 3, and 4. These components were purified further by a second PLC technique. Fremontii 4 was identified as a new sterol and named fremontol. The substance was chromatographed on thin layer plates with different solvents and compared with ergosterol. The R_F-values ($\times 100$) are reported below:

Solvent	$R_F \times 100$ Fremontol	Ergosterol
A	61	73
C	39	45
$CHCl_3$–MeOH(95:5)	45	60
Benzene–$CHCl_3$(1:1)	69	69

By recrystallizing the acetone-soluble product (II) of the original residue three times from diluted acetic acid, colourless and silky crystals were separated, yield 10.5 mg. (Found: C 81.97; H 11.23; O 6.26. Calc. for ergosterol, $C_{28}H_{44}O.\frac{1}{2}H_2O$: C 82.29; H 11.18; O 6.53). In day-light the product decomposes relatively fast in an acetone solution and the decomposition products were just the same as those obtained with authentic ergosterol. In the mass spectrum of the isolated ergosterol there are peaks for $(M-H_2O)$ *m/e* 378 and $(M-CH_3-H_2O)$ *m/e* 363 in addition to the molecular ion. IR of the isolated ergosterol is given on the same chart as that of fremontol (Fig. 1).

The part of the residue insoluble in cold acetone (III), was crystallized from boiling acetone, giving a faintly yellow product, yield 154 mg. (Found: C 70.91; H 11.24; O 17.72; C–CH_3 2.13. Calc. for $C_{33}H_{66}O_6$: C 70.92; H 11.90; O 17.18). The fraction was identified as a mixture of tetrahydroxy fatty acid alkyl esters.

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Cyclic Peptides of Sarcosine. Syntheses and Conformation

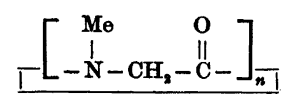
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A series of cyclic peptides of sarcosine with the general formula c-Sar$_n$, $n=2-8$, has been synthesized and conformational studies carried out both in solution and in the solid. The rings are conformationally very homogeneous and contain both *cis* and *trans* amide bonds. Their barriers to ring inversion are high; in the smaller rings this is attributed to steric hindrance, caused by the *N*-methyl-groups, whilst in the larger rings the folding of the chain in helical segments plays an important role.

Conformational studies have been reported for a large number of cyclic peptides from the simplest dipeptides to more complex biologically active molecules.[1] The majority of these investigations have been concerned with the common primary amino acids where both steric hindrance caused by the α-substituent, and hydrogen bonding, restrict the number of possible conformers, especially in the smaller rings. As we wanted to study cyclic peptides with a greater degree of freedom to take an unperturbed ring conformation, we chose sarcosine as the basic unit. In addition to excluding the aforementioned restrictions, the strong *trans* preference of the N—H amides is also nullified and therefore both *cis* and *trans* amide bonds become possible.*

A series of cyclic peptides of sarcosine with the general formula (I) has been synthesized and some results from the conformational studies communicated.[2-4]



I, $n=2, 3, 4, 5, 6, 7, 8$

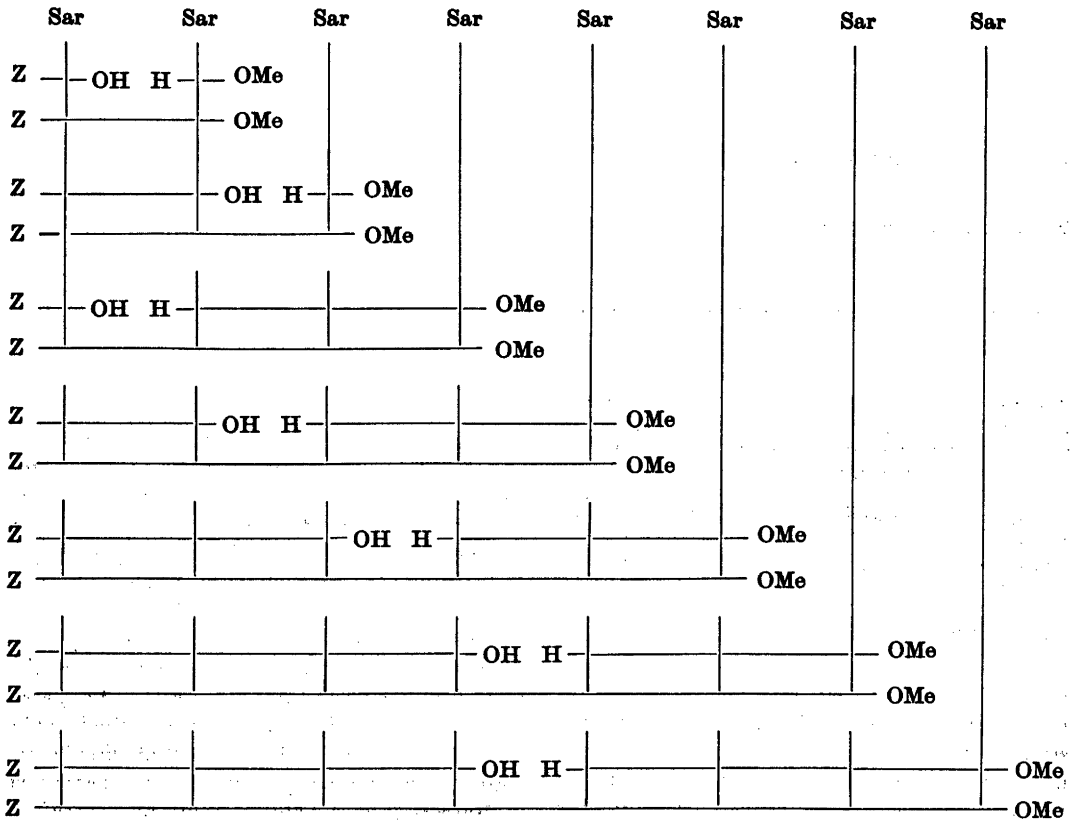
This paper describes in detail the syntheses together with additional results from the conformational investigation.

SYNTHESES

The different linear peptides were prepared by conventional methods (Scheme 1). The benzyloxycarbonyl group was used for protection of the amino function, the methyl ester for the carboxyl group and *N*,*N*′-dicyclohexylcarbodiimide as the coupling reagent. In the coupling reactions, which could all be carried out in dichloromethane, a tendency for acylurea products to form was observed but this could be minimised by using a low starting temperature (−15 °C) and by adding the carbodiimide in portions. It is well known that simple dipeptide esters readily form diketopiperazines on liberation of the amino group and therefore it was expected that molecules, with *N*-methyl substituent, would readily show this phenomenon. Indeed attempts to remove the Z-group from Z-Sar-Sar-OMe led to cyclic dimer, even in the presence of hydrochloric acid. Consequently the tripeptide was built up by coupling to the *N*-protected dipeptide acid. This complication has also been observed for larger peptides by Meienhofer,[5] who reported that the tripeptide D-valyl-L-prolyl-sarcosine splits off sarcosine and gives cyclo (D-valyl-L-prolyl) on storing, but was not encountered in the present study. Nevertheless, as a precaution all free amine peptides were used immediately on isolation.

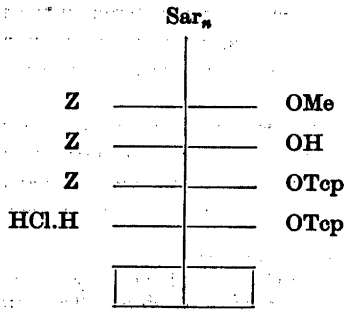
For the cyclisation (Scheme 2) the methyl ester group was removed by alkaline hydrolysis and replaced by the 2,4,5-trichlorophenyl ester group by the use of dicyclohexylcarbodiimide,

* For the assignment of stereochemistry around the amide partial double bond only the atoms of the peptide chain are considered. Thus *cis* corresponds to *E* and *trans* to *Z* for all molecules considered here.



Scheme 1.

whereafter the Z-group was removed by hydrogenolysis. The resulting peptide active ester was cyclized in pyridine using a modification of Schwyzer's method,[6] with relatively rapid addition (*ca.* 1 h) and subsequent refluxing for only 1 h. After passage through an ion-exchange column to remove non-cyclized material, the cyclic products were isolated by either vacuum sublimation or crystallisation.



Scheme 2. $n = 3,4,5,6,7,8$.

Each cyclisation reaction gave not only the corresponding cyclic compound but also other cyclic peptides of sarcosine with both larger and smaller rings. The yields which were relatively high, varied with size of the linear peptide but were unfortunately not particularly reproducible from experiment to experiment. Typical results are shown in Table 1. It can be seen that the cyclic dimer is the major byproduct in all cyclisations. The possibility that this arose *via* a transannular reaction in the cyclic products could be excluded since cyclic tri-, tetra-, and hexapeptides were recovered unchanged after heating at 150 °C for 3 h and also the tetrapeptide after boiling for 5 h in pyridine. Diketopiperazine formation was found to be very prominent during cyclisations of a series of heterodetic tetrapeptides.[7]

Table 1. Yields (%) of cyclic peptides formed upon cyclisation of the different linear peptides.

c-Sar_n $n=$	2	3	4	5	6	7	8
From HCl.H – Sar_3 – OTcp	20	*16*	1.5		10		
» HCl.H – Sar_4 – OTcp	21		*58*				3
» HCl.H – Sar_5 – OTcp	30		1	*65*			
» HCl.H – Sar_6 – OTcp	31		1		*42*		
» HCl.H – Sar_7 – OTcp	14		6			*38*	
» HCl.H – Sar_8 – OTcp	22		0.5				*55*

The yields of the cyclic dipeptide are calculated on the basis that only one molecule of cyclic dipeptide is formed per oligomer.

In some cases only cyclic dipeptides were isolated, especially where the second amino acid in the peptide chain was *N*-methylated. The mechanism was found to be the same as that reported by Meienhofer,[5] a nucleophilic attack by the free amino group on the second carbonyl carbon in the chain (Fig. 1). This removal of two units can obviously occur several times in the higher homologues; however, the yields in the table have been calculated on the basis that only one cyclic dipeptide is formed per oligomer. Minor quantities of cyclotetrasarcosyl were also isolated from every cyclisation. This probably originates from an analogous reaction but where the peptide chain is folded in such a way that the amino group comes close to the fourth carbonyl carbon, and is in accord with the observation that cyclotetrasarcosyl has a very stable conformation with a *cis,trans,cis,-trans* sequence of the amide groups. The tetramer isolated from the tripeptide reaction arises from the hexapeptide formed by doubling of the chain. No other cyclic peptides, such as tri- and pentapeptides, were formed by chain cleavage.

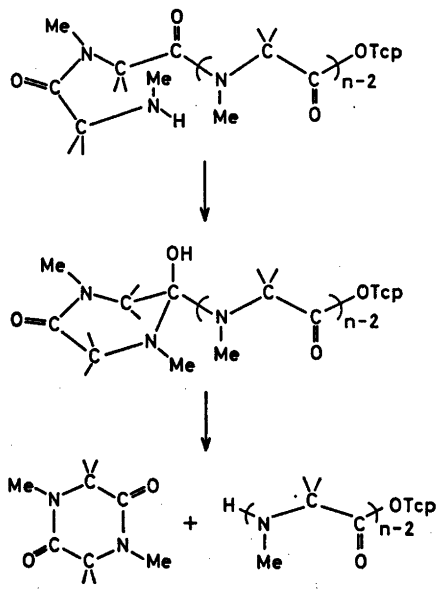


Fig. 1.

Doubling reactions are well known and are most marked for tripeptides with unsubstituted nitrogens, which normally give only cyclic hexamers. Doubling reactions were first observed with tri- and pentapeptides, and hydrogen bonding between two molecules, prior to cyclisation was suggested.[8] However, later studies with depsipeptides [9,10] and peptides which exclude hydrogen bonding [10,11] also demonstrated this phenomenon and indicated a simple coupling to the linear hexa- or decapeptide before cyclisation. Conformationally it is easier for cyclisation to occur with the larger peptides. The only exceptions where cyclic tripeptides have been found are when the amino acid is *N*-substituted and the peptides more readily allow *cis*-amide bonds. Thus only cyclic tripeptides from proline [12] and from combinations of proline and hydroxyproline [13] or sarcosine [14] have been described previously. However, even here dimerisations occurred. In the present study dimerisation was found to take place with tri-, tetra-, and penta-peptides, but the yields of doubling products were low and only the dimers from the tri- and tetrapeptides were isolable. Rothe,[14] on the other hand, has reported that cyclohexasarcosyl is formed in relatively high yield (38 %), together

Table 2. Properties of *c*-Sar$_n$.

n=	2	3	4	5	6	7	8
Sublim. temp. at 0.01 mmHg, °C	100	150	200	240	270	280	280
M.p., °C	147	221	>350	255	315	296	338
Mol ion in mass spectrum	142	213	284	355	426	497	586
C=O str.freq. IR. (cm^{-1}) in $CHCl_3$	1670	1650	1670, 1650	1675, 1640	1670, 1645[a]	1670(1650)	1675(1650)

[a] in KBr

with cyclonona- and dodecapeptide, when the tripeptide of sarcosine is cyclized using the pentachlorophenyl ester as active ester.

PROPERTIES OF THE PEPTIDES

The majority of the linear peptides remained as foamy solids. This indicates the presence of several conformers which may be due to the presence of both *cis* and *trans* amide bonds in the chain. It is possible to record the NMR spectra of the crystal conformations by dissolving the peptides at low temperature (see below), and, in fact, the crystalline tripeptide, Z-Sar-Sar-Sar-OMe, when dissolved at −70 °C, shows only one conformer containing each amide bond in a single configuration, *cis* or *trans*. On raising the temperature, the spectrum becomes more complex due to *cis-trans* isomerism of the peptide bonds. The corresponding non-crystalline heptapeptide shows a complex spectrum also when dissolved at low temperature.

The cyclic peptides (Table 2), however, are well defined solids with sharp melting points, sublimable and stable to heat, and they give the molecular ion in the mass spectrometer. They are soluble in chloroform, the cyclic di- and tripeptides also in benzene, in which solvent their dipole moments were measured. The infrared spectra show in several compounds separated carbonyl absorptions, but it was not possible to assign these unambiguously to *cis* and *trans* peptide bonds or to particular locations in the molecule.

The NMR spectra of the cyclic peptides show several common features and yielded considerable information. Different techniques were employed to aid spectral analysis and to provide information on the conformations of the individual cyclic peptides. Aromatic solvent induced shifts [15] were used to resolve overlapping signals. Dropwise addition of deuteriobenzene caused most of the signals to move to higher field to differing degrees, but did not alter the coupling constants or conformations. Surprisingly, the cyclic peptides in this series possess high conformational barriers to ring inversion which is coupled with *cis,trans* configuration exchange in the larger rings, and this enables an unambigous correlation between crystal and solution conformations. For example, a coalescence temperature of 30 °C (which is lower than for most of the cyclic peptides) will, according to the Eyring equation [16] correspond to an inversion barrier of about 15 kcal mol^{-1} and the life-time for a conformer at −70 °C will be about one hour. Whereas at the coalescence temperature the life-time of the conformer will be of the order of 10^{-3} s. Thus if the crystals are dissolved at −70 °C the spectrum can be recorded before any conformational change can take place. Furthermore, warming the sample and recording the spectrum will reveal such changes if any. This technique has previously been applied to simple amide systems.[17–18]

Since the NMR spectra could, due to the high barrier to ring inversion, be recorded below the coalescence temperature of the rings, analysis of the methylene quartets shows that the geminal coupling constants fall into two main categories, one with J of 14 Hz and the other with J of 18 Hz, the pairs of signals with the smaller coupling constants being usually more widely separated. The geminal coupling constant for a methylene group adjacent to a carbonyl is reported to depend on its relative orientation to the carbonyl group,[19] the largest value being found when the dihedral angle

of $R-C_{CO}-C_\alpha-R'$ is 0 or 180° and the smallest for 60 or 120°. The observed values in the sarcosine series indicates that the skeletal dihedral angles of $N-C_{CO}-C_\alpha-N$ display only a limited number of values and that these are repeated in all rings. This is confirmed by the crystal structure of cyclotetra-,[20] cyclopenta-,[4,21] cyclohepta-,[21a] and cyclooctasarcosyl [3,22] which have been determined by P. Groth, and which show that the $N-C_{CO}-C_\alpha-N$ dihedral angles follow an *anti-gauche* pattern.

DISCUSSION OF INDIVIDUAL CYCLIC PEPTIDES

Cyclodisarcosyl (Fig. 2) shows a simple NMR spectrum with one line for the *N*-methyl and

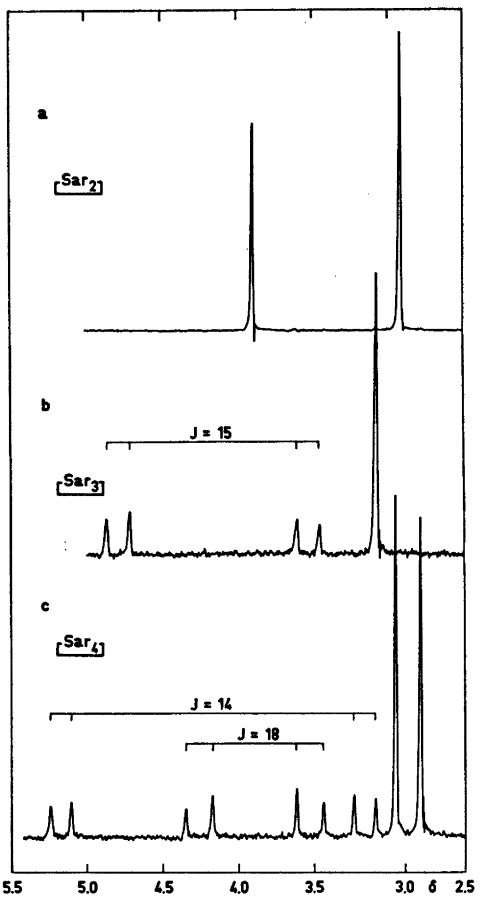


Fig. 2. The 100 MHz NMR spectrum of a, cyclodisarcosyl, b, cyclotrisarcosyl, and c, cyclotetrasarcosyl in $CDCl_3$ at 35 °C.

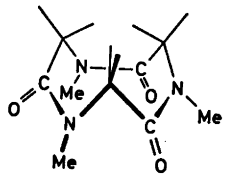


Fig. 3. The conformation of cyclotrisarcosyl.

one for the methylene protons. No broadening was observed down to −70 °C, and thus the ring is either inverting rapidly or is close to planar. The small dipole moment (1.13 D in benzene) suggests a boat type conformation in solution. The X-ray crystallographic analysis [23] revealed a flattened chair with a $CC_\alpha NC$ dihedral angle of 7° and the *N*-methyl carbons in a common plane with the amide groups.

Cyclotrisarcosyl (Fig. 2) shows one *N*-methyl signal and one methylene quartet, intensity 9:6, and the quartet coalesces to a single line at 135 °C (60 MHz, 1,2-dichlorobenzene solvent). This clearly shows that only one conformer is present in solution and that this contains only one type of amide bond. Since all in *trans* is excluded for a nine-membered ring, all must be *cis*. The coupling constant for the methylene protons is 15 Hz and is in accord with a *gauche* type dihedral angle for $C_{CO}-C_\alpha$, and the high dipole moment (4.66 D in benzene) conforms with the three carbonyl groups being on the same side of the ring. The crown conformation (Fig. 3) is the only one consistent with these data. The barrier to ring inversion,[24] (20.1 kcal mol^{-1} is in this case accidentally in the same region as for *cis-trans* isomerism of amides.

Cyclotetrasarcosyl (Fig. 2) shows two *N*-methyl singlets, intensity 6:6, and two methylene quartets, intensity 4:4, and therefore exists in one conformation in solution. By symmetry arguments a *cis*, *trans,cis,trans* configuration of the amide bonds in a centrosymmetric molecule was suggested.[3] The crystal structure [20] (Fig. 4) is virtually identical to that proposed, the dihedral angles of $N-C_{CO}-C_\alpha-N$ are 66 and 171° and correspond to the observed geminal coupling constants of 14 and 18 Hz. The NMR lines of this molecule coalesce only at a surprisingly high temperature, the CH_2-quartets start to broaden at 155 °C (60 MHz, quinoline solvent) and at

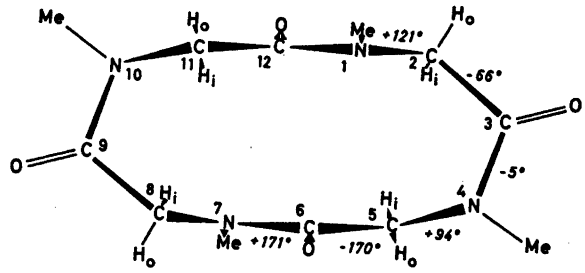


Fig. 4. The crystal conformation of cyclotetrasarcosyl.

about 200 °C the spectrum consists of a single line for the CH_2's and a single line for the *N*-methyl protons. In our preliminary communication [2] we suggested that this high resistance to ring inversion might be due to transannular interactions, such transannular interactions having been reported between the ester oxygen and ketone carbonyl in the crystal of the 10-membered-ring lactone of 9-hydroxy-6-oxononanoic acid [25] and between amino and keto-groups in some medium ring compounds.[26] However, the X-ray structure of cyclotetrasarcosyl [20] shows that the distance across the ring from carbonyl carbon to nitrogen is too large (3.08 Å) for such an interaction. Moreover, the analogous cyclic tetrapeptide, Sar-Gly-Sar-Gly, which adopts the same conformation as cyclotetrasarcosyl both in solution [27] and in the crystal [28] with the two NH-amide groups placed in the *trans* positions, has approximately the same transannular distance (3.00 Å). A longer distance was expected on replacing $N-CH_3$ with NH due to the lower basicity of the N–H. The barrier to ring inversion is now much lower (coalescence at *ca.* 20 °C). This indicates that the high barrier to ring inversion of cyclotetrasarcosyl is due more to the steric influence of the N-methyl groups.

Recently a naturally occurring cyclic tetrapeptide, tentoxin, cyclo(*N*-methyl-dehydrophenylalanyl-L-leucyl-glycyl-*N*-methyl-L-alanyl) has been found to take up an identical conformation with its two *N*-methylated amide groups in the *cis* positions.[29,29a]

Cyclooctasarcosyl (taken out of sequence since the conclusions form the basis for the discussion of cyclopenta-, cyclohexa-, and cycloheptasarcosyl) shows only one conformer in solution; four equally intense *N*-methyl lines and four CH_2-quartets (J 16 to 18 Hz) become resolved in the spectrum in chloroform (Fig. 5) on gradual addition of deuteriobenzene. The ring shows an unexpectedly high barrier to ring inversion, the CH_2-quartets begin to broaden at *ca.* 40 °C (60 MHz, chloroform solvent) and result in a sharp line together with a sharp line from the *N*-methyl protons at 120 °C, sealed tube. The solution conformation is shown to be the same as that in the solid by NMR and IR methods. Thus dissolution of the crystals in $CHFCl_2$ at −80 °C gives an NMR spectrum which does not change on warming to room temperature. Also the IR spectrum in the transparent regions of a chloroform solution is identical to that of the solid in KBr. The X-ray determination (Fig. 6) shows an open ring conformation [2,22] with a cluster of four water molecules, although the

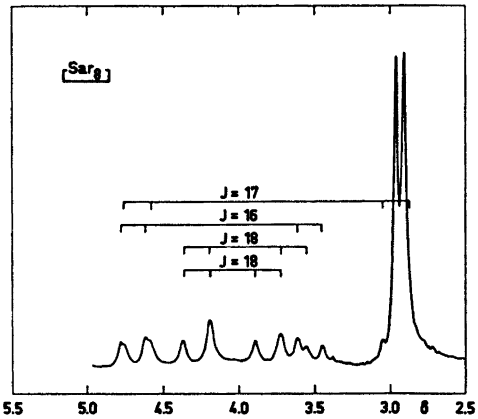


Fig. 5. The 100 MHz NMR spectrum of cyclooctasarcosyl in $CDCl_3$ (contg. a trace of TFA) at 35 °C. The splitting pattern was deduced by hexadeuteriobenzene addition and spin-decoupling.

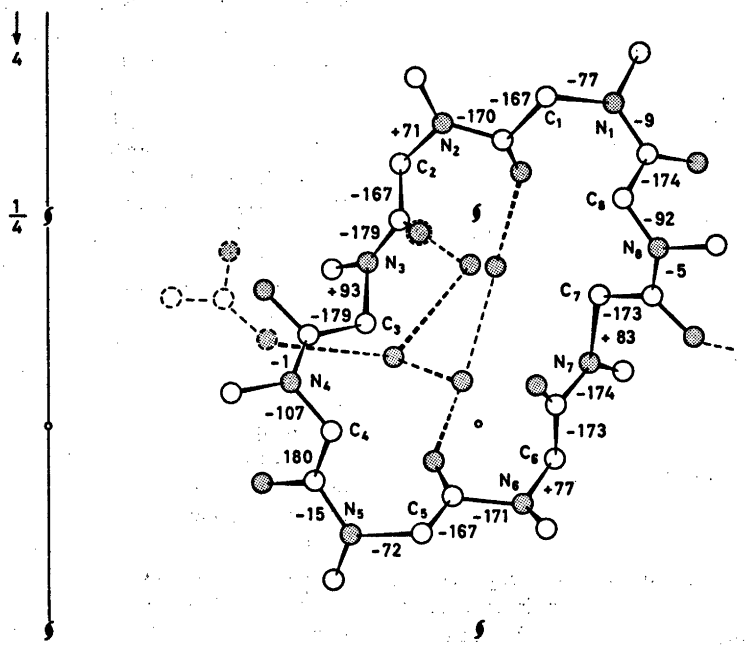


Fig. 6. The crystal structure of cyclooctasarcosyl. Open circles are carbon atoms, filled circles nitrogen (marked) or oxygen atoms. Dihedral angles refer to the ring skeleton.

crystals were grown from methanol. The molecule possesses approximate two-fold symmetry with the configuration sequence *cis,cis,-trans,trans,cis,cis,trans,trans* of the amide groups and with a deviation from planarity of up to 15°. The dihedral angles in the $N-C_{CO}-C_{\alpha}-N$ bonds are close to *anti* (between 167 and 180°) and correspond well with the large observed coupling constants, the $C_{CO}-C_{\alpha}-N-C_{CO}$ dihedral angles are relatively constants (71–107°), and moreover the peptide chain is folded in helical segments. The two *trans* portions [C(1) to N(4) and C(5) to N(8)] form a right-handed threefold screw axis with a residue repeat distance of 3.1 Å, which corresponds well to the left-handed helix found in poly-L-proline II,[30] where the amide bonds are *trans* and the translation 3.12 Å; a similar situation is found for polyglycine II.[31] The two *cis* portions [C(3) to N(6) and C(1) to N(2)] also form part of a right-handed helix with a threefold screw axis but with a shorter residue repeat distance, 2.3 Å; this resembles the helix found in poly-L-proline I[32] where the amide bonds are *cis* and the translation 1.9 Å.

This specific folding of the chain may explain the relatively high resistance to ring inversion. Transannular interactions as originally suggested were discounted when the X-ray analysis showed the transannular distance to be too great for such an interaction.

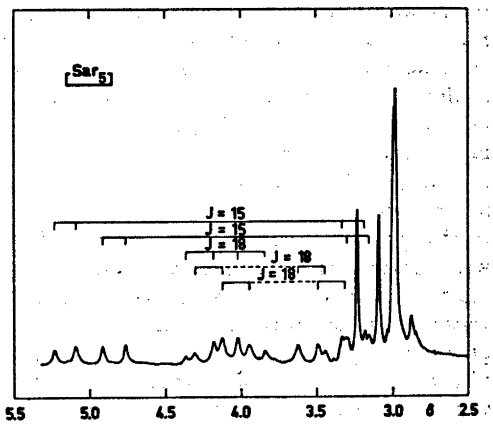


Fig. 7. The 100 MHz NMR spectrum of cyclopentasarcosyl in $CDCl_3$ at 35 °C. The splitting pattern was partially deduced from spin-decoupling and the two quartets shown by dotted lines are presumed to be as shown from the hexadeuteriobenzene addition.

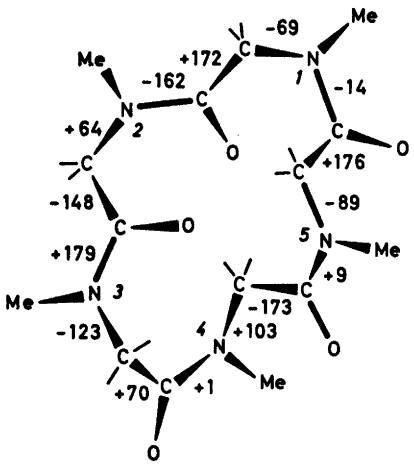


Fig. 8. The crystal conformation of cyclopentasarcosyl.

Cyclopentasarcosyl shows on dropwise addition of benzene to the chloroform solution (Fig. 7) five equally intense *N*-methyl lines and five methylene quartets, three with large coupling constant (18 Hz) and two with smaller coupling constants (14 and 15 Hz). Some weak additional lines (less than 10 %) are visible in the *N*-methyl region and may be assigned to a second conformer. Dissolution of the crystals in deuteriochloroform at −50 °C gives an NMR spectrum which shows only the lines corresponding to the dominant conformation in the room temperature spectrum. The additional signals begin to appear at about −30 °C and this confirms that these are due to a second solution conformation. The coalescence temperature is relatively high, the CH_2-quartets start to broaden at 40 °C (60 MHz, chloroform solvent) and at 130 °C the spectrum consists of a single line for the CH_2's and a single line for the *N*-methyl protons. The crystal structure [21] (Fig. 8) was determined on crystals grown from methanol but which contained two molecules of water. The *cis-trans* pattern of the amide groups was quite unexpected, with a succession of three *cis* and two *trans* where two of the *cis* and one *trans* deviate significantly from planarity, 9, 14, and 18°, respectively. Three of the dihedral angles in the $C_{CO}-C_\alpha$ bonds are close to *anti* and may be assigned to the three large coupling constants, and the two other 70 and 148° to the smaller ones. The dihedral angles in the $C_\alpha-N$ bonds vary between 64 and 123°. Again the chain is folded in helical segments and resembles those found in cyclooctasarcosyl. If the pentapeptide ring is cut open in the $C_{CO}-C_\alpha$ bond of residue 3 and the ends spread somewhat apart, the resulting chain 3,2,1,5,4 is directly superimposable on the corresponding part of the octapeptide ring skeleton (residues 3,2,1,8,7).

Cyclohexasarcosyl is less soluble in organic solvents than the others in this series. A sample for NMR can be dissolved in chloroform containing a trace of trifluoroacetic acid. The spectrum is complex with broad methylene and *N*-methyl lines and these do not become any simpler on cooling to −70 °C. On addition of benzene more than six *N*-methyl lines appear indicating that more than one conformer is present in solution. The spectrum of the crystal conformation (Fig. 9), is obtained by dissolving a sample in $CHFCl_2$ plus a trace of TFA at −80 °C, and shows three equally intense *N*-methyl signals and three CH_2-quartets with coupling constants of 18, 18, and 15 Hz, and no new lines being resolved on dropwise addition of benzene (added as a 1:1 mixture of $C_6D_6/CHFCl_2$ at −60 °C). This is consistent with either centro- or twofold-symmetry in the molecule, and the coupling constants show four of the $N-C_{CO}-C_\alpha-N$ dihedral angles to be *anti* and the other two to be smaller, which can

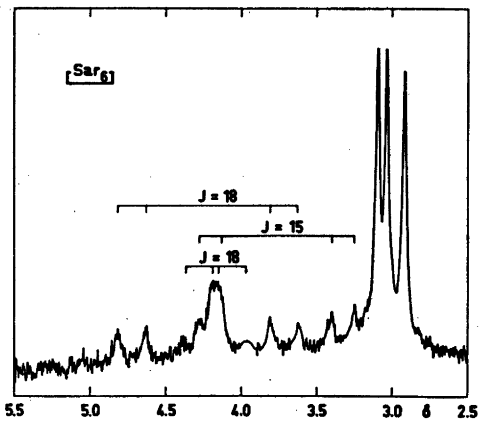


Fig. 9. The 100 MHz NMR spectrum of cyclohexasarcosyl in $CHFCl_2$ (contg. a trace of TFA) at −60 °C (dissolved at this temp.). The splitting pattern was deduced by spin-decoupling.

be accounted for by specific folding of the chain into helical segments as found for cycloocta- and cyclopentasarcosyl with either *cis,cis,-trans,cis,cis,trans* or *trans,trans,cis,trans,trans,cis* arrangement of the peptide units. On warming to about −30 °C the methylene region becomes unsharp and new *N*-methyl signals appear due to *cis-trans* interchange and development of new conformers. The coalescence of $N-CH_3$ and CH_3 take place between 50 and 100 °C and leaves one pair of singlets.

Cycloheptasarcosyl is easily soluble in $CDCl_3$ and has an NMR spectrum with broad methylene signals which do not sharpen upon cooling to −70 °C. Benzene addition resolves seven main *N*-methyl signals together with several less intense signals which indicates that one major conformer is present, together with one or more minor conformers. The NMR spectrum of the crystal conformation (Fig. 10) which is obtained in the usual way by dissolution in $CHFCl_2$ at −80 °C shows three *N*-methyl lines, intensity 9:9:3 and seven partially resolved quartets of which one has a small coupling constant (14 Hz) and the other six seem to have large coupling constants (18 Hz). No appreciable resolution of the spectrum was achieved by dropwise addition of benzene at −60 °C (added as a 1:1 mixture of $C_6D_6/CHFCl_2$). At about −30 °C new *N*-methyl lines appear and the methylene quartets begin to broaden, but two sharp lines are not obtained until 110 °C

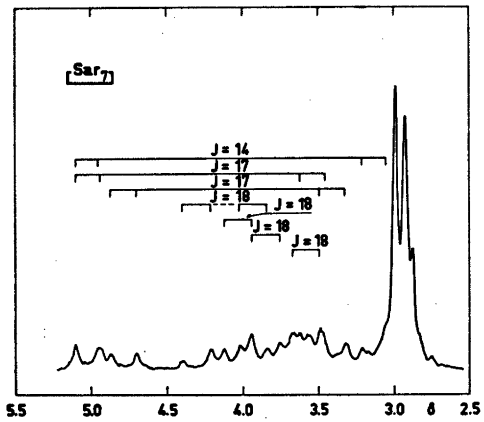


Fig. 10. The 100 MHz NMR spectrum of cycloheptasarcosyl in $CHFCl_2$ at −60 °C (dissolved at this temp.). Only limited spin-decoupling was feasible and therefore a complete interaction of signals not possible.

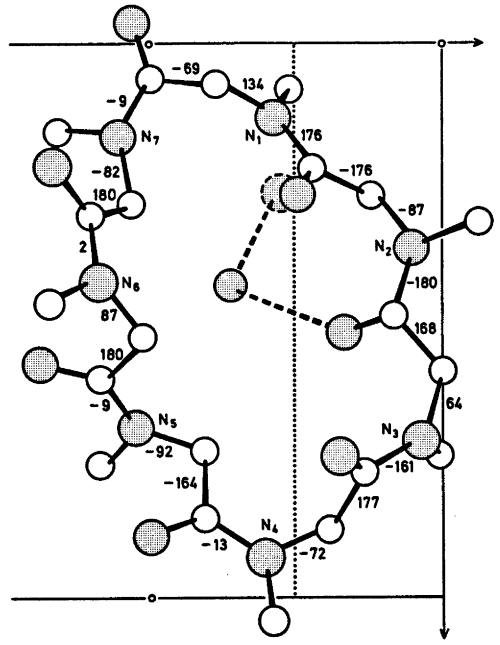


Fig. 11. The crystal structure of cycloheptasarcosyl. Open circles are carbon atoms, filled circles nitrogen (marked) or oxygen atoms. Dihedral angles refer to the ring skeleton.

(60 MHz, chloroform solution, sealed tube).

The crystal structure[21b] (Fig. 11) was determined on crystals grown from methanol but which contained one molecule of water. The *cis-trans* pattern of the amide groups was again somewhat unexpected with a succession of four *cis* and three *trans* where the largest deviation from planarity (19°) is found in a *trans* amide bond. Six of the dihedral angles in the $C_{CO}-C_\alpha$ bonds are close to *anti* (between 164 and 180°) and the seventh close to *gauche* (69°). The dihedral angles in the $C_\alpha-N$ bonds lie between 64 and 134° with the majority close to 90°. Again it appears as though the chain is folded into helical segments such that the four *cis* units form two independent similar helical fragments and two of the *trans* linked units form a third.

COMPLEXATION

Since it is well known that many cyclic compounds form complexes with alkali metal salts[33,34] some experiments were undertaken with cyclohexa-, cyclohepta-, and cyclo-

octasarcosyl to assess their complexing ability. Addition of hexamer to KNCS in methanol gave a precipitate which was insoluble in organic solvents. The NMR spectrum in D_2O was identical to that of pure hexamer, but here the complex may be expected to be largely dissociated. With the heptamer no tendency for complex formation was observed; for instance, KNCS did not dissolve in a chloroform-solution of cycloheptasarcosyl and no precipitate formed from acetone. The octamer, however, dissolved in acetone on addition of KNCS and shows after evaporation an NMR spectrum (in CD_3OD) which differs significantly from that of the free octamer, now with broad methylene lines which do not sharpen even at −60 °C. The barrier to ring inversion is also lower as the CH_2-quartets show one broad line at 30 °C (*cf.* uncomplexed at 100 °C). Apparently, the favoured conformation of cyclooctasarcosyl has been altered and now probably at least six of the carbonyl groups are orientated towards the metal atom as is found in the potassium complex of enniatin B [35] and of valinomycin.[36] The cyclic peptides were readily recovered by passing the complex through a mixed bed (strong acid and strong base) ion-exchange resin.

CONCLUSION

The high conformational homogeneity and high barriers to ring inversion found for the cyclic oligomers of sarcosine are at first sight surprising since the absence of α-substituents and hydrogen bonding, and the possibility for both *cis* and *trans* amide bonds, would be expected to result in a large number of competing conformations. This study has, hovewer, revealed a number of striking similarities between the various rings and suggests that other forces are significant in defining conformations. In the small rings steric effects due to the *N*-methyl groups seem to be dominant, but in the larger rings there appears to be a considerable tendency for residues to form helical segments. This latter effect is most clearly demonstrated in the cyclic octamer where the formation of the potassium complex lowers the barrier to ring inversion; it is virtually certain that the complexation alters the favoured conformation. It is also apparent that the three different types of bonds present in the ring skeleton ($C_{CO}-N$, $C_{CO}-C_\alpha$, and $C_\alpha-N$) take up only a limited number of orientations relative to one another, but at present it is not certain whether this is due solely to the helical influence or to some other factor. The observed dihedral angles for $N-C_{CO}-C_\alpha-N$ are either close to *anti* (180°) or *gauche* (60°), the only observed exception being that cyclopentasarcosyl has one intermediate value (148°); this is required to allow the remaining residue to form helices. The $C_\alpha-N$ bonds show a wider variation of dihedral angle but are, in the main, grouped around 90°. The amide bonds exist in both *cis*- and *trans*-forms, except in the smallest cyclo di- and trisarcosyl which are all *cis*. The maximum deviation from planarity found for the *cis*-amide group is 15° and for *trans* 19°. It is also noterworthy that all the carbonyl groups of the *cis* amide linkages are directed out of the ring whilst those from the *trans* are directed into the ring.

EXPERIMENTAL

Sarcosine was obtained from Merck Chemical Company. Solvents used in the reactions were of analytical grade, *N*,*N*-dimethylformamide and pyridine were dried over CaH_2 and distilled, the light petroleum had b.p. 40 – 60 °C. General methods of preparation are described where appropriate. Evaporations were performed in a rotary evaporator.

Several of the linear peptide derivatives were either viscous oils or noncrystalline solids. Their identity and purity were confirmed by NMR spectroscopy (Varian 60 A) and thin-layer chromatography performed on silica gel G in various solvent systems, ethylacetate-chloroform 1:2 (A), 10 % methanol-chloroform (B), 15 % methanol-chloroform (C), 20 % methanol-chloroform (D), methanol:chloroform: water 70:20:10 (E), ethanol:water:acetic acid 80:10:10 (F), detection in iodine vapor.

The cyclic peptides were separated from the linear ones by passage through a monobed ion-exchange column (Amberlite, MB-1, analytical grade), using methanol, or a mixture of methanol and water (1:1), as eluent.

Samples for elemental analysis of the methyl esters, $Z-Sar_n-OMe$, were purified on a silica gel column, eluted with chloroform followed by chloroform added increasing amount of methanol 2 – 10 %.

Purification of the acids, $Z-Sar_n-OH$, was attempted by extraction from an organic layer (dichloromethane or chloroform) into an alkaline

aqueous layer, acidification and re-extraction into an organic layer, followed by drying over $MgSO_4$ and evaporation, but with limited success. The dicyclohexylammonium salts were also prepared but in most cases without satisfactory results.

With the trichlorophenyl esters, $Z-Sar_n-OTcp$, a satisfactory purification on silica column could not be achieved due to transesterification with the methanol in the eluting solvent. Some of these esters contained dicyclohexylurea which was most readily removed after cyclisation.

Cyclotrisarcosyl

Benzyloxycarbonylsarcosylsarcosine methyl ester ($Z-Sar_2-OMe$). Sarcosine methyl ester hydrochloride[37] (28 g = 200 mmol) (prepared from sarcosine in methanol containing thionyl chloride) was suspended in dichloromethane (500 ml), triethylamine (20.5 g = 200 mmol) added and the precipitated triethylamine hydrochloride filtered off. Benzyloxycarbonylsarcosine[38] (44.5 g = 200 mmol) in dichloromethane (400 ml) was combined with the filtrate, stirred and cooled to −15 °C and dicyclohexylcarbodiimide (43.5 g = 210 mmol) added in portions over 30 min. The temperature was raised slowly to 0 °C and the reaction mixture kept in an icebath which was allowed to attain room temperature during 15 h. The precipitated dicyclohexylurea was filtered off, the filtrate diluted with dichloromethane (500 ml) and washed successively with 0.1 N HCl, water, 0.5 M $NaHCO_3$, water and finally dried over $MgSO_4$ and evaporated. The residual oil was dissolved in ether and precipitated with light petroleum to leave a viscous oil (58 g = 94 %). Homogeneous by TLC, R_F 0.3 (A), R_F 0.8 (B). 0.3 g was further purified on a silica gel column. (Found: C 58.31; H 6.49; N 8.71. $C_{15}H_{20}N_2O_5$ requires C 58.43; H 6.54; N 9.09).

Benzyloxycarbonylsarcosylsarcosine ($Z-Sar_2-OH$). To an ice-cooled, stirred solution of benzyloxycarbonylsarcosylsarcosine methyl ester (50 g = 160mmol) dissolved in a mixture of methanol (20 ml) and water (60 ml) was added 1 N sodium hydroxide (160 ml) in portions, and the solution kept at room-temperature (3 h) until the reaction was complete (TLC). The mixture was concentrated at 30 °C to *ca.* 40 ml, acidified with 2 N hydrochloric acid to pH 2 and extracted several times with dichloromethane. The organic layer was washed with a small amount of water, dried and evaporated to give an oil which was treated with ether and light petroleum. The resulted viscous oil (42 g = 88 %) was homogeneous by TLC, R_F 0.8 (F). (Found: C 56.74; H 6.30. $C_{14}H_{18}N_2O_5$ requires C 57.13; H 6.17).

The dicyclohexylammonium salt was prepared by dissolving 0.3 g acid in dichloromethane together with excess of dicyclohexylamine and evaporated. The residual oil crystallized from a mixture of acetone-ether-light petroleum to give a white solid m.p. 130 − 132 °C. (Found: C 65.64; H 8.41; N 8.87. $C_{26}H_{40}N_3O_5$ requires C 65.80; H 8.49, N 8.85).

Benzyloxycarbonyl(sarcosyl)$_2$sarcosine methyl ester ($Z-Sar_3-OMe$). Sarcosine methyl ester hydrochloride (16 g = 115 mmol) was suspended in dichloromethane (500 ml) and triethylamine (11.6 g = 115 mmol) added. The filtered solution was mixed with benzyloxycarbonylsarcosylsarcosine (33.8 g = 115 mmol), cooled to −15 °C and dicyclohexylcarbodiimide (25 g = 121 mmol) added in portions with stirring. The reaction conditions and way of isolating the protected peptide were as described for $Z-Sar_2-OMe$. The residual oil (36 g) crystallized from methanol-ether and was recrystallized from the same solvents (33 g = 75 %). M.p. 101 − 103°, TLC, R_F 0.55 (B). (Found: C 56.82; H 6.85; N 10.78. $C_{18}H_{25}N_3O_6$ requires C 56.98; H 6.64; N 11.08).

Benzyloxycarbonyl(sarcosyl)$_2$sarcosine ($Z-Sar_3-OH$). To an ice-cooled solution of benzyloxycarbonyl(sarcosyl)$_2$sarcosine methyl ester (14 g = 37 mmol) in methanol (20 ml) and water (60 ml) was added 1 N sodium hydroxide (38 ml) and kept at room temperature for 2.5 h. Worked up as described for $Z-Sar_2-OH$ to give a noncrystalline solid (11.5 g, 85 %) which softened at about 45 °C TLC, R_F 0.75 (F).

Benzyloxycarbonyl(sarcosyl)$_2$sarcosine 2,4,5-trichlorophenyl ester ($Z-Sar_3-OTcp$). To an ice-cooled and stirred solution of benzyloxycarbonyl(sarcosyl)$_2$sarcosine (2.9 g = 8 mmol) and 2,4,5-trichlorophenol (2 g = 10 mmol) in dichloromethane (50 ml) was added dicyclohexylcarbodiimide (1.8 g = 8.7 mmol) and the mixture was allowed to attain room temperature during 15 h. The precipitated dicyclohexylurea was filtered off, the filtrate evaporated and the residue treated twice with ether-light petroleum, which was decanted off, and the residue evaporated to dryness to give a white solid (4 g = 92 %), which had an NMR consistent with the structure, TLC, R_F 0.6 (B).

A sample (0.2 g) was purified on a silica gel column, eluted with chloroform followed by chloroform added 2 % methanol. (Found: C 51.00; H 4.47. $C_{13}H_{24}N_3O_6Cl_3$ requires C 50.71; H 4.44).

Cyclisation. A solution of benzyloxycarbonyl(sarcosyl)$_2$sarcosine 2,4,5-trichlorophenyl ester (2 g = 3.7 mmol) in methanol (100 ml) and concentrated hydrochloric acid (0.2 ml) was purged with nitrogen gas and 5 % palladium on charcoal (0.4 g) added. Hydrogen gas was passed through the mixture until evolution of CO_2 ceased (30 min) [tested with $Ba(OH)_2$]. The solution was filtered through Celite and evaporated at *ca.* 30 °C to yield a white solid (1.45 g = 88 %).

Part of the resulting (sarcosyl)$_3$sarcosine 2,4,5-trichlorophenyl ester hydrochloride (0.65 g = 1.46 mmol) in dimethylformamide (50 ml) was added dropwise under vigorous stirring to pyridine (500 ml) at 115 °C over a period of 2 h and stirred for another 1 h. After evaporation the residue was dissolved in methanol-water (1:1) (50 ml), passed through an ion-exchange column and eluted with methanol-water (only methanol can also be used). The first eluate (200 ml), which contained most of the cyclic peptides, was evaporated and the residue taken into methanol (5 ml). Undissolved material was filtered off (3 mg) which appeared to be pure cyclotetrasarcosyl. The filtrate was evaporated, acetone (5 ml) added and kept in a refrigerator overnight. The precipitate which now mostly contained cyclodisarcosyl together with some cyclohexasarcosyl was filtered off, the residue after evaporation washed with ether and sublimed in a glasstube at reduced pressure and gradually raising the temperature.[39] The different precipitations were then sublimed separately. Yields of isolated cyclic peptides: Cyclotrisarcosyl (16 %) sublim.temp. 150 °C/0.01 mmHg, m.p. 221 °C, TLC, R_F 0.5 (C), *m/e* 213. (Found: C 50.61; H 6.94; N 19.84. $C_9H_{15}N_3O_3$ requires C 50.69; H 7.09; N 19.71) and in addition cyclodisarcosyl (20 %), cyclotetrasarcosyl (1.5 %) and cyclohexasarcosyl (9.3 %).

Cyclotetrasarcosyl

Benzyloxycarbonyl(sarcosyl)$_3$sarcosine methyl ester (Z-Sar$_4$-OMe). Benzyloxycarbonyl(sarcosyl)$_3$sarcosine methyl ester (20 g = 528 mmol) in methanol (500 ml) was purged with nitrogen gas and 5 % palladium on charcoal (2 g) added. Hydrogen was bubled through the mixture (3 h), the product filtered through Celite and evaporated. The residual oil, (sarcosyl)$_2$sarcosine methyl ester (12 g = 49 mmol = 93 %) was examined on NMR and TLC and without storing dissolved together with benzyloxycarbonylsarcosine (11 g = 49 mmol) in dichloromethane (200 ml), cooled to −15 °C and dicyclohexylcarbodiimide (12 g = 58 mmol) added. The reaction conditions and way of isolation were as described for Z-Sar$_2$-OMe, and resulted in a viscous foam (19 g = 86 %) of the protected tetrapeptide. The NMR was consistent with the structure, and homogeneous by TLC, R_F 0.35 (B), R_F 0.75 (C).

Benzyloxycarbonyl(sarcosyl)$_3$sarcosine (Z−Sar$_4$−OH). Benzyloxycarbonyl(sarcosyl)$_3$sarcosine methyl ester (11 g = 25.2 mmol) was hydrolysed in methanol (20 ml) and water (40 ml) with 1 N sodium hydroxide (26 ml) for 2.5 h in the same way as described for Z−Sar$_2$−OH. After evaporation and acidification the aqueous layer was extracted with chloroform and worked up to give a white foam (9.5 g = 87 %) which displayed appropriate NMR sprctrum and TLC, R_F 0.65 (F).

Benzyloxycarbonyl(sarcosyl)$_3$sarcosine 2,4,5-trichlorophenyl ester (Z−Sar$_4$−OTcp). Benzyloxycarbonyl(sarcosyl)$_3$sarcosine (2.2 g = 5 mmol), 2,4,5-trichlorophenol (1.5 g = 7.6 mmol) and dicyclohexylcarbodiimide (1.2 g = 5.8 mmol) in dichloromethane (50 ml) were reacted together for 15 h as for Z−Sar$_3$−OTcp. The work up gave a white non-crystalline solid (2.9 = 94 %), one main spot on TLC, R_F 0.45 (B), R_F 0.8 (D) with traces of DC-urea.

Cyclisation. Benzyloxycarbonyl(sarcosyl)$_3$-sarcosine 2,4,5-trichlorophenyl ester (1.9 g = 3.1 mmol) in methanol (200 ml) and hydrochloric acid (0.15 ml) was hydrogenated over 5 % palladium on charcoal (0.3 g) for 1 h and worked up to yield a white solid (1.4 g = 2.7 mmol = 88 %) of (sarcosyl)$_3$sarcosine trichlorophenyl ester hydrochloride. This was dissolved in dimethylformamid (50 ml) and added dropwise to stirred pyridine (700 ml) at 115 °C over a period of 1 h, and stirred for another 1 h. After evaporation the residue was taken into methanol (10 ml) and undissolved material filtered off (380 mg), this appeared to be pure cyclotetrasarcosyl. The filtrate was diluted with methanol (100 ml), passed through an ion-exchange column and eluted with methanol. The first eluate (250 ml) contained most of the formed cyclic peptides while the next eluate (400 ml) also contained some dicyclohexylurea (20 mg). This was easily removed by sublimation (150 °C/0.01 mmHg) when the different cyclic peptides were isolated. Yields of cyclic compounds: Cyclotetrasarcosyl (58 %), sublim. temp. 200°/0.01 mmHg, m.p. > 350°, TLC, R_F 0.6 (C), *m/e* 284. (Found: C 50.76; H 6.96; N 19.94. $C_{12}H_{20}N_4O_4$ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (21 % calc. from one cyclisation per chain) and cyclooctasarcosyl (3 %).

Cyclopentasarcosyl

Benzyloxycarbonyl(sarcosyl)$_4$sarcosine methyl ester (Z−Sar$_5$−OMe). Benzyloxycarbonylsarcosylsarcosine (6.5 g = 22.1 mmol), (sarcosyl)$_2$-sarcosine methyl ester (5.5 = 22.4 mmol) and dicyclohexylcarbodiimide (4.9 g = 23.8 mmol) in dichloromethane (50 ml) were reacted together and worked up as described earlier. The isolated foam (9.8 g = 86 %) displayed appropriate NMR spectrum and one spot on TLC, R_F 0.35 (B) R_F 0.6 (D). An analytical sample (0.3 g) was purified on a silica gel column. (Found: C 54.78; H 6.98; N 14.07. $C_{24}H_{35}N_5O_8$ requires C 55.26; H 6.76; N 13.43).

Benzyloxycarbonyl(sarcosyl)$_4$sarcosine (Z−Sar$_5$−OH). Benzyloxycarbonyl(sarcosyl)$_4$sarcosine methyl ester (7.8 g = 14.9 mmol) in methanol (10 ml) and water (30 ml) was hydrolysed with 1 N sodium hydroxide (16 ml) in the usual way and worked up by concentrating to ca 20 ml, acidifying with 2 N

hydrochloric acid and extracting with chloroform. The acidic aqueous layer was then evaporated nearly to dryness and extracted again with chloroform, dried and evaporated. The two portions gave a solid (6.8 g = 90 %) which softened at *ca.* 75 °C, TLC R_F 0.55 (F).

Benzyloxycarbonyl(sarcosyl)$_4$sarcosine 2,4,5-trichlorophenyl ester (Z−Sar$_5$−OTcp). Benzyloxycarbonyl(sarcosyl)$_4$sarcosine (2 g = 4 mmol), 2,4,5-trichlorophenol (1 g = 5 mmol) and dicyclohexylcarbodiimide (0.9 g = 4.4 mmol) in dichloromethane (30 ml) were reacted in the usual way and the work up resulted in a white foam (2.3 g = 86 %), one main spot on TLC, R_F 0.7 (D) with traces of DC-urea. Purification on a silica gel column afforded the analysis. (Found: C 51.15; H 5.24. $C_{29}H_{34}N_5O_8Cl_3$ requires C 50.70; H 4.99).

Cyclisation. Benzyloxycarbonyl(sarcosyl)$_4$sarcosine 2,4,5-trichlorophenyl ester (1.3 g = 1.9 mmol) in methanol (100 ml) and hydrochloric acid (0.1 ml) was hydrogenated over 5 % Pd-C (0.3 g) for 1 h and worked up to give a solid (1 g = 1.7 mmol = 90 %) of the (sarcosyl)$_4$-sarcosine trichlorophenyl ester hydrochloride. This was dissolved in dimethylformamide and added dropwise to stirred pyridine (600 ml) at 115 °C over a period of 1 h and stirred for 1.5 h more. After evaporation the residue was dissolved in methanol (70 ml) and ion-exchanged. The first eluate (200 ml) was evaporated, the residue taken into acetone (10 ml) and undissolved material filtered off (200 mg) which on TLC and by sublimation was found to be almost pure cyclopentasarcosyl with traces of cyclotetrasarcosyl. The next eluate (600 ml) contained some DC-urea together with cyclic peptides. This was evaporated and the residue purified on a silica gel column eluted with chloroform followed by addition of methanol 2 − 10 %. The cyclic peptides may also be isolated by sublimation.

Yields of cyclic compounds: Cyclopentasarcosyl (65 %), sublim.temp. 240 °C/0.01 mmHg, m.p. 255 °C, TLC, R_F 0.4 (C), *m/e* 355. (Found: C 50.66; H 7.21; N 19.57. $C_{15}H_{25}N_5O_5$ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (30 %, calc. from one cyclisation per chain) and cyclotetrasarcosyl (1 %), some cyclic decapeptide was formed, but could not be isolated by these methods.

Cyclohexasarcosyl

Benzyloxycarbonyl(sarcosyl)$_5$sarcosine methyl ester (Z−Sar$_6$−OMe). Benzyloxycarbonyl(sarcosyl)$_2$sarcosine (6 g = 16.4 mmol), (sarcosyl)$_2$-sarcosine methyl ester (4.1 g = 16.7 mmol) and dicyclohexylcarbodiimide (3.7 g = 18 mmol) in dichloromethane (120 ml) were reacted together and worked up as described earlier. This gave a white powder (8.5 g = 87 %) m.p. 77 °C homogeneous by TLC R_F 0.65 (D). An analytical sample (0.3 g) was purified on a small silica gel column. (Found: C 54.75; H 7.13; N 14.01. $C_{27}H_{40}N_6O_9$ requires C 54.72; H 6.81; N 14.18).

Benzyloxycarbonyl(sarcosyl)$_5$sarcosine (Z−Sar$_6$−OH). Benzyloxycarbonyl(sarcosyl)$_5$sarcosine methyl ester (4 g = 6.7 mmol) in methanol (10 ml) and water (20 ml) was hydrolysed with 1 N sodium hydroxide (7 ml) for 2 h and worked up as described for Z−Sar$_5$−OH to yield a white amorphous powder (3.4 g = 87 %), softened at 98 °C, TLC, R_F 0.4 (F).

Benzyloxycarbonyl(sarcosyl)$_5$sarcosine 2,4,5-trichlorophenyl ester (Z−Sar$_6$−OTcp). Benzyloxycarbonyl(sarcosyl)$_5$sarcosine (2.7 g = 3.6 mmol), 2,4,5-trichlorophenol (1.1 g = 5.6 mmol) and dicyclohexylcarbodiimide (0.85 g = 4.1 mmol) in dichloromethane (60 ml) were reacted together and worked up as described earlier. The resulting white powder (3 g = 83 %) showed one main spot on TLC, R_F 0.8 (D) with traces of DC-urea.

Cyclisation. Benzyloxycarbonyl(sarcosyl)$_5$sarcosine 2,4,5-trichlorophenyl ester (1.7 g = 2.6 mmol) in methanol (120 ml) and hydrochloric acid (0.1 ml) was hydrogenated over 5 % Pd-C (0.4 g) for 50 min and worked up to give a white solid (1.2 g = 81 %) of the (sarcosyl)$_5$sarcosine trichlorophenyl ester hydrochloride. Part of it (0.6 g = 0.91 mmol) was dissolved in dimethylformamide (50 ml) and added dropwise to stirred pyridine (500 ml) at 115 °C over a period of ½ h and stirred for another 1 h. The residue after evaporation was dissolved in methanol (100 ml), ion-exchanged and the residue after evaporation taken into acetone (10 ml), filtered (130 mg) and the filtrate kept in a refrigerator overnight and filtered (10 mg). The precipitates contained mainly cyclohexasarcosyl with traces of cyclotetrasarcosyl. The solution was evaporated and the solid treated with ether and sublimed until the temperature was raised to 230 °C. The cyclic hexapeptide sublimes at 270 °C, but at this temperature the residue tends to decompose. It is therefore better to dissolve the residue in acetone, precipitate with ether and to crystallize the remaining cyclohexasarcosyl from methanol. Yields of cyclic compounds: Cyclohexasarcosyl (42 %), sublim.temp. 270 °C/0.01 mmHg, m.p. 315 °C, TLC, R_F 0.4 (D), *m/e* 426. (Found: C 50.67; H 7.45; N 19.80. $C_{18}H_{30}N_6O_6$ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (31 %, calc. one cyclisation per chain) and cyclotetrasarcosyl (0.8 %).

Cycloheptasarcosyl

Benzyloxycarbonyl(sarcosyl)$_6$sarcosine methyl ester (Z−Sar$_7$−OMe). Benzyloxycarbonyl)sarcosyl)$_3$sarcosine (3.3 g = 7.5 mmol), (sarcosyl)$_2$-sarcosine methyl ester (1.9 g = 7.7 mmol) and dicyclohexylcarbodiimide (1.7 g = 8.25 mmol) in dichloromethane (70 ml) were reacted together

and worked up as described earlier to give a white powder (4.5 g = 90 %), TLC R_F 0.4 (D). 0.3 g was purified on a small silica gel column, m.p. 90 °C. (Found: C 53.99; H 7.06; N 14.47. $C_{30}H_{45}N_7O_{10}$ requires C 54.29; H 6.83; N 14.77).

Benzyloxycarbonyl(sarcosyl)$_6$sarcosine (Z−Sar$_7$−OH). Benzyloxycarbonyl(sarcosyl)$_6$sarcosine methyl ester (4 g = 6 mmol) in methanol (10 ml) and water (20 ml) was hydrolysed with 1 N sodium hydroxide (6.3 ml) for 2 h and worked up as described for Z−Sar$_5$−OH. The resulting amorphous powder (3.8 g = 90 %) softened at 100 °C, homogeneous by TLC, R_F 0.3 (F).

Benzyloxycarbonyl(sarcosyl)$_6$sarcosine 2,4,5-trichlorophenyl ester (Z−Sar$_7$−OTcp). Benzyloxycarbonyl(sarcosyl)$_6$sarcosine (2.2 g = 3.4 mmol), 2,4,5-trichlorophenol (1 g = 5.1 mmol) and dicyclohexylcarbodiimide (0.8 g = 3.9 mmol) in dichloromethane (70 ml) were reacted together and worked up in the usual way. The resulting white powder (2.5 g = 89 %) softened at 90 °C, TLC R_F 0.7 (D). 0.3 g was purified on a small silica gel column. (Found: C 51.06; H 5.35; $C_{35}H_{44}N_7O_{10}Cl_3$ requires C 50.70; H 5.35).

Cyclisation. Benzyloxycarbonyl(sarcosyl)$_6$sarcosine 2,4,5-trichlorophenyl ester (1.8 g = 2.2 mmol) in methanol (120 ml) and hydrochloric acid (0.1 ml) was hydrogenated over 5 % Pd-C (0.4 g) for 50 min and worked up to give a white solid (1.3 g = 1.57 mmol = 82 %) of the (sarcosyl)$_6$sarcosine trichlorophenyl ester hydrochloride. This was dissolved in dimethylformamide (20 ml) and added dropwise to stirred pyridine (600 ml) at 115 °C over a period of 70 min and stirred for 70 min more. After evaporation the residue was dissolved in methanol-water and passed through an ion-exchange column, eluted with methanol-water and evaporated. The residue was dissolved in acetone (5 ml) and kept in a refrigerator overnight. The crystals (300 mg) were collected, and consisted mainly of cycloheptasarcosyl, but with traces of cyclodi- and cyclotetrasarcosyl and some DC-urea. The impurities were removed from the cycloheptasarcosyl by sublimation (heated to 230 °C/0.01 mmHg). The filtrate was evaporated, treated with ether and sublimed. Yields of cyclic compounds: Cycloheptasarcosyl (38 %), sublim.temp. 280 °C/0.01 mmHg, m.p. 296 °C, TLC, R_F 0.4 (E) *m/e* 497. (Found: C 49.88; H 6.97; N 19.89. $C_{21}H_{35}N_7O_7$ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (14 % calc. one cyclisatio per chain) and cyclotetrasarcosyl (6 %).

Cyclooctasarcosyl

Benzyloxycarbonyl(sarcosyl)$_7$sarcosine methyl ester (Z−Sar$_8$−OMe). Benzyloxycarbonyl)sarcosyl)$_3$sarcosine methyl ester (9 g = 20 mmol) in methanol (350 ml) was hydrogenated over 5 % Pd-C for 3 h and worked up to give an oil (6 g = 19 mmol = 95 %) of (sarcosyl)$_3$sarcosine methyl ester with an appropriate NMR spectrum. Without storing, the oil was dissolved together with benzyloxycarbonyl (sarcosyl)$_3$sarcosine (8 g = 18.3 mmol) in dichloromethane (200 ml) and dicyclohexylcarbodiimide (4.1 g = 20 mmol) added. The reaction conditions and work up was as usual, and resulted in a white powder (10 g = 80 %) m.p. 96 °C, TLC, R_F 0.45 (D). 0.3 g was purified on a small silica gel column. (Found: C 53.86; H 6.93; N 15.06. $C_{33}H_{50}N_8O_{11}$ requires C 53.94; H 6.86; N 15.25).

Benzyloxycarbonyl(sarcosyl)$_7$sarcosine (Z−Sar$_8$−OH). Benzyloxycarbonyl(sarcosyl)$_7$sarcosine methyl ester (4.5 g = 6.15 mmol) in methanol (10 ml) and water (20 ml) was hydrolysed with 1 N sodium hydroxide (6.5 ml) for 2 h. A non-crystalline solid (3.6 g = 82 %) was isolated which softened at 90 °C, TLC, R_F 0.25 (F).

Benzyloxycarbonyl(sarcosyl)$_7$sarcosine 2,4,5-trichlorophenyl ester (Z−Sar$_8$−OTcp). From benzyloxycarbonyl(sarcosyl)$_7$sarcosine (1.8 g = 2.5 mmol), 2,4,5-trichlorophenol (0.8 g = 4 mmol) and dicyclohexylcarbodiimide (0.6 g = 2.9 mmol) in dichloromethane (40 ml) was isolated a white powder (1.9 g = 85 %) m.p. 90 °C, TLC, R_F 0.55 (D).

Cyclisation. Benzyloxycarbonyl(sarcosyl)$_7$sarcosine 2,4,5-trichlorophenyl ester (1.9 g = 2.1 mmol) in methanol (90 ml) and hydrochloric acid (1.0 ml) was hydrogenated over 5 % Pd-C for 50 min. A white solid (1.6 g = 2 mmol = 94 %) of (sarcosyl)$_7$sarcosine 2,4,5-trichlorophenyl ester hydrochloride was isolated. This was dissolved in dimethylformamide (50 ml), added dropwise to stirred pyridine (500 ml) at 115 °C over a period of 30 min, stirred for 1½ h more and evaporated. The residue was taken into methanol (10 ml) and undissolved material filtered off (550 mg) which appeared to be pure cyclooctasarcosyl. The filtrate was ion-exchanged in methanol, evaporated and the residue taken into methanol (10 ml). More cyclic octapeptide, together with some DC-urea was filtered off (30 mg) and purified by sublimation (heated until the DC-urea had sublimed, 220 °C). The filtrate was evaporated, treated with ether and sublimed, only a few mg of cyclooctasarcosyl was left. Yields of cyclic compounds: Cyclooctasarcosyl (55 %) sublim. temp. 280 °C/0.01 mmHg, m.p. 338 °C, TLC, R_F 0.4 (E), *m/e* 568. (Found: C 49.90; H 6.88; N 19.92. $C_{24}H_{40}N_8O_8$ requires C 50.69; H 7.09; N 19.71), cyclodisarcosyl (22 %) and cyclotetrasarcosyl (0.35 %). The yields are in both cases calc. from one cyclisation per chain.

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Studies on Electrolytic Substitution Reactions. VIII.[1] The Effect of Anode Material on Anodic Acetoxylation; Synthesis of 2,4,6-Trimethylphenol

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Anodic oxidation of mesitylene in acetic acid containing acetate ion produces 2,4,6-trimethylphenyl acetate and 3,5-dimethylbenzyl acetate as the major products. The effect of anode material on the ratio of nuclear to side-chain acetates and the current yield of products has been studied for eleven different anode materials. The highest ratios were obtained on graphite based anodes. A considerable variation in the current yield was also observed. The highest yields were obtained with a rhodium (57 %) and an AUC graphite anode from Union Carbide (56 %). The latter anode material was used in a large-scale oxidation of mesitylene, 2,4,6-trimethylphenol being isolated after hydrolysis of the acetate in 40 % yield.

Electrochemical oxidations are generally carried out using only a few types of anode materials. Platinum is the most widely used due to its stability under anodic polarization. It can therefore be used repeatedly without loss of metal. The vast amount of knowledge of anodic reaction mechanism gathered by electroanalytical techniques is predominantly based on the use of platinum as the anode. Graphite, in various forms, is of great practical importance as an anode material, since the much lower cost compared to that of platinum and other noble metals makes it attractive for large-scale synthesis.

In selecting a suitable anode material for synthetic reactions it is important to know whether a specific reaction will follow the same path independent of anode material. Empirically, it is known that drastic changes may occur by substituting graphite for platinum. Thus, the Kolbe reaction, *i.e.* discharge of a carboxylate anion, leads to radical derived products (1 e-transfer) at a platinum anode [2] and carbonium ion derived products (2 e-transfer) at a graphite anode.[3] This is exemplified for the oxidation of acetic acid in eqn. 1. In a more recent investigation it was shown that radical

$$CH_3COO^- \xrightarrow[\text{ox.}]{\text{Pt}} \begin{cases} \xrightarrow{} CH_3^{\cdot} \longrightarrow CH_3-CH_3 \\ \xrightarrow{} CH_3^{+} \longrightarrow CH_3COOCH_3 \end{cases} \quad (1)$$

derived products could be obtained on special carbon anodes, such as vitreous carbon and baked carbon, the yields being similar to those obtained with platinum.[4]

Another interesting change in the product distribution takes place in the oxidation of ethyl vinyl ether in methanol (eqn. 2).[5,6]

$$EtO-CH=CH_2 \xrightarrow[\text{Pt}]{\text{MeOH}} EtO-\underset{\underset{\text{OMe}}{|}}{CH}-CH_2-OMe \quad \begin{matrix} \\ 68\ \% \\ - \end{matrix}$$

$$+ (EtO-\underset{\underset{\text{OMe}}{|}}{CH}-CH_2-)_2 \quad \begin{matrix} \\ 22\ \% \\ 51\ \% \end{matrix} \quad (2)$$

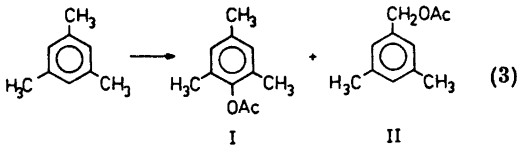
Similar drastic changes in the product yields occur in the anodic alkoxylation of *N*-methylamides, the substitution product being formed at a platinum anode but not at a graphite anode.[7] In the formyloxylation of *N*,*N*-dimethylformamide a high yield of *N*-formyloxy-

methyl-*N*-methylformamide is obtained at a platinum anode,[8] whereas a zero yield was experienced at a graphite anode.[9]

In this work anodic oxidation of mesitylene in acetic acid containing sodium acetate has been investigated at different anode materials with regard to the ratio of isomeric acetates and the yield of the products. This reaction has previously been studied from a mechanistic point of view, and is believed to follow the usual ECE type mechanism.[10]

RESULTS

Anodic oxidation of mesitylene in acetic acid containing sodium acetate resulted in the formation of 2,4,6-trimethylphenyl acetate (I) and 3,5-dimethylbenzyl acetate (II) as the major products (eqn. 3). The reaction was run at different anode materials until 0.2 F/mol of mesitylene



I II (3)

had passed. As usual in this type of study conversions were kept low in order to avoid further oxidation of the products formed.[11] The ratio of I to II and the yield of the products were determined by GLC. In order to ensure that similar reaction conditions prevail at the different materials the reaction was run at a constant current density of 10 mA/cm^2. The results are presented in Table 1. The variation in the ratio for the nuclear to side-chain acetates is surprisingly large, changing by a factor of six in going from the gold anode to the carbon based anodes. The product yields are also affected by the change in anode material, AUC quality graphite (Union Carbide) being the most favorable choice. Accordingly, a large-scale (1 mol of substrate) experiment was carried out using a slightly modified version of the concentric capillary gap cell previously described.[12,13] A solution of mesitylene, acetic acid, and potassium acetate (potassium acetate gives a better conductivity than sodium acetate) was electrolyzed at a constant current of 20 A. The product mixture was then hydrolyzed giving pure 2,4,6-trimethylphenol in 40 % yield.

Table 1. Anodic acetoxylation of mesitylene (0.2 M) in acetic acid-sodium acetate (1 M) at constant current density (i_d=10 mA/cm^2).

Anode	Ratio I to II	Product yield (%)
Gold	3.6	23
Platinum	4.4	19
Palladium	4.9	13
Rhodium	7.6	57
Platinum on graphite	14	38
Lead dioxide on graphite	9.6	37
Magnetite	10	22
Boron carbide	22	27
Carbon cloth	23	34
Glassy carbon	21	35
AUC graphite	23	56

In similar experiments with toluene as the substrate we have also experienced drastic changes. When a mixture of toluene, acetic acid and Bu_4NBF_4 was electrolyzed at either a platinum or a graphite anode, the major product was benzyl acetate, the yield being slightly higher (20 *vs.* 33 %) in the latter case.[14] On the other hand, when sodium acetate was used as the supporting electrolyte instead of Bu_4NBF_4, cresyl acetates were the major products at the platinum anode whereas no acetoxy derivatives of toluene were formed at all on graphite. The major anode process was oxidation of acetate ion with eventual formation of methyl acetate.[15] It thus seems as if the order of oxidation potentials for toluene and acetate ion is different on platinum and graphite anodes.

We do not at present wish to speculate in detail about the possible mode(s) of influence of the anode material upon the product distribution in anodic acetoxylation. Our synthetic goal, to optimize the reaction with respect to the yield of nuclear acetate, has been achieved to a reasonable extent. However, we would like to draw attention to the suggestion by Brennan and Brettle [4] that the *actual* current density at a porous electrode like graphite might be much lower than at a metallic surface like bright platinum and thus the prevailing concentration profiles near the anode surface might be significantly different in the two cases. This would certainly affect the relative concentrations of acetate ion and cation radical at the electrified interface.

EXPERIMENTAL

Anode materials. Gold, platinum, palladium, and rhodium were purchased from Engelhard Industries, USA, magnetite from KemaNord, Sweden, boron carbide (Norbide) from Norton Co, Worchester, Mass., USA, glassy carbon from Vitreous Carbon Ltd, England, carbon cloth and AUC graphite from Union Carbide Sweden. AUC graphite was plated with platinum by Degussa, Schwäbisch Gmund, Germany. Lead dioxide was plated on graphite according to a published procedure.[16]

Electrolysis conditions. A solution of mesitylene (0.01 mol) and acetic acid (50 ml) containing sodium acetate (0.05 mol) was electrolyzed at room temperature between the anode and a platinum cathode under stirring. The current was controlled by a Radiak 2 A/60 V instrument to give a constant current density of 10 mA/cm^2 (the electrode area was measured as the geometric area; in the case of the carbon cloth anode this value is of course uncertain due to difficulties in measuring the anode area). When 0.2 F/mol of mesitylene had passed, the electrolysis was stopped. Acetic acid was removed by evaporation *in vacuo*. The residue was treated with water and extracted with ether. The ether solution was washed with sodium bicarbonate solution and water and dried over anhydrous sodium sulfate. After filtration the ether solution was analyzed by GLC (Perkin-Elmer Model 880 in connection with Perkin-Elmer Integrator D 26 on a 2 m × 0.3 cm 5 % NPGS on Chromosorb P column). The isomer ratio and the product yield was calculated against a calibrated solution of authentic samples using an internal standard (pentamethylbenzene).

Synthesis of 2,4,6-trimethylphenol. A solution of mesitylene (1.0 mol) in acetic acid (1 l) containing potassium acetate (1.0 mol) was electrolyzed in the concentric capillary gap cell at a constant current of 20 A and an applied voltage of 20 V at a temperature of 25 °C until 2 F/mol of mesitylene had passed. After removal of acetic acid by evaporation *in vacuo* the residue was treated with water and extracted with ether. The ether solution was washed with sodium bicarbonate solution and water and dried over anhydrous sodium sulfate. After filtration and evaporation of the ether *in vacuo* the residue was hydrolyzed with a solution of potassium hydroxide in ethanol and water by boiling overnight. Most of the ethanol was removed by evaporation *in vacuo* and the residue extracted once with ether. The aqueous solution was cooled in an ice-bath and neutralized with dilute hydrochloric acid. The white precipitate was filtered off, washed with cold water and dried, giving pure (by GLC) 2,4,6-trimethylphenol (55.3 g; 40 % yield) identical with an authentic sample.

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The Crystal and Molecular Structure of (2-Hydroxyphenyl)alanine (*o*-Tyrosine)

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The crystal structure of D,L-(2-hydroxyphenyl)-alanine has been determined by X-ray methods using 1971 observed reflections. The crystals are monoclinic, space group $P2_1$, with four molecules in the unit cell of dimensions $a=6.32_5$ Å; $b=26.48_9$ Å; $c=5.36_7$ Å, and $\beta=98.1_8°$. The structure was solved by direct methods and refined to a conventional R-factor of 0.048; estimated standard deviations in bond lengths not involving hydrogen are 0.002—0.004 Å and in angles 0.1—0.2°.

Bond lengths and angles are in accordance with those found in tyrosine. Owing to an *intra*-molecular hydrogen bond between the ammonium group and the ring hydroxyl oxygen atom, however, the conformational angles differ from those found in several other phenylalanine derivatives. There are non-crystallographic centres of symmetry between pairs of enantiomeric molecules.

(2-Hydroxyphenyl)alanine (*ortho*-tyrosine) is the third of the monohydroxyphenylalanines to be examined in this laboratory in order to study the conformation of tyrosine and related compounds in different crystalline environments.[1-3] In *o*-tyrosine there is an additional opportunity to study possible interactions between the *ortho* phenolic group and the side chain; such interactions could be of importance for the biological and chemical activity of the molecule.

Even if the existence and activity of *o*-tyrosine in biological systems has been reported,[4] this compound seems to be far less involved in biological processes than *m*-tyrosine and tyrosine. *o*-Tyrosine does not seem to be a substrate for phenylalanine hydroxylase, whereas both phenylalanine and *m*-tyrosine are hydroxylated in the *para* position by this enzyme.[5] This may be explained by the presence of an *ortho* substituent or a different conformation of the molecule due to an interaction between the substituents and the side chain.

EXPERIMENTAL

o-Tyrosine is only sparingly soluble in methanol but dissolves readily when small amounts of aqueous ammonia are added. When the solution is left standing the ammonia evaporates and a slow crystallization of the compound is attained. This crystallization procedure yielded well formed single crystals of the title compound and has also been successfully employed for crystal formation of other amino acids when other methods have failed.

The specimen used for the X-ray experiments had the dimensions $0.6\times0.5\times0.2$ mm³.

Oscillation and Weissenberg photographs showed the crystal to be monoclinic; systematically absent reflections are k odd for ($0k0$) indicating the space group $P2_1$ or $P2_1/m$. Unit cell dimensions were obtained from a least-squares fitting of diffractometer 2θ values for 40 centered reflections with 2θ in the range 23 to 40° using Mo$K\alpha$ radiation ($\lambda=0.71069$ Å). The intensity data were recorded with the use of a SYNTEX P $\bar{1}$ diffractometer with graphite crystal monochromated Mo$K\alpha$ radiation. The $\omega-2\theta$ scanning mode was employed with the 2θ scan speed varying from 2 to 8° min^{-1} depending on the intensity. Reflections with $2\theta>40°$ were measured only if a quick scan gave an intensity larger than a preset value. The scan range was from 1° below $2\theta(\alpha_1)$ to 1° above $2\theta(\alpha_2)$ and background counts were taken for 0.35 times the scan time at each of the scan range limits. Two standard reflections were measured after every 50 reflections; the data set was adjusted according to a slow decrease in the intensity of the standard reflections during the data collection. The estimate of the standard deviation of the measurements was based on counting statistics with an additional term of 2 % of the intensity. 2685 reflections with $2\theta<75°$ were measured; of these 1971 were found to

have intensities larger than three times their estimated standard deviation and were regarded as observed. The remaining reflections were excluded from the refinement procedure. The data set was corrected for Lorentz and polarization effects, no absorption correction was carried out.

A description of the computer programs used is given in Refs. 6 and 7. In the full-matrix least-squares program the quantity minimized was $\sum w\Delta F^2$ where w is the inverse of the variance of the observed structure factors; for reflections with $\sin\theta/\lambda < 0.5$ w was multiplied by $(2 \sin\theta/\lambda)^2$ to give less weight to low-order reflections. Atomic form factors applied were those of Doyle and Turner [8] for oxygen, nitrogen, and carbon atoms and of Stewart, Davidson and Simpson [9] for hydrogen.

CRYSTAL DATA

D,L-(2-Hydroxyphenyl)alanine (*o*-tyrosine), $C_9H_{11}NO_3$, monoclinic, $a=6.325(1)$ Å; $b=26.489(3)$ Å; $c=5.367(1)$ Å; $\beta=98.18(1)°$. ($t=18$ °C) $V=890.13$ Å^3; M = 181.19; $F(000)=384$; $Z=4$; $D_{calc}=1.352$ g cm^{-3}; $\mu(MoK\alpha)=1.1$ cm^{-1}. Absent reflections: $(0k0)$ for k odd. Space group $P2_1$.

STRUCTURE DETERMINATION

The crystals were formed from a solution of the racemate and contain four molecules per

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations ($\times 10^5$). The temperature factor is given by $\exp-(B11h^2+B22k^2+B33l^2+B12hk+B13hl+B23kl)$.

	x	y	z	$B11$	$B22$	$B33$	$B12$	$B13$	$B23$
O1	4198	29377	87093	1881	203	3422	207	191	−341
	22		27	37	3	60	17	72	21
O2	36977	36435	59415	2146	154	1403	281	344	105
	20	5	21	36	2	35	15	58	15
O3	68759	32795	61797	1847	159	2096	94	997	−43
	20	5	22	34	2	41	15	61	17
N1	35837	36221	108298	2414	151	1342	293	790	32
	26	6	25	44	3	40	19	71	18
C1	34697	24595	81752	2100	97	2558	−21	612	184
	30	7	35	51	3	62	19	90	22
C2	13407	25735	74002	2076	129	2722	−80	721	−4
	31	7	36	54	3	68	21	97	24
C3	1800	23262	53585	2704	171	3505	−254	251	−134
	36	9	44	66	4	86	26	121	31
C4	11440	19604	40874	4133	149	3870	−476	739	−336
	48	9	47	92	4	95	31	151	31
C5	32423	18341	48505	4202	110	4705	−35	3021	−255
	48	8	51	93	3	105	28	166	30
C6	43883	20825	68640	2808	107	4368	101	1475	140
	37	8	47	64	3	94	24	129	28
C7	47980	27329	103272	2048	127	2290	114	116	302
	31	7	34	51	3	61	20	92	22
C8	52218	32967	98847	1563	130	1358	62	52	43
	26	7	27	44	3	45	18	71	19
C9	52701	34177	71078	1742	101	1390	−64	326	−54
	27	6	28	44	3	43	18	75	18

Table 2. Fractional atomic coordinates ($\times 10^4$) and B-values with estimated standard deviations for hydrogen atoms.

	x	y	z	B
HC3	−1439	2438	4876	6.6
	51	11	53	7
HC4	358	1808	2596	6.2
	51	11	62	8
HC5	3884	1619	3982	6.0
	45	10	53	7
HC6	5829	1985	7415	3.1
	38	8	40	5
H1C7	6174	2565	10666	4.0
	39	8	41	5
H2C7	4129	2727	11879	3.6
	37	8	44	5
HC8	6618	3394	10934	2.7
	36	8	40	5
H1N1	2131	3503	9989	7.2
	66	15	73	1.1
H2N1	3666	3939	10344	4.9
	44	11	50	6
H3N1	3681	3625	12651	3.3
	45	10	54	7
HO1	−835	3048	8022	7.4
	47	11	54	7

unit cell. It would thus seem natural to assume the space group to be the centric $P2_1/m$ rather than the non-centric $P2_1$. Intensity statistics calculations seemed also to indicate a centric structure. Nevertheless, a Patterson synthesis gave no indication of the presence of a mirror plane normal to b in the unit cell and we therefore decided to solve the structure assuming the space group to be the lower symmetric $P2_1$.

The structure was solved by application of non-centrosymmetric direct methods using the program assembly MULTAN.[7] All the non-hydrogen atoms of the two independent molecules appeared in the E-map based on the set of phases with the highest figure of merit, and the positions of the hydrogen atoms were calculated from stereochemical considerations after a preliminary refinement. Least-squares refinements yielded a conventional R-factor of 0.049 (R_w=0.050). The resulting bond lengths and angles were rather unsatisfactory, however, the distances deviating up to 0.08 Å from their expected values and corresponding distances in the chemically equivalent molecules being by as much as 0.12 Å different. Inspection of the correlation matrix revealed a very high correlation between corresponding parameters of the two molecules, and it became evident that there exists a non-crystallographic centre of symmetry between pairs of molecules. The centre of symmetry is situated in a general position and we have been unable to explain this in terms of space group symmetry. The existence of the extra symmetry was introduced as a constraining factor in the least-squares calculations which proceeded with only half the number of parameters. The refinement converged to a final conventional R-factor of 0.048 (R_w=0.052); the coordinates of the centre of symmetry are (0.6610,0.3899,0.8015).

The ratio of the weighted R-factors with and without the constraints is 1.03; the Hamilton test shows that the centrosymmetric model gives the best description of the structure.[10] The

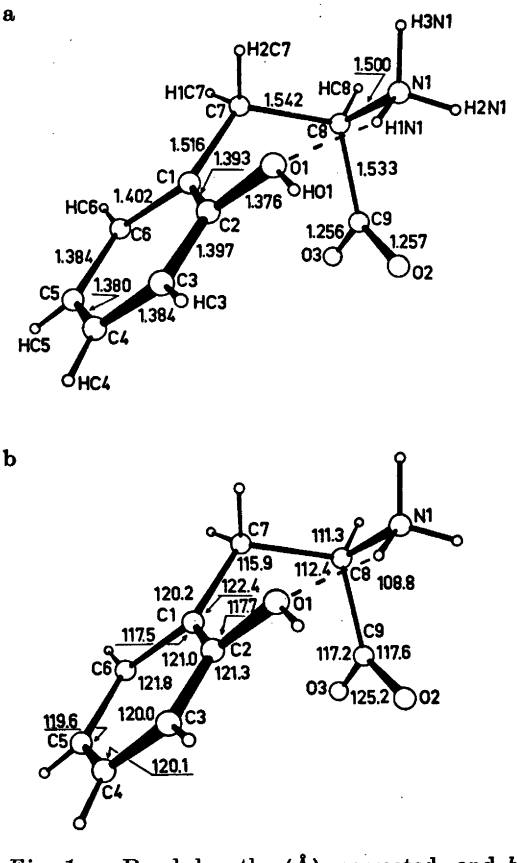


Fig. 1. a Bond lengths (Å), corrected, and *b* bond angles (°) in *o*-tyrosine.

Table 3. Bond lengths (Å) and angles (°).

Bond	Length	Corr.	Bond	Length
C1–C2	1.385(3)	1.393	HC3–C3	1.06
C2–C3	1.392(3)	1.397	HC4–C4	0.97
C3–C4	1.377(4)	1.384	HC5–C5	0.87
C4–C5	1.373(4)	1.380	HC6–C6	0.95
C5–C6	1.379(4)	1.384	H1C7–C7	0.97
C1–C6	1.395(3)	1.402	H2C7–C7	0.98
C1–C7	1.512(3)	1.516	HC8–C8	1.01
C7–C8	1.542(3)		H1N1–N1	1.01
C8–C9	1.529(2)	1.533	H2H1–N1	0.88
O1–C2	1.371(2)	1.376	H3N1–N1	0.97
N1–C8	1.491(2)	1.500	HO1–O1	0.88
O2–C9	1.249(2)	1.257		
O3–C9	1.250(2)	1.256		

	Angles		Angles
C1–C2–C3	121.0(2)	C7–C8–C9	112.4(1)
C2–C3–C4	120.0(2)	C7–C8–N1	111.3(1)
C3–C4–C5	120.1(2)	C9–C8–N1	108.8(1)
C4–C5–C6	119.6(2)	C8–C9–O2	117.6(1)
C5–C6–C1	121.8(2)	C8–C9–O3	117.2(1)
C2–C1–C6	117.5(2)	O2–C9–O3	125.2(1)
O1–C2–C1	117.7(2)	C2–O1–HO1	116
O1–C2–C3	121.3(2)	C8–N1–H1N1	107
C2–C1–C7	122.4(2)	C8–N1–H2N1	112
C1–C7–C8	115.9(1)	C8–N1–H3N1	114
C6–C1–C7	120.2(2)		

Table 4. Torsional angles and hydrogen bond data in *o*-tyrosine (°).

C2–C1–C7–C8	66.1	C1–C2–O1–HO1	168
C1–C7–C8–N1	−92.8	C7–C8–N1–H1N1	55
C1–C7–C8–C9	29.5	C7–C8–N1–H3N1	−69
C7–C8–C9–O3	73.9	C9–C8–N1–H1N1	−69
N1–C8–C9–O2	18.1		
N1–C8–C9–O3	−162.4		

Hydrogen bonds D–H···A	D···A	H···A	Angle D–H–A
O1–HO1···O4	2.61	1.74	170
N1–H1N1···O1 (intramol.)	2.82	1.92	146
N1–H2N1···O2	2.73	1.76	177
N1–N3N1···O3	3.04	2.33	137

structural implications of the centre of symmetry are discussed together with the description of the crystal packing.

The anisotropic thermal parameters were analysed in terms of rigid-motion both for the whole molecule and for the hydroxyphenyl and alanine parts separately. The results indicated that the entire molecule does not behave as a rigid body, whereas separating the molecule into two parts gave better results. The latter description was adopted when correcting bond lengths for libration effects.

The structure factor list may be obtained from the authors upon request. Final parameters for

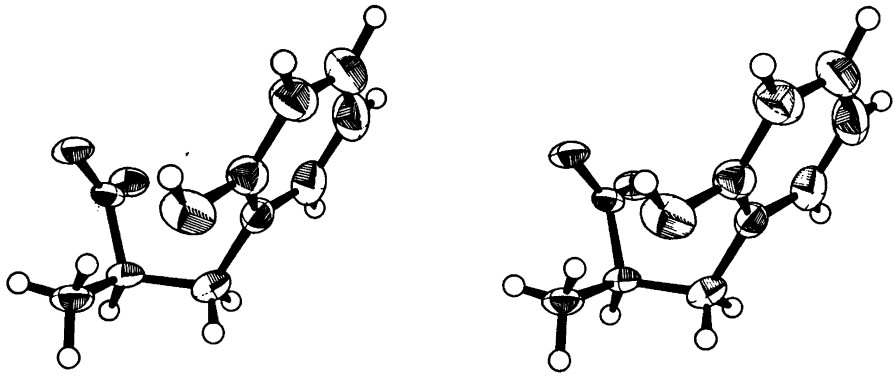


Fig. 2. Stereoscopic illustration of *o*-tyrosine.

non-hydrogen atoms are given in Table 1 and for hydrogen atoms in Table 2.

Standard deviations in interactomic distances and bond angles are calculated from the correlation matrix ignoring uncertainties in cell dimensions.

DISCUSSION

The bond lengths and angles are given in Table 3 as well as in Fig. 1 where the numbering of the atoms is also indicated. Conformational angles in the molecules and hydrogen bond data are given in Table 4. Dihedral angles are given as positive for a clockwise rotation and refer to the *R*-form.

The bond lengths found in *o*-tyrosine compare well with those in tyrosine [1,2] and *m*-tyrosine,[3] whereas there are some differences in some bond angles. Thus the angle C2—C1—C7 (122.4°) and C1—C7—C8 (115.9°) are significantly larger than the corresponding angles in the analogous compounds (120.4—120.9 and 114.0—114.4°, respectively). The C7—C8—C9 angle of 112.4° is larger than what is reported for this angle in L-DOPA [11] and *m*-tyrosine [3] but is close to that found in L- and D,L-tyrosine.[1,2] The cause of the opening of the angles may be found in the rather unusual conformation of the side chain brought about by an *intra*-molecular hydrogen bond between the *ortho* oxygen and the nitrogen atom. This hydrogen bond, in which the nitrogen

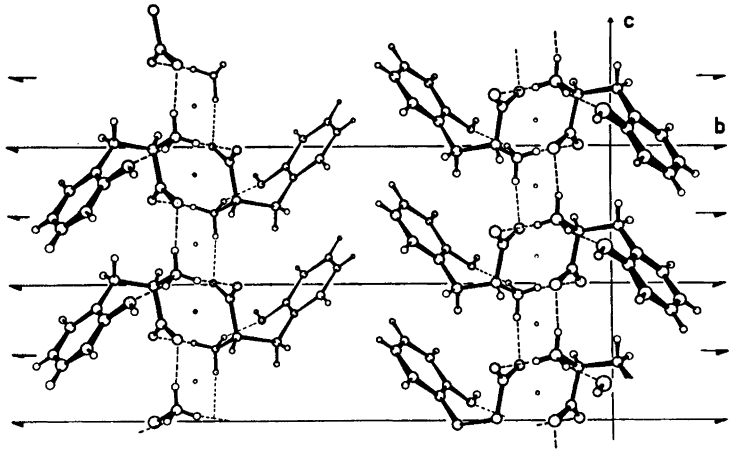


Fig. 3. The crystal structure of *o*-tyrosine as viewed down the *a* axis. The non-crystallographic centres of symmetry are drawn as small circles.

atom acts as the proton donor, is 2.82 Å with a H···O separation of 1.9 Å and a N—H···O angle of 146°. The existence of this hydrogen bond is probably also the reason for a considerable deviation from staggered conformation about the Cα—Cβ bond. The dihedral angle C1—C7—C8—C9 is 29.5° and the angle C1—C7—C8—N1 is 267.2°, whereas the corresponding torsional angles in L-tyrosine are 53.1 and 290.7°, respectively.[1] Furthermore, the torsion angle C2—C1—C7—C8 is 66° in the present compound as compared to 94° in tyrosine. The rotations about the Cα—Cβ and Cβ—Cγ bonds cooperate to bring a hydrogen atom of the ammonium group to a position favourable for the formation of the *intra*-molecular hydrogen bond to the phenolic oxygen atom. It is interesting to note, however, that there is practically no deviation from a staggered conformation about the C8—N1 bond nor in the usual features of the external angles at the C2 atom. The difference between the C3—C2—O1 and C1—C2—O1 angles is 3.6° with the larger angle on the same side of the C2—O1 bond as the phenolic hydrogen atom.

It seems reasonable that the special conformation of the molecule brought about by the *intra*-molecular hydrogen bond may account for the difference in biological activity as compared to that of the *meta* and *para* hydroxyphenylalanines.

The dihedral angle N1—C8—C9—O2 is 18.1°, the nitrogen atom being situated 0.43 Å out of the least-squares plane through the C8, C9, O2, and O3 atoms. This is the conformation usually found in amino acid groups. However, as distinct from the situation in most ionized amino acids [12] the two C—C—O angles are found to be equal in the present structure.

A stereo drawing of the molecule is presented in Fig. 2.

The packing of the molecules in the crystal is vizualized in Fig. 3. The hydrogen bonds are indicated in the figure, pertinent *inter*-molecular distances are listed in Table 4.

The four hydrogen atoms bonded to hetero atoms are all involved in hydrogen bonds, one of which is the *intra*-molecular hydrogen bond. Each molecule is donor in three and acceptor in three *inter*-molecular hydrogen bonds connecting each molecule to five neighbouring molecules through six such bonds. The N1—H3N1···O2 bonds produce chains of molecules in the *c* direction; these chains are connected through O1—HO1···O3 bonds to give layers parallel to the *a*—*c* plane. Each molecule of one layer is linked to a molecule of another layer by two N1—H2N1···O3 bonds related to each other by a non-crystallographic centre of symmetry. These centres of symmetry are operative within the double molecular layer only and have no effect on atoms outside these layers or on the two-fold screw axes. One layer is transferred to the next through the operation of the two-fold screw axes. The contacts between layers in the *b*-direction are only of the van der Waals type; the bonding is weak and the crystals have cleavage planes normal to the *b*-axis.

Owing to the *intra*-molecular hydrogen bond *o*-tyrosine is not as effectively packed as tyrosine and *m*-tyrosine. This results in a significantly lower density (1.352 g cm^{-3}) than that found for the other tyrosines (1.414—1.435 g cm^{-3}).

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Resolution and Configurational Assignment of α(-Benzotriazolyl-2)propionic Acid. Synthesis of (+)-α-(4,5,6,7-Tetrahydrobenzotriazolyl-2)propionic Acid

HÅKAN GUSTAFSSON

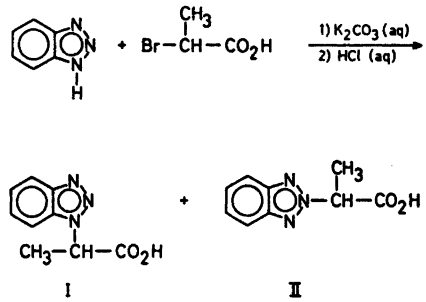
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α-(Benzotriazolyl-2)propionic acid (II) has been resolved into its optical antipodes. The dextrorotary acid was obtained by fractional crystallization of its cinchonidine salt in methanol-water (7:1) and the levorotary form from (+)-α-methylbenzylamine with ethyl acetate as solvent. The specific rotation of the antipodes was $[\alpha]_D^{25} = +33.4°$ and $-33.2°$ in acetone. The acid (+)-II was hydrogenated to give (+)-α-(4,5,6,7-tetrahydrobenzotriazolyl-2)propionic acid (+III) with $[\alpha]_D^{25} = +16.4°$ in acetone. The dextrorotary acids were found to have *R*-configuration by CD measurements.

In connection with work on synthetic auxins at this Institute α-(benzotriazolyl-2)propionic acid (II) has now been investigated. Both of its antipodes exhibit antiauxin activity,[1] with (−)-II being the strongest antiauxin.

Racemic II and racemic α-(benzotriazolyl-1)-propionic acid (I) have also been tested [2] for activity against *Plasmodium berghei* in mice. The mean survival times for infected mice treated with I and II were zero.

Preliminary experiments on resolution of II were performed with the common alkaloids and synthetic bases in methanol-water ethanol, ethyl acetate, chloroform, and acetone. 0.001 mol acid and 0.001 mol base were dissolved in a small amount of solvent and the salt obtained after cooling was filtered off and dried. The acid was then liberated from the salt by addition of diluted sulfuric acid and the specific rotation determined in acetone. In most cases only oily products were obtained, but salts were obtained with cinchonidine in methanol-water, strychnine in ethyl acetate, dehydroabietyl amine in ethanol, and (+)-α-methylbenzylamine in ethyl acetate giving acid with $[\alpha]_D^{25} = +15°$, $+13°$, $+13°$ and $-9°$, respectively. According to the results the resolution was performed with cinchonidine and (+)-α-methylbenzylamine as bases.



Scheme 1.

DETERMINATION OF THE ABSOLUTE CONFIGURATION

Experiments were carried out in order to determine the absolute configuration of II by cleavage to active alanine. Reaction of II with sodium in ethanol according to Gattermann [3] yielded alanine as indicated by the ninhydrin test and by paper chromatography and *o*-phenylenediamine as indicated by specific reactions with 2,4-pentanedione [4] and phenanthrenequinone.[5] No experiments with optically active II were done due to problems with the isolation of alanine. Reduction of II according to Cappel and Fernelius [6] was not successful.

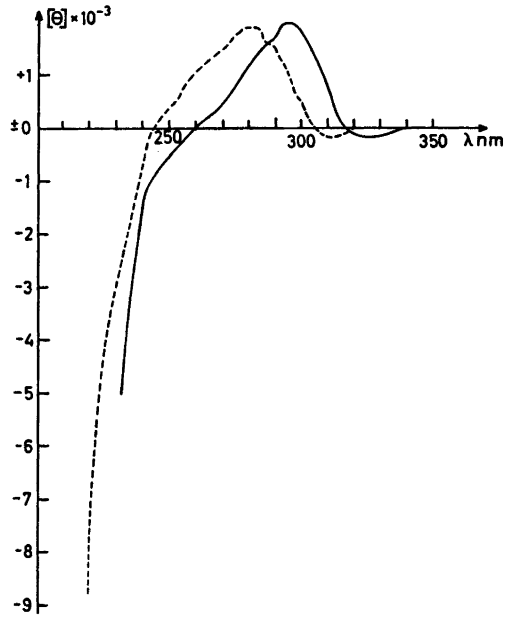


Fig. 1. CD curves of (+)-II ($c = 3.4 \times 10^{-2}$ g/100 ml) – – – –, and *R*-(+)-α-(indazolyl-2)-propionic acid ($c = 4.44 \times 10^{-2}$ g/100 ml) ——— in methanol.

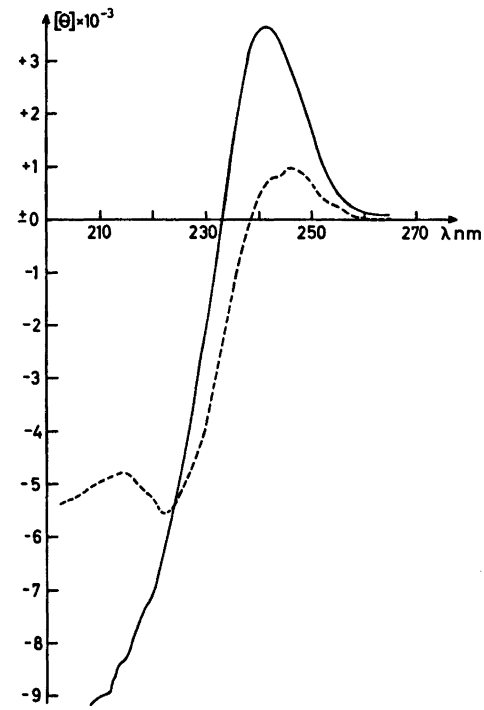
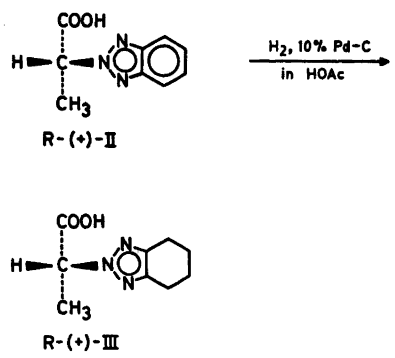


Fig. 2. CD curves of (+)-III ($c = 3.96 \times 10^{-2}$ g/100 ml) – – – and *R*-(+)-α-(4,5,6,7-tetrahydroindazolyl-2)propionic acid ($c = 3.56 \times 10^{-2}$ g/100 ml) ——— in methanol.

Attempts were also made to correlate the configuration of II with α-(indazolyl-2)propionic acid,[7] α-(thianaphthenyl-2)propionic acid [8] and the configuration of α-(4,5,6,7-tetrahydrobenzotriazolyl-2)propionic acid (III) with α-(4,5,6,7-tetrahydroindazolyl-2)propionic acid [7] by the quasi-racemate method.[9,10] No quasi-racemates were indicated by X-ray powder photograms, only solid solutions. (+)-III was obtained by hydrogenation of (+)-II (Scheme II). The CD curves of (+)-II and *R*-(+)-α-(indazolyl-2)propionic acid, however, show that (+)-II and (+)-III have *R*-configuration (Figs. 1 and 2).



Scheme 2.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Varian A-60 instrument with solutions of about 10 % using tetramethylsilane as internal standard. The mass spectra were recorded at 70 eV with an LKB 9000 instrument. The optical activity was measured with a Perkin-Elmer 141 spectropolarimeter in micro cells of 10 cm length or in cells of 20 cm length with an older instrument built at this institute. The CD curves were recorded in 1 ml cells in methanol solutions with a Cary 60 spectropolarimeter equipped with a CD accessory and the UV spectra on a Spectronic 505 spectrophotometer.

Thin layer chromatograms were run on non-activated plates (E. Merck) coated with silica gel F 254 with a nominal thickness of 0.25 mm. The chromatograms were examined

with UV light or developed with iodine vapour.

The melting points were determined with a hot stage microscope and are uncorrected. The microanalyses were carried out in the analytical department of the institute.

Racemic α-(benzotriazolyl-2)propionic acid (II). A mixture of II and its isomer α-(benzotriazolyl-1)propionic acid (I) was prepared according to Fredga and Lindgren[11] in 62 % yield. The isomers were separated utilizing their differing basicities[12] in a way described by Sparatore and Pagani.[13] 73 % I and 27 % II were obtained calculated on the total amount of acid. The separation was followed using the characteristic UV absorptions of I and II. The m.p. of II was 212–214 °C. Yield 17 % (lit. 20 %, 210–212 °C). R_F of I and II was 0.63 and 0.33, respectively, in 20 % acetic acid-benzene. ^{1}H NMR (DMSO-d_6) δ 7.19–7.86 (m, Ar-4 H, wt. 4), δ 5.76 (q, α-H, wt. 1), δ 1.90 (d, $-CH_3$, wt. 2).

R-(+)-α-*(Benzotriazolyl-2)propionic acid (+II).* 20.0 g (0.105 mol) α-(benzotriazolyl-2)-propionic acid and 30.8 g (0.105 mol) cinchonidine were dissolved in 320 ml boiling methanol-water (7:1). After one day in a refrigerator the salt obtained was filtered off. The acid was liberated from a small sample of the salt after each recrystallization and the rotary power was determined. The salt was recrystallized several times until the optical activity remained constant.

After nine recrystallizations the acid was liberated by addition of 2 M sulfuric acid. The acid obtained was extracted with ether and the ether evaporated *in vacuo*. 1.27 g (13 %) (+)-II was isolated, which was recrystallized from 170 ml boiling water and finally dried over concentrated sulfuric acid in a desiccator. M.p. 230–231 °C. $[\alpha]_D^{25} = +33.4°$, $[\alpha]_{365}^{25} = +74.4°$ ($c = 0.548$ g/100 ml $l = 1$ dm, $\alpha_D^{25} = +0.183°$, $\alpha_{365}^{25} = +0.408°$ in acetone). $[\theta]_{max}$ is +1945° at 280 nm in methanol solution.

S-(−)-α-*(Benzotriazolyl-2)propionic acid* [(−)*II*]. The mother liquor from the first crystallization was evaporated to dryness and the acid isolated as before. 7.5 g (0.04 mol) acid and 4.8 g (0.040 mol) (+)-α-methylbenzylamine was dissolved in 280 ml boiling ethyl acetate. The solution was cooled gently and then put in a refrigerator. 8.5 g salt was obtained, which was recrystallized until the optical activity remained constant.

The acid was isolated as described above. 2.33 g (11 %) acid was obtained. The acid was recrystallized from boiling water and dried over sulfuric acid in a desiccator. M.p. 230–231 °C. The rotatory power of the (−)-II acid in various solvents is given in Table 1.

Racemic α-(4,5,6,7-tetrahydrobenzotriazolyl-2)-propionic acid (III). Method A. 0.50 g (2.6 mmol) II was dissolved in 3 ml glacial acetic acid and 0.70 g 10 % palladium-on-charcoal was added. The reaction was carried out in a Parr catalytic hydrogenation apparatus in the usual way with hydrogen gas of 6 atm. pressure at room temperature during 15 h. The catalyst was then filtered off and the residue was evaporated to dryness and recrystallized from water. M.p. 148 °C.

Table 1. The optical activity of (−)-II in some different solvents. g = grams acid dissolved in the solvent to 10.0 ml. l = the tube length in dm.

Solvent	$[\alpha]_D^{25}$	l	g
Dimethylformamide	−42.6°	1	0.352
Acetone	−33.2°	1	0.750
Abs. ethanol	−7.7°	2	0.325
Water (neutral.[a])	+41.0°	2	0.305

[a] The acid was dissolved in 10.0 ml of 1.5 M ammonia solution.

Method B. 0.60 g (3.1 mmol) II was dissolved in 15 ml 99 % ethanol and 1.0 g 10 % palladium-on-charcoal was added. The hydrogenation was performed at 15 atm. hydrogen pressure and 75 °C with continuous shaking. After 18 h very little II remained. Another 0.50 g catalyst was added and the hydrogenation was continued for 5 h more. The catalyst was then filtered off and thoroughly washed with ethanol. After evaporating off the solvent 0.55 g of a faint yellow semisolid product remained. ^{1}H NMR, TLC, and MS analysis of the product showed that a minor amount of ethyl α-(4,5,6,7-tetrahydrobenzotriazolyl-2)propionate also was formed in addition to III. The product was dissolved in a small amount of diluted sodium hydroxide and extracted with chloroform. The acid was precipitated by addition of hydrochloric acid to pH 2 and then extracted with chloroform. The chloroform solution was washed with a small amount of water, dried with magnesium sulfate and then evaporated to dryness. The residue was dissolved in 2 ml chloroform. The acid III was crystallized out after addition of 5 ml ligroin. After another recrystallization in the same way, 0.33 g (55 %) III was obtained with m.p. 147.5–149 °C. $R_F = 0.57$ in 96 % ethanol. UV λ_{max} 236 nm (log ε 3.94). ^{1}H NMR ($CDCl_3$): $-CH_2-$ and CH_3- (multiplet with a doublet at δ 1.84, wt. 7), $-CH_2-\underset{|}{C}=$ (multiplet with the highest peak at δ 2.70, wt. 4), α-H (δ 5.35, wt. 1). (Found: (195.2, MS): C 54.69; H 6.63; N 21.12. Calc. for $C_9H_{13}N_3O_2$ (195.2): C 55.37; H 6.71; N 21.53).

R-(+)-α-*(4,5,6,7-Tetrahydrobenzotriazolyl-2)-propionic acid* (+*III*). 0.30 g *R*-(+)-II was hydrogenated according to method B to give 0.09 g (29 %) of (+)-III. M.p. 169–170.5 °C. $[\alpha]_D^{25} = +16.4°$, $[\alpha]_{365}^{25} = +41.5°$ ($\alpha_D^{25} = +0.053°$, $\alpha_{365}^{25} = +0.134°$, $c = 0.0323$ g/100 ml, $l = 1$ cm, acetone).

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Reaction of Sugar Esters with Hydrogen Fluoride. XIII. Preparation of 1,6-Anhydro-β-D-gulopyranose Derivatives

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Reaction of methyl 2,3,6-tri-*O*-benzoyl-4-*O*-*p*-nitrobenzoyl-α-D-galactopyranoside with hydrogen fluoride leads to formation of 2-*O*-benzoyl-4-*O*-*p*-nitrobenzoyl-1,6-anhydro-β-D-gulopyranose, which was converted into tri-*O*-acetyl-1,6-anhydro-β-D-gulopyranose. The reaction in hydrogen fluoride takes place *via* dioxolanylium ions which were observed by ^{13}C NMR spectroscopy.

In view of the results obtained previously from the reaction of glucopyranose or mannopyranose pentabenzoates with anhydrous hydrogen fluoride it might be expected that treatment of galactopyranose pentabenzoate with hydrogen fluoride should lead to formation of talose derivatives.[1,2] Rearrangement of galactopyranose pentaacetate into talopyranose derivatives has been shown to take place with antimony pentachloride.[3] Conversion of galactofuranose derivatives into talofuranose derivatives has been achieved with hydrogen fluoride.[4]

We have found that prolonged treatment of penta-*O*-benzoyl-D-galactopyranose with hydrogen fluoride gives mainly galactose derivatives. It was shown previously that methyl tetra-*O*-benzoyl-α-D-glucopyranoside gave a good yield of 1,6-anhydro-β-D-altropyranose when treated with hydrogen fluoride for a few days. Methyl tetra-*O*-benzoyl-D-galactopyranose was found to give a complex mixture of products by the same treatment and the reaction was therefore not investigated further.

The results obtained previously by treatment of mixed esters of cyclohexanediol [5] and of 1,5-anhydro-arabinitol [6] with hydrogen fluoride indicated that it would be of interest to study the reactions of benzoylated carbohydrates, which had *p*-nitro- or *p*-methoxy-benzoyl groups in selected positions. For this purpose methyl 2,3,6-tri-*O*-benzoyl-4-*O*-*p*-nitrobenzoyl-α-D-galactopyranoside (*1*) was prepared from the readily available methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside.

When *1* was dissolved in anhydrous hydrogen fluoride at 0 °C it reacted rapidly to give the 1,2-benzoxonium ion (*3*) as seen from a ^{1}H NMR spectrum of the hydrogen fluoride solution. This ion is probably in equilibrium with the fluoride (*2*),[2] but the latter could not be seen in the spectrum. When the hydrogen fluoride solution was kept at room temperature further reactions took place for *ca.* 48 h. A ^{1}H NMR spectrum of the solution at this stage was not well resolved and could not be analyzed. Good ^{13}C NMR spectra could, however, be obtained when the reaction was carried out in deuterium fluoride solution. A spectrum measured within 1 h after *1* had been dissolved in deuterium fluoride gave the ^{13}C chemical shifts of *3*. A ^{13}C spectrum after 48 h (Table 1) showed that the benzoxonium ion (*8*) was almost the only species present since only a few other small signals could be seen. The low field position of C1 and C2 in *3* shows that a 1,2-benzoxonium ion is present. In *8* C2 and C3 are found at relatively low field in agreement with the structure. The chemical shifts of the ionic carbon atoms in the benzoxonium ions (187.17 and 185.35) are in agreement with results found for other dioxolanylium ions.[7] The signal at 60.64 ppm is due to methanol cleaved off from *1* whereas the signal at 64.02 arises from methyl benzoate, formed in a secondary reaction.[2] The absence of $^{13}C—^{19}F$ couplings shows that glycosyl fluorides or ortho-

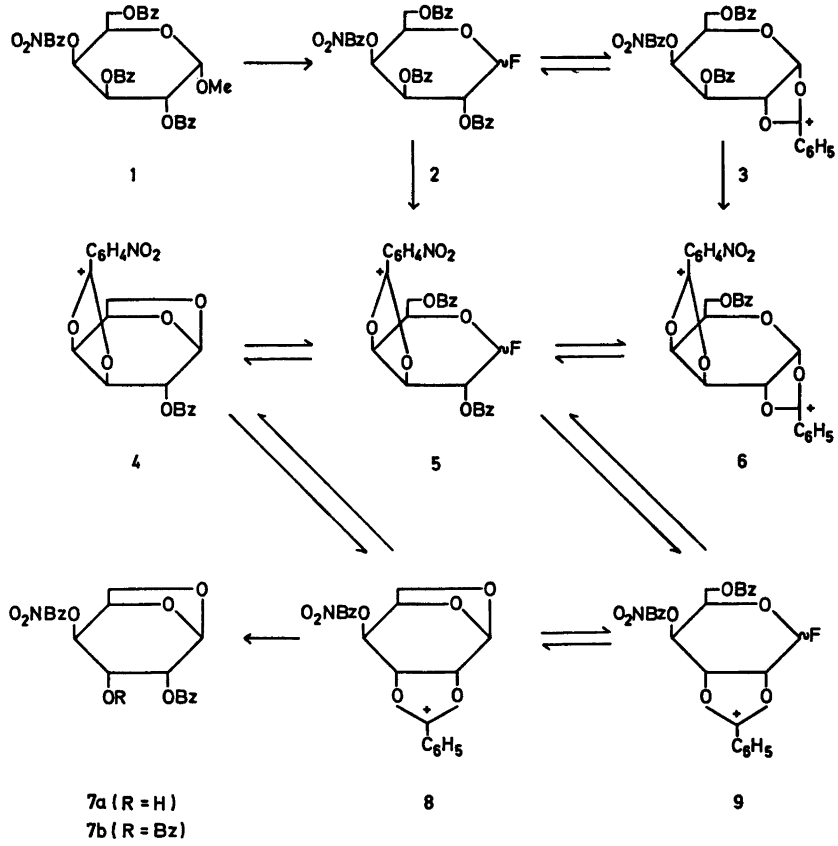


Table 1. Chemical shifts in $CDCl_3$ (ppm) and observed first-order coupling constants (Hz).

Compound	H1	H2	H3	H4	H5	H6	H6′	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
1	5.30	5.68	5.99	6.06		4.7–4.3		3.5	10.8	3.4				
7a	5.58	5.25	4.30	5.38	4.72	4.10	3.72	2.5	4.5	9.0	5.0	0	5.0	−8.0
7b	5.77	5.72	5.92	5.68	4.93	4.34	3.89	2.2	4.6	9.3	4.0	0	4.8	−8.1
[a]	5.43		5.1–5.3		4.62	4.10	3.72	2.0			2.6	0	4.6	−8.0

^{13}C NMR data in deuterium fluoride (ppm values)

	C1	C2	C3	C4	C5	C6	$-C^{a}-$	OMe
3	117.56	84.81	76.56	72.92	76.43	69.47	187.17	60.64
8	101.06	90.79	88.06	75.58	75.19	69.41	185.35	64.02

[a] Tri-*O*-acetyl-1,6-anhydro-β-D-gulopyranose.

acid fluorides are not present.

The 1,2-benzoxonium ion (*3*), which is the first product formed in hydrogen fluoride, could rearrange to a 2,3-benzoxonium ion with the *talo*-configuration by attack of the benzoyloxy group of C3 upon C2. This would be analogous to the rearrangement of glucopyranose derivatives into mannopyranose derivatives.[2] Such a rearrangement did, however, not take place to any detectable extent. Since *1* has a pair of 3,4-*cis*-oriented acyloxy-group it could, alternatively, give a 3,4-dioxolanylium ion, analogous to the reactions of esters of 1,2-*cis*-cyclohexanediol. According to the results found with mixed esters [5] (*3*) would be expected to give the *p*-nitrobenzoxonium ion (*6*). The latter ion would be in equilibrium with the fluoride (*5*) and probably also with the 1,6-anhydride (*4*).[2] The *p*-nitrobenzoxonium ions (*4* and *5*) would undoubtedly rearrange to the more stable benzoxonium ions (*8* and *9*), in analogy with the results obtained with derivatives of 1,5-anhydro-arabinitol.[6] The equilibrium between *8* and *9* is probably shifted rather strongly towards *8* since a methyl glycoside (*1*) was used.[2]

Hydrolysis of *8* should give *7a*, with an axial *O*-benzoyl group,[8] and this compound was actually obtained in 55 % yield when the hydrogen fluoride solution was hydrolyzed. The structures of *7a* and of the benzoylated product *7b* were evident from the ^{1}H NMR spectra (Table 1). In a separate experiment the crude product, obtained by treating *1* with hydrogen fluoride, was deacylated and then acetylated. In this manner a 40–50 % yield of the known tri-*O*-acetyl-1,6-anhydro-β-D-gulopyranose could be isolated.

EXPERIMENTAL

^{1}H NMR spectra and thin layer chromatography was performed as described previously.[2] ^{13}C NMR spectra were measured at 0 °C with deuterium fluoride as solvent in Teflon sample tubes on a Bruker WH-90 instrument. Positions of signals are given in ppm relative to internal $(CH_3)_3SiCH_2CH_2CH_2SO_3Na$.

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-p-nitrobenzoyl-α-D-galactopyranoside (1).* Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside [9] (3.52 g) was dissolved in pyridine (15 ml) and cooled in ice while *p*-nitrobenzoyl chloride (1.0 g) was added. The mixture was stirred over night at room temperature and it was then worked up in the usual way to give 3.73 g of product, m.p. 99–102 °C. Recrystallization from ethanol gave *1*, m.p. 128–129 °C, $[\alpha]_D^{25}$ +185.3° (*c* 1.4, $CHCl_3$). (Found: C 64.12; H 4.46; N 2.12. Calc. for $C_{35}H_{25}NO_{12}$: C 64.12; H 4.43; N 2.14).

Reaction of 1 *with hydrogen fluoride. A.* A solution of *1* (600 mg) in anhydrous hydrogen fluoride (2 ml) was kept at room temperature for 48 h in a polyethylene bottle. It was then diluted with dichloromethane and poured on ice. The organic phase was washed with water and with aqueous sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated. The residue (600 mg) was purified by preparative TLC with benzene-ether (1:1) as eluent. The main fraction gave 205 mg (55 %) of 2-*O*-benzoyl-4-*O*-*p*-nitrobenzoyl-1,6-anhydro-β-D-gulopyranose (*7a*) as a syrup, which was characterized through its spectrum only (Table 1). The product was benzoylated with benzoyl chloride in pyridine to give 2,3-di-*O*-benzoyl-4-*O*-*p*-nitrobenzoyl-1,6-anhydro-β-D-gulopyranose (*7b*), which was crystallized from ethanol, yield 233 mg, m.p. 150–151 °C, $[\alpha]_D^{25}$ +225° (*c* 1.0, $CHCl_3$).

B. In a second experiment 2.13 g of *1* was treated with hydrogen fluoride (10 ml) as described above. The crude product (1.97 g) was benzoylated with benzoyl chloride (3 ml) in pyridine (10 ml). This gave 2.13 g of a product which was crystallized from ethanol to give 600 mg (36 %) of *7b*, m.p. 153–153.5 °C, $[\alpha]_D^{25}$ +229.5° (*c* 1.9, $CHCl_3$). (Found: C 62.25; H 4.03; N 2.69. Calc. for $C_{27}H_{21}NO_9$: C 62.42; H 4.05; N 2.70).

C. In a third experiment 9.3 g of *1* was treated with hydrogen fluoride. The crude product was dissolved in methanol (36 ml) containing 0.3 % sodium methoxide and kept over night. The solution was then deionized with Amberlite IR-120, filtered and evaporated. The residue was dissolved in water and extracted twice with ether. The water was evaporated and the residue was acetylated with acetic anhydride in pyridine. Work up in the usual way and crystallization from ether-pentane gave 1.634 g (40 %) of tri-*O*-acetyl-1,6-anhydro-β-D-gulopyranose, m.p. 95–102 °C. Recrystallization gave a product with m.p. 110–111 °C, $[\alpha]_D^{25}$ +22.1° (*c* 2.2, $CHCl_3$) (recorded [10] m.p. 114–115 °C, $[\alpha]_D^{25}$ +22.1°).

Microanalyses were performed by Dr. A. Bernhardt, Mikroanalytisches Laboratorium.

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Reaction of Some Pentofuranose Derivatives with Dibromomethyl Methyl Ether. Preparation of Some 2-Bromo-2-deoxy-pentofuranoside Derivatives

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When methyl tri-*O*-acetyl-α-D-arabinofuranoside, or tetra-*O*-acetyl-β-D-ribofuranose, was treated with dibromomethyl methyl ether and zinc bromide di-*O*-acetyl-2-bromo-2-deoxy-α-D-arabinofuranosyl bromide was formed. Analogous treatment of tri-*O*-acetyl-1-*O*-benzoyl-β-D-xylofuranose gave di-*O*-acetyl-2-bromo-2-deoxy-β-D-xylofuranosyl bromide. Benzoylated arabino- and xylo-furanoses also yielded 2-bromo-2-deoxy-furanosyl bromides. Benzoylated ribo- and lyxo-furanoses did not react with dibromomethyl methyl ether. The unstable 2-bromo-2-deoxy-furanosyl bromides were converted into the corresponding methyl furanosides by treatment with methanol.

In a previous paper the reaction of acetylated and benzoylated pentopyranoses with dibromomethyl methyl ether (DBE) was described.[1] It was found that with zinc bromide as a catalyst 2-bromo-2-deoxy-xylopyranose derivatives were obtained from acylated xylo-, arabino-, and lyxo-pyranoses. Acetylated ribopyranose on the other hand, gave 2-bromo-2-deoxy-arabinopyranose derivatives. This investigation has now been extended to pentofuranoses.

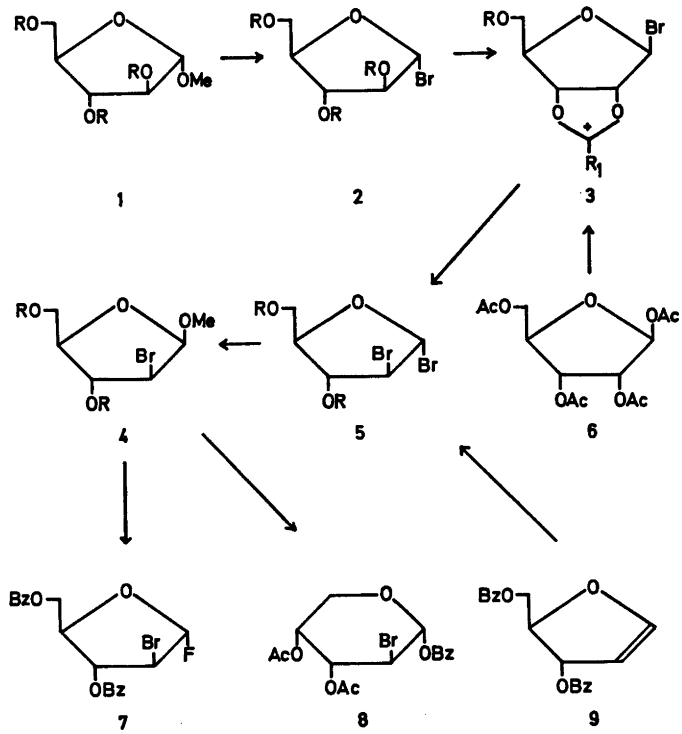
Treatment of methyl tri-*O*-benzoyl-α-D-arabinofuranoside (*1b*) with DBE and zinc bromide in chloroform for 15 min gave a high yield of the furanosyl bromide (*2b*) and a similar result was obtained with tetra-*O*-benzoyl-α-D-xylofuranose (*10*). Acetylated methyl furanosides also gave the corresponding furanosyl bromides when treated briefly with DBE-$ZnBr_2$ as seen from ^{1}H NMR spectra of the reaction mixtures. The bromides were not isolated because of their instability.

More prolonged treatment with DBE led to formation of 2-bromo-2-deoxy-derivatives, analogous to the results found in the pyranose series.[1] Thus reaction of *1b* with DBE-$ZnBr_2$ for 16 h gave a rather unstable product which, as seen from a ^{1}H NMR spectrum (Table 1), was the 2-bromo-2-deoxy-D-arabinosyl bromide (*5b*). Treatment with methanol gave the methyl furanoside (*4b*) in 50 % yield. In the same manner *10* gave a crude bromo-deoxy-bromide (*14b*), which was converted into a mixture of the anomeric methyl furanosides (*15b*), obtained in *ca.* 50 % yield. The tribenzoylated ribo- or lyxo-furanosyl bromides did not give 2-bromo-2-deoxy-compounds on prolonged treatment with DBE-$ZnBr_2$.

The acetate *1a*, when treated in the same way, gave *5a* (Table 1) which was converted into *4a*, contaminated with a small amount of the α-anomer. The same product was obtained in *ca.* 50 % yield by treating tetra-*O*-acetyl-β-D-ribofuranose (*6*) with DBE-$ZnBr_2$.

The xylofuranose derivative (*12*) analogously yielded the unstable bromide (*14a*) and subsequently a mixture of the anomeric methyl furanosides (*15a*). These could not be separated and were therefore only characterized through their ^{1}H NMR spectra (Table 1).

In order to prove the structure of *4b* the unsaturated compound *9* was treated with bromine and the product was reacted with methanol. This gave two products one of which was *4b* and thus proves the configuration at C3 and C4 of *4b*. The $J_{1,2}$ value (Table 1) shows that the substituents at C1 and C2 are

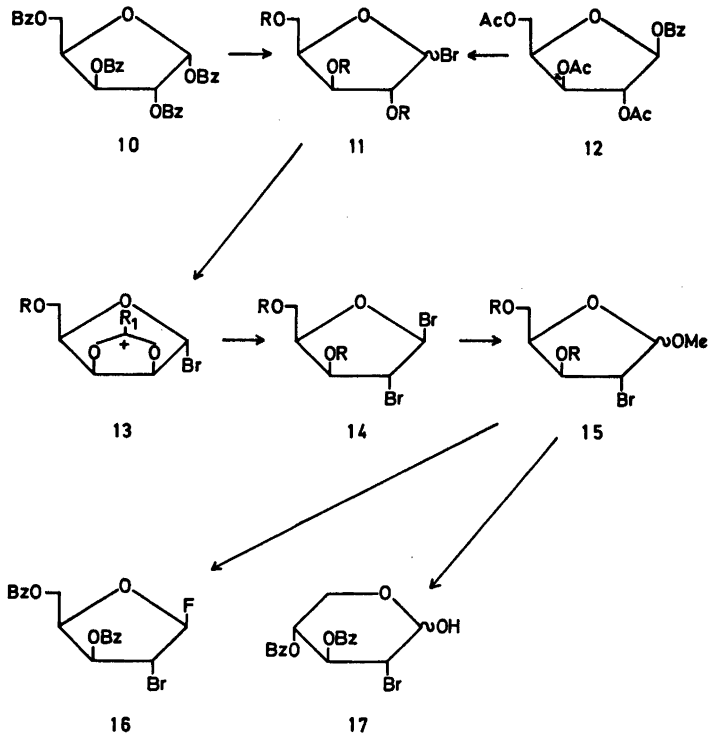


a) R = CH_3CO

b) R = C_6H_5CO

Table 1. Proton chemical shifts and coupling constants of pentofuranose derivatives.

Compound	δ-values H1	H2	H3	H4	H5	H5′	Coupling constants (Hz) $J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{4,5}'$	$J_{5,5}'$	
2b	6.63	5.97	5.66		4.7–5.0		0.7	0	3.5				$J_{13} \simeq 0.5$
5b	6.72	4.92	5.72		4.6–5.0		~0.5	0	3.5				$J_{13} \simeq 0.5$
5a	6.66	4.78	5.3		4.3–4.7		0	0.5	4.0				$J_{13} \simeq 0.5$
7	6.07	4.44	5.66		4.6–4.9		0	0.8	3.0				J_{1F} 61; J_{2F} 7.9; J_{4F} 0
4b	5.00	4.42	5.84	4.4	4.74	4.57	4.4	8.6	5.6	4.6	6.0	11.9	
4a	4.96	4.24	5.44		4.0–4.5		4.4	8.5	5.2				
14b	6.67	4.94	5.97	5.21	4.77	4.77	0	0	5.3	5.3	5.3		
14a	6.61	4.85	5.66	4.99	4.5	4.5	0	0	5.1	6.3	6.3		
16	6.03	4.42	5.85	5.14	4.71	4.71	0	~0	5.0	5.8	5.8		J_{1F} 65; J_{2F} 6.0; J_{4F} 6.0
β-15b	5.27	4.30	5.77	5.02	4.67	4.58	~0.5	1.6	5.5	5.5	5.5	11.8	
α-15b	5.10	4.44	5.96	4.81	4.5	4.5	4.2	6.9	6.7	4.8	4.8		
β-15a	5.20	5.45					0.5	2.0	5.4				
α-15a	5.03	5.59					4.1	7.2	7.2				
12	6.44	5.41	5.52	4.76	4.4	4.3	0	1.3	5.4	5.2	6.8	11.8	



a) R = CH_3CO

b) R = C_6H_5CO

cis-oriented. Brief treatment of *4b* with anhydrous hydrogen fluoride gave the furanosyl fluoride (*7*) which has $J_{1,2}=0$ Hz and therefore must have bromide and fluorine *trans*-oriented. It furthermore has $J_{4,F}=0$ Hz, which shows that fluorine and H4 are *cis*-oriented.[2] There results are only compatible with the structure *4b*. It was further confirmed by its conversion to the known[1] pyranose derivative *8*. The structure of *4a* was proved by its preparation from *4b* *via* a debenzoylation and acetylation.

The *β*-anomer of *15b* has physical data identical with those of a known product.[3] The *α*-anomer was converted into the known pyranose derivative (*17*),[1] thus proving the configuration at C2, C3, and C4. Treatment of *α*-*15b* with hydrogen fluoride gave the *β*-fluoride 16, the anomeric structure of which was found from the $J_{1,2}$ and $J_{4,F}$ values of 0 and 6.0 Hz, respectively.

The anomeric acetates *15a* were not obtained pure, but their structures are evident from the ^{1}H NMR spectra which are very similar to those of *15b* (Table 1).

The 2-bromo-2-deoxy-bromides (*5* and *14*) are probably formed *via* the 2,3-dioxolanylium ions (*3* and *13*), respectively, by an intramolecular migration of the bromine from C1 to C2 as proposed previously in the pyranose series.[1] The ions *3* and *13* may be formed by two different mechanisms depending on the stereochemistry at C2 and C3. When these are *trans*-oriented, as in arabino- and xylo-furanose derivatives, the reaction with DBE probably proceeds *via* a 1,2-dioxolanylium ion which subsequently rearranges to a 2,3-ion, (*3* or *13*). When C2 and C3 are *cis*-oriented the 2,3-ion is probably formed by a mechanism similar to that by which *cis*-1,2-diacetoxycyclohexane reacts with hydrogen fluoride.[4]

The mechanism will be discussed further in a forthcoming paper, in which the reactions of model compounds with DBE are described.

EXPERIMENTAL

Melting points are uncorrected. Preparative TLC was performed on 1 mm layers of silica gel (Merck PF_{254}). ^1H NMR spectra were measured on Varian A-60 and HA-100 instruments or on a Bruker HX-90E instrument in deuteriochloroform solution with tetramethylsilane as internal reference. ^{19}F NMR spectra were obtained on the Bruker instrument. Optical rotations were measured on solutions in chloroform.

2,3,5-Tri-O-*acetyl-1*-O-*benzoyl-β*-D-*xylofuranose* (12). 3,5-Di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose [5] (1.50 g) in acetyl bromide (1.5 ml) was kept at room temp. for 1.5 h. Evaporation gave a mixture of the anomeric tri-*O*-acetyl-D-xylofuranosyl bromides as a syrup. A ^1H NMR spectrum showed H1 of the β-anomer as a singlet at δ 6.3 and H1 of the α-anomer as a doublet at δ 6.85, $J_{1,2}$ 4.6 Hz.

The bromide was treated with silver benzoate in acetonitrile to give 1.5 g of a product which was purified by preparative TLC (ether-pentane 3:1) to give pure *12* as a syrup, $[\alpha]_D^{25}$ −46.8° (*c* 4.8). (Found: C 56.87; H 5.34. Calc. for $C_{18}H_{20}O_9$: C 56.84; H 5.26).

Tri-O-*benzoyl*-α-D-*arabinofuranosyl bromide* (2b). Methyl tri-*O*-benzoyl-α-D-arabinofuranoside [6] (1.09 g) in chloroform (2 ml) was stirred with dibromomethyl methyl ether [7] (DBE) (1.0 ml) and anhydrous zinc bromide (100 mg) for 15 min at room temp. The mixture was then diluted with dichloromethane and washed with 4 N hydrochloric acid until the organic phase was clear; it was then washed with aqueous sodium hydrogen-carbonate, dried ($MgSO_4$) and evaporated. The residue was crystallized from ether-pentane to give 1.06 g (89 %) of *2b*, m.p. 97—100 °C. An additional recrystallization gave m.p. 101—102 °C, $[\alpha]_D^{20}$ +80.1° (*c* 1.7) (reported [6] m.p. 103—104 °C (corr.), $[\alpha]_D^{20}$ +85°). A ^1H NMR spectrum of the material in the mother liquor showed that no β-bromide was present.

Tri-O-*benzoyl*-D-*xylofuranosyl bromide* (11). Treatment of tetra-*O*-benzoyl-α-D-xylofuranose [8] (530 mg) with DBE (1.0 ml) and $ZnBr_2$ (85 mg) in chloroform (1.5 ml) as described above gave 505 mg of a syrup. A ^1H NMR spectrum showed that the product was a mixture of the two anomeric bromides *11* containing *ca.* 80 % α-anomer. The two products could not be separated.

Methyl di-O-*benzoyl-2-bromo-2-deoxy-β*-D-*arabinofuranoside* (4b). *A.* A solution of *1b* (5.0 g) in chloroform (10 ml) was stirred with DBE (10 ml) and $ZnBr_2$ (300 mg) for 16 h at room temp. Work-up as described above gave a syrup (a ^1H NMR spectrum is shown in Table 1) which was dissolved in methanol (200 ml) and stirred for 24 h with silver carbonate (8 g). The mixture was then filtered and evaporated. The residue was dissolved in ether, filtered through carbon and evaporated. Crystallization from ether-pentane gave 2.27 g (50 %) of 4b, m.p. 107—108 °C, $[\alpha]_D^{20}$ −98.0° (*c* 2.8). One additional recrystallization gave a product with m.p. 109—109.5 °C, $[\alpha]_D^{20}$ −98.6° (*c* 2.7). (Found: C 55.29; H 4.55; Br 18.21. Calc. for $C_{20}H_{19}BrO_6$: C 55.19; H 4.37; Br 18.39).

B. Freshly prepared di-*O*-benzoyl-1,2-dideoxy-D-*erythro*-pent-1-enofuranose (*9*) [9] (0.57 g) in benzene (15 ml) was cooled to +5 °C and bromine (0.5 ml) in benzene (10 ml) was added during 15 min. Evaporation of the solvent gave a residue which, as seen from a ^1H NMR spectrum, was a 1:1 mixture of the two *trans*-addition products. The mixture was stirred with methanol (20 ml) and silver carbonate (1.0 g) for 1 h. Filtration through carbon and evaporation gave a residue which was purified by preparative TLC (ether-pentane 1:2) to give 446 mg (58 %) of a 1:1 mixture of two methyl glycosides as seen from ^1H NMR spectra.

Crystallization from ether-pentane gave 129 mg of (4b), m.p. 106—107 °C, $[\alpha]_D^{20}$ −96.0° (*c* 2.5). A ^1H NMR spectrum and a mixed m.p. proved the identity with the product described above.

The material in the mother liquor was a mixture which could not be separated completely by chromatography. A ^1H NMR spectrum showed that methyl 3,5-di-*O*-benzoyl-2-bromo-2-deoxy-α-D-ribofuranoside was the main component. It gave the following spectral data: δ 5.13 (H1), 4.38 (H2), 5.55 (H3). $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 6.9 Hz, $J_{3,4}$ 3.1 Hz.

Methyl di-O-*acetyl-2-bromo-2-deoxy-β*-D-*arabinofuranoside* (4a). *A.* Methyl tri-*O*-acetyl-α-D-arabinofuranoside [10] (587 mg) in chloroform (1.5 ml) was treated with DBE (1.5 ml) and $ZnBr_2$ (200 mg) for 16 h at room temp. An additional 1.5 ml of DBE was then added and the mixture was stirred for 24 h. Work-up as described above gave 0.8 g of a dark syrup which was treated with methanol (50 ml) and silver carbonate (1.0 g). The product (427 mg) was purified by preparative TLC (ether-pentane 3:1) to give 280 mg (45 %) of a mixture of anomeric glycosides. A ^1H NMR spectrum showed that 90—95 % was the β-anomer (*4a*). Crystallization from ether-pentane gave 175 mg of *4a*, m.p. 79—80 °C, $[\alpha]_D^{21}$ −107° (*c* 2.5).

B. Tetra-*O*-acetyl-β-D-ribofuranose (1.01 g) in chloroform (1.5 ml) was treated with DBE (1.5 ml) and $ZnBr_2$ (317 mg) for 16 h. After treatment with methanol and silver carbonate the product (750 mg) was purified by preparative TLC (ether-pentane 3:1) to give 503 mg (51 %) of a mixture of anomers. Crystallization from ether-pentane gave 427 mg of *4a*, m.p. 80—81 °C, $[\alpha]_D^{21}$ −107° (*c* 2.0). Further recrystallization did not change these values.

(Found: C 38.40; H 4.92; Br 25.70. Calc. for $C_{10}H_{15}BrO_6$: C 38.59; H 4.87; Br 25.68).

The α-anomer could not be obtained pure, but a ^{1}H NMR spectrum of the material in the mother liquor gave the following data: δ 5.20 (H1), 5.26 (H3); $J_{2,3}$ 2 Hz, $J_{3,4}$ 4 Hz, in agreement with reported values.[11]

*Methyl di-*O*-benzoyl-2-bromo-2-deoxy-*D*-xylofuranoside* (15b). A solution of *10* (1.07 g) in chloroform (2.0 ml) was treated with DBE (2.0 ml) and $ZnBr_2$ (567 mg) for 16 h. This gave 1.1 g of crude di-*O*-benzoyl-2-bromo-2-deoxy-β-D-xylofuranosyl bromide (*14*) (^{1}H NMR spectral data are shown in Table 1) which was at once treated with methanol and silver carbonate as described above. The product (630 mg) was separated into two fractions by preparative TLC (ether-pentane 1:2).

The fast moving fraction gave 97 mg (12 %) of *β-15b* which was crystallized from ether-pentane, m.p. 66—67 °C, $[\alpha]_D^{21}$ −4.8° (*c* 1.5) (reported [3] m.p. 67.5—68 °C, $[\alpha]_D$ −6°). (Found: C 55.08; H 4.84; Br 18.17. Calc. for $C_{20}H_{19}BrO_6$: C 55.19; H 4.36; Br 18.36).

The next fraction contained 351 mg (43 %) of *α-15b* as a syrup, $[\alpha]_D^{21}$ +163.1° (*c* 5.9). (Found: C 54.99; H 4.34; Br 18.20).

*Methyl di-*O*-acetyl-2-bromo-2-deoxy-*D*-xylofuranoside* (15a). Reaction of *12* (605 mg) in 1 ml of chloroform with DBE (1.5 ml) and $ZnBr_2$ (200 mg) for 16 h gave di-*O*-acetyl-2-bromo-2-deoxy-β-D-xylofuranosyl bromide (*14a*) as an unstable, dark syrup (Table 1). The bromide was treated with silver carbonate in methanol and the product thus obtained was purified by preparative TLC (ether-pentane 1:1). This gave 218 mg (44 %) of *15a* as a mixture of anomers in an α:β ratio of 3:1. They could not be separated, but ^{1}H NMR data were obtained from the mixture (Table 1).

*Di-*O*-benzoyl-2-bromo-2-deoxy-α-D-arabinofuranosyl fluoride* (7). A solution of *4b* (244 mg) in anhydrous hydrogen fluoride (5 ml) was kept at 0 °C for 15 min. Dichloromethane was then added and the mixture was poured on ice. The organic phase was washed with aqueous sodium hydrogen carbonate, dried and evaporated. The residue (230 mg) was purified by preparative TLC (ether-pentane 1:3) to give 164 mg (70 %) of *7*, m.p. 92—93 °C. One recrystallization from ether-pentane gave a product with m.p. 94—95 °C, $[\alpha]_D^{20}$ −15.4° (*c* 2.1). (Found: C 54.08; H 3.93; Br 18.71. Calc. for $C_{19}H_{16}BrFO_5$: C 53.92; H 3.81; Br 18.88). ^{1}H NMR data are given in Table 1. A ^{19}F NMR spectrum gave ϕ = −106.5 ppm (using internal trichlorofluoromethane).

*Di-*O*-benzoyl-2-bromo-2-deoxy-β-D-xylofuranosyl fluoride* (16). Treatment of 335 mg of (*α-15b*) with hydrogen fluoride in the same way, followed by preparative TLC gave 135 mg (41 %) of *16*. Recrystallization from ether-pentane gave the pure product, m.p. 68—70 °C, $[\alpha]_D^{20}$ +10.4° (*c* 3.3). (Found: C 53.84; H 3.98; Br 18.70). ^{19}F NMR data: ϕ = −103.0 ppm.

Conversion of 4b *into 3,4-di-*O*-acetyl-1-*O*-benzoyl-2-bromo-2-deoxy-α-*D*-arabinopyranose* (8). Sodium (200 mg) was dissolved in methanol (10 ml) and 400 mg of *4b* was added. The mixture was stirred for 30 min and it was then neutralized with Amberlite IR-120 and evaporated. The residue was boiled for 5 h with 0.1 N HCl and the solution was neutralized with Amberlite IR-4B and evaporated. After two evaporations with pyridine the residue was acetylated with acetic anhydride (2 ml) in pyridine (10 ml). Work-up in the usual way gave 204 mg of tri-*O*-acetyl-2-bromo-2-deoxy-D-arabinopyranose which was treated with DBE (0.3 ml) and $ZnBr_2$ (20 mg) in chloroform (0.5 ml) for 1 h. Work-up as described above and treatment of the crude product with silver benzoate (1.0 g) in acetonitrile (10 ml) yielded 170 mg of a product which was purified by preparative TLC (ether-pentane 1:1). This gave 83 mg (20 %) of *8* which was recrystallized from ether-pentane, m.p. 144—145 °C, $[\alpha]_D^{20}$ −5.57° (*c* 2.3). A ^{1}H NMR spectrum and a mixed m.p. proved its identity with a product described previously.[1]

Conversion of α-15b *into 3,4-di-*O*-benzoyl-2-bromo-2-deoxy-*D*-xylopyranose* (17). 350 mg of *α-15b* was debenzoylated and hydrolyzed with 0.1 N HCl as described above. The 2-bromo-2-deoxy-D-xylose thus obtained was benzoylated with benzoyl chloride in pyridine to give tri-*O*-benzoyl-2-bromo-2-deoxy-D-xylopyranose. Treatment of this with DBE and $ZnBr_2$ gave the pyranosyl bromide, which was hydrolyzed with aqueous acetone in the presence of silver carbonate. This gave crude *17* which was purified by preparative TLC and recrystallized to yield 49 mg of the pure product, m.p. 141—143 °C, $[\alpha]_D^{20}$ −44.2° (5 min) → −32.5° (5 days) (*c* 0.2). The product was identical with that described previously.[1]

Conversion of the benzoate 4b *into the acetate* 4a. 538 mg of *4b* was debenzoylated with methanolic sodium methoxide as described above. The product, obtained after evaporation of the methanol, was extracted with pentane to remove most of the methyl benzoate. Acetylation with acetic anhydride in pyridine gave 409 mg of crude *4a* which was crystallized from ether-pentane, 272 mg (71 %), m.p. 79.5—80.5 °C, $[\alpha]_D^{20}$ −106.5° (*c* 4.0). A mixed m.p. and a ^{1}H NMR spectrum proved its identity with the product described above.

Microanalyses were performed by Dr. A. Bernhardt, Mikroanalytisches Laboratorium.

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Chemistry of *gem*-Dihalocyclopropanes. IX. The Stereospecific Synthesis of Conjugated *meso*-Diallenes

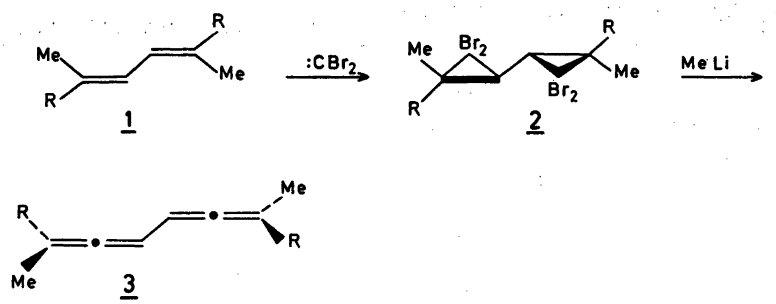
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Addition of dibromocarbene to the conjugated *E,E*-dienes *1b* and *1c* occurred stereoselectively in two cases to give the corresponding bis-adducts *2b* and *2c* with *meso*-configuration. Treatment of the bis-adducts with methyllithium proceeded with stereospecific ring-opening to the corresponding *meso*-diallenes *3b* and *3c*. The stereoselectivity of the addition reaction is rationalized by discussing the possible conformations of the intermediate vinyl-dibromocyclopropane derivatives. The second reaction is explained in terms of a preferred mode of ring-opening of a cyclopropylidene-like intermediate.

Diallenes represent a unique diene system, and they have been shown to undergo interesting intramolecular [1,2] and intermolecular [3,4] thermal reactions. In order to study the stereochemistry of these reactions it is necessary to use a chiral diallene of known configuration. A synthesis of diallenes which has some general applicability involves the addition of dibromocarbene to a diene followed by reaction of the bis-adduct with methyllithium; cyclic[2,5,6] and noncyclic diallenes [3,7] have been prepared by this method. The present work describes the stereospecific synthesis of conjugated *meso* diallenes by this twostep reaction sequence [8] (Scheme 1).

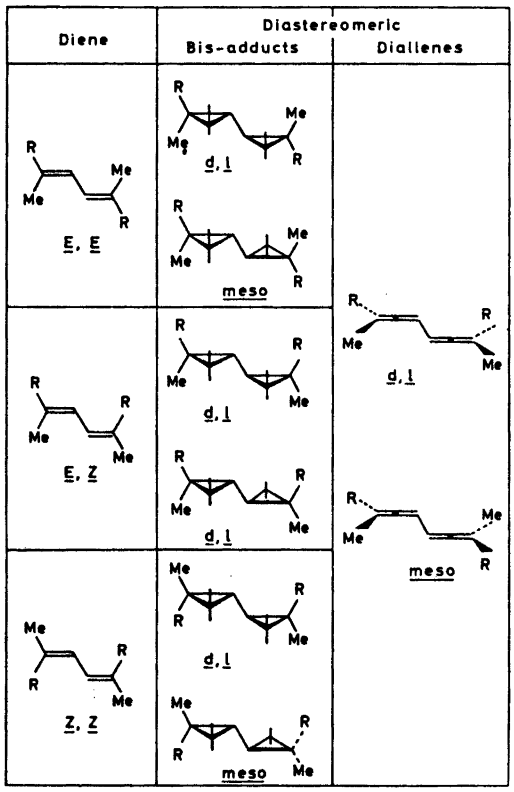
The addition of dibromocarbene to 2,5-dimethyl-2,4-hexadiene (*1a*) exhibited some stereoselectivity in that *meso* and racemic bis-adducts *2a* were formed in a ratio of 2.3:1;[9] both reacted with methyllithium to give exclusively 2,7-dimethyl-2,3,5,6-octatetraene (*3a*), a non-chiral diallene. However, by replacing one methyl group at each end of the diene *1a* with another substituent three possible stereoisomeric dienes could result and the addition of dibromocarbene could lead to six different bis-adducts as shown in Table 1. Each of these bis-adducts could react with methyllithium to *meso*- and racemic diallenes. It was therefore essential to use one of the geometric isomers of *1* as starting material and preferably the *E,E*- or *Z,Z*-isomer because if necessary the separation of *meso*- and racemic forms of the bis-adduct was expected to be easier than that of two racemates. Recently, the cuprous salt-catalyzed coupling of vinylic lithium [10] or Grignard [11] derivatives have been shown to yield the corresponding conjugated dienes with a high degree of stereospecificity, and this procedure was attempted for the preparation of the *E,E*-isomers of *1*.



1 2 3

Scheme 1.

Table 1.



The lithium derivative from *E*-1-bromo-2,3,3-trimethyl-1-butene [12] was successfully coupled in 87 % yield to the diene-2,2,3,6,7,7-hexamethyl-3*E*,5*E*-octadiene (*1b*). Coupling of the Grignard derivative of *E*-1-bromo-2-phenyl-1-propene [13] in tetrahydrofuran gave a 48 % yield of crystalline 2,5-diphenyl-2*E*,4*E*-hexadiene (*1c*) [14] besides some α-methylstyrene and 1-phenyl-1-propyne; the lithium derivative of the above bromide could not be used because it rearranges rapidly to an equilibrium mixture of *E*- and *Z*-1-lithio-2-phenyl-1-butene and 1-phenyl-1-propyne.[13] On the other hand, we were not able to obtain pure *E*-1-bromo-2-methyl-1-butene, the starting material for the preparation of 3,6-dimethyl-3*E*,5*E*-octadiene (*1d*); consequently, the diene was prepared by a conventional synthetic route.

Reaction of 1-bromo-3-methyl-2-pentene and triphenyl phosphine afforded the phosphonium salt, and the corresponding ylid reacted with 2-butanone to yield the diene *1d* as a mixture of 50 % *E,E*-, 40 % *E,Z*-, and 10 % *Z,Z*-isomers. These could unfortunately not be separated by fractional distillation.

Table 2. Tetrabromobicyclopropyl derivative (*2*) from dienes (*1*) and dibromocarbene.

Diene *1*	R	Diadduct *2* Racem. %	*meso* %	Yield %
a	Me	30	70	92
b	*t*-Bu	0	100	27
c	Ph	0	100	44
d	Et[a]			75[b]

[a] Mixture of geometric isomers. [b] Complex mixture of diastereomers.

The addition of dibromocarbene, generated from bromoform and potassium *t*-butoxide, to the dienes gave the corresponding bis-adducts in varying yields (Table 2). The structures were assigned on the basis of spectral data. It is worth noting that the mass spectral data provide little information; in no case was the molecular ion observed and usually the highest observable mass was a result of extensive fragmentation. Both products *2a* and *2d* were stereoisomeric mixtures, but on the other hand compounds *2b* and *2c* were homogeneous as shown by thin layer chromatography. Their configurations were established by crystallographic measurements; space group determinations demanded that both molecules have a centre of symmetry, a requirement which is only satisfied by the *meso*-form. Hence, a highly selective addition of the carbene had taken place. Unfortunately, the yields of these bis-adducts were not good; compound *2c* was accompanied by some unreacted starting material

Table 3. Conjugated diallenes (*3*) from tetrabromobicyclopropyl derivatives (*2*) and methyllithium.

Bis-adduct *2*	Diallene *3*	Yield %
a	$[Me_2C=C=CH-]_2$	93[3]
b	$[t\text{-}Bu(Me)C=C=CH-]_2$	91
c	$[Ph(Me)C=C=CH-]_2$	90
d	$[Et(Me)C=C=CH-]_2$	82

while from the reaction of *1b* a liquid was actually obtained as the major product. It consisted mainly of two isomeric bromides in a ratio of approximately 4:1, but decomposition on attempted separation by preparative gas chromatography has hampered the structural elucidation; further work is in progress and will be reported in due course. The product from the addition of dibromocarbene to the diene *1d* consisted of a mixture of stereoisomeric bis-adducts, as expected, since the diene was stereochemically heterogeneous.

Treatment of the bis-adducts with methyllithium afforded in each case the corresponding diallene in high yield (Table 3). From both *meso-2b* and *meso-2c* single products were obtained as indicated by the NMR spectra and the configurations were shown in both cases to be *meso* since the crystallographic space group demanded a centre of symmetry.

A complete X-ray analysis of the diallene *3c* [15] confirmed this conclusion. The conversion of the dibromocyclopropane ring to the corresponding allenic linkage had occurred with complete stereospecificity. As expected, the diallene *3d* obtained from the mixture of bis-adducts *2d* consisted of a mixture of stereoisomers as indicated by the NMR spectrum, but all attempts to separate them were unsuccessful.

DISCUSSION

Addition of dibromocarbene to symmetrically substituted conjugated *E,E*-dienes can only lead to a single vinylcyclopropane derivative independent of which double bond reacts; however, the vinylcyclopropane derivative can react further with a second mole of carbene to give either *meso* or racemic bis-adduct depending on the direction of addition to the double bond. This has been verified experimentally; 1,3-butadiene and dichlorocarbene afforded a mixture of almost equal amounts of the two stereoisomeric bis-adducts;[16] dibromocarbene and 2,5-dimethyl-2,4-hexadiene yielded a mixture consisting of 70 % *meso* and 30 % racemic bis-adducts, whereas from 2,3-dimethyl-1,3-butadiene the *meso*-bis-adduct was obtained exclusively.[8] Cyclic conjugated dienes add dihalocarbenes preferentially *anti* with formation of racemic bis-adducts.[16,17] This selectivity must essentially result from steric factors which would also be expected to determine the selectivity in additions to the dienes *1* of the present work. Consequently, knowledge about possible conformations of the intermediate vinylcyclopropane derivatives could provide an explanation for the preferred formation of *meso* bis-adducts.

In the gas phase vinylcyclopropane itself exists as an equilibrium mixture of approximately 75 % *anti* and 25 % *gauche* conformations with a free energy difference at 20 °C of about 1 kcal/mol.[18] NMR spectroscopy indicates that such an equilibrium is also present in solution.[19,20] However, a similar study on 1-isopropenyl-1-methylcyclopropane indicates that the *anti* conformation does not contribute significantly to the rotational equilibrium; steric repulsion between the cyclopropyl methyl group and the vinylic hydrogen, estimated to about 1 – 1.5 kcal/mol, is sufficient to shift the equilibrium towards a *gauche* conformation.[21] For 1,1-dibromo-2-vinylcyclopropane NMR studies indicate a preferred conformation in which the double bond is rotated about 90° from the *anti* position owing to hydrogen-bromine repulsion.[20] Molecular models suggest that both the hydrogen-methyl and the hydrogen-bromine interactions described above would be

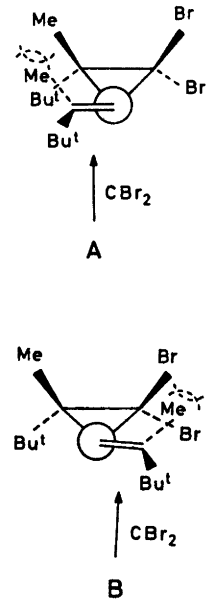


Fig. 1.

important in the *anti* conformation of the vinylcyclopropane precursors of the bis-adducts *2b* and *2c*. We therefore assume that these compounds will prefer a *gauche* conformation as depicted in Fig. 1 for the case of adduct *2b*. The double bond can rotate clockwise or counterclockwise. The former mode will first encounter van der Waals contact between the methyl and *t*-butyl groups and having passed this barrier serious methyl-methyl interactions are apparent after about 70 – 80° of rotation (Fig. 1, A). The latter mode will result in van der Waals contact between a bromine atom and a methyl group after about 70 – 80° (Fig. 1, B). It seems reasonable that the conformer B will be of lower energy, an assumption which is also supported by the observed conformational equilibrium of 2-bromobutane in which a methyl-bromine interaction is preferred over a corresponding methyl-methyl interaction.[22] A similar argument can be presented for adduct *2c*. The least hindered approach to the double bond of conformer B would be from the bottom phase, and consequently the addition of dibromocarbene would be expected to yield the *meso* bis-adduct. The same approach is also the least hindered one in the case of conformer A, but in this case the racemic bis-adduct would result.

Extensive experimental results indicate that reactions of *gem*-dibromocyclopropane derivatives with methyllithium lead to a carbene-like intermediate which subsequently can undergo ring-opening to an allene. The geometry of the allene demands that this ring-opening is conrotatory [24] or monorotatory.[25] Substituted cyclopropylidenes with an *E*-configuration will have two distinctly different modes of ring-opening, *a* and *b*: (Fig. 2).

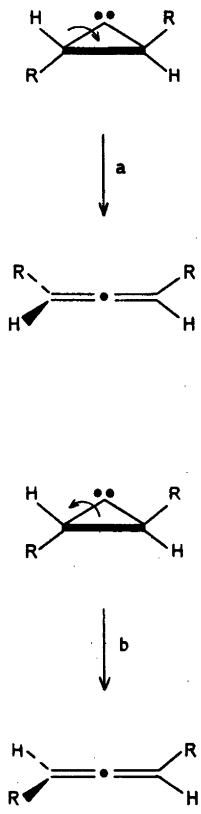
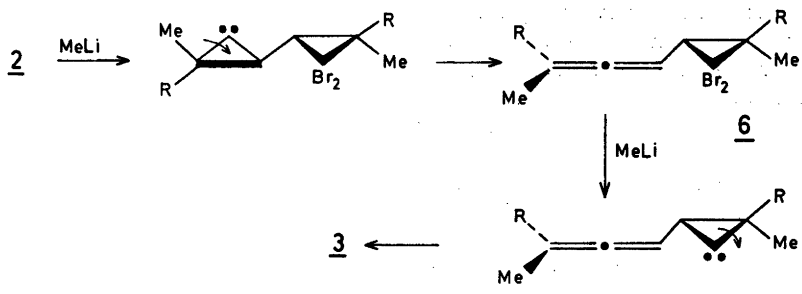


Fig. 2.

Mode *a* creates the least steric interaction during the bond rotations because the substituents R and R′ are moving away from each other, and experiments [24,23] have shown that this is indeed the preferred mode. Applying this knowledge to our present system provides an explanation for the observed stereospecificity



Scheme 2. R = Bu^t or Ph

of the bis-adduct to diallene conversion; *meso* bis-adduct *2* is converted *via* the allene *6* to *meso* diallene *3* by employing twice the *a* mode of ring-opening as shown in Scheme 2. Preference for mode *a* can probably only be important if the steric bulk of group R is considerably larger than that of a methyl group. The stereochemistry of the ring-opening to diallenes of other bis-adducts is under study.

EXPERIMENTAL

NMR spectra were recorded on Varian Associates A60A and HA100-15D spectrometers. Mass spectral data were obtained using an A.E.I. MS 902 mass spectrometer. A Perkin-Elmer model 457 spectrophotometer was used for obtaining IR spectra, and UV spectra were recorded on a Cary 14 spectrophotometer. Gas chromatographic analyses were performed with Varian Aerograph Models 90P and 711. Elemental analyses were carried out by Ilse Beetz Microanalytical Laboratory, 8640 Kronach, West Germany. All reactions were carried out under pure nitrogen.

E,E-*2,2,3,6,7,7-Hexamethyl-3,5-octadiene* (1b) A solution of 18.0 g (0.1 mol) of *E*-1-bromo-2,3,3-trimethyl-1-butene [12] in 25 ml of dry ether was added to 2.0 g (0.28 g at.) of lithium in 75 ml of dry ether with vigorous stirring and cooling (ice). After 3 h stirring at room temperature the solution of the lithium derivative was added during 15 min to a suspension of 40 g (0.4 mol) of finely powdered anhydrous cuprous chloride in 50 ml of dry ether kept at −60 °C. The green-coloured suspension was stirred at this temperature for 1 h and then allowed to attain room temperature overnight. The reaction mixture was finally heated with reflux for 1 h, cooled and the solid separated by centrifugation. Water was added, the product extracted with ether and dried ($MgSO_4$). Evaporation of the ether and distillation of the residue gave 8.5 g (87 %) of crude (92 %) *1b*. Fractionation through a spinning-band column gave pure *1b* (99 %); b.p. 76 °C (3.5 mmHg), m.p. 43 °C; ν_{max} 1595 m, 880 cm^{-1} (C=C); $\delta(CCl_4)$ 1.09 (s, 18 H, *t*-Bu), 1.72 (s, 6 H, Me), 6.00 (s, 2 H, olef. H). (Found: C 86.44; H 13.45. Calc. for $C_{14}H_{26}$: C 86.51; H 13.49.)

E,E-*2,5-Diphenyl-2,4-hexadiene* (1c). A solution of 5.0 g (2.5 mmol) of *E*-1-bromo-2-phenyl-1-propene [13] in 10 ml of dry THF was added to 0.61 g (2.5 mmol) of magnesium and catalytic amounts of mercuric chloride in 25 ml THF. The reaction temperature was kept below 40 °C during the addition of the bromide (15 min). After 30 min at room temperature and 1 h reflux the reaction mixture appeared as a grey-coloured suspension. Hydrolysis of an aliquot gave 95 % α-methylstyrene. An additional 10 ml THF was added and the reaction mixture was cooled to −60 °C. 40 g (0.4 mol) of finely powdered anh. cuprous chloride was added portionwise with stirring. The reaction mixture was further stirred for 2 h at −60 °C and allowed to attain room temperature overnight. The reaction was worked up as described under *1b*. Evaporation of the solvents gave 1.4 g (48 %) of pure (99 %) *1c*, m.p. 134−137 °C (lit.[14] m.p. 138 °C) λ_{max} 1590, 860 cm^{-1} (C=C); $\delta(CCl_4)$ 2.25 (s, 6 H, Me), 6.75 (s, 2 H, olef. H), 7.8 (m, 10 H, ar. H).

3,6-Dimethyl-3,5-octadiene (1d). To a suspension of 213 g (0.5 mol) of 3-methyl-2-pentenyltriphenylphosphonium bromide prepared from 1-bromo-3-methyl-2-pentene and triphenylphosphine in 1000 ml of dry ether was added with stirring during 90 min 245 ml of 2.17 M ethereal methyllithium (0.52 mol). The deep red-coloured reaction mixture was stirred at room temperature for another 1 h and then cooled (ice). A solution of 37.4 (0.52 mol) of 2-butanone was added dropwise with stirring during 2 h. The reaction mixture was left stirring at room temperature overnight and then heated under reflux for 2 h. Triphenylphosphine oxide was filtered, and the ether solution was washed with water and dried ($MgSO_4$). Evaporation of ether and fractionation of the residue gave 58.6 g (85 %) of *1d*,[26] b.p. 62 °C (14 mmHg); GLC (20 % SE-30) showed the presence of 10 % *Z,Z*-, 40 % *Z,E*-, and 50 % *E,E*-isomers.

Preparation of bis-adducts. General. Solid potassium *t*-butoxide was prepared from potassium and *t*-butyl alcohol as described in the literature.[27] The diene and dry pentane were added, and to the stirred slurry the calculated amount of bromoform was added dropwise at about −15 °C. The mixture was stirred overnight at room temperature, hydrolyzed with dil. HCl and worked up in the usual way.

E,E-*2,2,2',2'-Tetrabromo-3,3'-di-t-butyl-3,3'-dimethylbicyclopropyl* (2b). Reaction of potassium *t*-butoxide [from 3.9 g (0.1 g at.) of potassium], 25 g (0.1 mol) of bromoform and 5.5 g (28 mmol) of the diene *1b* in 40 ml pentane gave 4.0 g (27 %) of the *meso* bis-adduct *2b*, m.p. 143 °C from methanol; $\delta(CCl_4)$ 1.29 (s, 18 H, *t*-Bu), 1.53 (s, 6 H, Me), 1.70 (s, 2 H cyclopropyl H). (Found: C 35.89; H. 4.99. Calc. for $C_{16}H_{26}Br_4$: C 35.71; H 4.87).

From another experiment on twice the scale 6.6 g (25 %) of *2b* was obtained, and distillation of the mother liquor gave 6.6 g of a liquid, b.p. 80−86 °C (0.5 mmHg) which consisted essentially of two isomers $C_{15}H_{25}Br$ in a ratio of 4:1 as shown by mass spectrometry and GLC. The isomers could not be separated by prep. GLC without decomposition.

E,E-*2,2,2',2'-Tetrabromo-3,3'-dimethyl-3,3'-diphenylbicyclopropyl* (2c). Reaction of potassium *t*-butoxide [from 8.0 g (0.2 g at.) potassium], 51 g (0.2 mol) of bromoform and 12 g (0.05 mol) of the diene *1c* in 200 ml pentane gave 12.8 g (44 %) of the *meso* bis-adduct *2c*, m.p. 180 °C (decomp.) from petroleum ether-benzene; $\delta(CCl)_4$

1.83 (s, 6H, Me), 2.08 (s, 2 H, cyclopropyl H), 2.4 (m, 10 H). (Found: C 41.52; H 3.43. Calc. for $C_{20}H_{18}Br_4$: C 41.55; H 3.31).

2,2,2',2'-Tetrabromo-3,3-'diethyl-3,3'-dimethylbicyclopropyl (2d). Reaction of potassium *t*-butoxide (from 35 g (0.9 g at.) of potassium), 200 g (0.9 mol) bromoform and 41 g (0.3 mol) of the diene *1d* in 250 ml pentane gave 112 g (75 %) of the bis-adduct *2d*, m.p. 83–85 °C from methanol. (Found: C 29.93; H 3.91. Calc. for $C_{12}H_{18}Br_4$: C 29.91; H 3.76.) Thin layer chromatography on silicagel (pentane) revealed the presence of at least three isomers.

Reactions of bis-adducts with methyllithium. General. The procedure was essentially as described previously.[3] An ethereal solution or suspension of the bis-adduct was cooled to −70 °C and an ethereal solution of methyllithium was added drop-wise with stirring. After an hour, whereby the temperature rose to about −20 °C, water was added and the product isolated in the usual way.

2,2,3,8,9,9-Hexamethyl-3,4,6,7-decatetraene (3b). From reaction of 5.4 g (10 mmol) of adduct *2b* and methyllithium (40 mmol) in ether was obtained 2.0 g (91 %) of the *meso* diallene *3b*, m.p. 72 °C from methanol; ν_{max} 1945, 810 cm^{-1} (C=C=CH−); λ_{max} (heptane) 214 nm (42 000); $\delta(CCl_4)$ 1.08 (s, 18 H, *t*-Bu), 1.76 (m, 6 H, Me), 5.53 (m, 2 H, olef. H). (Found: M.w. 218; C 88.05; H 11.98. Calc. for $C_{16}H_{26}$ (218): C 88.00; H 12.00.)

2,7-Diphenyl-2,3,5,6-octatetraene (3c). From reaction of 7.0 g (12 mmol) of the adduct *2c* and methyllithium (40 mmol) in ether was obtained 2.8 g (90 %) of the *meso* diallene *3c*, m.p. 139 °C (decomp.) from petroleum ether; ν_{max} 1930, 810 cm^{-1} (C=C=CH−); λ_{max} ($CHCl_3$) 261 nm (53.500); $\delta(CCl_4)$ 2.18 (m, 6 H, Me), 5.97 (m, 2 H, olef. H), 7.2 (m, 10 H, ar. H). (Found: M.w. 258; C 92.69; H 7.29. Calc. for $C_{20}H_{18}$ (258): C 92.98; H 7.02.) The structure has been confirmed by X-ray crystallography.[15]

3,8-Dimethyl-3,4,6,7-decatetraene (3d). From reaction of 14.5 g (30 mmol) of the adduct *2d* and methyllithium (80 mmol) in ether was obtained 4.0 g (82 %) of the diallene *3d*, b.p. 45–47 °C (0.5 mmHg); ν_{max} 1955, 820 cm^{-1} (C=C=CH−); λ_{max} (heptane) 212 nm (42 600); $\delta(CCl_4)$ 1.01 (t, 6 H) and 1.95 (q, 4 H)(Et), 1.70 (m, 6 H, Me), 5.42 (m, 2 H, olef. H). (Found: M.w. 162; C 88.78; H 11.17. Calc. for $C_{12}H_{18}$ (162): C 88.82; H 11.18.)

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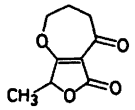
Naturally Occurring Lactones and Lactams. VI.* A Novel Synthetic Route to Carolic Acid

A. SVENDSEN and P. M. BOLL

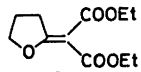
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In a reinvestigation of the total synthesis of carolic acid the problem of the side chain construction has been solved *via* the preparation of the hitherto unknown compound ethyl 6-chloro-3-oxohexanoate *4*. When condensed with 2-bromopropionyl bromide *4* gave an intermediate bromo ester which could be cyclized to the natural product with 2.5 N potassium hydroxide. Cyclization of *4 per se* with sodium hydride afforded the tetrahydrofurylidene acetate *8*.

In an attempt to prepare the mold tetronic acid carolic acid *1* Haynes, Plimmer and Stanners [2] condensed 4-bromobutyryl chloride with ethoxymagnesiomalonic ester in order to obtain the carolic acid side chain, but instead a solid product, $C_{11}H_{16}O_5$, of m.p. 52.5—54.0 °C was isolated, to which they assigned the structure *2*.



1



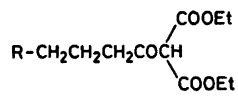
2

Later Sudo *et al.*[3] in their synthesis of carolic acid circumvented the problem of a labile halogen atom by means of the benzyloxy ester *3*.

R-$CH_2CH_2CH_2COCH_2COOEt$

3 R=$C_6H_5CH_2O$
4 R=Cl

In connection with our studies on the synthesis of naturally occurring tetronic acids we have investigated the possibility of preparing the chloro ester *4* as a useful building block since a chloro derivative might be less labile than the corresponding bromo derivative. At first the synthetic route of Haynes, Plimmer and Stanners was reexamined. In our hands, when 4-bromobutyryl bromide was condensed with diethyl ethoxymagnesiomalonate no solid product was obtained. Instead a 60 % yield of a viscous oil was isolated. Upon standing for months at room temp. or at −15 °C with agitation and scratching from time to time this oil showed no tendency to solidify. Since elemental analysis and spectroscopic data of this liquid product were in agreement with the structure and formula ($C_{11}H_{16}O_5$) of *2*, we were led, initially, to think that the original structure report might be erroneous. In any case it was obvious that the bromo compound *5* could not be prepared by this method. In contrast the chloro derivative *6* from 4-chlorobutyryl chloride and diethyl ethoxymagnesiomalonate was isolated without difficulty.



5 R=Br
6 R=Cl

This established that the chloro diester was less prone to spontaneous cyclization than the bromo derivative. More vigorous conditions were demanded for this reaction and the transformation of *6* to the tetrahydrofurylidene malonate *2* was achieved with sodium hydride in refluxing

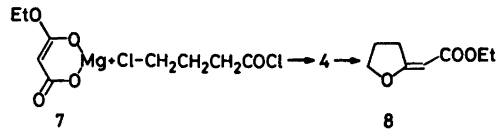
* Part V, see Ref. 1.

benzene to give only liquid material. On the other hand, when performed in abs. ethanol with potassium hydroxide acting as a base the cyclization led to a crystalline sample of m.p. 55–56 °C. When any of the aforementioned oily samples of *2* were seeded with solid *2* a rapid crystallization took place to solid products with m.p. 55–56 °C. The identity of our material with that previously prepared and assigned the structure *2* was thus fully established.

The decarboxylation of *6* would offer a convenient route to the key compound *4*. In an attempt to carry out this degradation *6* was refluxed in dilute acetic acid for 4 h, after which time a quantitative amount of carbon dioxide gas had been collected. Careful work-up afforded only traces of organic material indicating progressive destruction of *6* to watersoluble fragments.

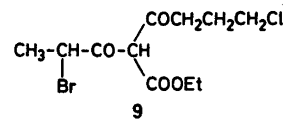
By analogy with the application of *t*-butyl ethyl malonate in the preparation of other β-keto esters, *e.g.* diethyl β-keto adipate,[4,5] we condensed this mixed ester with 4-chlorobutyryl chloride and obtained an almost quantitative yield of a colourless oil which when pyrolyzed at 130 °C (0.2 mmHg) with *p*-TsOH as catalyst only sluggishly evolved gas. The prolonged subjection to heating (10 h) was damaging and distillation of the pyrolysis product afforded no *6*. Finally modification of earlier synthetic procedures for β-keto esters gave us *6* in hand.

It has been reported [6] that methylene chloride may serve well as medium of reaction for Grignard compounds if these are prepared first in ether, but this possibility does not seem to have been considered very much since. The condensation of ethyl hydrogen malonate with acid chlorides or anhydrides and subsequent hydrolysis and decarboxylation is an existing route to β-keto esters. The magnesium complex *7* has been prepared previously by treatment of the mono ester with magnesium ethoxide or isopropylmagnesium bromide in tetrahydrofuran.[7,8] We prepared *7* as a soluble complex in methylene chloride from ethyl hydrogen malonate and two equivalents of ethylmagnesium bromide in the same solvent. Addition of 4-chlorobutyryl chloride, hydrolysis with concentrated hydrochloric acid and work-up by distillation provided analytically pure *4* in good yield. In analogy with *6* cyclization of *4* by treatment with sodium hydride in benzene gave the tetrahydrofurylidene acetate *8*. The indicated geometry of this compound rests upon the identity of *8* with a compound whose structure has been unambigously assigned from shift reagent studies.[9]



7 8

Recently we have demonstrated the cyclization of γ-bromo-β-keto esters by means of 2.5 N potassium hydroxide to tetronic acids.[1] Among other tetronic acids the naturally occurring carolinic acid was obtained. Following this route 2-bromopropionyl bromide was condensed with *4* in methylene chloride with ethylmagnesium bromide acting as base. The condensation product *9*



9

was not purified by any means since TLC revealed only trace amounts of impurities. Treatment of *9* with 2.5 N potassium hydroxide for 24 h simultaneously induced cyclization and replacement of the side chain chlorine atom by hydroxide, just as NMR and TLC data clearly indicated the presence of carolic acid in the resulting oily product, but the isolation of pure material was not straightforward. Preparative layer chromatography on silica gel with abs. ethanol as eluent separated two bands of almost equal intensity but reextraction was unsuccessful. Finally, when the oil was left standing with a mixture of ether/light petrol for two days crystals were produced. This material was identical in all respects with carolic acid.

EXPERIMENTAL

All m.p.'s are uncorrected. ^{1}H NMR spectra were recorded on a JEOL C-60 HL spectrometer with TMS as internal standard. The chemical shifts are expressed in δ-values (ppm downfield from TMS). Coupling constants are expressed in Hz. UV spectra were recorded on a Beckman

ACTA III spectrophotometer. Microanalysis was performed at the microanalytical department of the University of Copenhagen. The progression of the reactions was monitored conveniently on TLC with ether as eluent.

4-Bromobutyryl bromide.[10] Butyrolactone (86 g, 1 mol) and phosphorus tribromide (67 ml) were mixed at room temp. and heated at 140 °C for 2 h. The temp. was raised to 180 °C and all volatile material distilled off; b.p. 65–110 °C (13 mmHg). Redistillation afforded 89 g (39 %) of the title compound; b.p. 90–93 °C (13 mmHg) [lit.[10] b.p. 90–91 °C (13 mmHg)]; n_D^{20}: 1.5300; NMR (CCl_4): 2.21 (2 H, m, $CH_2CH_2CH_2$), 3.21 (2 H, t, $J=6.5$, CH_2CH_2CO), 3.44 (2 H, t, $J=6$, $BrCH_2CH_2$).

4-Chlorobutyryl chloride. Prepared in 80 % yield by the method of Reppe *et al.*;[11] b.p. 67–69 °C (13 mmHg); n_D^{19}: 1.4600.

Diethyl 4-chlorobutyrylmalonate (6). To a solution of diethyl ethoxymagnesiomalonate from Mg (25 g, 1 mol) abs. ethanol (105 ml) and diethyl malonate (160 g, 1 mol) in dry ether (300 ml) was added 4-chlorobutyryl chloride (141 g, 1 mol) in ether (100 ml) as rapidly as possible with ice cooling. After complete addition the mixture was refluxed briefly giving a greenish viscous complex. The reaction mixture was chilled with ice and poured into ice-cooled 4 N HCl (500 ml). After vigorous shaking the ether layer was separated, washed with sat. $NaHCO_3$ aq., then water, dried and the solvent stripped off to give 265 g of a slightly coloured oil. Distillation afforded the title compound (234 g, 88 %), b.p. 145–147 °C (0.5 mmHg); n_D^{21}: 1.4670; NMR ($CDCl_3$): 1.31 (6 H, t, $J=7$). 1.85–2.40 (2 H, m, $CH_2CH_2CH_2$), 2.50–3.08 (2 H, m, CH_2CH_2CO), 3.61 (2 H, t, $J=6$, $ClCH_2CH_2$), 4.31 (4 H, q, $J=7$), 4.51 (1 H, s, $COCHCO$). (Found: C 50.15; H 6.56; Cl 13.05. Calc. for $C_{11}H_{17}ClO_5$: C 49.91; H 6.48; Cl 13.39).

Diethyl tetrahydro-2-furylidenemalonate (2) (a) From diethyl 4-bromobutyrylmalonate. Diethyl ethoxymagnesiomalonate was prepared from Mg (7.5 g), abs. ethanol (7.5 ml), and CCl_4 (0.5 ml) in ether (100 ml). Excess abs. ethanol was removed by azeotropic distillation with benzene (100 ml). The salt was redissolved in ether (80 ml) and 4-bromobutyryl bromide (69.0 g, 0.3 mol) in ether (50 ml) added as rapidly as possible with ice cooling. The resulting green complex was very viscous. Careful hydrolysis with 4 N HCl (300 ml) at 0 °C gave 61.0 g of crude oily product. Distillation afforded 43.0 g (60 %) of analytically pure *2* as a colourless liquid; b.p. 127–129 °C (0.1 mmHg); [lit.[2] b.p. 129 °C (0.1 mmHg)]; n_D^{21}: 1.4891; NMR ($CDCl_3$): 1.26 (3 H, t, $J=7.5$), 1.30 (3 H, t, $J=7.5$), 1.75–2.33 (2 H, m, $CH_2CH_2CH_2$), 3.14 (2 H, t, $J=7.5$), CH_2CH_2CO), 4.16 (2 H, q, $J=7.5$), 4.19 (2 H, q, $J=7.5$), 4.34 (2 H, t, $J=7.5$, OCH_2CH_2). (Found: C 58.10; H 7.11. Calc. for $C_{11}H_{16}O_5$: C 57.88; H 7.07).

(b) From diethyl 4-chlorobutyrylmalonate and NaH in benzene. NaH (5.0 g of a 55–60 % dispersion in oil, 0.11 mol) was washed thoroughly with dry benzene (3×25 ml) and suspended in benzene (60 ml). A solution of *6* (26.5 g, 0.1 mol) in benzene (40 ml) was added at room temp. under vigorous evolution of H_2. The reaction was exothermic and a final temp. of 60 °C was reached. When the gas evolution ceased the mixture was refluxed for 2 h, after which time all NaH had been consumed. After cooling at 0 °C 4 N HCl (100 ml) was added, the benzene layer separated, washed with sat. $NaHCO_3$ aq. (100 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the remaining oil distilled to yield 18.3 g (80 %) of *2* as a colourless liquid; n_D^{20}: 1.4880; b.p. 139–141 °C (0.15 mmHg); NMR data as above. (Found: C 58.00; H 7.06).

(c) From diethyl 4-chlorobutyrylmalonate *6* and potassium hydroxide in abs. ethanol. To a solution of *5* (52.9 g, 0.2 mol) in abs. ethanol (100 ml) was added a solution of potassium hydroxide (13.0 g of 86 % pellets) in abs. ethanol (150 ml) at room temp. During the addition of potassium hydroxide a white precipitate was formed. After stirring for 15 h, the mixture was chilled to 0 °C and the precipitated potassium chloride (12.0 g) was filtered off and washed with ether. The filtrate was concentrated to 100 ml *in vacuo*, water (300 ml) was added and organic material extracted with ether (3×50 ml). The pooled ether extracts were dried (Na_2SO_4) and evaporated *in vacuo* to leave 42.0 g of an oil which rapidly solidified to a material with m.p. 54–55 °C. Recrystallization from light petroleum (boiling range 50–70 °C) afforded 39.9 g (83 %) of pure *2* as a solid m.p. 55–57 °C; NMR (CCl_4): 1.25 (6 H, t, $J=7.5$), 1.81–2.38 (2 H, m, $CH_2CH_2CH_2$), 3.10 (2 H, t, $J=7.5$, CH_2CH_2CO), 4.09 (2 H, q, $J=7.5$), 4.11 (2 H, q, $J=7.5$), 4.32 (2 H, t, $J=7.5$, OCH_2CH_2). (Found: C 57.70; H 7.00).

Ethyl 6-chloro-3-oxohexanoate (4). A solution of ethylmagnesium bromide was prepared from Mg flakes (12.5 g) and ethyl bromide (60 g) in ether (200 ml). The ether was evaporated *in vacuo* and replaced by methylene chloride (100 ml). Freshly distilled ethyl hydrogen malonate (13.2 g, 0.1 mol) was dissolved in methylene chloride (75 ml) and cooled to 0 °C. To this solution a freshly prepared solution of ethylmagnesium bromide in methylene chloride was added with instantaneous evolution of gas. During the addition the reaction mixture became very viscous and efficient mechanical stirring was imperative. Extra methylene chloride (25 ml) was added and when the calculated amount of gas (~2.4 l) had been collected the addition of ethylmagnesium bromide was stopped. The clear, moderately viscous complex was stirred for further 5 min, when a solution of 4-chlorobutyryl chloride (14.3 g, 0.1 mol) in methylene chloride (50 ml) was added dropwise, still at 0 °C. The mixture was left for 10 h at ambient temp. and then chilled at 0 °C. Concentrated HCl (100 ml) was added dropwise

with vigorous stirring. The organic layer was separated, shaken thoroughly with sat. $NaHCO_3$ aq. (100 ml) and dried (Na_2SO_4). Evaporation of the solvent *in vacuo* left 19.3 g of oily product. The oil was distilled twice affording 10.9 g (56 %) of analytically pure *4*; b.p. 80–85 °C (0.15 mmHg); n_D^{20}: 1.4575; NMR (CCl_4): 1.28 (3 H, t, $J=7.5$), 1.75–2.36 (2 H, m, $CH_2CH_2CH_2$), 2.70 (2 H, t, $J=6$, CH_2CH_2CO), 3.35 (2 H, s, $COCH_2CO$), 3.54 (2 H, t, $J=6$, $ClCH_2CH_2$), 4.13 (2 H, q, $J=7.5$). (Found: C 49.70; H 6.93; Cl 18.25. Calc. for $C_8H_{13}ClO_3$: C 49.88; H 6.80; Cl 18.40).

Ethyl α-(tetrahydro-2-furylidene)acetate (*8*). Sodium hydride (4.0 g of a 55–60 % dispersion in mineral oil, ~9.1 mmol) was liberated from dispersing agent by repeated washing with dry benzene (3×15 ml) and suspended in benzene (50 ml). A solution of *4* (16.0 g, 8.3 mmol) in benzene (25 ml) was added dropwise at 0° with evolution of H_2. When the addition was finished the mixture was refluxed for 2 h, after which time a white slurry had been formed. After cooling at 0 °C 4 N HCl (40 ml) was added and the organic layer was separated, washed with sat. $NaHCO_3$ aq. and dried. Evaporation of the solvent *in vacuo* left 13.7 g of slightly coloured oil. Distillation yielded 7.3 g (56 %) of *8* as a colourless liquid; b.p. 62 °C (0.15 mmHg); n_D^{20}: 1.4868; [lit.[9] b.p. 50 °C (0.05 mmHg)]; the NMR spectrum was identical in all respects with that recently published.[9]

(±)-Carolic acid (1). A solution of *4* (9.65 g, 0.05 mol) in methylene chloride (50 ml) chilled to 0° was treated with a solution of ethylmagnesium bromide in methylene chloride, prepared as described above, until the calculated amount of gas (~1.2 l) had been collected. The resulting clear solution of magnesium complex was then added dropwise at 0 °C to a solution of freshly distilled 2-bromopropionyl bromide (11.1 g, 0.051 mol) in methylene chloride (50 ml). No immediate colour reaction was observed, but on standing at room temp. for 12 h the reaction mixture turned wine red. After this time 4 N HCl (100 ml) was added at 0 °C to hydrolyse the magnesium complex and possibly unreacted acid chloride. The organic layer was separated, washed with sat. $NaHCO_3$ aq. and dried (Na_2SO_4). Evaporation of the solvent *in vacuo* left 16.0 g of brown oil. TLC (silica gel, elution with ether) revealed the presence of one major component ($R_F=0.61$) and traces of 2 other components. The crude product (8.0 g) was dissolved in ether (25 ml) at 0 °C and 2.5 N potassium hydroxide (25 ml) was added with vigorous stirring in 30 min. The stirring was continued for 12 h and the ether layer containing 0.6 g of unidentified material discarded. The water phase was acidified with 4 N HCl and extracted with chloroform (3×25 ml). The combined chloroform extracts were dried (Na_2SO_4) and the solvent distilled off *in vacuo* to give 5.0 g of viscous brown oil. TLC (silica gel, elution with abs. ethanol) revealed two spots $R_F=0.70$ and $R_F=0.82$ of equal intensity. A mixture of ether/light petroleum 1:1 (50 ml) was added and the whole left at room temp. for two days. Impurities went into solution and beautiful rosettes of crystals appeared. The solvent was decanted and the crystals collected had m.p. 111–112 °C [lit.[3] m.p. 113 °C]; UV 227 nm (log ε 3.47) and 272 nm (log ε 4.11). The NMR spectrum was completely in accord with that published.[12]

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Ion Pair Extraction in Preparative Organic Chemistry. IX. Kinetic Evidence for an Ion Pair Mechanism in the Halogen Exchange of Alkyl Halides Catalyzed by Tetrabutylammonium Halides

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The kinetics of the reaction between a methylene chloride solution of benzyl chloride and an aqueous solution of sodium iodide in the presence of tetrabutylammonium iodide has been studied. When the mixture is properly stirred, the rate-determining step occurs within the organic layer. The dominating nucleophile was found to be the ion pair. An excellent preparative method is given for the conversion of alkyl iodides into alkyl chlorides, as well as a method for the reverse reaction.

The halogen exchange in alkyl halides with an alkali halide has been studied under a variety of conditions. In most cases, the main reaction is proved, or believed, to be an attack on the alkyl halide by the free halide anion. A careful discussion of the problems involved in the identification of the nucleophile has recently been given by Beronius, Isacsson and Nilsson.[1] We have studied the reaction of benzyl chloride RCl in methylene chloride with an aqueous solution of sodium iodide, in the presence of tetrabutylammonium iodide QI, to benzyl iodide RI by the formula

$$RCl_s + I_w^- \xrightarrow{QI_s} RI_s + Cl_w^-$$

where the subscripts w and s indicate that the molecule or ion is present in water or solvent.

The reaction was followed by taking samples from the aqueous layer and analyzing for Cl^- by potentiometric argentimetry. When the

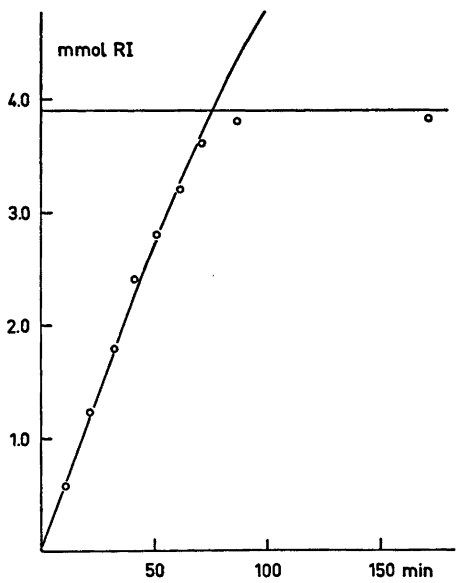


Fig. 1. The sudden stop. The *circles* are the experimental quantities of benzyl iodide formed from a mixture of 25 ml of a 0.4 M solution of benzyl chloride in methylene chloride and 25 ml of a solution 0.156 M in NaI and 0.25 M in NaCl with 4.00 g of tetrabutylammonium iodide as a catalyst. The *curve* represents the values that should be obtained with an excess of sodium iodide in the aqueous layer. The *horisontal line* is the total quantity of NaI available.

Table 1. Reaction of benzyl chloride with aqueous sodium iodide in the presence of 4.000 g of tetrabutylammonium iodide.

t min	Aqueous volume ml	a ml	$a+b$ ml	mmol Cl^-	mmol RCl	RCl calc
0	25.0	0	–	0	10.00	10.00
15	25.0	0.080	0.787	0.99	9.01	9.05
30	24.5	0.143	0.788	1.75	8.25	8.18
45	24.0	0.210	0.783	2.57	7.43	7.40
60	23.5	0.257	0.740	3.29	6.71	6.69
75	23.0	0.325	0.773	3.95	6.05	6.05
90	22.5	0.380	0.781	4.53	5.47	5.47
105	22.0	0.429	0.782	5.06	4.94	4.94
120	21.5	0.474	0.780	5.56	4.44	4.47
137	21.0	0.516	0.783	5.98	4.02	3.99
150	20.5	0.556	0.786	6.36	3.64	3.65
180	20.0	0.618	0.785	6.99	3.01	2.99

$k[QI]_s = 67.1 \times 10^{-4}$ min^{-1}. S.E.$(k[QI]_s) = 0.41 \times 10^{-4}$ min^{-1}.

The calculated value of RCl was obtained from this constant. a and b are the ml of $AgNO_3$ used to titrate Cl^- and I^-.

Table 2. Reaction of benzyl chloride with aqueous sodium iodide in the presence of different quantities of tetrabutylammonium iodide.

QI g	$[QI]_s$ M	$k[QI]_s$ min^{-1}	S.E. (k) min^{-1}	k M^{-1} min^{-1}
0.250	0.0271	4.16×10^{-4}	2.6×10^{-4}	1.56×10^{-2}
1.000	0.1083	16.3×10^{-4}	0.6×10^{-4}	1.51×10^{-2}
2.309	0.2500	38.0×10^{-4}	1.4×10^{-4}	1.52×10^{-2}
4.000	0.4332	67.1×10^{-4}	1.0×10^{-4}	1.55×10^{-2}

mixture was properly stirred, the reaction showed the following features.

1. The quantity of QI used remains in the organic layer throughout the reaction.

2. For a given quantity of QI, the reaction velocity follows first order kinetics with the rate proportional to the concentration of RCl in the organic layer.

3. The pseudo first-order velocity constant according to point 2 is directly proportional to the quantity of QI added to the organic layer.

4. The velocity of the reaction is independent of the concentration of I^- in the aqueous layer, provided that this concentration is above a low critical value (about 10^{-3} M).

5. As soon as the concentration of I^- in the aqueous layer has fallen below the critical value, the reaction suddenly stops.

The results of the kinetic measurements are given in Tables 1 and 2 and in Fig. 1. From these experimental facts we can draw the following conclusions:

The rate-determining step occurs within the organic layer between RCl and a nucleophile dissolved in the organic layer. As will be demonstrated, this nucleophile is the ion pair QI. The rate determining step is therefore

$$RCl_s + QI_s \xleftarrow[\text{slow}]{} RI_s + QCl_s$$

This reaction is coupled with a very rapid ion exchange step.

$$QCl_s + I_w^- \underset{\text{rapid}}{\rightleftharpoons} QI_s + Cl_w^-$$

The equilibrium constant for this exchange has been independently determined to be about 4×10^3. This means that the equilibrium is displaced far to the right unless the concentration of I^- in the water layer is very low. The

concentration of QCl in the organic layer is thus very low and the reverse reaction

$$RI_s + QCl_s \longrightarrow RCl_s + QI_s$$

is so slow that it can be neglected as long as there is enough I^- present in the aqueous layer. When I^- in this layer has been consumed to a low critical value, however, the reverse reaction suddenly becomes of importance and the reaction stops.

The extraction equilibrium for QI has been studied by Gustavii.[2] He describes the total equilibrium by the equations:

$$Q_w^+ + I_w^- \rightleftharpoons QI_s \qquad [QI]_s = E_{QI}[Q^+]_w[I^-]_w$$
$$QI_s \rightleftharpoons Q_s^+ + I_s^- \qquad [Q^+]_s[I^-]_s = K_{diss}[QI]_s$$
$$2QI_s \rightleftharpoons Q_2I_{2s} \qquad [Q_2I_2]_s = K_2[QI]_s^2$$

with the constants $E_{QI} = 3200$

$K_{diss} = 7.6 \times 10^{-5}$ and $K_2 = 6.3$.

In his calculations, the deviation from unity of the activity coefficient γ_s in the organic layer was not taken into consideration. It was found that an introduction of the activity coefficients obtained by the equations of Debye, Hückel or Marshall and Grünwald [3] gave rise to only minor changes in the constants.

The dimerization describes the behaviour in the high concentration range in a very satisfactory way, especially when the activity coefficients γ_s are considered to be unity. The physical existence of this dimer can be highly questioned, however. The dimerization constant calculated by either method is so low that the lifetime of the dimer must be of the same magnitude as the contact time in molecular collisions, and the physical significance of the dimer is therefore lost.[4]

An alternative explanation of the increased extraction in the high concentration region is a small medium effect. (A methylene chloride solution of QI is a better extraction solvent than pure methylene chloride). This medium effect becomes detectable in solutions stronger than about 10^{-2} M and is rather low even at high concentrations. There is thus no reason to introduce other nucleophiles than the ion pair QI and the free anion I^- into the discussion.

Denoting the total concentration of QI in the organic layer by C_{QI}, we have in the concentration range used in the kinetic experiments

$$[QI]_s \simeq C_{QI} \text{ and } [Q^+]_s = [I^-]_s \simeq (K_{diss}C_{QI})^{\frac{1}{2}}/\gamma_s$$

Assuming second order kinetics, we obtain the velocity for the formation of RI

$$\frac{d[RI]}{dt} = k_{QI}[QI]_s[RCl]_s \simeq k_{QI}C_{QI}[RCl]_s$$

$$\frac{d[RI]}{dt} = k_I[I^-]_s[RCl]_s \simeq k_I \times \frac{(K_{diss}C_{QI})^{\frac{1}{2}}}{\gamma_s} \times [RCl]_s$$

depending on whether the ion pair or the anion is the dominating nucleophile. Since the velocity has been found to be directly proportional to C_{QI} and not to $C_{QI}^{\frac{1}{2}}$, we can conclude that the ion pair is the dominating nucleophile in this concentration range.

As a complement, we have also briefly studied the reaction

$$RI_s + QCl_s \longrightarrow RCl_s + QI_s$$

in an homogenous methylene chloride solution. The reaction was rapid and the quenching procedure far from satisfactory. The experimental values therefore showed a considerable scattering. It was obvious, however, that the reaction was of second order with the equilibrium shifted far to the right. The velocity constant was about 200 times higher than that of the reverse reaction.

When making a comparison of the two reactions, it should be remembered that, in the presence of a water layer, QCl is extracted together with several molecules of water of solvation which may decrease the reactivity of the ion pair, whereas QI is extracted without any water of this type. The kinetic facts open the curious possibility of an excellent method to convert alkyl iodides to alkyl chlorides, as well as a good method for the reverse reaction.

EXPERIMENTAL

The kinetic measurements in a two-layer mixture

A carefully weighed quantity of pure tetrabutylammonium iodide was added to a 100 ml flask with a very efficient stirrer and a replacable stopper. The flask was placed in a water thermostat at 25.0 °C. 25.00 ml of a prethermostated 0.3902 M solution of NaI in water was added, followed by 25.00 ml of a 0.400 M solution of benzyl chloride in methylene chloride. The time was taken from the moment when the last mentioned solution was added. At intervals the stirrer was stopped for a few seconds, the

layers were allowed to separate and 0.500 ml of the upper layer was withdrawn. This was added to a few ml of dilute nitric acid and the contents of Cl^- and I^- were determined by potentiometric argentimetry.

Calculations. Due to the difficulties in removing 0.500 ml samples accurately and rapidly, the concentration of Cl^- in the sample was calculated in the following way: The apparent extraction constant of QI is about 4000 for all the solutions used. The concentration of Q^+ in the aqueous layer is thus $< 10^{-2}$ M, as long as the concentration of I^- in the water layer is above 0.025 M. This is valid during the first 95 % of the reaction. Since the concentration of Q^+ is low, the total concentration of halide ions in the sample is almost exactly equal to that of sodium ions, which is 0.3902 M. Assume that the volume of $AgNO_3$ used to titrate Cl^- is a and that used to titrate I^- is b. The concentration of Cl^- in the sample is thus

$$[Cl^-]_w = \frac{a}{a+b} \times 0.3902$$

Since the extraction constant of QCl is very low (about 1 under the present conditions), and the concentration of Q^+ in the water layer is very low, the fraction of Cl^- present in the organic layer is very low, < 1 %, and can thus be neglected in the calculations. The total quantity of Cl^- formed is thus equal to the concentration of Cl^- found in the sample, multiplied by the aqueous volume from which the sample was withdrawn, plus the quantity of Cl^- in all samples previously taken.

Since the velocity of the reaction is proportional to the constant concentration $[QI]_s$ of QI and to the concentration $[RCl]_s$ of RCl, the following equation is valid

$$[RCl]_s = [RCl]_s^0 \, e^{-k[QI]_s t}$$

where $[RCl]_s^0$ is the starting concentration of RCl. We can expect that the error in $[RCl]_s$ is normally distributed. Since the reaction is followed to a high degree of conversion, the normal way of calculating the velocity constant ($k[QI]_s$) from the linear transform $\ln [RCl]_s = \ln[RCl]_s^0 - k[QI]_s t$ by the method of least squares should *not* be used. (The errors in $\ln[RCl]_s$ are far from normally distributed). $k[QI]_s$ was instead calculated by a least squares computer program for nonlinear regression based on normal distribution of the error in $[RCl]_s$ and the iterative method of Gauss.[5] This method gives both $k[QI]_s$ and the standard error S.E. of this constant.

A typical run is given in Table 1 and a summary of 4 runs at different $[QI]_s$ values is given in Table 2. Fig. 1 demonstrates the sudden stop in the reaction that occurs when an insufficient quantity of NaI is used.

The kinetic measurements in a homogeneous solution

A carefully weighed quantity of pure anhydrous tetrabutylammonium chloride was dissolved in methylene chloride to make a standard solution, about 0.16 molar. A second solution of about the same molarity was prepared by dissolving benzyl iodide in methylene chloride. The two solutions were placed in a water thermostat at 25.0 °C. Ten ml portions of each solution were rapidly mixed. The progress of the reaction was followed by taking 1.00 ml samples which were rapidly added to 10 ml of water contained in centrifuge tubes, and the mixture was immediately vigorously shaken for one minute. The layers were then rapidly separated by centrifugation. A 5.00 ml sample of the aqueous layer was analyzed for Cl^- by potentiometric argentimetry. A calculation of the phase equilibria shows that less than 1 % of the Cl^- was present in the organic layers.

Calculations. Since the concentrations of the two reacting species are almost equal to the mean concentration A, we obtain, according to Widequist,[6] the following equation

$$\frac{1}{A - [RCl]_s} = \frac{1}{A} + kt$$

which is valid provided that the reverse reaction can be neglected. This, in fact, was found to be the case.

The errors in $[RCl]_s$ can be expected to be normally distributed and the constants A and k were calculated by a least squares computor program for nonlinear regression considering this fact. The results are given in Tables 3 and 4.

Preparation of alkyl chlorides from alkyl iodides. 0.1 mol of an alkyl iodide and 0.108

Table 3. The reaction of benzyl iodide with tetrabutylammonium chloride in methylene chloride $[RI]_s^0 = 0.0904$ M. $[QCl]_s^0 = 0.0814$ M. Mean concentration $A = 0.0859$ M.

t min	$[QCl]_s$ M	$[RCl]_s$ M	$[RCl]_{calc}$ M
0	0.0814	0	0
0.38	0.0700	0.0115	0.0079
0.82	0.0670	0.0145	0.0154
1.17	0.0610	0.0205	0.0204
1.47	0.0550	0.0265	0.0241
1.73	0.0530	0.0285	0.0270
2.02	0.0530	0.0285	0.0300
2.30	0.0485	0.0330	0.0325
2.63	0.0435	0.0380	0.0353

$k = 3.16$ M^{-1} min^{-1}. $SE(k) = 0.19$ M^{-1} min^{-1}.
$A = 0.0851$ M. $SE(A) = 0.0016$ M.
$[RCl]_{calc}$ is calculated from these constants.

Table 4. The reaction of benzyl iodide with tetrabutylammonium chloride in methylene chloride $[RI]_s^0 = [QCl]_s^0$.

t min	$[QCl]_s$ M	$[RCl]_s$ M	$[RCl]_{calc}$ M
0	0.0833	0	0
2.10	0.0532	0.0301	0.0305
3.92	0.0416	0.0417	0.0432
6.05	0.0314	0.0519	0.0521
8.02	0.0251	0.0582	0.0574
10.02	0.0211	0.0622	0.0612

$k = 3.28$ M^{-1} min^{-1}. $SE(k) = 0.102$ M^{-1} min^{-1}.
$A = 0.0835$ M. $SE(A) = 0.0012$ M.
$[RCl]_{calc}$ is calculated from these constants.

mol of anhydrous tetrabutylammonium chloride were mixed in a flask arranged for distillation. The mixture was heated and distilled directly from the reaction mixture, eventually at reduced pressure, so that the temperature does not exceed 140 °C. In this way over 90 % of the n-alkyl chloride is obtained in a few minutes. For the preparation of butyl chloride, the distillation started at atm. pressure at ≤ 72 °C and was finished at 50 °C/190 Torr. For the preparation of octyl chloride, the distillation was performed at 7 Torr, and the product was collected up to 70 °C. Both products contained a small droplet of water that was readily removed. Apart from this, they were pure when tested by gas chromatography.

Preparation of iodoaceto-2,6-xylidide. A mixture of 19.77 g (0.1 mol) of chloroaceto-2,6-xylidide, 16.49 g (0.11 mol) of sodium iodide, and 5 g (0.014 mol) of tetrabutylammonium iodide was added to 100 ml of chlorobenzene and 50 ml of water. The resulting suspension was refluxed for 90 min. The mixture was chilled to 0 °C and the product collected by filtration, washed with cold water and cold methylene chloride yielding 26.5 g (92 %) of iodoacetoxylidide. The product was pure and was readily recrystallized from 1,2-dichloroethane. The melting point was 170 °C.

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N-Quaternary Compounds. Part XXXVIII.* Syntheses and Chiroptical Properties of Some 2-Trimethylammoniopropan-1-ols

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The CD-spectra of some dimethylamino- and trimethylammoniopropan-1-ols have been discussed in terms of conformational preferences. The CD-spectra of 1,1-*gem*-diphenyl derivatives contain, in addition to the usual aromatic absorption at 210 – 220 nm and 250 – 270 nm, a third dichroic band at 230 nm. The origin of this band is discussed.

The circular dichroic absorption of some α-trimethylammonio carboxylic acids has recently been reported upon;[2] such studies have also been extended to α-trimethylammonio aldehydes.[3] In this report we describe chiroptical data for stereochemically related hydroxy derivatives; the latter are homologues of the biologically important choline molecule.

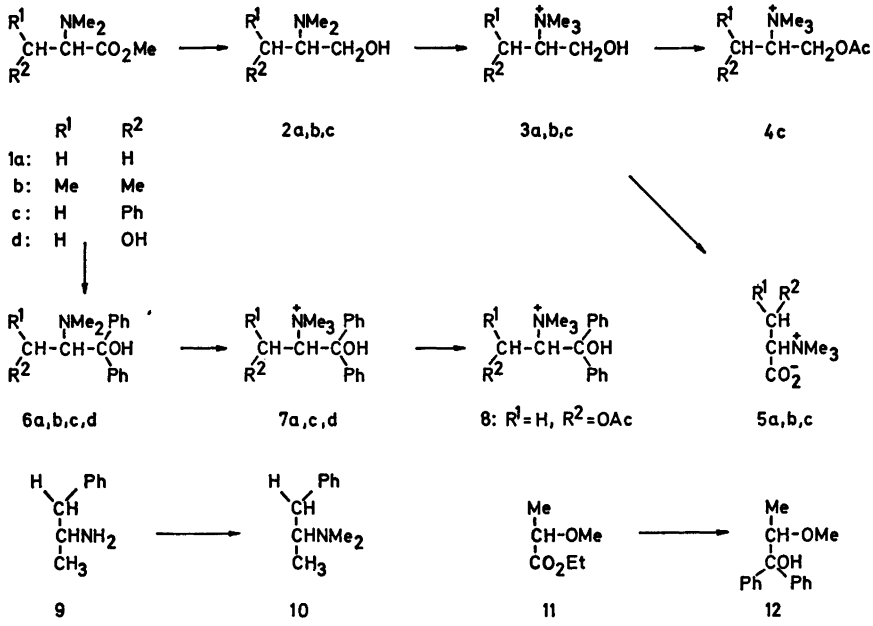
The syntheses of hydroxy analogues for the dichroic absorption studies are outlined in Scheme 1. α-Dimethylamino acids and their esters (*1*) are suitable starting materials as they are available in an enantiomerically pure form by reductive methylation of the respective optically active amino acid.[4,5] Lithium aluminium hydride reduction of (*S*)-*1* gave the alcohol (*2*) which was *N*-quaternised (*3*) by means of methyl iodide; acetylation gave the choline ester (*4c*). Acid permanganate oxidation of the aliphatic cholines (*3a*, *3b*) gave the corresponding α-trimethylammonio acids (*5a*, *5b*) with the same D-line rotation as previously found on direct quaternisation of the respective amino acids.[2] The specific rotation for the aromatic acid (*5c*) prepared from *3c*, however, was only half of that obtained after permethylation of phenylalanine.

The *gem*-diphenyl derivatives (*6*) were prepared from the esters (*S*)-*1* by treatment with phenyl lithium. The methiodides (*7a*, *7c*, *7d*) were available from the reaction of *6* with methyl iodide. The valine derivative (*6b*), however, failed to react presumably because of the added steric interference from the isopropyl group.

Quaternisation of *6d* with methyl iodide in acetic anhydride furnished the sterically less hindered mono-acetate (*8*).

The CD-curves for the aromatic cholines (*S*)-*3c* and (*S*)-*4c* were almost identical; therefore only the CD-curve for the acetate (*4c*) is reproduced (Fig. 1). The multiple Cotton effect due to the phenyl group in the 250 – 270 nm region is negative while the sign for its dimethylamino precursor (*S*)-*2c* is positive (Fig. 2). For comparison it is pointed out that the dichroic absorption is positive in the desoxy-analogues (*S*)-α-methyl-β-phenethylamine (*9*), its *N*,*N*-dimethyl derivative (*10*) as well as in acid salts and in the methiode.[6] By the definition of absolute configuration the actual group arrangements around the chiral carbon in the latter corresponds to that of their (*R*)-choline analogues; thus reduction of (*S*)-phenylalaninol gives (*R*)-α-methyl-β-phenethylamine. In agreement with the published report[6] (*R*)-α-*N*,*N*-trimethyl-β-phenethylamine (*10*) has

* Part XXXVII, see Ref. 1.



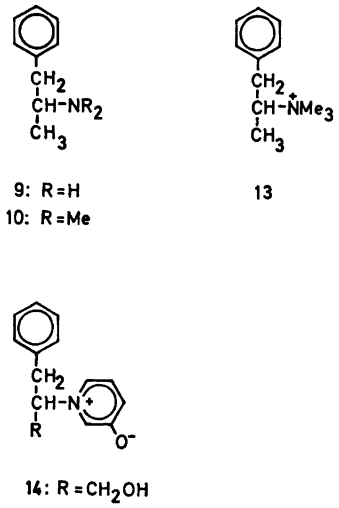
Scheme 1.

negative absorption in the 250 – 270 nm region and is thus opposite to that of its hydroxy analogue (*S*)-*2c*; *10* was prepared from (*R*)-(−)-amphetamine by reductive methylation.

A quadrant sector rule has been proposed for the prediction of the sign for the longest wavelength Cotton effect with the stereochemistry of α-phenylalkylamines. The rule has been established empirically by correlating the observed sign with preferred conformers in suitable model compounds.[7,8] The observed CD-sign for (*R*)-*10* is predicted by the rule (Fig. 2).[8] The conformational mobility in open chain structures, however, is considerable; hydroxylation such as in *2c* may well affect the conformational equilibrium which in its turn may alter the overall rotational sign. Besides the conformational aspects, the introduced hydroxy group may also directly interfere with the chiral absorption of the phenyl ring.

The considerable non-bonded interaction from the ammonium group in *3c* and *4c* is expected to favour *anti* conformational equilibria with respect to the phenyl group; this corresponds to the preferred conformational equilibrium of α-*N*,*N*-trimethyl-β-phenethylamine. The quadrant sector rule for the latter then predicts the observed longest wavelength rotation for *3c* and *4c*. Assignments of preferred conformations to be considered in the quadrant sector rule, however, are complicated by the conformational situation at the choline part of the molecule. It is known from conformational studies of choline and acetyl choline in solutions that an overwhelming predominance of the *gauche* form exists.[9] The *gauche* forms are sterically the most unfavourable but the electrostatic attraction between the cationic nitrogen atom and the oxygen atom is the more important. The conformational equilibrium, however, is sensitive to further steric interaction and α-substitution leads to increased population of *trans* forms.[10] Both *3c* and *4c* are to be regarded as α-substituted cholines and therefore probably will show little conformational preference for this part of the molecule.

A negative multiple Cotton effect in this region has also been found for the (*S*)-α-pyridinium analogue (*14*) of *3c*.[11] The strongly negative absorption for the pyridinium derivative in the 220 nm region has been reversed for the methylamino derivatives (*2c*, *3c*, *4c*). The intensity for the absorption below 220 nm is



Scheme 2.

much higher than at any other wavelength and in all cases gives the sign of the optical rotation in the visible part of the spectrum; this observation generally appears to be true.[8,12]

The CD-curves for the *gem*-diphenyl-dimethylamines (*6*) contain a negative multiple Cotton effect in the 250–270 nm region (Fig. 2). The CD-sign has been reversed in the methiodides (*7a* and *7d*) but has been retained in the phenyl analogue (*7c*). The sign for the latter is the same as in its phenylalaninol analogues (*3c* and *4c*) (Fig. 1). All the *gem*-diphenyl derivatives have strongly positive dichroic absorption at or below 220 nm. This represents the usual strong $\pi \rightarrow \pi^*$ absorption in phenyl derivatives. In addition dichroic absorption bands are present in the 230–235 nm region. The sign is negative for the dimethylamines (*6*) and is reversed for the methiodides (*7a* and *7d*). In the acetate (*8*), prepared from *7d*, the dichroic absorption is again negative and the maximum position has undergone a slight hypsochromic shift. The spectrum of the triphenyl derivative (*7c*) has no specific band seen at this wavelength. The absorption in this case, however, may well be hidden under the broad and overlapping 220 nm band (Fig. 1). The UV spectra for *6* and *7* are very similar to that of the α-methoxy analogue shown in Fig. 3; in no case was a separate UV-band detectable in the 230 nm region.

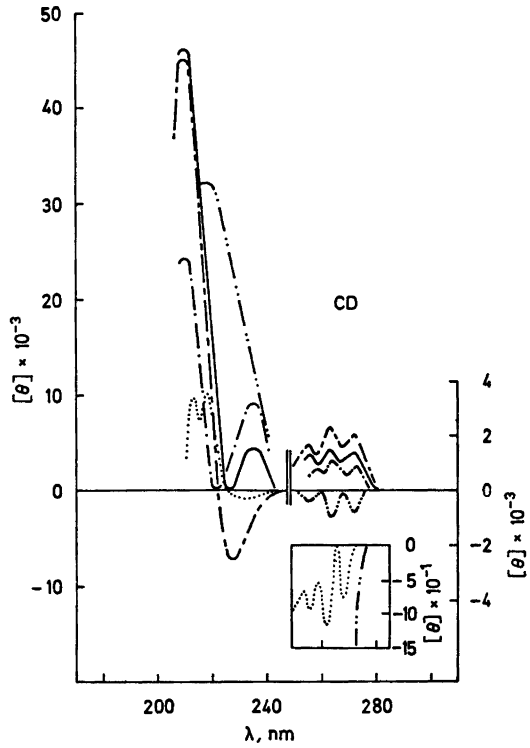


Fig. 1. CD-curves in methanol for (*S*)-3-Phenyl-2-*N*,*N*,*N*-trimethylammoniopropyl-1-acetate iodide (*4c*) · · · ; (*S*)-3-acetoxy-1,1-diphenyl-2-*N*,*N*,*N*-trimethylammoniopropan-1-ol iodide (*8*) – – – –; (*S*)-1,1-diphenyl-2-*N*,*N*,*N*-trimethylammoniopropan-1-ol iodides: (*7a*) –· – ·; (*7c*) – · · – · · and (*7d*) ———.

In the phenylalaninol derivatives (*2c*, *3c*, and *4c*) weakly dichroic bands are present in this region. These absorption bands are broad and have been shifted to about 240 nm. A common feature for these molecules is the presence of an aliphatic hydroxy group. On the other hand the spectra of a series of 1-phenyl-3-dialkylamino-propan-1-ols do not contain any aromatic dichroic absorption outside the 210–220 nm and the 250–270 nm regions.[13] The phenethylamine analogue (*10*) shows no dichroic absorption in this area nor has such absorption been reported in simple phenethylamines.[6,8] This absorption is also absent in such close analogues as the *gem*-diphenyl derivative 1,1,2-triphenylethylamine hydrochloride and in 2,3-diphenyl-1-methylpropylamine hydrochloride.[8] The hydroxy group itself does not absorb

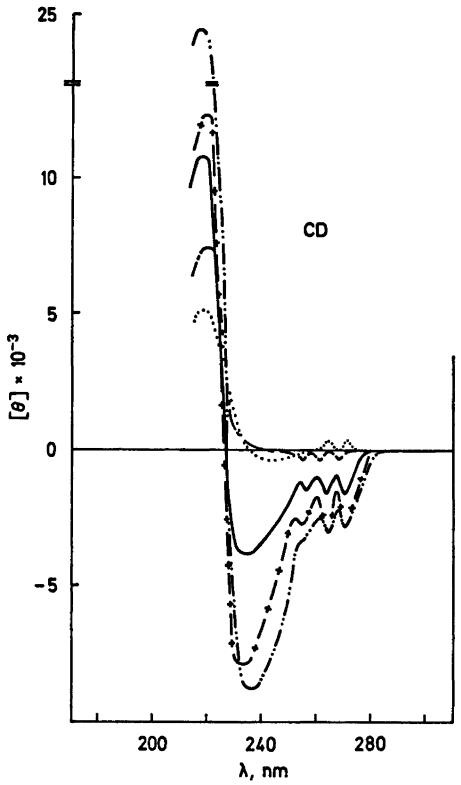


Fig. 2. CD curves in methanol for (*S*)-2-*N*,*N*-dimethylamino-3-phenylpropan-1-ol (*2c*) ····; (*R*)-α-*N*,*N*-trimethyl-β-phenethylamine (*10*) – – – –; (*S*)-1,1-diphenyl-2-*N*,*N*-dimethylaminopropan-1-ols: (*6a*) – + – +, (*6c*) – ·· – ·· and (*6d*) ———.

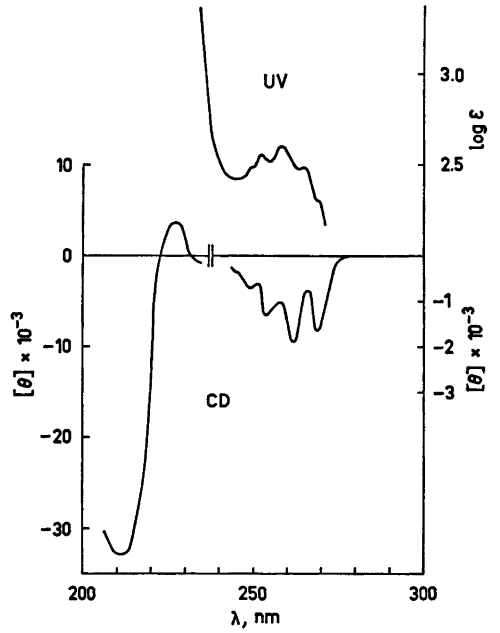


Fig. 3. CD and UV curves in methanol for (*S*)-1,1-diphenyl-2-methoxypropan-1-ol (*12*).

in this wavelength region. The amino function may be replaced by other groups; thus the α-methoxy analogue (*12*) shows dichroic absorption which relative to that of its closest analogue (*6a*) has undergone a hypsochromic shift (233→226 nm). The methoxy analogue (*12*) has the (*S*)-configuration (Scheme 1); it has the same sign for the 250 nm bands as (*S*)-*6a* but at the other maxima the signs are reversed. It is concluded that the dichroic band at about 230 nm is due to the two aromatic chromophores in the *gem*-diphenyl derivatives. The Cotton effect may be associated with some form of dipole-dipole interaction between the two aromatic chromophores. This is reminiscent of the interaction between a benzoate group and another aromatic chromophore but should split the dichroic absorption into two curves of opposite sign.[14] Inspection of the spectra shows that the other half of the band could well be hidden under the strong 210–220 nm band. A closer inspection of the spectra also shows that the intensities and the positions of the maxima varies slightly, despite the fact that the aromatic chromophore is the same. Any vicinal interaction by homoconjugation between the *gem*-phenyl groups over the two sigma bonds[8] does not alter the fact that the aromatic chromophore is the same in these derivatives. It is therefore suggested that the 230 nm band arises as a result of the dichroic absorption of a second major conformer in solution. Support for this view was sought in the studies of the effect of temperature on the 233 nm dichroic band for *6a* and *7a*. The absorption intensity was found to increase slightly with decrease in temperature while the positions of the maxima were hardly affected. The data for *6a* were: $[\theta]_{233} = -5.7 \times 10^3$ (+58 °C), $= -7.6 \times 10^3$ (+25 °C), $= -1.1 \times 10^4$ (−28 °C); for *7a*: $[\theta]_{233} = +7.4 \times 10^3$ (+62 °C), $= +9.8 \times 10^3$ (+25 °C), $+1.3 \times 10^4$ (−28 °C). The data can be interpreted in terms of changes in conforma-

tional equilibria; alteration in solvation equilibria, which may vary from compound to compound, also has to be considered.

The Cotton effects are dependent on the relative orientation of the phenyl groups with respect to one another (screwness) and in this respect the *gem*-diphenyl derivatives resemble optically active biphenyls.[15] The preferred orientation of the phenyl groups are further controlled by the group sequence through steric or electronic interaction with the other groups attached to the chiral centre. It is thus understandable that the preferred orientation of the phenyl groups will be sensitive to major alteration in the groups attached to the chiral carbon. This is illustrated by the *N*-quaternisation of *6* to the ammonium analogues (*7*); the CD-spectra are different.

EXPERIMENTAL

The CD measurements were carried out with a Jasco Automatic Spectropolarimeter Model J-10. The cell lengths were 1, 2, and 10 mm and the temperature 27 °C. The concentrations were in the range 0.2 – 0.4 g/l.

The NMR data were recorded on a 60 MHz Varian A-60A instrument.

Syntheses: Syntheses of the esters (*1a*, *1b*, *1c*) have previously been described.[2]

(S)-N,N-Dimethylserine methyl ester (1d). (*S*)-Serine methyl ester (0.1 mol) and 35 % formalin (30 ml) were added to water (180 ml) and hydrogenated over 5 % Pd/C (5 g) for 16 h at atmospheric pressure. Conc. HCl (15 ml) was then added, the catalyst filtered off and the filtrate evaporated at reduced pressure. The residue was triturated with acetone/ether before dissolution by heating in methanol. A little undissolved material was removed by filtration before the filtrate was concentrated to about 40 ml. To this solution, cooled in an ice-bath, was added trimethylamine (12 g) in methanol (20 ml) followed by ether (400 ml). The precipitated salt was removed by filtration, the filtrate evaporated and the residue distilled to give the title compound in 51 % yield, b.p. 90 °C/11 mmHg (lit.[16] for DL-isomer gives b.p. 108 – 109 °C/25 mmHg). (Found: C 48.76; H 8.92. Calc. for $C_6H_{13}NO_3$: C 48.96; H 8.90); $[\alpha]_D = +4.0°$ ($c = 1.9$ in 2 N HCl); τ(CDCl$_3$) 7.6 (NMe), 6.3 (OMe).

(S)-2-N,N-Dimethylamino-3-methylbutan-1-ol.HI (2b). A solution of (*S*)-*N,N*-dimethylvaline methyl ester (0.023 mol) in anhydrous ether (50 ml) was added dropwise over 10 min to lithium aluminium hydride (0.048 mol) in anhydrous ether (550 mol). The reaction mixture was heated under reflux for 5 h before the excess hydride was destroyed by dropwise addition of saturated sodium sulfate solution with ice-cooling. The ether phase was washed and dried and gave on evaporation 52 % of the alcohol. This material was used in the consecutive reaction without further purification.

For analytical purposes part of this material was dissolved in 10 % HI (50 ml) and the solution filtered before evaporation. The residual material was crystallized from acetone, m.p. 135 °C. (Found: C 32.26; H 6.78. Calc. for $C_7H_{17}NO.HI$: C 32.45; H 6.61); $[\alpha]_D = +6.2°$ ($c = 1.2$ in 2 N HCl).

(S)-2-N,N-Dimethylaminopropan-1-ol.HI (2a) was synthesised from (*S*)-*N,N*-dimethylalanine methyl ester as (*2b*) above, m.p. 110 – 112 °C (acetone/ether). (Found: C 26.02; H 6.18. Calc. for $C_5H_{13}NO.HI$: C 25.98; H 6.10); $[\alpha]_D = +13.7°$ ($c = 0.7$ in 2 N HCl).

(S)-2-N,N-Dimethylamino-3-phenylpropan-1-ol (2c) was prepared in the same way from the corresponding methyl ester; properties as described.[4]

(S)-1,1-Diphenyl-2-N,N-dimethylamino-3-methylbutan-1-ol (6b). The free base of (*S*)-*N,N*-dimethylvaline methyl ester was initially prepared from its HCl-salt as follows: The salt (0.061 mol) in methanol solution (30 ml) was ice-cooled and a solution of anhydrous trimethylamine (11 g) in methanol (20 ml) added gradually with stirring. Ether (400 ml) was then added, the precipitated salt was filtered off, the filtrate was evaporated and the residual ester distilled, b.p. 84 – 85 °C/75 mmHg.

Part of the methyl ester thus obtained (0.022 mol) was dissolved in anhydrous ether and a 0.33 M solution (150 ml) of phenyl lithium in ether [17] added dropwise with stirring under N_2-atmosphere. The reaction mixture was then heated under reflux for 4 h. The cold etheral solution was extracted with 6 N HCl (6 × 15 ml), the aqueous extracts made alkaline by addition of solid sodium carbonate and the title compound extracted into chloroform. Evaporation of the washed and dried chloroform solution yielded a solid (94 %), m.p. 65 °C. This material was used in the consecutive reaction step without further purification.

For analyses part of the product was dissolved in methanol and converted to its HCl-salt by addition of 5 N HCl. Evaporation left a solid material which was recrystallized from methanol/acetone, m.p. 212 °C. (Found: C 71.36; H 8.03. Calc. for $C_{19}H_{25}NO.HCl$: C 71.33; H 8.19); $[\alpha]_D = +48.0°$ ($c = 1.1$ in 2 N HCl/MeOH (1:1)); τ (TFA) 7.0 and 6.8 (NMe), 2.4 (Ph).

(S)-1,1-Diphenyl-2-N,N-dimethylaminopropan-1-ol (6a) was prepared from *N,N*-dimethylalanine methyl ester as above in 56 % yield; m.p. 61 °C (isoPrOH/water) [lit.[18] for DL-isomer m.p. 65 – 66 °C]. (Found: C 80.25; H 8.19. Calc. for $C_{17}H_{21}NO$: C 79.98; H 8.29); $[\alpha]_D = +54.5°$ ($c = 1.2$ in 2 N HCl); τ (CDCl$_3$), 7.9 (NMe), 2.6 (Ph).

*(S)-1,1,3-Triphenyl-2-*N,N*-dimethylaminopropan-1-ol (6c)* was prepared from *N,N*-dimetylphenylalanine methyl ester as above in 81 % yield. For analysis part of the product was crystallized as its HCl-salt, m.p. 209 °C (MeOH/acetone/pet. ether). (Found: C 74.96; H 7.19. Calc. for $C_{23}H_{25}NO.HCl$: C 75.09; H 7.12); $[\alpha]_D = +67.1$ ($c = 0.6$ in MeOH); τ ($CDCl_3$) 7.9 (NMe), 2.7 (Ph).

*(S)-1,1-Diphenyl-2-*N,N*-dimethylaminopropan-1,3-diol (6d)* was prepared as above from *N,N*-dimethylserine methyl ester in 40 % yield, m.p. 147–148 °C (isoPrOH). (Found: C 74.75; H 7.71. Calc. for $C_{17}H_{21}NO_2$: C 75.23; H 7.79); $[\alpha]_D = +11.3°$ ($c = 1.2$ in 2 N HCl/MeOH (1:1); τ ($CDCl_3$) 7.7 (NMe), 2.7 (Ph).

N-Quaternisation of the dimethylamines

A solution of the *N,N*-dimethylamino derivative (0.015 mol) and methyl iodide (0.03 mol) in nitromethane (20 ml) was kept at 40 °C in a pressure bottle for 20 h. The solution was then concentrated to about 5 ml and ether (150 ml) added. The precipitated methiodide was collected and recrystallized as described for each individual compound below.

*(S)-2-*N,N,N*-Trimethylammoniopropan-1-ol iodide (3a)*. Yield 50 %, m.p. 289 °C (acetone) [lit.[19] m.p. 299 °C]; $[\alpha]_D = -2.6°$ ($c = 1.1$ in 2 N HCl); τ (TFA) 6.7 (NMe).

*(S)-3-Methyl-2-*N,N,N*-trimethylammoniobutan-1-ol iodide (3b)*. Yield 97 %, m.p. 224–225 °C (acetone) [lit.[20] for DL-isomer 191–195 °C]; (Found: C 35.04; H 7.48. Calc. for $C_8H_{20}INO$: C 35.17; H 7.38); $[\alpha]_D = +8.6°$ ($c = 1.0$ in 2 N HCl); τ (TFA) 6.6 (NMe).

*(S)-3-Phenyl-2-*N,N,N*-trimethylammoniopropan-1-ol iodide (3c)* was prepared as above; properties as described.[4]

*(S)-1,1-Diphenyl-2-*N,N,N*-trimethylammoniopropan-1-ol iodide (7a)*. Yield 90 %, m.p. 220 °C (isoPrOH: MeOH) [lit.[18] for DL-isomer m.p. 205 °C]. (Found: C 54.09; H 6.17. Calc. for $C_{18}H_{24}INO$: C 54.38; H 6.09); $[\alpha]_D = +18.7°$ ($c = 1.1$ in 2 N HCl/MeOH (1:1); τ (TFA): 6.9 (NMe), 2.6 (Ph).

*(S)-1,1,3-Triphenyl-2-*N,N,N*-trimethylammonio-propan-1-ol iodide (7c)*. Yield 59 %, m.p. 138–140 °C (H_2O). (Found: C 60.40; H 5.90. Calc. for $C_{24}H_{28}INO$: C 60.89; H 5.96); $[\alpha]_D = +23.5°$ ($c = 0.6$ in MeOH); τ (TFA/$CDCl_3$) 6.9 (NMe), 2.7 (Ph).

*(S)-1,1-Diphenyl-2-*N,N,N*-trimethylammoniopropane-1,3-diol iodide (7d)*. Yield 74 %, m.p. 192 °C (isoPrOH/Ether). (Found: C 51.90; H 5.76. Calc. for $C_{18}H_{24}INO_2$: C 52.31; H 5.85; $[\alpha]_D = +39.7°$ ($c = 1.1$ in 2 N HCl/MeOH (1:1); τ (TFA) 6.7 (NMe).

*(S)-3-Phenyl-2-*N,N,N*-trimethylammoniopropyl-1-acetate iodide (4c)*. (*S*)-2-*N,N*-Dimethylamino-3-phenylpropan-1-ol (0.0035 mol) and methyl iodide (0.007 mol) in acetic anhydride (40 ml) were heated in a pressure bottle at 80 °C for 4 h. The solution was then evaporated at reduced pressure almost to dryness and the product precipitated by ether (200 ml) addition; yield 97 %, m.p. 238 °C (EtOH); (Found: C; 46.17; H 6.05. Calc. for $C_{14}H_{22}INO_2$: C 46.29 H 6.10); $[\alpha]_D = +18.5°$ ($c = 1.0$ in 2 N HCl); τ (TFA) 7.7 (MeCO), 6.5 (NMe), 2.6 (Ph); (KBr) 1721 cm^{-1} (CO).

*(S)-3-Acetoxy-1,1-diphenyl-2-*N,N,N*-trimethylammoniopropan-1-ol iodide (8)* was prepared as (*4c*) above from (*6d*); yield 74 %, m.p. 203 °C (EtOH); (Found: C 52.55; H 5.48. Calc. for $C_{20}H_{26}INO_3$: C 52.76; H 5.75); $[\alpha]_D = +25.1°$ ($c = 1.1$ in 2 N HCl/MeOH (1:1); τ (TFA) 7.8 (MeCO), 6.7 (NMe), 2.6 (Ph); (KBr) 1745 cm^{-1} (CO).

(*S*)-*N,N,N*-Trimethylammoniopropionic acids (5)

The *N*-quaternary amino-alcohol (*3*) (0.001 mol) and potassium permanganate (0.002 mol) were added to 4 N H_2SO_4 (20 ml) and the resultant solution kept at 60 °C for 40 min. An aqueous solution of barium chloride was added dropwise to the cold reaction mixture to remove the sulfate ions. The reaction mixture was further worked up as previously described; properties: (*5a*), m.p. 202–204 °C; $[\alpha]_D = -19.5°$ (2 N HCl), lit.[2] −20.3°; (*5b*), m.p. 196–199 °C; $[\alpha]_D = +18.7°$ (2 N HCl), lit.[2] +18.9°; (*5c*), m.p. 192–194 °C; $[\alpha]_D = +27.5°$ (2 N HCl), lit.[2] +51.0°.

*(R)-α-*N,N*-Trimethyl-β-phenethylamine (10)*. L-Amphetamine (0.05 mol) in a solution of methanol (75 ml), water (30 ml) and 35 % formaline (40 ml) was hydrogenated at atmospheric pressure over 5 % Pd/C (5 g) until the hydrogen uptake had ceased. The catalyst was then removed by filtration, conc. HCl (5 ml) added and the solution evaporated to dryness at reduced pressure. The residual material was redissolved in methanol (20 ml) containing triethylamine (6 ml) and the solution poured into ether (200 ml). The precipitate was removed by filtration and the filtrate evaporated and the residual material distilled; b.p. 88 °C/9 mmHg [lit.[21] b.p. 72–74 °C/2.5 mmHg], yield 47 %. (Found: C 81.21; H 10.60. Calc. for $C_{11}H_{17}N$: C 80.95; H 10.50); $[\alpha]_D = +7.8°$ ($c = 2.6$ in MeOH).

(S)-1,1-Diphenyl-2-methoxypropan-1-ol (12) was prepared according to the procedure described for (*6*) above by treatment of ethyl L(−)-α-methoxy-lactate[22] with phenyl lithium in ether; yield 40 %, m.p. 72 °C (pet. ether) (Found: C 79.55; H 7.48. Calc. for $C_{16}H_{18}O_2$: C 79.31; H 7.49); $[\alpha]_D = -91°$ ($c = 1.4$ in MeOH); τ ($CDCl_3$) 9.0 (Me), 6.7 (OMe), 5.8 (CH), 2.8 (Ph).

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Mass Spectrometry of Onium Compounds. XXVIII.* Pyrolysis of Methonium Salts

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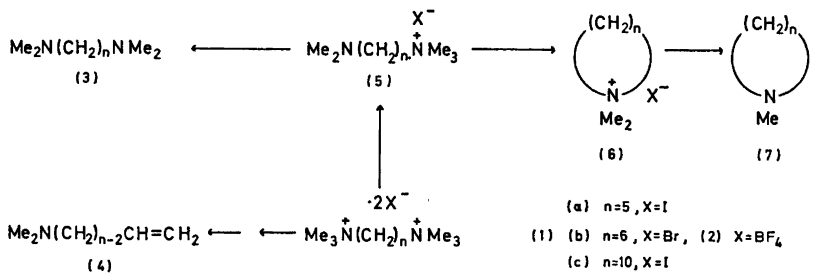
Pyrolytic reactions of methonium salts in the mass spectrometer have been analysed. Formation of the corresponding *bis*-dimethylamines and dimethylamino-olefins are characteristic in the higher mass range of the spectra. Their formation is aided by moderately nucleophilic anions. Electron impact fragmentations are discussed.

The mass spectra from involatile *N*-quaternary salts are formed through superimposition of the fragmentation spectra from the volatile pyrolysis products. A complication in spectral analysis may be variations in relative peak intensities with recording conditions and with time in accordance with the relative volatilities of the components. The major pyrolytic fragmentation paths, however, can be rationalised in terms of the nature of the substituents attached to the quaternary nitrogen atoms.[2-6] This report extends earlier studies of onium compounds in the mass spectrometer to bis-*N*-quaternary compounds; such knowledge is of interest for analytical studies of "methonium" type drugs [7] and related compounds by mass spectrometry.

The penta-, hexa-, and decamethonium salts (*1*) in which the nitrogen atoms are bridged by five, six, and ten methylene groups, respectively, were available for studies. The pyrolytic behaviour of the bromide (*1b*) and the iodides (*1a*, *1c*) were compared with the corresponding fluoroborates (*2*). The fluoroborate anion is partially pyrolysed to the strongly basic fluoride anion; Hofmann elimination reactions will thus be promoted. The more nucleophilic bromide and iodide ions are likely to favour substitution reactions.[3] The expected differences are apparent by comparison of the spectra for each pair. One or two major pyrolytic pathways are desirable to ease interpretation of the spectra and increase sensitivity. An important factor is here the nature of the anion.

Several possibilities exist for product formation from the *N*-quaternary salts. In the present series major volatile products were the bis-dimethylamines (*3*) and the dimethylamino-olefins (*4*); methyl halides and trimethylamine are concurrently formed. Especially formation of trimethylamine may at first sight complicate interpretation of the spectra; its fragmentation,

* Part XXVII, see Ref. 1.



Scheme 1.

Table 1. Relative intensities of the major ions in the mass spectra of tertiary amines.

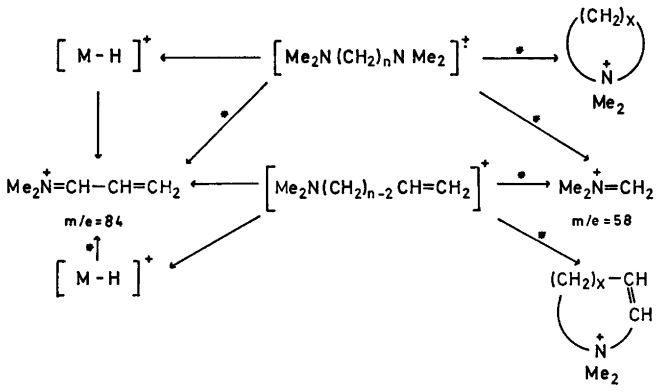
(*3a*) *m/e*	%	(*3b*) *m/e*	%	(*3c*) *m/e*	%	(*4a*) *m/e*	%	(*4b*) *m/e*	%
158	0.5	172	1	228	2	113	3	127	4
114	2	128	3	213	1	98	8	99	0.5
98	5	127	4	184	1	84	12	98	0.5
84	4	114	2	142	0.5	71	3	84	4
70	2	84	7	128	1	58	*100*	71	1
58	*100*	58	*100*	114	0.5	42	8	58	*100*
42	3	42	5	84	3	30	4	42	5
30	3	30	3	58	*100*			30	3
				42	3				
				30	3				

however, has been studied in some detail.[8] The mass spectral behaviour of the bisdimethylamines and the dimethylamino-olefins on the other hand, had to be ascertained; these compounds with the exception of (*4c*) were therefore synthesised separately and their spectra recorded.

Bridged diamines on electron induced fragmentation are often characterised by low intensity molecular ion;[9] a short bridge between the amino functions and *N*-substitution such as in 1,4-tetramethylaminobutane may increase the molecular ion intensity considerably.[10] In the present series the base peak for both the diamines (*3*) and the olefins (*4*) was at *m/e* 58 $[CH_2NMe_2]^+$; this fragment is formed primarily by α-cleavage of a bridge carbon-carbon bond. The molecular ion intensities were of the order 0.5—2 %.

The elemental compositions of the fragments discussed have been determined by high resolution. Expulsion of the dimethylamine radical is seen in the spectra of the bis-dimethylamines but not in the spectra of the dimethylamino-olefins. The process is therefore thought to be caused by the second amino group leading to a cyclic ammonium structure; accordingly this path is important for (*3a*) which can form a six-membered ring and was absent for (*3c*). The functional groups may also interact in other ways. Thus the diamines show loss of the dimethylaminomethylene radical $[Me_2NCH_2]^\cdot$ presumably with formation of cyclic ammonium structures. In general dimethylamino-alkyl radical expulsions are less important if the other amino function can be formulated as part of a medium size ring but signals corresponding to six-, five-, and three-membered rings are present. The dimethylamino-olefins show similar fragmentation patterns.

Hydrogen rearrangements are common phenomena in unsaturated aliphatic hydrocarbons.



Scheme 2.

In the dimethylamino-olefins the observed radical expulsions can be rationalised in terms of the various rearranged molecular ions; formation of unsaturated cyclic ammonium structures are postulated for the cations.

By far the most dominating peak in the spectra is due to the *N,N*-dimethylimmonium ion at *m/e* 58. The base peak in the spectrum of trimethylamine is also at *m/e* 58 $[M-H]^+$; the relative intensity of the trimethylamine molecular ion is 45 %. Accordingly the base peak in the spectra of the methonium salts is also found at *m/e* 58 (Fig. 1 and 2). The relative intensities of the trimethylamine peak in the spectra of the halides were 25 (*1a*), 16 (*1b*), and 8 % (*1c*), the figures for the fluoroborates were 35 (*2a*), 24 (*2b*), and 20 (*2c*). The fluoroborates had to be heated at 310—330 °C in the probe to give a sufficient ion current while the other salts were kept a 220 °C. The figures show that fragmentation of the bisdimethylamine and the dimethylamino-olefin is a major contributor to the mass number 58. More trimethylamine is formed from the compound with the shortest methylene bridge. More trimethylamine is also formed in the fluoroborate series. The relatively nucleophilic bromide and iodide ions cause extensive dealkylations at the lower temperature while the more electronegative fluoride ion possibly together with the fluoroborate ion, requires higher activation and then acts more as a base in Hofmann elimination. The relative intensities of the molecular ions of the diamines support this conclusion; the figures for the bromide and iodides were 0.5 (*1a*), 1 (*1b*), and 2 % (*1c*); and 0 (*2a*), 0 (*2b*), and 1 % (*2c*) for the fluoroborates. Corresponding figures for the molecular ions of the dimethylamino-olefins

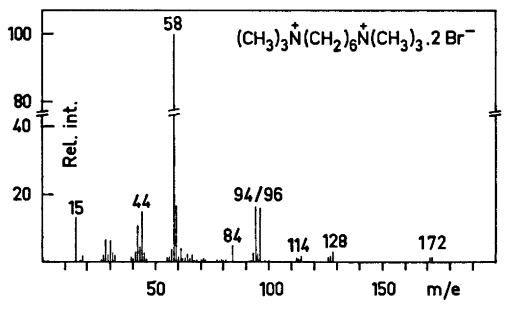


Fig. 1.

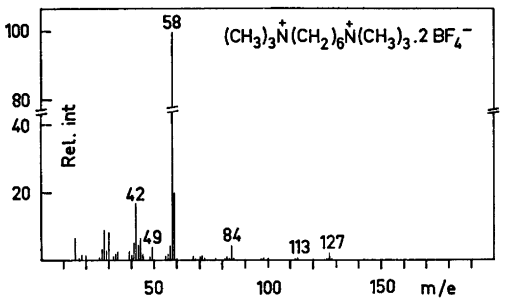


Fig. 2.

were 2 (*1a*), 2 (*1b*), and 0.5 (*1c*) which are to be compared with 0.5 (*2a*), 2 (*2b*), and 3 (*2c*).

After demethylation of the one amino group another demethylation or Hofmann elimination must occur. Alternatively the Hofmann elimination occurs initially. The products from two Hofmann eliminations, however, were not seen.

Ions with mass number corresponding to the dihalides of the methylene bridge or the corresponding halo-olefins were absent. Long-chain aliphatic halides, however, may display very weak molecular ions but contain significant signal for ions after halogen expulsion;[11] the absence of corresponding signals in the present series excludes such structures as major pyrolytic components. Nor are signals due to amino-halides present. In this case expulsion of the halogen and formation of a cyclic ammonium structure would be expected to show up in the spectra. The mass number for these ions is the same as after dimethylamine radical expulsions from the diamines (*3*). A comparison of the relative intensities for this mass number in the spectra of the diamines (*3*) and the spectra from the methonium salts suggest that amino halides are not important pyrolytic products. These observations show that the nucleophilic attack by the halide ion is mainly on the methyl carbon rather than on the α-carbon of the methylene bridge.

Another pyrolytic reaction to be considered is internal cyclisation with trimethylamine displacement after initial demethylation in the other amino function. The involatile, cyclic ammonium product may then undergo a Hofmann elimination to the dimethylamino-olefin or undergo demethylation; cyclisation was found important for the pentamethonium iodide lead-

ing to a six-membered ring; thus the intensity of the mass number corresponding to *N*-methylpiperidine was 13.5 % with $[M-H]^+$ 40 %. Support for the former being a molecular ion was forthcoming by the substantial increase in the relative intensity of this species in low-energy spectra. The relative intensities of the corresponding peaks in the spectra of the hexamethonium bromide were 1 (*m/e* 113) and 1.5 % (*m/e* 112); and the relative intensity of the higher mass number was increased to 8 % in the low energy spectra. Corresponding signals in the spectra of the decamethonium iodide were absent. These results reflect the relative tendencies for ring formation. This reaction path will also depend on the availability of a tertiary amino function and is therefore favoured in the presence of nucleophilic anions. Thus in the spectra of the fluoroborates the signal intensity for the cyclic product from the methonium salts was reduced to 1 % and were absent in the spectra of the other isomers.

EXPERIMENTAL

The mass spectra were recorded on an AEI MS902 mass spectrometer attached to an AEI DS 30 data system. The electron energy was 70 eV and the ionising current 100 μA. The compounds were introduced by the heated direct insertion probe; the iodide and bromide methonium salts at 220 °C and the fluoroborates at 310–330 °C. The source temperature was 220 °C.

Commercial methonium bromide or iodides were used. The fluoroborates were available from concentrated aqueous solutions of these salts by addition of 40 % fluoroboric acid; the fluoroborates crystallised directly or after concentration of the solution.

The bisdimethylamines (*3*) were synthesised from the corresponding dibromides.[12] The dimethylamino-olefins (*4*) were synthesised from piperidine and hexamethylenimine by quaternisation with methyl iodide and the products ground together with solid sodium hydroxide and pyrolysed.[13]

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Supporting Electrolyte in Indirect Electrolytic Reactions

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With LiCl as supporting electrolyte electron transfer in solution may take place between a stable anion radical $A^{\cdot -}$ and a compound BX, forming an unstable anion radical, even if the polarographic wave of BX is masked by the reduction of Li^+. With tetrabutylammonium iodide (TBAI) as supporting electrolyte, electron transfer from an anion radical to the cation may occur with formation of tetrabutylammonium radical which decomposes slowly into tributylamine and butyl radical; the butyl radical may couple with the anion radical. Indirect reduction of a difficultly reducible compound BX may, however, take place, if the radical anion $BX^{\cdot -}$ decomposes much faster than the tetraalkylammonium radical.

In connection with the present series on indirect electrolytic reactions of organic compounds [1] some aspects of the rôle of supporting electrolyte in such reactions have been investigated. At a mercury electrode, amalgam formation often is the cathodic potential-limiting reaction, and the idea of the work described below is to investigate the possible circumvention of this potential-limiting reaction by use of stable anion radicals $A^{\cdot -}$ as reagents for transferring electrons to compounds (BX), which form unstable anion radicals.

Lithium chloride and tetrabutylammonium iodide (TBAI) have been chosen as examples of supporting electrolytes; the solvent was in all cases (nominally) anhydrous *N,N*-dimethylformamide (DMF).

RESULTS AND DISCUSSION

Lithium chloride. The half-wave potential of 2-chloropyridine (I) is in DMF containing 0.1 m TBAI −2.27 V *vs.* aqueous saturated calomel electrode (aq. S.C.E.), which is more negative than the foot (~ −2.1 V (aq. S.C.E.)) of the reduction wave of 0.1 m LiCl in DMF. A polarographic wave of 2-chloropyridine is thus not visible, when the dropping mercury electrode (DME) is used in DMF containing LiCl.

The polarographic half-wave potential of anthracene (II) in DMF/TBAI is −1.91 V *vs.* aq. S.C.E.; an anion radical which is stable in the absence of protons is formed in the electrode reaction; anthracene may thus be used as an A-compound [1] in the scheme below. 2-Chloropyridine on electron transfer forms an unstable anion radical which rapidly decomposes into chloride ion and pyridine radical, thus acting as a BX-compound [1] in the scheme below.

$$A + e^- \rightleftharpoons A^{\cdot -} \quad (1)$$
$$A^{\cdot -} + BX \rightleftharpoons A + BX^{\cdot -} \quad (2)$$
$$BX^{\cdot -} \rightleftharpoons B^{\cdot} + X^- \quad (3)$$
$$A^{\cdot -} + B^{\cdot} \rightleftharpoons A + B^- \quad (4)$$
$$A^{\cdot -} + B^{\cdot} \rightleftharpoons AB^- \quad (5)$$
$$A^{\cdot -} + A^{\cdot -} \rightleftharpoons A^{2-} + A \quad (6)$$
$$B^{\cdot} + B^{\cdot} \rightleftharpoons B-B \quad (7)$$
$$B^{\cdot} + e^- \rightleftharpoons B^- \quad (8)$$
$$H^+ + B^-(AB^-) \rightleftharpoons BH(ABH) \quad (9)$$

2-Chloropyridine is reduced at a potential of about 0.4 V more negative than that of anthracene, so the equilibrium (2) is shifted far to the left; the rapid decomposition of the 2-chloropyridine anion radical (3) forces, however, the reaction (2) to proceed from left to right.

In the polarography of anthracene (II) in DMF/0.1 m TBAI in the presence of 2-chloropyridine (I) an indirect reduction of I is indicated by an increase of the limiting current of II, a so-called catalytic wave. A rate constant of the catalyzing reaction of 75 m^{-1} s^{-1} was

found by a calculation according to Koutecký.[2] A similar catalytic wave is found in DMF containing LiCl and the rate constant is, within the experimental uncertainty, the same; if the small difference found is significant, the rate constant in DMF/LiCl is slightly higher than in DMF/TBAI.

A preparative reduction of 2-chloropyridine by means of electrolytically generated (and regenerated) anthracene radical anions produced pyridine in high yield (83 – 86 %), regardless of whether LiCl of TBAI was the supporting electrolyte. (4) rather than (5) is followed by 2-chloropyridine as with all aromatic BX-compounds investigated so far.

These results show that with a lithium salt as supporting electrolyte electron transfer is possible from an A-compound to a BX-compound, even if the polarographic wave of the BX-compound is masked by the reduction wave of lithium ions.

The electrode reaction of lithium ions at a mercury electrode is the formation of lithium amalgam which takes place at a potential much less negative than the reduction potential of Li^+ at an inert eletrode. In solution the tendency of electron transfer from an anion radical $A^{\cdot -}$ to a lithium ion (possibly participating in some kind of ion-pairing) is determined by the difference in redox potentials (at an inert electrode) of $A/A^{\cdot -}$ and Li^+/Li; this tendency is extremely small when the potential difference is one volt or more, which is the case when A gives a polarographic wave with LiCl as supporting electrolyte. The use of an electron transfer reagent, such as an anion radical, thus makes it possible by the indirect method to reduce at a mercury electrode certain substrates with a cheap, amalgam-forming cation, such as Li^+ or Na^+, as supporting electrolyte, even if a direct electrolytic reduction at a mercury electrode in that medium does not seem possible judging from polarographic data.

The present paper is not concerned with whether the electron transfer from the anion radical to the BX compounds is following (2) or the electron donating species is A^{2-}, formed in (6), or *via* some other route; this will be discussed in a later communication.

Tetrabutylammonium iodide (TBAI). Tetrabutylammonium iodide is usually an "inert" supporting electrolyte, but in the presence of a strong base it may act as a proton donor; the loss of a proton results in a Hofmann elimination with formation of butylene and tributylamine.

In the present context it is also of interest to know whether the tetrabutylammonium ion may accept an electron from an anion radical;[3] the resulting neutral radical would be expected either to lose the electron again or to decompose into tributylamine and a butyl radical, the salt thus acting as a BX-compound in the scheme.

It has been found [4] that in an indirect reduction, where the BX-compound is aliphatic, the aromatic anion radical $A^{\cdot -}$ and the aliphatic radical $B^{\cdot}$, formed in (3), may couple to AB^- (5), which then accepts a proton to ABH.

An anion radical could then conceivably react with TBAI in two ways, it could abstract a proton from it or it could transfer an electron to it. The outcome of this competition would depend on factors such as basicity of the anion radical, decomposition rate of the quaternary ammonium radical, and redox potential of the compounds in question. Again, a similar situation is found if the aromatic dianion (6) is the electron-transferring species.

The limiting current of an A-compound and the coulometric data might thus depend on the concentration and the nature of the supporting electrolyte; with 2-methylnaphthalene ($E_{\frac{1}{2}} = -2.42$ V *vs.* S.C.E. in DMF/TBAI) only a very small increase, if any, of the limiting current is observed on increasing the concentration of TBAI and this might just as well be due to an increase in the concentration of the proton donor, TBAI; a catalytic reaction could thus not be proved from polarographic data.

A preparative reduction of 2-methylnaphthalene in DMF containing 0.2 m TBAI produced a 3:1 mixture of 2-methyl-1,2,3,4-tetrahydronaphthalene and 6-methyl-1,2,3,4-tetrahydronaphthalene together with a small amount (~1 %) of some compound(s) with $m/e = 202$, assumed to be butylated methyltetrahydronaphthalene. The low yield of butylated compounds is not surprising in view of the finding,[4] that the coupling reaction (5) competes much less effectively with the electron transfer reaction for B = n-C_4H_9 than it does when B = *t*-C_4H_9.

The reductive alkylation of an aromatic hydrocarbon, such as 2-methylnaphthalene, by

a radical mechanism (5) suggests thus the electron transfer in solution between the 2-methylnaphthalene anion radical (or possibly through participation of the dianion) and the tetrabutylammonium ion. It would be of interest to investigate whether this reaction effectively blocked an electron transfer to another BX-compound, which was reduced at so negative a potential that it did not give a wave at the dropping mercury electrode in DMF/TBAI.

Benzyl isopropyl ether does not give a polarographic wave in DMF/TBAI; a preparative indirect reduction of benzyl isopropyl ether, using electrolytically generated 1-methylnaphthalene anion radical in DMF/TBAI, gave, however, toluene in high yield. The electron consumption corresponded to $n=2$ for benzyl isopropyl ether and $3<n<4$ for 1-methylnaphthalene, which is present in a concentration less than one third of the concentration of the ether.

These results may be explained, if it is assumed, that the rate of decomposition of benzyl isopropyl ether anion radical is much faster than that of the tetrabutylammonium radical, or that the tendency of electron transfer in solution to the tetrabutylammonium ion is considerably smaller than the polarographic results suggest; probably both effects are operating with the results that the available potential region is enlarged.

In this context it does not matter whether the electron on its way from the 1-methylnaphthalene anion radical to the benzyl isopropyl ether has resided on one or more tetrabutylammonium entities as long as the tetrabutylammonium radical loses the electron again before it decomposes. The electron consumption observed does not allow the reduction of more than a very small amount TBAI. The excessive electron consumption observed in some cases [3,5] was thus not found here.

In this connection it might be mentioned that the anion radical also could have exchanged the electron with other methylnaphthalene molecules before it finally reached the benzyl isopropyl ether molecule, the anion radical of which decomposed. This possibility is open to all anion or cation radicals where the irreversible reaction of the radical ion (protonation, deprotonation, cleavage) is slow compared to the rate of electron transfer between species of the same kind; this rate, which is known from the line-broadening in the EPR spectra,[6] is of the order of 10^8 m^{-1} s^{-1}. In the process of electron transfer in solution the heterogeneous nature of the electrode reaction is lost and perhaps such an electron transfer in solution between species of the same kind is responsible for the lack of steric influence of the electrode found in most electrolytic reactions in aprotic media.

EXPERIMENTAL

Materials. Benzyl isopropyl ether, prepared from benzyl chloride and sodium isopropylate in isopropyl alcohol, was shaken with a solution of iron(II) sulfate to remove peroxides, dried and distilled immediately before use.

Reduction of 2-chloropyridine (a). A mixture of 2-chloropyridine (0.50 g) and anthracene (0.10 g) was reduced at −2.10 V (aq. SCE) in DMF/0.1 m TBAI. The electron consumption corresponded to a 2-electron reduction of 2-chloropyridine. In the reduced solution pyridine (84 − 86 %) was determined by GLC on a 15 % polyethyleneglycol 4000 column at 70 °C with *o*-xylene as internal standard. (*b*) A similar reduction ($n=2$) was carried out at −2.08 V with 0.1 m LiCl as supporting electrolyte; yield of pyridine 85 %.

Reduction of 2-methylnaphthalene (a). 2-Methylnaphthalene (5 g) was reduced at −2.65 V (aq. SCE) in DMF/0.3 m TBAI; $n=7.4$, which indicates reduction of other compounds besides 2-methylnaphthalene. The catholyte was diluted with water and extracted with distilled petrol ether. The organic layer was washed several times with water to remove DMF, dried and evaporated *in vacuo*. The residue was analyzed by GLC on 5 % SE 30 at 120 °C, which gave 2 major peaks, one of them corresponding to 2-methyl-1,2,3,4-tetrahydronaphthalene and the other one to 6-methyl-1,2,3,4-tetrahydronaphthalene; a minor peak at longer retention time (1 % of the major peaks) was attributed to a butylated tetrahydromethylnaphthalene due to the fact that mass spectrometry of the crude product showed peaks up to and including *m/e* 202 and none at higher *m/e*. Attempted separation of the major components by preparative GLC gave the two components in about 95 % purity; ^1H and ^{13}C NMR-spectroscopy, however, readily identified the major compounds. ^1H NMR. 2-Methyl-1,2,3,4-tetrahydronaphthalene (CCl_4): $\delta=1.03$, doublet, $J=6.5$ Hz, $\sum H=3$; $\delta=1-3$, series of multiplets, $\sum H=7$; $\delta=6.93$, singlet, $\sum H=4$. 6-Methyl-1,2,3,4-tetrahydronaphthalene (CCl_4): $\delta=1.6-1.9$, multiplet, $\sum H=4$; $\delta=2.20$, singlet, $\sum H=3$; $\delta=2.5-2.8$, multiplet, $\sum H=4$; $\delta=6.78$, $\sum H=3$. The two multiplets resemble the corresponding ones of tetraline.

Indirect reduction of benzyl isopropyl ether. A mixture of 0.5 ml (0.513 g) 1-methylnaphthalene

and 2.0 ml (1.88 g) benzyl isopropyl ether in *N,N*-dimethylformamide (DMF) containing 0.1 m tetrabutylammonium iodide (TBAI) was reduced at -2.1 V *vs.* Ag/AgI-electrode (-2.6 V *vs.* aq. SCE). The solution turned green and remained so until the reduction was finished. The electron consumption could be accounted for by assuming $n=2$ for the reduction of the ether and n between 3 and 4 for the methylnaphthalene. The yield of toluene was determined (93 %) directly in the reduction mixture by GLC on a 15 % polyethylene glycol 4000 column at 80 °C with *p*-xylene as internal standard.

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Mass Spectrometry of Onium Compounds. XXIX.* Ionisation Potential in Structure Analysis of Valence Isomers

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The ionisation potentials of 1-substituted 1a,6a-dihydroindeno[1,2-*b*]azirin-6(1*H*)-one and 2-substituted isoquinolinium-4-oxide valence isomeric pairs show that thermal intermolecular isomerisation does not occur. The appearance potentials for the $[M-N_2]$ species from 3-substituted 3a,8a-dihydroindeno[1,2-*d*]triazol-8-(3*H*)-ones are in accordance with thermal formation of the corresponding indanoaziridines.

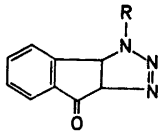
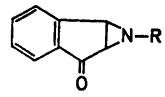
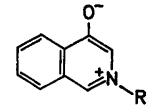
Simple pyridinium betaines are readily evaporated in the mass spectrometer.[2,3] The volatility could possibly be explained by isomerisation of the pyridinium-3-oxides to the corresponding non-charged 6-azabicyclo[3,1,0]hex-3-en-2-one. This electronic rearrangement is analogous to the reversible thermal valence isomerisation of phenyl substituted epoxides of cyclopentadienones and indenone to the corresponding pyrylium-3-oxide and the benzopyrylium-4-oxide.[4,5] We have therefore prepared some valence isomer pairs;[6,7] their mass spectral behaviour is discussed in this paper. The isomer pairs studied have incorporated a condensed benzene ring for stability reasons and thus consist of the indanones (II) and the isoquinolinium salts (III), in Table 1. The thermal bicyclic ring formation (III→II) or ring opening (II→III) is formally not permitted by the geometry of the system since the aziridine is constrained in a bicyclic structure of medium size (five-membered ring). During our work, however, it has been shown that closely related 6-imino- and 6-keto-1,1a,-6,6a-tetrahydroindeno[1,2-*b*]azirines could be reversible isomerised to isoquinolinium ylides.[8,9]

Structurally isomeric molecules may often have very similar mass spectra and cannot with certainty be differentiated by their fragmentation patterns alone. Energetically, however, the isomers may differ sufficiently for this to be detected in their ease of ionisation. We have on several occasions shown that measurements of ionisation potentials (IP) is a useful technique in structure analysis in the gas phase;[10–12] a recent paper reviews the use of IP and appearance potential (AP) in structure analysis.[13]

The IP's and AP's were arrived at by the semilog plot of the ionisation efficiency curves.[14] It is immediately apparent from a comparison of the IP data in Table 1 for the dihydroindeno-azirin-6-ones (II) and the isoquinolinium-4-oxides (III) that no thermal interconversion between the two series occurs in the mass spectrometer. Thus the IP's for the isoquinolinium betaines (about 7 eV) are at least 1 eV lower than the values for their aziridine isomers. It is further seen that the *N*-benzyl and *N*-phenyl isoquinolinium derivatives have very similar IP's which suggest that the primary ionisation is in the heterocycle. This suggestion is supported by the relatively weak influence of the phenyl *para* substituents on the ease of ionisation; *N*-phenylpyridinium-3-oxides have been found to behave similarly.[15] The phenyl aziridines can be considered as *N*,*N*-dialkylanilines in which case the variation in IP with the *para* substituent would show the expected pattern for ionisation in the phenyl ring.[16] Therefore the IP of *N*,*N*-dimethylaniline was measured and found to be 7.78 eV which agrees reasonably well with the value (8.13 eV) for the phenyl aziridine (IIa). Primary ionisation in the aromatic part of the indanone system would

* Part XXVIII, see Ref. 1.

Table 1. Ionisation potentials.

 (I)	AP[M−N_2]	 (II) IP	 (III) IP
(a) R=Ph	8.1±0.1	(a) 8.13±0.05 eV	(a) 7.10±0.05
(b) =*p*-MeOPh	7.8±0.1	(b) 7.68±0.05 »	(b) 6.93±0.05
(c) =*p*-NO_2Ph	8.8±0.1	(c) 8.71±0.05 »	(c) 7.29±0.05
(d) =CH_2Ph	8.1±0.1	(d) 8.15 [6]	(d) 7.10 [6]
(e) =Me	8.8±0.2		
(f) =Bu	7.8±0.2	(f) 7.90±0.1 eV	

appear to be excluded since the IP of indanone was found to be considerably higher (9.31 eV). No definite conclusion can be drawn, however, since the IP's of both the *N*-benzyl and *N*-butyl derivatives are also about 8 eV, and this can be interpreted in favour of initial ionisation on the aziridine nitrogen atom.

The *N*-phenyl aziridines (IIa−IIc) are formed in a heterogeneous mixture on pyrolysis of the corresponding 3a,8a-dihydroindeno[1,2-*d*]-triazol-8(3*H*)-ones (I); preparatively photolysis was superior.[7] In the mass spectra of the phenyl triazolines the highest mass number peak was that of a species having expelled two nitrogen atoms, in fact the mass spectrum was that of the corresponding aziridine (II). Dominating pyrolytic formation of aziridines was confirmed by measurements of the AP for the [M−N_2] species; the values showed good correlation with the IP values for the respective aziridines (II). Preparative pyrolysis of the benzyl derivative (Id), but not of the alkyl derivatives (Ie, If), yielded the respective aziridines. The mass spectra and the AP [M−N_2] for the benzyl derivative show aziridine formation as the principal pyrolytic pathway. The AP [M−N_2] for the butyl and methyl derivatives was variable and both spectra contained signals of *ca.* 1 % intensity corresponding to the molecular ion of the parent compound. As the alkyl aziridines were not available for comparison the butyl derivative (IIf) was separately synthesised by a Gabriel reaction from *trans*-2-bromo-3-butyl-*N*-aminoindan-1-one as described for the benzyl analogue (IId).[6] Both the IP value and its spectrum were similar to AP [M−N_2] for the triazoline (If) and its spectrum. As pointed out above, however, a weak molecular ion is present in its spectrum and a metastable for the [M−N_2] formation was seen by the defocusing technique. The closeness between the AP and IP values discussed indicates that pyrolysis is the major contributor to the [M−N_2] species. The AP

Table 2. Relative intensities of higher mass ions in the spectra.

(IIa)		(IIb)		(IIc)		(IIf)		(IIIa)		(IIIb)		(IIIc)	
m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
221	74	251	*100*	266	*100*	201	8	221	*100*	251	71	266	*100*
220	*100*	250	73	265	35	172	7	220	40	250	29	238	33
193	17	236	18	220	65	160	13	193	26	223	23	220	12
165	18	223	8	219	51	159	*100*	192	14	222	13	208	10
118	14	208	32	192	23	145	31	165	19	208	*100*	192	27
		118	20	118	17	131	58						
						118	14						

$[M-N_2]$ for the methyl derivative is significantly higher. Part of the increase may be due to the change from the butyl to the methyl group,[17] but it must be concluded that electron induced fragmentation of the evaporated parent molecule is here a major source for the species with mass number $[M-N_2]$. Comparisons of relative fragment intensities for the isomeric pairs in Table 2 show great similarities in the fragmentation patterns. Higher intensities for the [M−CO] fragments are generally found in the isoquinolinium series. The molecular ion is the base peak or a strong peak in all spectra except for the butylaziridine (IIf) where the base peak is formed by propylene expulsion (*m/e* 159) from the molecular ion. [M−H] appears more important for the aryl aziridines than for the isoquinolinium analogues, but this may be affected by the phenyl substituents. The fragmentation patterns are in general analogous to those briefly discussed for the benzyl pairs (IId/IIId) [6] and for the *N*-phenylpyridinium-3-oxides.[15] It suffices to point out that a characteristic signal for the indano-aziridines arises by expulsion of the whole NR-group to the species *m/e* 118; the mass number corresponds to that of indenone.

EXPERIMENTAL

The mass spectra and ionisation efficiency (i.e.) curves were recorded on an AEI-902 mass spectrometer as previously described.[14] The i.e. curves were interpreted by the semilog plot method.[18] The AP's and IP's are the average of three determinations.

Syntheses of the compounds have previously been described [6,7] with the exception of the butylaziridine (IIf).[19]

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The Synthetic Utility of Heteroaromatic Azido Compounds. I. Preparation and Reduction of Some 3-Azido-2-substituted Furans, Thiophenes, and Selenophenes

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The reaction of 3-bromo-2-formylfuran, -thiophene and -selenophene with sodium azide in dimethyl sulfoxide gave the corresponding 3-azido-2-formyl substituted heterocycles, which were transformed to the azido-cyanides and azido-carboxylic acids. Reduction of the 3-azido-2-formyl derivatives with hydrogen sulfide gave the hitherto unknown 3-amino-2-formyl derivatives, which were quite stable, and constitute excellent starting materials for the synthesis of fused heterocyclic systems.

The 3-amino-2-formyl derivatives exist in the hydrogen bonded *trans* conformations, which is evident from the long-range coupling between the formyl proton and the proton in the 4-position.

The azido group of arylazido compounds is a versatile synthon in organic synthesis. Its value depends largely on two synthetic transformations. The first one is its easy reduction by various means to the corresponding primary amino group. This reaction is especially important in those cases where the amino derivatives are hard to obtain in other ways. However, it should be borne in mind that arylazido compounds are often prepared *via* diazotization of the corresponding amino derivatives. The second transformation is its decomposition, thermally or photolytically, to give a nitrene. In general, nitrenes are generated photolytically as triplets and thermally as singlets, which can then decay to triplets. Nitrenes are highly reactive species which can react in a number of different ways, for example by intramolecular isomerization to imines, by dimerization to azo compounds, by insertion into a C—H bond, by addition to unsaturated systems yielding heterocyclic compounds, *etc.* As a special case there are also decompositions which proceed *via* a synchronous intramolecular mechanism. This means that no free nitrenes are generated along the reaction pathway.

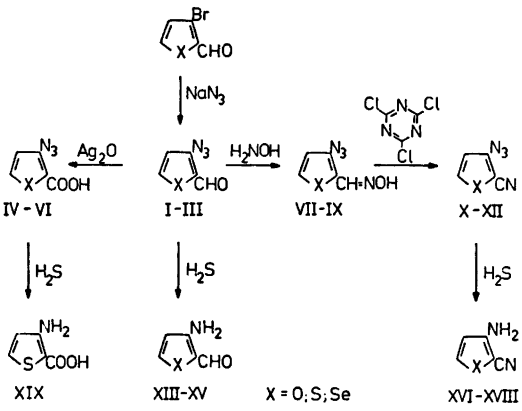
The distinction between these two synthetic transformations is not a sharp one since there are borderline cases, but this division seemed to us to be a practical approach. (Fo review *cf.* Ref. 1.) This paper will deal with the synthesis and reduction of some 3-azido-2-substituted furans, thiophenes and selenophenes. The use of the resulting 3-amino-2-formylfuran, -thiophene, and -selenophene for synthesis of furo-, thieno- and selenolo[3,2-*b*]pyridines will be reported in a following paper. Work on the synthetic utility of thermal decompositions of the azido compounds described in this paper is under way.

Preparation of some 3-azido-2-substituted furans, thiophenes and selenophenes

A sensible approach to this class of compounds was to use aromatic nucleophilic substitution with appropriate 2-substituted 3-bromofurans, -thiophenes and -selenophenes as substrates. The 3-bromo-2-formyl derivatives in these series were all known,[2-4] and they were chosen as suitable starting materials. The formyl group was expected to facilitate the nucleophilic substitution owing to its electron-attracting power.

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Thus the 3-bromo-2-formyl derivatives were treated with sodium azide in dimethyl sulfoxide at 60—65 °C for 1—2 days (see Scheme 1).



Scheme 1.

This gave the expected 3-azido-2-formylfuran (I), 3-azido-formylthiophene (II) and 3-azido-2-formylselenophene (III) in moderate yields (45—60 %). These azido compounds are quite stable, crystalline solids. A sample of 3-azido-2-formylfuran (I) showed no sign of decomposition after standing in air, light and at room temperature for four weeks. This is in marked contrast with the observed instability of 5-azido-2-formylfuran, prepared from 5-nitro-2-formylfuran, which is the only example in the literature of an azido derivative in these heterocyclic series.[5] This compound is reported to be stable for a longer time only at 0—4 °C. Samples of 3-azido-2-formylthiophene (II) and 3-azido-2-formylselenophene (III) gave a slight discoloring under the conditions described above. However, no change in the IR spectra of these compounds was evident.

Unfortunately, the scope of this nucleophilic aromatic substitution seems to be quite limited. 2-Bromo-3-formylthiophene, 5-bromo-2-formylthiophene and 4-bromo-3-formylthiophene did not give azides under the above-mentioned conditions, nor did 3-bromo-2-cyano-, 2-acetyl-3-bromo- or 3-bromo-2-carboxythiophene. Greater success, however, was achieved with 3-bromo-2-nitrothiophene and 3-bromo-4-formyl-2-nitrothiophene, which smoothly gave the expected azides (unpublished results).

Since the aromatic azido group is known to be quite stable in non-reducing environment, it was possible to make some transformations which retained or raised the oxidation level of the formyl group, leaving the azido function intact.

Thus it was possible to oxidize the formyl group to a carboxylic group using silver(I) oxide (see Scheme 1). This gave 3-azido-2-carboxyfuran (IV), 3-azido-2-carboxythiophene (V) and 3-azido-2-carboxyselenophene (VI) in good yields (65—90 %).

The 3-azido-2-formyl derivatives were easily transformed to the corresponding aldoximes (VII, VIII, IX) in the usual manner (see Scheme 1). These could be dehydrated to nitriles by a slight variation of the method of Chakrabarti and Hotten,[6] using 2,4,6-trichloro-s-triazine. They used a small excess of 2,4,6-trichloro-s-triazine calculated for reaction of all three chlorine atoms (0.36—0.50 mol triazine/mol aldoxime). In this work this proportion was found to give a gummy precipitate, which was difficult to separate and wash. If, however, a larger proportion of triazine was used (*ca.* 0.75 mol triazine/mol aldoxime), a precipitate, which was easy to separate and wash, was formed. Thus 3-azido-2-cyanofuran (X), 3-azido-2-cyanothiophene (XI) and 3-azido-2-cyanoselenophene (XII) were obtained in good yields (80—87 %).

These 3-azido-2-substituted derivatives are quite stable compounds. However, no regular melting points could be obtained for the 2-aldoximes (VII, VIII, IX) or the 2-carboxylic acids (IV, V, VI) since these decomposed slowly on heating above *ca.* 110 °C without melting. The 2-cyano derivatives, on the other hand, had distinct melting points.

Preparation of some 3-amino-2-substituted furans, thiophenes and selenophenes

It was of interest to try to prepare some simple, previously unknown, 3-amino-2-substituted derivatives from the corresponding 3-azido derivatives. Several methods for achieving this are known. However, we found that a sparingly used method for reducing azido compounds to the corresponding primary amines consisting in treatment with hydrogen sulfide in alkaline or neutral solutions [7–10] was the superior one. This is a reaction which compares favourably with many of the more currently used

methods for this transformation, namely the use of sodium borohydride,[11] lithium aluminium hydride,[12] sodium dithionite,[13] alkaline arsenite,[14] or different catalytical methods.[15,16] The advantages of using hydrogen sulphide in this respect can be summarized as follows.

(1) The preparative procedure is easy to carry out.

(2) The method is very mild and the reaction can be carried out in neutral solutions at or slightly below room temperature. It should be pointed out that the reaction rate is accelerated in the presence of base.

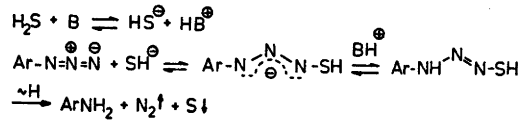
(3) The reducing agent is not prone to react with other parts of the molecule under these conditions.

(4) Excess of reducing agent can be removed simply by blowing a stream of air through the solution for relatively short time.

(5) The reaction gives high yields of the corresponding amines, at least in those cases where no secondary reactions can take place, and it is so "clean" that the reaction mixture after removal of precipitated sulfur can be used directly for further reactions without isolating the amino compounds.

The observations recorded above have been made on reductions of the azido compounds described in this work. However, it seem quite reasonable that they can be generalized to other systems.

Scheme 2 suggests a possible mechanism for this kind of reduction in an alkaline medium.



Scheme 2.

This mechanistic proposal is mainly based on analogy with the mechanism for reduction of azido compounds with alkaline arsenite, proposed by Ugi and co-workers.[14]

Thus the 3-azido-2-formyl compounds were treated with hydrogen sulfide in ethanol and in presence of a small amount of some base such as piperidine (see Scheme 1). This gave 3-amino-2-formylfuran (XIII), 3-amino-2-formylthiophene (XIV) and 3-amino-2-formylselenophene (XV) in good yields (83—89 %). All three compounds were crystalline solids, even though it was rather difficult to obtain 3-amino-2-formylfuran (XIII) in a crystalline form. A sample of 3-amino-2-formylfuran (XIII) had completely disintegrated on standing in air, light and at room temperature for four weeks. The 3-amino-2-formylthiophene (XIV) became rather heavily discoloured under the same conditions. However, no change in the IR spectrum of the compound was evident. 3-Amino-2-formylselenophene (XV), in contrast to those above, showed no sign of decomposition under these conditions.

The synthesis of 3-amino-2-formylthiophene (XIV) has been claimed by Büttner in his Ph.D. thesis.[17] He obtained the alleged compound by condensation of α,β-dichloropropionitrile with mercaptoacetaldehyde. He reports the substance to be a yellow oil with a boiling point of 79 °C/0.001 mmHg and that it very rapidly decomposes in air. This indicates that Büttner did not obtain 3-amino-2-formylthiophene (XIV).

ortho-Amino aldehydes are compounds of great synthetic value as starting materials for many different fused systems. Through the Friedländer reactions (for review *cf.* Ref. 18) they give quinolines or isosteric molecules. The use of the 3-amino-2-formyl derivatives described in this paper in the Friedländer synthesis is the subject of a following paper.

Since none of the simple 3-amino-2-cyano derivatives in these heterocyclic series was known earlier, and since they should be valuable starting materials, especially for annelating pyrimidine rings to the furan, thiophene or selenophene nucleus,[19–21] similar reductions as described above were carried out with the 3-azido-2-cyano derivatives as substrates (see Scheme 1). This gave 3-amino-2-cyanofuran (XVI), 3-amino-2-cyanothiophene (XVII) and 3-amino-2-cyanoselenophene (XVIII) in excellent yields (90—95 %).

It was also of interest to try to prepare the 3-amino-2-carboxylic acids in these series. These compounds are analogues of anthranilic acid, which gives them a potential pharmacological interest. 3-Amino-2-carboxythiophene (XIX) was known earlier and had been prepared by hydrolyzing the corresponding methyl ester obtained by the Fiesselmann ring-closure reaction.[22,23] When 3-azido-2-carboxythiophene (V)

was subjected to hydrogen sulfide as described above in slightly alkaline medium, 3-amino-2-carboxythiophene (XIX) could be isolated in medium yield (61 %) (see Scheme 1). When the two other azides (IV and VI) were exposed to the same conditions they were also reduced, which was evident from the evolution of nitrogen. However, no well-defined products could be isolated from these reactions. The reason for these failures, at least in the case of the furan derivative, might be the easy ring-opening of compounds of this type,[24,25] and other complex reactions.

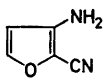
Spectral data

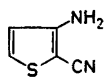
The IR spectra of the azido compounds prepared in this work showed N_3-asymmetric stretching absorption in the region of *ca.* 2090—2140 cm^{-1}. All the amines showed characteristic absorptions in the region of 3120—3470 cm^{-1} due to NH stretching vibrations.

The ^1H NMR data for most of the prepared compounds are given in the experimental part. The NMR data for the amino aldehydes and two of the amino nitriles are given in Tables 1 and 2.

The unsubstituted 3-amino derivatives of these heterocyclic series are known to be quite unstable. The reason for this instability seems to be their tendency to isomerize to the corresponding imines, which then can react in a number of ways. This tendency is greatest in the furan owing to its lesser aromatic stabilization. However, if electron-attracting groups are substituted into these rings, the stability of the amino compounds increases markedly. The reason for this is that the substituent acts as an "electron sink",[26] or in certain cases can form intra- or intermolecular hydrogen bonds with the amino group. It is well known from early NMR investigations that simple aminothiophenes exist in the amino form.[27] 5-Aminophenyl-2-phenyl-3-amino-4-thiophene carboxhydrazides are an exception, and are claimed to exist in the imino form.[28] In no case do the 3-amino-2-substituted derivatives described in this paper give rise to NMR spectra which could be interpreted as due to imino compounds, at least not in a measurable degree. Wie *et al.*[29] have reported that the NMR spectrum of for example 2-amino-3-cyano-4,5-dimethylfuran in trifluoroacetic acid indicates ring protonation. The ring protonation was evident from the observed coupling with the methyl protons. In order to find out if this was also the case for 3-amino-2-cyanofuran (XVI) and 3-amino-2-cyanothiophene (XVII), their NMR spectra were recorded in trifluoroacetic acid. The results are given in Table 1. No formation of additional lines or couplings was observed in this solvent. The only differences were that the bands attributed to the amino protons were absent and that a larger downfield shift of the ring protons compared to that in deuteriochloroform had occurred in this medium. The last effect is to be expected when the strongly electron-

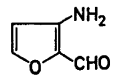
Table 1. ^1H NMR chemical shifts (δ) and coupling constants (Hz) of 3-amino-2-cyanofuran (XVI) and -thiophene (XVII) in deuteriochloroform and trifluoroacetic acid at 60 MHz.

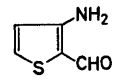


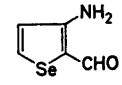


δ	XVI $CDCl_3$	CF_3COOH	XVII $CDCl_3$	CF_3COOH
NH_2	~4.2	not detectable	~4.7	not detectable
H-4	6.17	6.95	6.56	7.43
H-5	7.23	7.80	7.28	7.98
$J_{4,5}$	2.00	2.00	5.30	5.30

Table 2. ^1H NMR chemical shifts (δ) and coupling constants (Hz) of 3-amino-2-formylfuran (XIII), -thiophene (XIV), and -selenophene (XV) in deuteriochloroform at 60 MHz.







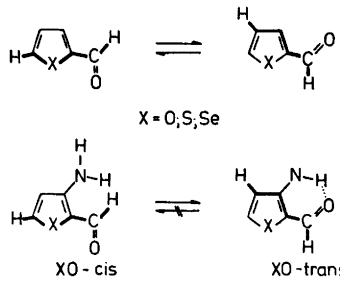
δ	XIII	XIV	XV
NH_2	~5.4	~6.4	~6.5
CHO	9.58	9.55	9.57
H-4	6.11	6.94	6.89
H-5	7.29	7.20	8.17
$J_{4,5}$	1.80	5.25	5.7
$J_{5,CHO}$	—	—	—
$J_{4,CHO}$	0.75	0.7	0.65

donating amino group is replaced by the strongly electron-attracting ammonium group. This seems to indicate that 3-amino-2-formylfuran (XVI) and 3-amino-2-formylthiophene (XVII) in their protonated forms exist largely as ammonium salts.

Several studies of the conformation of 2-formyl derivatives of furan, thiophene and selenophene have been made.[30–34] At room temperature 2-formylfuran exists in 70 % as O,O-*cis* and 30 % as O,O-*trans*,[31] 2-formylthiophene completely as S,O-*cis*.[32] and 2-formylselenophene exists most probably also completely as the Se,O-*cis* form [34] (*cf.* Scheme 3). However, Russian workers [33] have claimed 2-formylselenophene to be a 1:1 mixture of *cis* and *trans* conformers. The increased stability of the *cis* isomer in 2-formylthiophene and 2-formylselenophene has been ascribed to bonding interaction through space between the *d*-orbital of the heteroatom and the electron pair of the carbonyl group.[35,36] Indication for such bonding has recently been obtained by ^{77}Se NMR for 2-formylselenophene.[36]

Substituents in the rings can alter the proportions between *cis* and *trans* conformers in different ways. The NMR spectra of such compounds, especially those unsubstituted in the 4- and 5-positions of the ring, can give an estimate of the amount of each conformer present. The long-range couplings between the aldehyde proton and the ring protons are here extremely useful. In the X,O-*cis* conformation, the aldehyde proton couples with the 5-proton, and in the X,O-*trans* conformation with the 4-proton according to the "zig-zag" convention (see Scheme 3). All three 3-amino-2-formyl derivatives described in this paper show exclusively a long-range coupling to the 4-proton (see Table 2), that is to say that they all exist largely in an X,O-*trans* conformation in deuteriochloroform. The chemical shift assignments are based on the known substituent-caused shifts in 3-amino and 2-formyl derivatives and their approximate additivity, and on the known differences between the α- and β-proton resonances in these heterocyclic systems.

The reason for the dominance of the *trans* conformation is that the aldehyde group is locked in this position by strong intramolecular hydrogen bonding (see Scheme 3). Only two examples of such an intramolecular association in these heterocyclic series, leading exclusively to the X,O-*trans* conformation have been previously reported in the literature, namely the case of 2-formyl-3-hydroxythiophene and 2-formyl-2-hydroxyselenophene.[37,38] However, it has been shown that in the former case the intramolecular hydrogen bond could be broken by using acetone as solvent.[31] No such change in the NMR spectra of 3-amino-2-formylthiophene (XIV) could, however, be observed on going from deuteriochloroform to deuterioacetone as solvent.



Scheme 3.

EXPERIMENTAL PART

3-Azido-2-formylfuran (I). 5.0 g (0.029 mol) of 3-bromo-2-formylfuran [2] and 5.0 g (0.077 mol) of sodium azide were dissolved in 100 ml of dimethyl sulfoxide. The reaction mixture was slowly stirred for 48 h at 65 °C, whereupon it was cooled and poured into 150 ml of water. The water solution was extracted with four 30 ml portions of ether. The combined ether phases were dried over magnesium sulfate and evaporated to dryness. The crude product was recrystallized from methanol which gave 1.8 g (45 %) of product, which melted with slight decomposition at about 115 °C. IR spectrum (KBr): N_3 = 2140 cm^{-1}, CO = 1650 cm^{-1}, NMR spectrum ($CDCl_3$): δ 6.53 (H-4), 7.60 (H-5), 9.67 (CHO), $J_{4,5}$ 2.00 Hz, $J_{5,CHO}$ 0.60 Hz. [Found: C 43.9; H 2.21; N 30.6; m.wt. 137. Calc. for $C_5H_3N_3O_2$: C 43.8; H 2.21; N 30.7; m.wt. 137.11.]

3-Azido-2-formylthiophene (II) was prepared as described above for I from 45.0 g (0.236 mol) of 3-bromo-2-formylthiophene [3] and 45.0 g (0.692 mol) of sodium azide in 600 ml of dimethyl sulfoxide. The reaction temperature was 65 °C and the reaction time 24 h. The yellow crude product was recrystallized from methanol, giving 17.5 g (48 %) of 3-azido-2-formylthiophene (II), m.p. 56.6–57.2 °C. IR spectrum (KBr): N_3 = 2120 cm^{-1}, CO = 1660 cm^{-1}, NMR spectrum ($CDCl_3$): δ 7.02 (H-4), 7.73 (H-5), 9.88 (CHO), $J_{4,5}$ 5.15 Hz, $J_{5,CHO}$ 1.20 Hz. [Found: C 39.4; H 1.90; N 27.2; S 20.8; m.wt. 153. Calc. for $C_5H_3N_3OS$: C 39.2; H 1.98; N 27.4; S 20.9; m.wt. 153.17.]

3-Azido-2-formylselenophene (III) was prepared as described above for I from 33.0 g (0.138 mol) of 3-bromo-2-formylselenophene[4] and 26.0 g (0.406 mol) of sodium azide in 600 ml of dimethyl sulfoxide. The reaction temperature was 60 °C and the reaction time 24 h. The brown-red crude product was recrystallized from methanol, giving 16.7 g (60 %) of the product, m.p. 69.0–70.5 °C. IR spectrum (KBr): N_3= 2110 cm^{-1}, CO=1640 cm^{-1}, NMR spectrum ($CDCl_3$): δ 7.35 (H-4), 8.42 (H-5), 9.84 (CHO), $J_{4,5}$ 5.75 Hz, $J_{5,CHO}$ 1.00 Hz. [Found: C 29.9; H 1.75; Se 39.2; m.wt. 201. Calc. for $C_5H_3N_3OSe$: C 30.0; H 1.51; Se 39.5; m.wt. 200.07.]

3-Azido-2-furan carboxylic acid (IV). 1.9 g (0.014 mol) of 3-azido-2-formylfuran (I) was added with stirring to a suspension prepared from 20 ml of water, 4.8 g (0.028 mol) of silver nitrate and 2.3 g (0.058 mol) of sodium hydroxide cooled to 10 °C. The reaction mixture was allowed to attain room temperature and the stirring continued for another 30 min. The solid material was filtered off and washed with 6 ml of water. The combined aqueous phases were acidified with conc. hydrochloric acid, whereupon a white voluminous precipitate was formed, which was filtered off. The crude product was dissolved in warm ether and the resulting solution filtered. The ether solution was dried over magnesium sulphate and concentrated, giving 1.7 g (79 %) of the acid. An analytical sample which was obtained by recrystallization from toluene showed an IR spectrum identical with that of the crude product, m.p. 110 °C (decomp.). IR spectrum (KBr): N_3=2120 cm^{-1}, CO=1670 cm^{-1}. NMR spectrum (DMSO-d_6): δ 6.73 (H-4), 7.87 (H-5); $J_{4,5}$=2.14 Hz. [Found: C 39.2; H 2.05; O 31.0; m.wt. 153. Calc. for $C_5H_3N_3O_3$: C 39.2; H 1.98; O 31.4; m.wt. 153.11.]

3-Azido-2-thiophene carboxylic acid (V) was prepared as described above for IV from 5.0 g (0.033 mol) of 3-azido-2-formylthiophene (II), 44 ml of water 11.5 g (0.0676 mol) of silver nitrate, and 5.4 g (0.14 mol) of sodium hydroxide, yielding 5.0 g (90 %) of the product, m.p. 110 °C (decomp.). An analytical sample which was obtained by recrystallization from toluene showed an IR spectrum identical with that of the crude product. IR spectrum (KBr): N_3= 2110 cm^{-1}, CO=1680 cm^{-1}; NMR spectrum (DMSO-d_6): δ 7.08 (H-4), 7.87 (H-5), and $J_{4,5}$=5.25 Hz. [Found: C 35.9; H 1.80; S 19.0; m.wt. 169. Calc. for $C_5H_3N_3O_2S$: C 35.5; H 1.79; S 19.0; m.wt. 169.17.]

3-Azido-2-selenophene carboxylic acid (VI) was prepared as described above for IV form 1.7 g (0.0085 mol) of 3-azido-2-formylselenophene (III), 11 ml of water, 2.9 g (0.017 mol) of silver nitrate, and 1.4 g (0.035 mol) of sodium hydroxide, giving after recrystallization from toluene 1.2 g (65 %) product with m.p. 110 °C (decomp.). IR spectrum (KBr): N_3=2090 cm^{-1}, CO=1660 cm^{-1}. NMR spectrum (DMSO-d_6): δ 7.34 (H-4), 8.44 (H-5); $J_{4,5}$ 5.65 Hz. [Found: C 27.9; H 1.55; Se 36.6; m.wt. 217. Calc. for $C_5H_3N_3O_2Se$: C 27.8; H 1.40; Se 36.5; m.wt. 216.07.]

3-Azido-2-formylfuran aldoxime (VII). 10.0 g (0.142 mol) of hydroxylamine hydrochloride was dissolved in 60 ml of water, whereupon 40 ml of 10 % sodium hydroxide solution was added. To this solution, 4.0 g (0.029 mol) of 3-azido-2-formylfuran (I) in 10 ml of ethanol was added. The reaction mixture was heated to 50 °C with stirring, whereupon it was allowed to attain room temperature and kept at this temperature for another 45 minutes. After cooling to 0 °C the precipitate was filtered off, giving 4.0 g (91 %) of the product. An analytical sample which was obtained by recrystallization from water/ethanol gave an IR spectrum identical with that of the crude product, m.p. 110 °C (decomp.). IR spectrum (KBr): N_3=2110 cm^{-1}. NMR spectrum (DMSO-d_6): δ 6.72 (H-4), 7.69 (H-5), 7.84 (CH), 11.39 (OH); $J_{4,5}$ 2.00 Hz. [Found: C 39.4; H 2.83; O 21.0; m.wt. 152. Calc. for $C_5H_4N_4O_2$: C 39.5; H 2.66; O 21.0; m.wt. 152.13.]

3-Azido-2-formylthiophene aldoxime (VIII) was prepared as described above for VII from 15.0 g (0.0980 mol) of 3-azido-2-formylthiophene (II) in 25 ml of ethanol, 37.0 g (0.529 mol) of hydroxylamine hydrochloride in 225 ml of water and 150 ml of 10 % sodium hydroxide solution, yielding 15.1 g (92 %) of the product. An analytical sample which was obtained by recrystallization from water/ethanol showed an IR spectrum identical with that of the crude product, m.p. 110 °C (decomp.). IR spectrum (KBr): N_3=2120 cm^{-1}. NMR spectrum (DMSO-d_6): δ 7.20 (H-4), 7.80 (H-5), 7.60 (CH), 11.2 (OH); $J_{4,5}$ 5.40 Hz. [Found: C 35.8; H 2.53; S 19.2; m.wt. 168. Calc. for $C_5H_4N_4OS$: C 35.7; H 2.40; S 19.1; m.wt. 168.19.]

3-Azido-2-formylselenophene aldoxime (IX) was prepared as described above for VII from 2.0 g (0.010 mol) of 3-azido-2-formylselenophene (III) in 30 ml of ethanol, 5.0 g (0.071 mol) of hydroxylamine hydrochloride in 30 ml of water and 20 ml of 10 % sodium hydroxide solution, yielding 1.9 g (88 %) of the product. An analytical sample which was obtained by recrystallization from water/ethanol showed an IR spectrum identical with that of the crude product, m.p. 110 °C (decomp.). IR spectrum (KBr): N_3=2110 cm^{-1}. NMR spectrum (DMSO-d_6): δ 7.44 (H-4), 8.45 (H-5), 7.75 (CH), 12.00 (OH); $J_{4,5}$ 6.05 Hz, and $J_{5,CH}$ 0.85 Hz. [Found: C 27.9; H 1.89; Se 36.6; m.wt. 216. Calc. for $C_5H_4N_4OSe$: C 27.9; H 1.88; Se 36.7; m.wt. 215.09.]

3-Azido-2-cyanofuran (X). 3.5 g (0.023 mol) of 3-azido-2-formylfuran aldoxime (VII) was dissolved in 55 ml of methylene chloride and 2.0 g of pyridine. The reaction mixture was treated with nitrogen gas for 10 min, whereupon 31.2 g (0.0168 mol) of 2,4,6-trichloro-s-triazine was added in one batch with stirring and continued nitrogen inlet. The stirring and nitrogen inlet

were then continued for 90 minutes and the temperature kept at 30 °C with a water bath. The reaction mixture was filtered and the solid phase was washed twice with 10 ml portions of methylene chloride. The crude product obtained by concentration of the filtrate was recrystallized from methanol/water giving 2.7 g (87 %), m.p. 61.5—62.5 °C. IR spectrum (KBr): CN= 2200 cm^{-1} and N_3=2110 cm^{-1}. NMR spectrum ($CDCl_3$): δ 6.50 (H-4), 7.51 (H-5); $J_{4,5}$ 2.10 Hz. [Found: C 44.6; H 1.61; O 12.2; m.wt. 134. Calc. for $C_5H_2N_4O$: O 44.8; H 1.51; O 11.9; m.wt. 134.11.]

3-Azido-2-cyanothiophene (XI) was prepared as described above for X from 13.0 g (0.0774 mol) of 3-azido-2-formylthiophene aldoxime (VIII), 175 ml of methylene chloride, 6.1 g of pyridine and 10.6 g (0.057 mol) of 2,4,6-trichloro-s-triazine. Recrystallization of the crude product from methanol gave 10.0 g (86 %) of the product, m.p. 77.5—79.0 °C. IR spectrum (KBr): CN=2215 cm^{-1} and N_3=2130 cm^{-1}. NMR spectrum ($CDCl_3$): δ 6.96 (H-4), 7.61 (H-5); $J_{4,5}$ 5.20 Hz. [Found: C 39.9; H 1.56; N 36.6; m.wt. 150. Calc. for $C_5H_2N_4S$: C 40.0; H 1.35; N 37.3; m.wt. 150.17.]

3-Azido-2-cyanoselenophene (XII) was prepared as described above for X from 3.3 g (0.015 mol) of 3-azido-2-formylselenophene aldoxime (IX), 35 ml of methylene chloride, 1.2 g pyridine and 2.1 g (0.011 mol) of 2,4,6-trichloro-s-triazine. Recrystallization from methanol gave 2.4 g (80 %) of the product, m.p. 108.0—109.0 °C. IR spectrum (KBr): CN=2190 cm^{-1} and N_3=2100 cm^{-1}. NMR spectrum ($CDCl_3$): δ 7.19 (H-4), 8.27 (H-5); $J_{4,5}$ 5.90 Hz. [Found: C 30.5; H 1.20; Se 40.3; m.wt. 198. Calc. for $C_5H_2N_4Se$: C 30.5; H 1.03; Se 40.1; m.wt. 197.07.]

3-Amino-2-formylfuran (XIII). 1.8 g (0.013 mol) of 3-azido-2-formylfuran (I) was dissolved in 20 ml of methanol and cooled to 10 °C, whereupon 2 drops of piperidine was added. With continued cooling, hydrogen sulfide was bubbled through the reaction mixture, whereupon an exothermic reaction took place with evolution of nitrogen. The rate of the hydrogen sulfide inlet was adjusted so that the reaction temperature was kept below 20 °C. After about 20 min, the evolution of nitrogen had ceased, the solution was cooled to 0 °C and the precipitated sulfur filtered off. The filtrate was poured into 100 ml of water and the water solution was extracted five times with chloroform. The combined chloroform solutions were dried over magnesium sulfate and concentrated; giving 1.2 g (83 %) of an oil which partly crystallized after standing in a freezer for 2 days. An analytical sample which was obtained by recrystallization from ether showed an IR spectrum identical to that of the crude oil, m.p. 45—50 °C. IR spectrum (KBr): NH=3450, 3340, 3210 cm^{-1}, CO=1630 cm^{-1}. NMR spectrum ($CDCl_3$): see Table 2. [Found: C 54.3; H 4.46; O 28.3; m.wt. 111. Calc. for $C_5H_5NO_2$: C 54.1; H 4.55; O 28.8; m.wt. 111.11.]

3-Amino-2-formylthiophene XIV was prepared as described above for XIII from 6.2 g (0.041 mol) of 3-azido-2-formylthiophene (II), 50 ml of ethanol, 2 drops of piperidine and hydrogen sulfide, yielding after precipitation with ice 4.4 g (85 %) of the product. An analytical sample showing an IR spectrum identical with that of the crude product was obtained by recrystallization from ethanol/water, m.p. 68.0—70.0 °C. IR spectrum (KBr): NH=3430, 3320, 3180 cm^{-1}, CO=1610 cm^{-1}. NMR spectrum ($CDCl_3$): see Table 2. [Found: C 47.3; H 4.10; S 25.5; m.wt. 127. Calc. for C_5H_5NOS: C 47.2; H 3.97; S 25.2; m.wt. 127.17.]

3-Amino-2-formylselenophene (XV) was prepared as described above for XIII from 0.92 g (0.0046 mol) of 3-azido-2-formylselenophene (III), 20 ml of ethanol, 1 drop of piperidine and hydrogen sulfide, yielding 0.71 g (89 %) of crude product. An analytical sample showing an IR spectrum identical with that of the crude product was obtained by recrystallization from toluene, m.p. 82.0—84.0 °C. IR spectrum (KBr): NH=3440, 3340—3160 cm^{-1}, CO=1620 cm^{-1}. NMR spectrum ($CDCl_3$): see Table 2. [Found: C 34.7; H 2.92; Se 45.1; m.wt. 175. Calc. for C_5H_5NOSe: C 34.5; H 2.90; Se 45.4; m.wt. 174.07.]

3-Amino-2-cyanofuran (XVI). 1.5 g (0.011 mol) of 3-azido-2-cyanofuran (X) was dissolved in 40 ml of ethanol, 6 drops of piperidine were added and the reaction mixture cooled to 10 °C. With continued cooling, hydrogen sulfide was bubbled through the reaction mixture, whereupon an exothermic reaction took place with evolution of nitrogen. The rate of the hydrogen sulfide inlet was adjusted so that the reaction temperature was kept below 30 °C. After about 30 min the evolution of nitrogen had ceased, whereupon the reaction mixture was cooled to 0 °C and the precipitated sulfur filtered off. The filtrate was poured into 150 ml of water and the water solution was extracted four times with chloroform. The combined chloroform solutions were dried over magnesium sulfate and concentrated, giving 1.1 g (93 %) of the product. An analytical sample showing an IR spectrum identical with that of the crude product was obtained by recrystallization from toluene, m.p. 32.5—36.0 °C. IR spectrum (KBr): NH=3440, 3340 and 3210 cm^{-1}, CN=2200 cm^{-1}. NMR spectrum ($CDCl_3$ and CF_3COOH): see Table 1. [Found: C 54.4; H 3.74; O 15.0; m.wt. 108. Calc. for $C_5H_4N_2O$: C 55.6; H 3.74; O 14.8; m.wt. 108.11.]

3-Amino-2-cyanothiophene (XVII) was prepared as described above for XVI from 0.95 g (0.0063 mol) of 3-azido-2-cyanothiophene (XI), 25 ml of ethanol, 2 drops of piperidine, and hydrogen sulfide, yielding 0.75 g (95 %) of the product. An analytical sample showing an IR spectrum identical with that of the crude product was obtained by recrystallization from toluene, m.p. 47.5—50.0 °C. IR spectrum (KBr): NH=3440, 3340 and 3220 cm^{-1}, CN=2190

cm^{-1}. NMR spectrum ($CDCl_3$ and CF_3COOH): see Table 1. [Found: C 48.0; H 3.47; S 25.8; m.wt. 124. Calc. for $C_5H_4N_2S$: C 48.4; H 3.25; S 25.8; m.wt. 124.17.]

3-Amino-2-cyanoselenophene (XVIII) was prepared as described above for XVI from 1.1 g (0.0056 mol) of 3-azido-2-cyanoselenophene (XII), 2.5 ml ethanol, 2 drops of piperidine, and hydrogen sulfide, yielding 0.90 g (93 %) of the product. An analytical sample showing an IR spectrum identical with that of the crude product was obtained by recrystallization from toluene, m.p. 72.5—74.0 °C. IR spectrum (KBr): NH = 3420, 3310—3120 cm^{-1}, CN = 2170 cm^{-1}. NMR spectrum ($CDCl_3$): δ 6.89 (H-4), 7.98 (H-5), 4.7 (NH_2); $J_{4,5}$ 6.00 Hz. [Found: C 35.0; H 2.44; Se 46.3; m.wt. 172. Calc. for $C_5H_4N_2Se$: C 35.1; H 2.36; Se 46.2; m.wt. 171.07.]

3-Amino-2-thiophene carboxylic acid (XIX). 2.7 g (0.016 mol) of 3-azido-2-thiophene carboxylic acid (V) was dispersed in 30 ml of ethanol, and 1.7 g (0.017 mol) of triethylamine was added. The reaction mixture was cooled to 10 °C with an ice-bath and hydrogen sulfide was bubbled through the solution. An exothermic reaction took place with evolution of nitrogen gas. The rate of the hydrogen sulfide inlet was adjusted so that the reaction temperature was kept below 30 °C. After about 45 min the evolution of nitrogen had ceased, whereupon the reaction mixture was poured into 150 ml of water. Precipitated sulfur was filtered off and the filtrate was acidified with dilute hydrochloric acid (pH 2—3). The acid was filtered off and dried, yielding 1.4 g (61 %). An analytical sample showing an IR spectrum identical with that of the crude product was obtained by recrystallization from toluene, m.p. 82 °C (decomp.). (Lit. value 83 °C.[23]) IR spectrum (KBr): NH = 3465 and 3360 cm^{-1}, CO = 1640 cm^{-1}. NMR spectrum (DMSO-d_6): δ 6.62 (H-4), 7.48 (H-5), 8.0 (NH_2); $J_{4,5}$ 5.40 Hz.

The ^1H NMR spectra were obtained with a Varian A-60 high resolution spectrometer. The IR spectra were recorded on a Perkin-Elmer model 257 instrument. Mass spectra were obtained with an LKB 9000 mass spectrometer. Elemental analyses were carried out at the Analytical Department of the Chemical Institute and by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mühlheim/Ruhr.

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The Synthetic Utility of Heteroaromatic Azido Compounds. II. Preparation of Some Furo-, Thieno-, and Selenolo[3,2-*b*]pyridines

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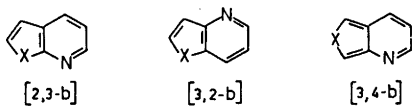
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A very convenient method for the synthesis of the parent furo-, thieno-, and selenolo[3,2-*b*]-pyridine, and of their 5- and 5,6-substituted derivatives has been found applying the Friedländer reaction to 3-amino-2-formylfuran, -thiophene, and -selenophene. The NMR spectra of these systems have been analyzed, and excellent inverse linear correlations between the electronegativity of the heteroatom and the 7-proton shifts in these systems were found.

The current interest in furo-, thieno-, and selenolopyridines depends largely on two facts. The first is that in these systems a π-electron excessive nucleus (furan, thiophene, selenophene) and a π-electron deficient nucleus (pyridine) are fused together. This ultimately raises the question of how the annelation will perturb the electronic structure of each individual nucleus and how this will be manifested for example in the substitution pattern obtained in electrophilic or nucleophilic aromatic substitution on these substrates. Furthermore, these systems are isosteric with quinolines and isoquinolines, which gives them potential pharmacological interest.

A detailed study of some of the thiophene analogues of isoquinoline (thieno[2,3-*c*]pyridine and thieno[3,2-*c*]pyridine) has recently been carried out.[1-3] There are three different isomers (A–C) for each unsubstituted heterocyclic analogue of quinoline (see Scheme 1.)

In the furan series, derivatives of all these forms are known from the literature. However,



Scheme 1.

only one of the parent compounds, namely A (see Scheme 1) is known. In the thiophene series, on the other hand, all three parent compounds and derivatives of them are known (for review *cf.* Ref. 4). In the selenophene series two of the parent compounds (A and B) and some derivatives of them are known. A short summary of the most important methods described in the literature for obtaining the quinoline analogues B, is given below.

The furo[3,2-*b*]pyridine system has exclusively been prepared from suitably substituted pyridines, and mainly from reaction of 2-iodo-3-hydroxypyridines with various copper acetylides.[5-7] Recently, another reaction route starting from *ortho*-substituted pyridines was described.[8] None of these methods lead to the parent compound.

For the preparation of the thieno[3,2-*b*]pyridine system there are two important synthetic methods, one of which utilizes 3-vinylpyridines as starting materials and involves treatment with hydrogen sulfide at high temperatures.[9,10] The parent compound has been prepared in this way, but in low yield (27 %).[10] The other method starts from the hexachlorostannates or

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hydrochlorides of 3-aminothiophenes which are reacted with various 1,3-dicarbonyl compounds.[11–17] This method has suffered from the unavailability of the 3-amino compounds, but recently an alternative method for their preparation *via* 3-acetylthiophene has made this route more efficient.[17]

There is only one method described for preparing the selenolo[3,2-*b*]pyridine system.[17] This method is completely analogous to the second one described above for the synthesis of the thiophene analogue and it suffers from the same limitation. The modification described above which gave good yields of the thieno[3,2-*b*] parent compound does not seem to give the corresponding selenolo[3,2-*b*] parent compound under the same conditions.[17]

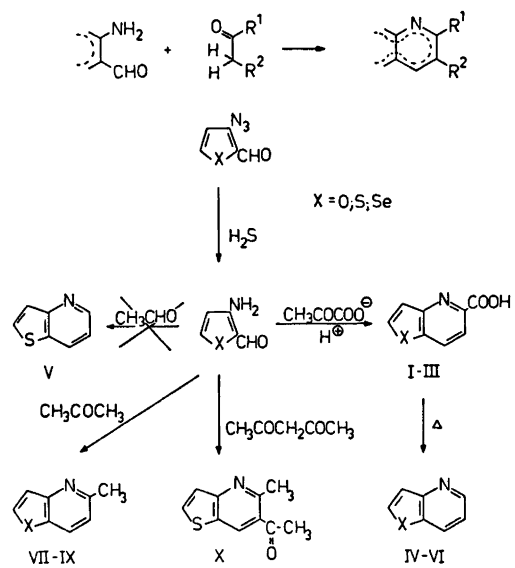
SYNTHESIS

This paper describes a new general way to prepare all three [3,2-*b*] systems utilizing the Friedländer reaction.[18] The reason that the Friedländer scheme, with one recent exception,[19] has not been used in the synthesis of furo-, thieno-, and selenolopyridines is probably the lack of suitable starting materials. The preparation of such suitable starting materials, namely 3-amino-2-formylfuran, -thiophene, and -selenophene, were described in the previous paper in this series.[20]

The Friedländer method utilizes the reaction in alkaline or acidic media between an aromatic *ortho*-aminocarbonyl compound and a carbonyl compound containing an active methylene group α to the carbonyl group (see top of Scheme 2). The amino compounds used in this study are 3-amino-2-formylfuran, 3-amino-2-formylthiophene and 3-amino-2-formylselenophene, which were obtained by reducing the corresponding 3-azido derivatives with hydrogen sulphide in alkaline medium.[20] However, it proved unnecessary to isolate these *ortho*-aminoformyl compounds before treating them with the appropriate carbonyl compound. Thus the reaction solutions obtained from the reductions of the azido compounds could be used directly after removal of precipitated sulfur and adjustment of the volumes. The carbonyl compounds used in this study are acetone, pyruvic acid and to a limited extent acetylacetone and acetaldehyde.

Initial trials to react acetaldehyde with 3-amino-2-formylthiophene in alkaline ethanol were unsuccessful. No thieno[3,2-b]pyridine could be isolated under these conditions (see Scheme 2). Since it is known [18] that aldehydes in certain cases can give little or no product, other synthetic paths leading to the parent compounds were sought. Quinoline-2-carboxylic acid is known to decarboxylate easily on heating and give high yields of quinoline.[21] In order to obtain the corresponding carboxylic acids in these series, the *ortho*-amino aldehydes were treated with freshly distilled pyruvic acid in alkaline ethanol/water (see Scheme 2). This gave 5-carboxyfuro[3,2]pyridine (I), 5-carboxythieno[3,2-b]pyridine (II) and 5-carboxyselenolo[3,2-b]pyridine (III) in moderate yields (43–48 %). The yield did not improve even if a large excess of pyruvic acid was used. These 5-carboxy compounds could be smoothly decarboxylated simply by heating above their melting points (see Scheme 2). Thus the parent compounds, furo[3,2-*b*]pyridine (IV), thieno[3,2-*b*]pyridine (V), and selenolo[3,2-*b*]pyridine (VI) were obtained in good to excellent yields (78–99 %).

When the *ortho*-amino aldehydes were treated with excess of acetone in alkaline ethanol/water, 5-methylfuro[3,2-*b*]pyridine (VII), 5-methylthieno[3,2-*b*]pyridine (VIII), and 5-methylselenolo[3,2-*b*]pyridine (IX) were formed in good yields (71–87 %) (see Scheme 2.)

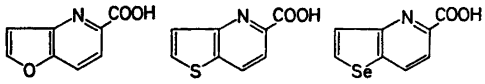


Scheme 2.

3-Amino-2-formylthiophene was also treated with acetylacetone in alkaline ethanol. This gave 6-acetyl-5-methylthieno[3,2-*b*]pyridine (X) in good yield (67 %) (see Scheme 2.)

The results of the reactions presented above and the availability of a great number of other carbonyl compounds capable of reacting in a similar manner show clearly that the Friedländer scheme is a powerful complement to existing methods for annelating pyridine rings to the furan, thiophene or selenophene nucleus.

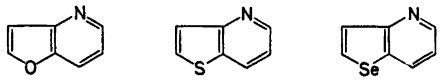
Table 1. ^{1}H NMR chemical shifts (δ) and coupling constants (Hz) in 5-carboxy substituted furo-, thieno-, and selenolo[3,2-*b*]pyridines in hexadeuterio dimethyl sulfoxide solution at 60 MHz.



I II III

	I	II	III
δ (H-2)	8.51	8.33	8.78
δ (H-3)	7.33	7.76	7.96
δ (H-6)	8.21	8.11	8.02
δ (H-7)	8.21	8.70	8.80
$J_{2,3}$	2.30	5.50	5.95
$J_{3,7}$	0.6	0.6	0.6
$J_{6,7}$	—	8.35	8.20

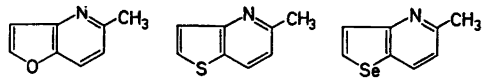
Table 2. ^{1}H NMR chemical shifts (δ) and coupling constants (Hz) in furo-, thieno-, and selenolo[3,2-*b*]pyridines in carbon tetrachloride solution at 60 MHz.



IV V VI

	IV	V	VI
δ (H-2)	7.99	7.60	8.14
δ (H-3)	6.95	7.49	7.74
δ (H-5)	8.46	8.59	8.57
δ (H-6)	7.17	7.04	7.04
δ (H-7)	6.64	8.00	8.09
$J_{2,3}$	2.20	5.60	5.95
$J_{5,6}$	4.70	4.65	4.60
$J_{5,7}$	1.30	1.60	1.50
$J_{6,7}$	8.25	8.75	8.10
$J_{2,6}$	≤ 0.2	0.40	0.30
$J_{3,7}$	1.00	0.75	0.70

Table 3. ^{1}H NMR chemical shifts (δ) and coupling constnats (Hz) in 5-methyl substituted furo-, thieno-, and selenolo[3,2-*b*]pyridines in carbon tetrachloride solution at 60 MHz.



VII VIII IX

	VII	VIII	IX
δ (H-2)	7.73	7.57	8.09
δ (H-3)	6.84	7.41	7.67
δ (H-6)	6.91	6.92	6.88
δ (H-7)	7.50	7.87	7.93
δ (CH_3)	2.57	2.58	2.58
$\alpha_{2,3}$	2.25	5.60	5.90
$J_{6,7}$	8.45	8.40	8.20
$J_{3,7}$	0.95	0.70	0.70
$_{2,6}J$	0.40	0.35	≤ 0.2

^{1}H NMR SPECTRA

The NMR data for compounds I to IX are given in Tables 1, 2, and 3. The NMR data for compound X are given in the experimental part. The assignment of different bands to different protons in the new compounds has been based on knowledge of shifts and coupling constants in furans, thiophenes, selenophenes, and quinolines, and on comparison with literature data for previously prepared compounds in these series. Thus, the NMR spectra of thieno[3,2-*b*]pyridine (V), selenolo[3,2-*b*]pyridine (VI), and their 5-methyl derivatives (VIII, IX) have been reported earlier.[16,17]

The analyses of the spectra were made on the assumption that it was possible to treat the molecules as consisting of two isolated systems of protons, one consisting of the protons in the five-membered ring and the other of the protons in the pyridine nucleus. This assumption is not strictly valid since there are long-range couplings between the rings. However, since few

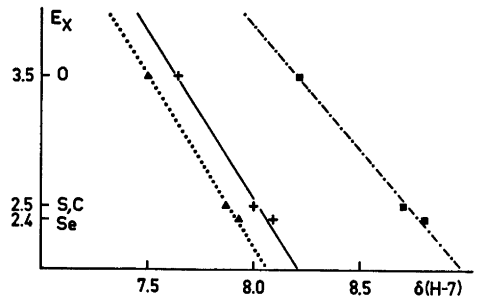
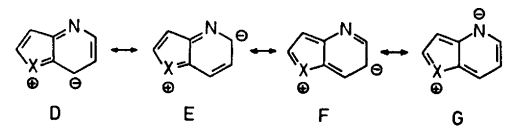


Fig. 1. Correlations between the electronegativity of the heteroatom and the 7-proton chemical shifts for furo-, thieno- and selenolo[3,2-*b*]pyridine (+ ———, $r=0.99$) and their 5-methyl (▲ · · ·, $r\approx1.0$) and 5-carboxy (■ — · —, $r\approx1.0$) derivatives. The corresponding values for quinoline and 2-methylquinoline are included.

of the AB systems involved showed signs of strong coupling, it seemed reasonable that this assumption should lead only to minor errors. In order to determine if this was the case, the NMR spectra of the three parent compounds (IV, V, VI) were also analyzed as 5-spin systems in an iterative mode utilizing an extended version of the QCPE program UEAITR (No. 188).[22] The numerical differences for the two types of analyses were in the same range as the experimental error. The NMR data given in Table 2 are those obtained from the iterative analyses.

It should be pointed out that the 7-proton absorptions for the fused pyridine derivatives are systematically shifted to lower field on going from the furan-fused to the selenophene-fused systems. This order O > S > Se is the same as that of the electron-donating mesomeric effect of the heteroatom. Excellent linear correlations with the electronegativity of the heteroatoms (O = 3.5, S = 2.5, and Se = 2.4) were obtained both for the parent compounds and for the 5-methyl and 5-carboxy derivatives (Fig. 1). For comparison the shifts for quinoline [23] and 2-methylquinoline [24] were also included (C = 2.5), and were found to fit the correlation well. The effects of the heteroatom on the 5- and 6-proton shifts are very small and for the 6-proton shifts perhaps even of the opposite direction.

It appears that electron donation from the five-membered ring to the six-membered ring, as expressed in resonance form D, is important, although it is evident from resonance forms E



to G that the electron-donation from the five-membered ring can reach all positions in the pyridine ring, especially the nitrogen.

Similar observations were made a few years ago [25] in a study of the ^{1}H NMR parameters of 2-methylbenzoxazole, -benzothiazole, and -benzoselenazole. Linear correlations between the electronegativity of the heteroatoms and several ^{1}H and ^{13}C NMR parameters have also been observed in monocyclic furans, thiophenes, selenophenes, and tellurophenes.[26]

It should be pointed out that in the discussion above no consideration of the effect of the magnetic anisotropy of the heteroatom has been taken into account.

The long-range couplings found in this work are consistent with the so-called "zig-zag" path hypothesis, and similar cases have been previously noted in these types of systems.[3,17,27]

EXPERIMENTAL

*Furo[3,2-*b*]pyridine-5-carboxylic acid (I).* 5.7 g (0.042 mol) of 3-azido-2-formylfuran [20] was dissolved in 50 ml of ethanol containing three drops of piperidine. The solution was cooled to 10 °C and hydrogen sulfide was bubbled through the solution with continued cooling. An exothermic reaction with evolution of nitrogen gas took place. The rate of addition of hydrogen sulfide was such that the temperature was kept below 25 °C. After about 45 min, the evolution of nitrogen had ceased and the reaction mixture was cooled to 0 °C, whereupon precipitated sulfur was filtered off. The filtrate was concentrated to about 10 ml, by evaporation, and treated with 33 ml of 15 % sodium hydroxide solution containing 7.4 g (0.084 mol) of freshly distilled pyruvic acid. The reaction mixture was carefully heated to 60 °C and stirred at this temperature for 3 h, after which it was allowed to attain room temperature and placed in a refrigerator over night. The resulting sodium salt of the acid was filtered off and dissolved in the smallest possible amount of hot water, followed by acidification with dilute hydrochloric acid (pH = 4). After slow cooling to 5 °C the precipitated acid was filtered off. The crude product was dried in a desiccator over

conc. sulfuric acid for 24 h, giving 3.0 g (43 %) of the acid. An analytical sample obtained by recrystallization from toluene showed an IR spectrum identical with that of the crude product, m.p. 174.0 – 176.0 °C. IR spectrum (KBr): CO = 1700 cm^{-1}. NMR spectrum (DMSO-d_6): see Table 1. (Found: C 58.7; H 3.19; O 29.2; m.wt. 163. Calc. for $C_8H_5NO_3$: C 58.9; H 3.10; O 29.4; m.wt. 163.14).

Thieno[3,2-b]pyridine-5-carboxylic acid (II) was prepared as described for I from 3.0 g (0.020 mol) of 3-azido-2-formylthiophene,[20] 20 ml of ethanol, two drops of piperidine, hydrogen sulfide, 3.5 g (0.040 mol) of pyruvic acid, and 18 ml of 15 % sodium hydroxide solution, yielding 1.7 g (48 %) of the product. An analytical sample obtained by recrystallization from toluene showed an IR spectrum identical with that of the crude product; m.p. 201.5 – 202.0 °C. IR spectrum (KBr): CO = 1700 cm^{-1}. NMR spectrum (DMSO-d_6): see Table 1. (Found: C 53.6; H 2.89; S 17.9; m.wt. 179. Calc. for $C_8H_5NO_2S$: C 53.6; H 2.82; S 17.9; m.wt. 179.20).

Selenolo[3,2-b]pyridine-5-carboxylic acid (III) was prepared as described above for I from 5.4 g (0.027 mol) of 3-azido-2-formylselenophene,[20] 40 ml of ethanol, two drops of piperidine, hydrogen sulfide, 4.8 g (0.055 mol) of pyruvic acid, and 60 ml of 15 % sodium hydroxide solution, yielding after recrystallization from toluene 2.6 g (42 %) of the product; m.p. 167.0 – 169.0 °C. IR spectrum (KBr): CO = 1670 cm^{-1}. NMR spectrum (DMSO-d_6): see Table 1. (Found: C 42.7; H 2.37; Se 34.9; m.wt. 227. Calc. for $C_8H_5NO_2Se$: C 42.5; H 2.23; Se 34.9; m.wt. 226.10).

Furo[3,2-b]pyridine (IV). 1.6 g (0.0098 mol) of furo[3,2-*b*]pyridine-5-carboxylic acid (I) was transferred to a Claisen flask, which was then heated with a gas burner. The substance melted with sublimation. The heating was continued until evolution of carbon dioxide had ceased. The resulting liquid was allowed to cool, whereupon it was distilled at reduced pressure. 0.9 g (78 %) of a light yellow oil with a smell of quinoline was obtained; b.p. 79 – 80 °C/12 mmHg, $n_D^{19} = 1.5753$. NMR spectrum (CCl_4): see Table 2. (Found: C 70.4; H 4.41; O 13.6; m.wt. 119. Calc. for C_7H_5NO: C 70.6; H 4.24; O 13.4; m.wt. 119.13).

Thieno[3,2-b]pyridine (V) was prepared as described above for IV from 2.7 g (0.015 mol) of thieno[3,2-*b*]pyridine-5-carboxylic acid (II), yielding 2.0 g (99 %) of the crude product, which by GLC (BDS 10 % Chrom. W. 2 m) showed only one component. Its IR spectrum was identical with that of the distilled product (1.1 g); b.p. 107 – 108 °C/10 mmHg, $n_D^{21} =$ 1.6391. (Lit. value[16] b.p. 82 – 84 °C/2 mmHg). NMR spectrum (CCl_4): see Table 2.

Selenolo[3,2-b]pyridine (VI) was prepared as described above for IV from 2.8 g (0.012 mol) of selenolo[3,2-*b*]pyridine-5-carboxylic acid (III), yielding 2.2 g (98 %) of the crude product, showing a gas chromatogram (BDS 10 % Chrom. W. 2 m) with only one peak and an IR spectrum identical with that of the distilled fraction (1.6 g), b.p. 127 – 129 °C/10 mmHg, m.p. 35.5 – 37.0 °C. (Lit. value[17] m.p. 30 °C). NMR spectrum (CCl_4): see Table 2.

5-Methylfuro[3,2-b]pyridine (VII). 7.0 g (0.051 mol) of 3-azido-2-formylfuran[20] was dissolved in 75 ml of ethanol containing three drops of piperidine. The solution was cooled to 10 °C whereupon hydrogen sulfide was bubbled through it with continued cooling. An exothermic reaction with evolution of nitrogen gas took place. The rate of the addition of hydrogen sulfide was such that the temperature was kept below 20 °C. After about 45 min the evolution of nitrogen had ceased, the reaction mixture was cooled to 0 °C, and precipitated sulfur was filtered off. The filtrate was treated with a mixture of 25 ml of acetone and 25 ml of water, followed by the addition of 10 ml of 15 % sodium hydroxide solution. The reaction mixture, which turned dark, was stirred at 55 °C for 16 h, whereupon it was diluted with 150 ml of water and extracted four times with ether. The combined ether phases were extracted four times with 1 N hydrochloric acid, and the acidic phases were made alkaline with pellets of sodium hydroxide, and then extracted with ether. The combined ether phases were dried over magnesium sulfate and concentrated, giving 4.8 g (71 %) of a red-brown oil showing only one peak in GLC (OV 17, 3 % Chrom. Q, 3 m) and an IR spectrum identical with that of the distilled fraction, b.p. 81.5 – 82.0 °C/8 mmHg, $n_D^{21} = 1.5593$. NMR spectrum (CCl_4): see Table 3. (Found: C 70.3; H 5.32; O 12.1; m.wt. 133. Calc. for C_8H_7NO: C 72.2; H 5.31; O 12.0; m.wt. 133.16).

5-Methylthieno[3,2-b]pyridine (VIII) was prepared as described above for VII from 4.9 g (0.032 mol) of 3-azido-2-formylthiophene,[20] 40 ml of ethanol, two drops of piperidine, hydrogen sulfide, 20 ml of acetone in 20 ml of water, and 10 ml of 15 % sodium hydroxide solution. Upon evaporation, 3.9 g (81 %) of a red-brown oil was obtained, showing only one peak in GLC (OV 17, 3 % Chrom. Q, 3 m) and an IR spectrum identical with that of the distilled fraction, b.p. 114.5 – 116.5 °C/8 mmHg, $n_D^{21} = 1.6284$. (Lit. value:[16] b.p. 95 – 98 °C/5 mmHg). NMR spectrum (CCl_4): see Table 3.

5-Methylselenolo[3,2-b]pyridine (IX) was prepared as described above for VII from 6.0 g (0.030 mol) of 3-azido-2-formylselenophene,[20] 40 ml of ethanol, two drops of piperidine, hydrogen sulfide, 25 ml of acetone in 25 ml of water, and 10 ml of 15 % sodium hydroxide solution. Upon evaporation, 5.2 g (87 %) of a red-brown oil was obtained, showing only one peak in GLC (OV 17, 3 % Chrom. Q, 3 m) and an IR spectrum identical with that of the distilled fraction, b.p. 135 – 140 °C/15 mmHg, m.p. 46.0 – 48.0 °C. (Lit. value:[17] m.p. 47 – 48 °C). NMR spectrum (CCl_4): see Table 3.

5-Methyl-6-acetyl[*3,2*-b]*pyridine (X)* was prepared as described above for I and VII from 3.2 g (0.021 mol) of 3-azido-2-formylthiophene,[20] 40 ml of ethanol, two drops of piperidine, and hydrogen sulfide. After filtration of precipitated sulfur, 2.7 g (0.027 mol) of acetoacetone and another seven drops of piperidine were added. The reaction mixture was refluxed for 18 h, whereupon the volume of the solution was concentrated to a third and poured into 100 ml of ice and water with vigorous stirring. The precipitate was filtered off, giving 2.7 g (67 %) of the product. An analytical sample obtained by recrystallization from toluene showed an IR spectrum identical with that of the crude product; m.p. 131.5–132.5 °C. IR spectrum (KBr): CO = 1675 cm^{-1}. NMR spectrum ($CDCl_3$): δ 7.50 (H-3), 7.91 (H-2), 8.50 (H-7), 2.65 and 2.90 (CH_3 or $COCH_3$); $J_{2,3}$ 5.30 Hz, $J_{3,7}$ 0.7 Hz. (Found: C 62.6; H 4.70; S 16.9; m.wt. 191. Calc. for $C_{10}H_9NOS$: C 62.8; H 4.75; S 16.8; m.wt. 191.26).

The ^1H NMR spectra were obtained with a Varian A-60 high resolution spectrometer. The IR spectra were recorded on a Perkin-Elmer model 257. The gas chromatograph used was a Perkin-Elmer 900 analytical instrument. Mass spectra were obtained with an LKB 9000 mass spectrometer. Elementary analyses were carried out at the Analytical Department of the Chemical Institute and by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mühlheim/Ruhr.

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Structural Studies of Metabolic Products of Dopamine. III.* Crystal and Molecular Structure of (—)-Adrenaline

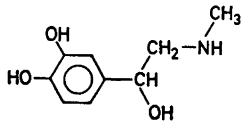
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The crystal structure of (—)-adrenaline has been determined by X-ray methods, using 831 observed reflections collected by counter methods. The crystals are monoclinic, space group $P2_1$ with $a=7.873(2)$, $b=6.790(2)$, $c=8.638(2)$ Å and $\beta=98.02(2)°$. Least-squares refinements yielded a conventional R-factor of 0.053. Standard deviations in bond lengths are 0.005—0.006 Å and in bond angles 0.4°. The adrenaline molecules were found to exist as zwitterions in the crystals. The conformation of the adrenaline molecule corresponds closely to that usually encountered among the salts of the sympathomimetic amines. The crystals consist of molecular double layers parallel to (100). The molecules within a layer are linked through hydrogen bonds of the types N—H···O and O—H···O, whereas the layers are connected by van der Waals interactions.

The hormone adrenaline (epinephrine) is a sympathomimetic catecholamine acting on both α- and β-adrenergic receptors. The natural $R(-)$-form is considerably more active than the $S(+)$-isomer. Adrenaline is easily oxidized, and its metabolic path has during the recent years been of substantial interest to research workers in various fields.[1]

Information concerning the molecular geometry and conformation pattern of the sympathomimetic amines is essential for structure-activity relationship studies. The molecular structure of the salts of several sympathomimetic amines has been determined by X-ray diffraction methods,[2] among them (—)-adrenaline hydrogen (+)-tartrate.[3] To the author's knowledge, none of the free amines belonging to this class of compounds have yet been subjected to X-ray investigations. This may be due to the difficulty in obtaining suitable crystals. It could therefore prove valuable to compare the molecular geometry and conformation of the free base form of (—)-adrenaline with the results of the structure determinations earlier mentioned.



EXPERIMENTAL

Commercially obtained (—)-adrenaline was dissolved in a 1 M nitric acid solution. At the interface between this solution and a 1 M ammonia solution, thin colourless crystal plates appeared. The infra-red spectrum of these crystals was identical to that of the original compound.

Oscillation and Weissenberg photographs indicated monoclinic symmetry. Systematically absence of reflections $0k0$ with k odd determined the space group to be $P2_1$ since the adrenaline molecule is optically active.

Unit cell parameters were obtained from a least-squares treatment of diffractometer measurements on 15 general reflections. The three-dimensional intensity data were recorded on a SYNTEX $P\bar{1}$ automatic diffractometer with graphite crystal monochromated Mo$K\alpha$ radiation. Intensities of 956 reflections with $2\theta < 65°$ were measured using the $\omega-2\theta$ scan technique, the scan speed varied from 1 to 2° min^{-1} depending on the peak intensity. Background counts were taken at each end of the scan range, the latter being 1.4°. The ratio of the summarized background counting time to scan time was 0.7. 831 reflections having net intensities greater than $2\sigma(I)$ were considered to be ob-

* The previous publication in this series: *Acta Chem. Scand. B 29* (1975) 45.

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations for non-hydrogen atoms ($\times 10^4$). The temperature factor is given by $\exp-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)$.

Atom	x	y	z	$B11$	$B22$	$B33$	$B12$	$B13$	$B23$
O1	9522(4)	3012(8)	8318(3)	121(6)	137(8)	47(4)	−39(12)	9(8)	−0(9)
O2	6554(4)	876 (8)	8417(3)	141(8)	288(12)	69(5)	−154(16)	−20(9)	62(13)
O3	8423(4)	1137(7)	1423(3)	174(7)	154(8)	53(4)	107(14)	−6(8)	−4(11)
N	8213(5)	5230(7)	510(4)	115(7)	116(9)	60(5)	28(15)	11(10)	7(11)
C1	8157(5)	2055(7)	4130(4)	84(8)	113(11)	65(6)	29(16)	31(11)	10(13)
C2	9092(6)	2769(9)	5509(5)	101(8)	118(11)	66(6)	−35(17)	15(12)	14(15)
C3	8609(6)	2390(8)	6972(4)	105(9)	96(12)	66(6)	14(17)	26(12)	−4(13)
C4	7128(5)	1266(10)	7018(4)	116(9)	151(12)	64(6)	−1(20)	31(12)	47(16)
C5	6205(6)	504(9)	5661(5)	100(9)	213(16)	91(7)	−114(20)	10(13)	12(17)
C6	6729(6)	904(9)	4236(5)	114(9)	184(14)	61(6)	10(21)	−22(11)	5(16)
C7	8680(6)	2642(8)	2585(5)	109(9)	118(12)	55(5)	45(18)	10(11)	7(13)
C8	7604(7)	4369(9)	1924(5)	124(9)	145(12)	68(6)	5(18)	78(13)	−1(14)
C9	6876(8)	6354(10)	−490(6)	159(12)	190(16)	120(8)	81(24)	7(16)	112(20)

Table 2. Fractional atomic coordinates ($\times 10^3$) with estimated standard deviations for hydrogen atoms. The isotropic thermal parameter is $B=3.2$ Å^2.

Atom	x	y	z
HC2	1006(7)	348(8)	549(5)
HC5	528(6)	−26(8)	571(5)
HC6	610(6)	37(7)	337(5)
HC7	994(6)	306(8)	272(5)
H1C8	757(5)	553(7)	265(5)
H2C8	641(6)	396(7)	169(5)
H1C9	736(6)	693(7)	−138(5)
H2C9	648(6)	737(8)	6(5)
H3C9	597(6)	550(7)	−91(5)
H1N	858(6)	430(7)	−14(5)
H2N	920(6)	604(8)	91(5)
HO2	722(6)	111(9)	909(5)
HO3	917(6)	34(8)	169(5)

served, the remaining 125 were excluded from the further calculations.

The intensity data were corrected for Lorentz and polarization effects. The intensities were scaled to an absolute level by methods described by Karle and Hauptman,[4] and normalized structure factor magnitudes were calculated.

Atomic form factors used were those of Doyle and Turner [5] for oxygen, nitrogen, and carbon atoms, and of Stewart *et al.*[6] for hydrogen atoms.

Computer programs employed during the present study are described in Refs. 7 and 8.

CRYSTAL DATA

Adrenaline, $C_9H_{13}O_3N$

Space group $P2_1$, monoclinic

$a=7.873(2)$ Å; $b=6.790(2)$ Å, $c=8.638(2)$ Å; $\beta=98.02(2)°$ $V=457.2$ Å^3; $M=183.22$; $F_{(000)}=196$; $Z=2$; $D_{calc}=1.331$ g cm^{-3}

STRUCTURE DETERMINATION

The structure was determined by use of the MULTAN program system,[8] starting with $|E|$ values of 149 reflections ($|E|\geq 1.30$). The program produced 16 sets of phases as possible solutions. An E map calculated from the set of phases with the highest absolute figure of merit revealed the positions of all non-hydrogen atoms. One Fourier refinement, based on these positional parameters, yielded an R-factor of 0.14. Coordinates of six hydrogen atoms bonded to carbon atoms were calculated. Methyl hydrogen atoms were not included. A few cycles of least-squares refinement of positional and anisotropic thermal parameters of the non-hydrogen atoms lowered the R-factor to 0.078. In three successive difference Fourier maps the seven remaining hydrogen atoms were located in positions showing a zwitterionic structure of the adrenaline molecule. The zwitterionic structure was further indicated by the infra-red spectrum of the crystals which showed a broad absorption band centered about 2500 cm^{-1}. Subsequent

Table 3. Bond lengths (Å) and bond angles (°) with estimated standard deviations in parantheses.

Bond length		Corrected	Bond angle	
C1 – C2	1.397(5)	1.399	C2 – C1 – C6	118.4(4)
C2 – C3	1.394(5)	1.396	C1 – C2 – C3	122.1(4)
C3 – C4	1.398(6)	1.405	C2 – C3 – C4	117.4(4)
C4 – C5	1.390(6)	1.392	C3 – C4 – C5	121.3(4)
C5 – C6	1.379(6)	1.381	C4 – C5 – C6	119.6(5)
C1 – C6	1.382(6)	1.389	C5 – C6 – C1	121.2(4)
C1 – C7	1.504(6)	1.507	C6 – C1 – C7	122.3(4)
C3 – O1	1.346(5)	1.348	C2 – C1 – C7	119.2(4)
C4 – O2	1.374(5)	1.376	C2 – C3 – O1	123.0(4)
C7 – C8	1.512(6)		O1 – C3 – C4	119.6(4)
C8 – N	1.492(6)		C3 – C4 – O2	120.5(4)
N – C9	1.478(6)		O2 – C4 – C5	118.2(4)
C7 – O3	1.426(5)		C1 – C7 – O3	113.9(4)
C2 – HC2	0.90(5)		O3 – C7 – C8	105.8(3)
C5 – HC5	0.90(5)		C1 – C7 – C8	109.4(4)
C6 – HC6	0.91(5)		C7 – C8 – N	112.7(4)
C7 – HC7	1.02(5)		C8 – N – C9	113.4(4)
C8 – H1C8	1.01(5)			
C8 – H2C8	0.98(5)			
C9 – H1C9	0.98(5)			
C9 – H2C9	0.91(5)			
C9 – H3C9	0.95(5)			
N – H1N	0.91(5)			
N – H2N	0.97(5)			
O2 – HO2	0.75(5)			
O3 – HO3	0.81(5)			

least-squares refinements including the hydrogen atoms with a common isotropic thermal parameter yielded a conventional R-factor of 0.053 and a weighted R-factor of 0.038. A total difference Fourier map demonstrated irregular electron density with the largest peaks less than 0.3 e Å^{-3}.

The final parameters for non-hydrogen atoms are listed in Table 1 and for hydrogen atoms in Table 2.

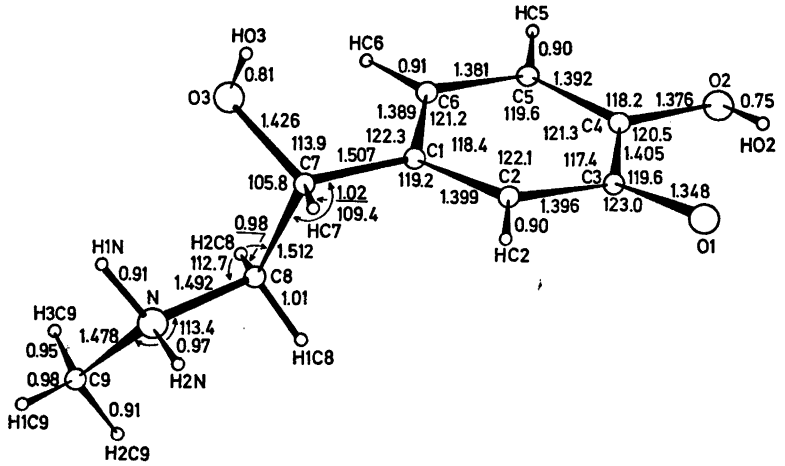


Fig. 1. Bond lengths (corrected) (Å) and angles (°).

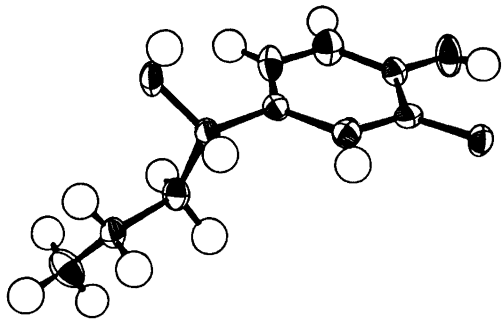


Fig. 2. The ellipsoids of thermal motion with a probability of 50 %. (The drawing was prepared using the computer program ORTEP [15]).

An analysis of the molecular motions in terms of rigid body vibrations indicated that the catechol part of the adrenaline molecule could be regarded as a rigid unit with the axis of the largest oscillation amplitude nearly parallel to the C1—C3 direction. The bond lengths in this part of the molecule were corrected for thermal vibration effects.

Bond lengths and angles are given in Table 3. The numbering of the atoms is indicated in Fig. 1, which also presents bond lengths (corrected) and angles. Fig. 2 shows the *R*-configuration of the adrenaline molecule including the ellipsoids of thermal motion.

The structure factor list may be obtained from this institute upon request.

DISCUSSION

Molecular structure. An interesting feature of this crystal structure is the appearance of the adrenaline molecules as zwitterions, with one proton transfered from the *meta* phenolic hydroxyl group to the nitrogen atom. The zwitterionic structure is indicated by the tetrahedral bonding arrangement around the nitrogen atom. The two C—N distances (1.478 and 1.492 Å) are both in the range normally reported for $C-N^+_i$ single bonds,[9-12] and the two N—H distances (0.91 and 0.97 Å) are in agreement with that usually found for this type of bond in X-ray analyses. The O1 oxygen atom appears to be the carrier of the negative charge of the zwitterion. No hydrogen atom could be located in the vicinity of this oxygen atom, and furthermore, the C3—O1 bond length of 1.348 Å

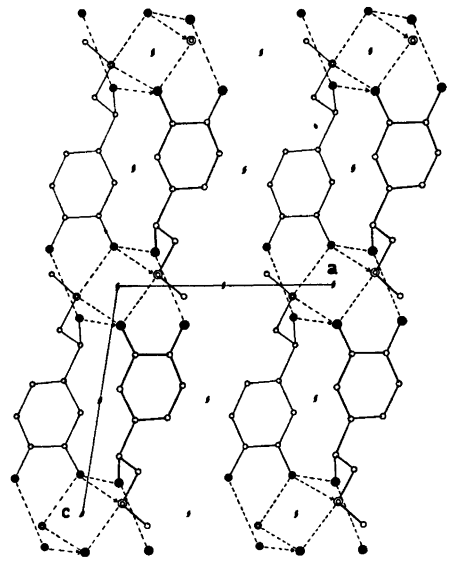


Fig. 3. The structure as viewed down the *b*-axis.

is significantly shorter than the C4—O2 bond of 1.376 Å. The latter distance, as well as the O2—HO2 distance of 0.75 Å, represents a normal value for phenols. However, the C3—O1 separation is larger than would be expected for a $C-O^-$ bond. This relative lengthening may be attributed to the formation of three fairly short hydrogen bonds having the O1 oxygen atom as a hydrogen acceptor (see below). The other bond lengths and bond angles are in agreement with corresponding values found in (—)-adrenaline

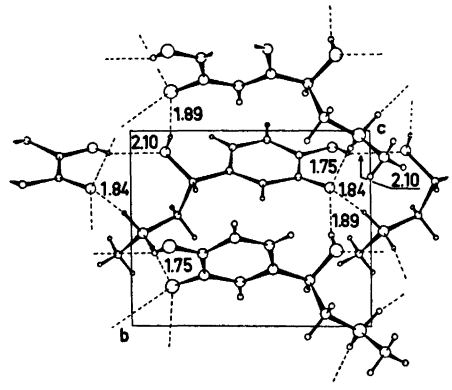


Fig. 4. The structure as viewed down the *a*-axis showing the hydrogen bonds (Å).

Table 4. Hydrogen bonded interactions X—H···Y.

X	Y		X···Y (Å)	H···Y (Å)	X—H···Y (°)
N	O1	(x , y , $z-1$)	2.732	1.84	165
N	O1	($-x+2$, $y+\frac{1}{2}$, $-z+1$)	2.695	1.75	163
O3	O1	($-x+2$, $y-\frac{1}{2}$, $-z+1$)	2.657	1.89	160
O2	O3	(x , y , $z+1$)	2.806	2.10	157

hydrogen (+)tartrate[3] and related compounds.[9–11]

The catechol part of the adrenaline molecule is nearly planar. The deviations of the ring carbon atoms from a least-squares plane through these atoms are in the range 0.004—0.012 Å. The exocyclic atoms are situated —0.065 Å (O1), 0.042 Å (O2) and 0.114 Å (C7) out of the ring plane. Likewise, the ethylamine side chain is nearly planar, the dihedral angle C1—C7—C8—N (τ_2) is 171.6°. Such a fully extended side chain has always been found in the crystal structures of the salts of the sympathomimetic amines.[2] The plane of the side chain of the adrenaline molecule is approximately perpendicular to the ring plane, the dihedral angle C6—C1—C7—C8 (τ_1) is 80.5°. In the crystal structures of the salts of the closely related amines, the τ_1 torsion angle is with only a few exceptions[2,3,13] found to have a value about ±90°. (—)-Adrenaline hydrogen (+)tartrate[3] is one of the few related compounds with a different arrangement of the ring and side chain in the crystals, having a τ_1 torsion angle close to 0°. However, the planarity of the latter molecule is assumed to be an effect of the particular molecular packing arrangement and hydrogen bond system of this crystal structure. The conformation of the adrenaline molecule in the present investigation is furthermore in agreement with the quantum mechanical calculations performed by Pullman *et al.* on free *N*-protonated adrenaline.[14]

The hydroxyl group of the side chain is found to be in a *trans* position relative to the *meta* oxygen atom of the phenyl ring. The dihedral angle C6—C1—C7—O3 is —37.7°. In (—)-adrenaline hydrogen (+)-tartrate[3] and (—)-noradrenaline hydrochloride,[10] both with an *R*-configuration, the two hydroxyl groups are in a *cis* position. These latter molecules are thus reported to have a hydrophobic and a hydrophilic side in contrast to the adrenaline molecule in the present crystals.

Molecular packing and hydrogen bonding. The arrangement of the molecules in the crystals is illustrated in Fig. 3 and Fig. 4. The structure is characterized by double layers parallel to (100), containing hydrogen bonded molecules. The molecular double layers are held together by van der Waals interactions. This molecular arrangement resembles that found in the crystal structure of DL-*o*-tyrosine.[13] The shortest intermolecular C···C and C···O contacts between the double layers in the present structure are 3.89 and 3.40 Å, respectively.

The adrenaline molecules within a double layer are linked through hydrogen bonds of the types N—H···O and O—H···O. The hydrogen bond network is arranged in infinite spirals about the *b*-axis. Hydrogen bond distances are given in Table 4 and also indicated in Fig. 4. The negatively charged oxygen atom is acting as an acceptor in three relatively short hydrogen bonds from two nitrogen atoms and one hydroxyl oxygen atom in neighbouring molecules. The N···O separations are 2.732 Å and 2.695 Å and the O···O separation 2.657 Å. The arrangement about the charged oxygen atom is approximately tetrahedral. The phenol oxygen atom acts as hydrogen donor in a hydrogen bond with length 2.806 Å to a hydroxyl oxygen atom in a neighbouring molecule.

Each adrenaline molecule is thus bonded to four other molecules within a double layer through hydrogen bonds, and to the neighbouring molecules in the adjacent double layer by van der Waals interactions.

It is a pleasure to thank Arvid Mostad and Chr. Rømming for their helpful suggestions and their interest in this study.

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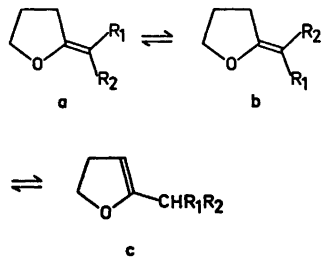
Thermodynamics of Vinyl Ethers. XII.* The Relative Stabilities of 2-Alkylidenetetrahydrofurans and 5-Alkyl-2,3-dihydrofurans

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The relative stabilities of 2-alkylidenetetrahydrofurans and their *endo*-cyclic isomers, 5-alkyl-2,3-dihydrofurans (alkyl = Et, i-Pr, i-Bu) have been determined by chemical equilibration in cyclohexane solution. At 298.15 K, the *endo*-cyclic compounds are thermodynamically more stable than their *exo*-cyclic isomers except for the case alkyl = i-Pr. Of the two geometric isomers of 2-ethylidenetetrahydrofuran and 2-isobutylidenetetrahydrofuran, the *Z* form (with Me or i-Pr *cis* to ring oxygen) is the more stable one. The standard entropy change in each *exo*→*endo* isomerization is positive; comparison of the value of $\Delta S°$ for alkyl = Et with that for alkyl = i-Bu suggests that the rotation of the i-Pr group about the C—C(sp^2) bond in 2-isobutylidenetetrahydrofuran is hindered by the proximity of the ring atoms.

The relative stabilities of 2-alkylidenetetrahydrofurans and 5-alkyl-2,3-dihydrofurans, and of 2-alkylidenetetrahydropyrans and 6-alkyl-3,4-dihydro-2*H*-pyrans were treated in a recent study by the present author.[1] The isomeric compounds were equilibrated in diethyl ether solution with *p*-toluenesulfonic acid as catalyst, and their relative concentrations at equilibrium were determined by GLC. When the experimental work was practically completed, Rhoads *et al.*[2] reported that iodine in an inert solvent had been found to be a good catalyst-solvent combination for equilibration experiments on α,β-unsaturated ethers. To test the applicability of this method for equilibration of the heterocyclic ethers concerned, a few of the six-membered *exo-endo* pairs were equilibrated in hexane with iodine as catalyst. The values of $\Delta H°$ and $\Delta S°$ of isomerization were found to differ considerably from those obtained by using diethyl ether as solvent. In a later study however,[3] the effect of solvent on the equilibrium between the acyclic compounds 2-methoxy-3-methyl-1-butene and 2-methoxy-3-methyl-2-butene was found to be small. In view of these opposing results it was considered necessary to make a reinvestigation of the relative stabilities of the five-membered heterocyclic ethers concerned, now in an inert solvent. Besides for the clarification of the solvent effect, the study was justified by a need of "basic" thermodynamic data of isomerization, relevant to an inert solvent, for these simple heterocyclic ethers for comparative purposes in some future investigations dealing with more complex unsaturated ethers. Thus the compounds II—IV were prepared (compound III was not included in the previous study [1]) and the thermodynamics



(1)

I: $R_1=R_2=H$ (Ia≡Ib). III: R_1=i-Pr, $R_2=H$. II: R_1=Me, $R_2=H$. IV: $R_1=R_2$=Me (IVa≡IVb).

* Part XI: *Tetrahedron. In press.*

Table 1. Thermodynamic data for the reactions quoted in eqn. 1 in the liquid phase at 298.15 K. The errors are twice the standard errors.

Reaction	$\Delta G°$/kJ mol^{-1}	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
IIa→IIc[a]	−3.99±0.03	−1.9±0.2	7.0±0.4
IIIa→IIIc[b]	−6.56±0.08	−3.0±0.5	12.0±1.5
IVa→IVc[c]	4.60±0.05	6.3±0.3	5.6±0.7
IIb→IIc[a]	−0.44±0.04	1.9±0.2	7.7±0.7
IIIb→IIIc[b]	−3.13±0.04	−0.1±0.3	10.2±0.7
IIa→IIb[a]	−3.54±0.04	−3.8±0.2	−0.7±0.7
IIIa→IIIb[b]	−3.42±0.05	−2.9±0.3	1.9±0.9

[a] The reaction was studied between 282 and 403 K. [b] Studied between 282 and 400 K. [c] Studied between 282 and 428 K.

Table 2. Thermodynamic data for the reactions quoted in eqn. 1 in the gas phase at 298.15 K.

Reaction	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
IIa→IIc	−5.0±0.4	3.5±0.6
IIIa→IIIc	−5.9±0.6	8.9±1.6
IVa→IVc	2.6±0.4	1.7±0.9
IIb→IIc	−0.1±0.4	5.4±0.9
IIIb→IIIc	−2.0±0.4	8.2±0.9
IIa→IIb	−4.8±0.4	−1.9±0.9
IIIa→IIIb	−3.9±0.4	0.8±1.0

of their isomeric interconversion was studied. As it turned out that the effect of solvent on the values of the thermodynamic parameters was negligible, investigation of reaction Ia⇌Ic was not repeated, since analysis of the equilibrium mixtures of Ia and Ic by GLC is laborious, due to the easy isomerization of Ia to Ic.

EXPERIMENTAL

Materials. The following synthetic route was employed:[1] $ClCH_2CH_2CH_2COCl \rightarrow ClCH_2CH_2CH_2COCHR_1R_2 \xrightarrow{\Delta} HOCH_2CH_2CH_2COCHR_1R_2 \rightarrow$ a,b,c. The chloro ketones were now prepared from 4-chlorobutyryl chloride and the appropriate organomagnesium halide (instead of the corresponding organozinc halide [4]) by the method suggested by Cason and Kraus [5] for the preparation of aliphatic ketones. The yields and boiling temperatures of the ketones prepared were: R_1=Me, R_2=H: 42 %, b.t. 334.7 K at 0.8 kPa (lit.[1] 39 %, b.t. 348 to 358 K at 1.6 to 2.1 kPa); R_1=i-Pr, R_2=H: 65 %, b.t. 358 K at 1.1 kPa; R_1=R_2=Me: 45 %, b.t. 348 to 350 K at 1.5 kPa (lit.[1] 12 %, b.t. 356 to 357 K at 2.1 kPa). The physical constants of I, II, and IV have been published previously.[1] IIIc: b.t. 338 K at 6.0 kPa. *NMR* (60 MHz, CCl_4, TMS): I, II, and IV, see Ref. 1; IIIc: τ 5.78 (CH_2-O), 7.45 (C-CH_2-C in the ring), 5.53 (=C−H), 8.09 (CH_2 in the side chain), 9.08 (CH_3), the signal of the methine proton of the i-Bu group was not detected; J_{vic} (CH_2-CH_2) 9.5 Hz, J_{vic} (CH_2−CH=) 2.0 Hz. The *normal boiling temperatures* of the isomeric vinyl ethers studied were determined by the method described previously.[6] IIa, 401.8 K; IIb, 395.2 K; IIc, 382.7 K; IIIa, 433.0 K; IIIb, 426.7 K; IIIc, 414.8 K; IVa, 417.4 K; IVc, 394.6 K.

Procedure. The equilibrations were carried out in cyclohexane with iodine as catalyst.[2,3]

RESULTS

The values of the thermodynamic parameters of isomerization in the liquid phase at 298.15 K are shown in Table 1, and the values of $\Delta H°$(g) and $\Delta S°$(g) at 298.15 K, evaluated from the liquid phase data and the normal boiling temperatures,[6] in Table 2.

DISCUSSION

The change of solvent from diethyl ether to cyclohexane has no practical effect on the values of the thermodynamic functions of isomerization as can be seen from the following examples (the errors are twice the standard errors):

Reaction	Solvent	$\Delta H°$/ kJmol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
IIa→IIb	Et_2O (Ref. 1)	−3.7±0.7	−1.6±2.6
	C_6H_{12}	−3.8±0.2	−0.7±0.7
IIb→IIc	Et_2O (Ref. 1)	2.0±0.5	8.0±2.0
	C_6H_{12}	1.9±0.2	7.7±0.7
IVa→IVc	Et_2O (Ref. 1)	6.4±0.5	5.5±1.8
	C_6H_{12}	6.3±0.3	5.6±0.7

Thus it is reasonable to assume that the values of $\Delta H°$(l) and $\Delta S°$(l) (-4.7 ± 0.6 kJ mol^{-1} and 8.0 ± 2.0 J K^{-1} mol^{-1},[1] respectively, the errors being twice the standard errors) for reaction Ia→Ic in diethyl ether solution should, within experimental error, correspond to those obtainable from experiments performed in an inert solvent, such as cyclohexane. In the gas phase therefore, the values of $\Delta H°$ and $\Delta S°$ for Ia→Ic should be about -7.8 kJ mol^{-1} and 4.2 J K^{-1} mol^{-1},[7] since the normal boiling temperatures of Ia and Ic are about 373 and 354 K,[1] respectively.

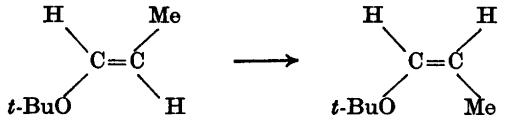
The conclusions extractable from the values of the changes of the thermodynamic parameters for reactions involving the isomeric forms of I, II, and IV have been discussed in detail previously,[1] but some essential features will be briefly treated here together with new findings on the basis of gas-phase data, unavailable in the previous study.

The relative stability of the *exo* isomers (a,b) increases with increasing number of alkyl groups attached to the double bond of the *exo* form. For example, the values of $\Delta H°$(g) for Ia→Ic and IIa→IIc are -7.8 and -5.0 kJ mol^{-1}; the methyl group of IIa thus stabilizes the double bond by about 3 kJ mol^{-1}. For comparison, the standard enthalpies of isomerization of methylenecyclopentane to 1-methylcyclopentene and of ethylidenecyclopentane to 1-ethylcyclopentene are about -16 and -5 kJ mol^{-1}, respectively, in acetic acid solution.[7] Hence the methyl group of ethylidenecyclopentane stabilizes the double bond by about 11 kJ mol^{-1}. Possible reasons for the weak stabilizing effect of alkyl groups on the double bond of vinyl ethers have been suggested previously.[1,3] The same phenomenon seems to be responsible for the fact that the value of $\Delta H°$(g) for Ia→Ic (-7.8 kJ mol^{-1}) is about 8 kJ mol^{-1} less negative than that for methylenecyclopentane → 1-methylcyclopentene, *i.e.* about -16 kJ mol^{-1} (since the normal boiling temperatures of the two olefins concerned are equal within 1 K,[8,9] the values of $\Delta H°$(l) and $\Delta H°$(g) for the latter reaction should be practically equal, see Ref. 6). In each *exo*→*endo* reaction in question, the number of alkyl substituents attached to the double bond increases by one, and since the stabilization due to a single alkyl group should be about 11 kJ mol^{-1} in olefins (*cf.* ethylidenecyclopentane above), but only 3 kJ mol^{-1} in vinyl ethers, the value of $\Delta H°$(g) in Ia→Ic might be expected to be about $-16+(11-3)=-8$ kJ mol^{-1}, which agrees well with the experimental value of about -7.8 kJ mol^{-1}.

The values of $\Delta H°$(g) and $\Delta S°$(g) for IIa→IIb are interesting in comparison with those for reaction (2), for which $\Delta H°$(g)$=-2.9\pm0.3$ kJ

Table 3. Experimental data for reactions (1) and (3). The data for reaction (3) are from Ref. 12. Gas phase, 298.15 K.

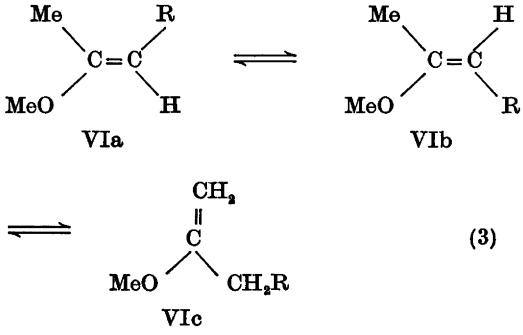
Reaction	$\Delta H°$(g)/kJ mol^{-1}	$\Delta S°$(g)/J K^{-1} mol^{-1}
IIIa→IIIc	$-5.9(\pm0.6)$	$8.9(\pm1.6)$
IIa→IIc	$-5.0(\pm0.4)$	$3.5(\pm0.6)$
	$\Delta=-0.9$	$\Delta\cong5$
VIa→VIc, R=i-Pr	$-0.6(\pm0.4)$	$6.4(\pm0.8)$
R=Me	$-0.5(\pm0.4)$	$1.6(\pm0.8)$
	$\Delta=-0.1$	$\Delta\cong5$
IIIb→IIIc	$-2.0(\pm0.4)$	$8.2(\pm0.9)$
IIb→IIc	$-0.1(\pm0.4)$	$5.4(\pm0.9)$
	$\Delta=-1.9$	$\Delta\cong3$
VIb→VIc, R=i-Pr	$-12.2(\pm0.4)$	$-9.1(\pm0.9)$
R=Me	$-10.3(\pm0.6)$	$-11.8(\pm1.5)$
	$\Delta=-1.9$	$\Delta\cong3$



(2)

mol^{-1} and $\Delta S°(g) = 5.3 \pm 0.6$ J K^{-1} mol^{-1}.[10] The enthalpy change in IIa→IIb is about 2 kJ mol^{-1} more negative than that in reaction (2). Since it seems unlikely that the difference, at least totally, could be ascribed to steric repulsions between the methyl group and ring hydrogens in IIa (the corresponding strain is less than 1 kJ mol^{-1} in the analogous compound ethylidenecyclopentane [11]), it is possible that attractive forces between the methyl group and oxygen atom are more pronounced in IIb than in Vb. Inspection of molecular models shows that the shape of the five-membered ring in IIb leads to a slightly increased distance, relative to that in Vb, between the methyl group and oxygen atom, which appears to be advantageous for enhanced stability.

Some of the results of the present work are compared with data for reaction (3) in Table 3. The enthalpy data show that the *cis*



(3)

interaction between Me and R in VIa is the same for R=Me and R=i-Pr. The data also suggest that the corresponding interaction in IIIa might be slightly greater than that in IIa, although the values of $\Delta H°(g)$ for IIIa→IIIc and IIa→IIc are, in fact, equal if the experimental uncertainty in the values of $\Delta H°(g)$ is taken into account. However, the stabilizing *cis* interaction between MeO and R in VIb is definitely greater (by about 1.9 kJ mol^{-1}) for R=Me than for R=i-Pr. This result is confirmed by enthalpy data for IIIb→IIIc and IIb→IIc.

The value of $\Delta S°(g)$ for IIIa→IIIc is about 5 J K^{-1} mol^{-1} more positive than that for IIa→IIc; this compares well with the difference in the corresponding values for reaction VIa→VIc in cases R=i-Pr and R=Me. On the other hand, the difference in the values of $\Delta S°(g)$ for IIIb→IIIc and IIb→IIc is 3 J K^{-1} mol^{-1}, which is the same as the corresponding difference for reaction VIb→VIc in cases R=i-Pr and R=Me. These results probably show that rotation of the i-Pr group about the C(sp^3)—C(sp^2) bond in, for instance, IIIa is restricted by the *cis* substituent (ring hydrogens in IIIa), which decreases the entropy of the *a* isomer relative to that of the *c* isomer, where the i-Pr group should be free to rotate.

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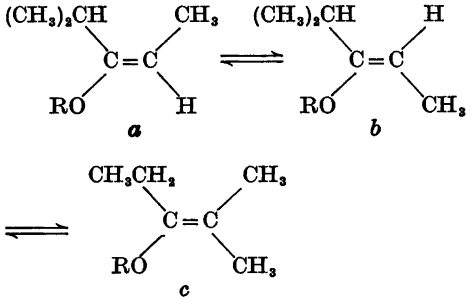
Thermodynamics of Vinyl Ethers. XIII.* Effect of the Alkoxy Group on the Relative Stabilities of Isomeric Alkoxyolefins

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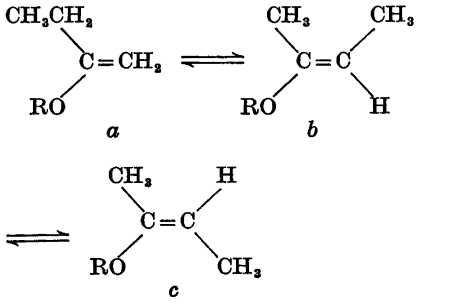
The effect of the size of the alkoxy group on the relative stabilities of various isomeric alkyl-substituted vinyl alkyl ethers has been studied by chemical equilibration. Bulky alkoxy groups favor the relative stability of molecular structures with an alkyl group in a *cis* position with respect to the alkoxy group. The results are explained in terms of rotational isomerism about the $C(sp^2)-O$ bond. The enthalpy of the *gauche* rotamer of methyl vinyl ether is probably about 12 kJ mol^{-1} higher than that of the s-*cis* rotamer.

The present paper reports the results of a study of the effect of the size of the alkoxy group on the following equilibria:



(1)

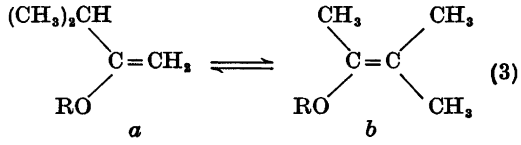
I: R = Me (Ref. 1); II: R = Et; III: R = Et_2CH



(2)

IV: R = Me (Ref. 2); V: R = Et; VI: R = $(Me)_3CCH_2$; VII: R = Bz; VIII: R = Et_2CH

Except for the interconversion of geometric isomers, the reaction studied involve changes in the number of methyl groups attached to the β carbon of the vinyl group: for instance, in IVa→IVb a methyl group is formally transferred to the β *trans* position (*trans* to RO) and in IVa→IVc to the β *cis* position. The variation with RO of the enthalpy of reaction reflects the dependence on RO of the apparent stabilizing effect of the β *cis* or β *trans* methyl group. The results achieved are used to estimate the variation with RO of the enthalpy change for reaction (3) involving a formal transfer of two methyl groups to the β carbon.



(3)

IX: R = Me (Refs. 3,4); X: R = Et (Ref. 4); XI: R = Et_2CH (Ref. 4); XII: R = $(Me)_3CCH_2$

EXPERIMENTAL

Materials. The isomeric vinyl ethers studied were prepared by the standard methods illustrated in the previous parts of this series. II: boiling temperature (b.t.) 397 to 415 K at 100.0 kPa; III: b.t. 447 to 461 K at 100.0 kPa; V: b.t. 355 to 363 K at 100.2 kPa; VI: b.t. 409 to 411 K at 101.1 kPa; VIIa: b.t. 368 to 371 K at 1.6 kPa; VIIIa: b.t. 319 K at 1.7 kPa; XII: b.t. 424 to 426 K at 100.7 kPa. The *normal*

* Part XII: Taskinen, E. *Acta Chem. Scand. B 29* (1975) 245.

boiling temperatures were determined as described previously.[1] IIa, 395.0 K; IIb, 398.9 K; IIc, 404.7 K; IIIa, 445.2 K; IIIb, 450.9 K; IIIc, 455.9 K; Va, 361.1 K; Vb, 373.3 K; Vc, 368.9 K; VIa, 400.0 K; VIb, 411.0 K; VIc, 411.0 K; VIIa, 484.0 K; VIIb, 492.2 K; VIIc, 488.4 K; VIIIa, 412.2 K; VIIIb, 422.4 K; VIIIc, 422.4 K; XIIa, 414.4 K; XIIb, 435.0 K. The *NMR spectra* are available on request.

Equilibration procedure. The equilibrations were carried out in cyclohexane solution with iodine as catalyst.[3] The range of temperature covered by the experiments and the number of temperatures at which the experiments were carried out were: II, 284 to 405 K, 13; III, 284 to 415 K, 14; V, 273 to 404 K, 9; VI, 273 to 421 K, 8; VII, 282 to 426 K, 8; VIII, 258 to 402 K, 15; XII, 273 to 415 K, 9.

RESULTS

To save space, the values of the mean equilibrium constant K at various temperatures are not given here but are available on request. The value of $\Delta G°$ was calculated for each K value, and then $\Delta G°$ was plotted as a function of temperature T. If $\Delta G°$ proved to be a linear function of temperature ($\Delta C_p° = 0$), the values of $\Delta G°$, $\Delta H°$, and $\Delta S°$ at 298.15 K were obtained by a linear least-squares treatment of $\Delta G°$ against T.[3] If however $\Delta G°$ was a nonlinear function of T, the values of K were fitted to the equation [5]

$$\ln K = A + B/T + C \ln T \qquad (4)$$

Table 1. Values of $\Delta G°$, $\Delta H°$, $\Delta S°$ and $\Delta C°_p$ in the liquid phase at 298.15 K for the isomerization reactions studied in this work together with some literature data. The errors are twice the standard errors.

Reaction	Compound	$\Delta G°$/kJ mol^{-1}	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}	$\Delta C_p°$/J K^{-1} mol^{-1}
$a \rightarrow b$	I (Ref. 1)	5.55 ± 0.04	11.2 ± 0.2	19.0 ± 0.7	–
	II	4.72 ± 0.08	10.0 ± 0.5	17.7 ± 1.3	–
	II[a]	4.74 ± 0.07	10.6 ± 1.1	19.6 ± 3.4	−12 ± 23
	III	2.80 ± 0.02	3.9 ± 0.1	3.6 ± 0.3	–
	III[a]	2.80 ± 0.02	3.7 ± 0.3	3.1 ± 1.0	3 ± 6
	IV (Ref. 2)	−0.38 ± 0.07	−1.8 ± 0.2	−4.7 ± 0.7	–
	V	−0.45 ± 0.05	−1.4 ± 0.3	−3.1 ± 1.0	–
	VI	−0.10 ± 0.02	−0.8 ± 0.2	−2.3 ± 0.4	–
	VI[a]	−0.10 ± 0.03	−0.9 ± 0.3	−2.6 ± 0.5	2 ± 4
	VII	−0.37 ± 0.04	−0.6 ± 0.2	−0.8 ± 0.6	–
	VIII	0.36 ± 0.03	0.1 ± 0.2	−0.9 ± 0.6	–
	VIII[a]	0.37 ± 0.03	−0.1 ± 0.3	−1.5 ± 1.2	6 ± 10
	XII	1.40 ± 0.02	6.2 ± 0.2	16.1 ± 0.7	−24 ± 5
$a \rightarrow c$	I (Ref. 1)	1.66 ± 0.03	7.8 ± 0.2	20.7 ± 0.4	–
	II[a]	0.35 ± 0.04	7.0 ± 0.6	22.2 ± 2.0	−26 ± 13
	III	−1.13 ± 0.02	0.8 ± 0.1	6.5 ± 0.3	–
	III[a]	−1.13 ± 0.02	1.0 ± 0.3	7.1 ± 1.0	−4 ± 6
	IV (Ref. 2)	5.70 ± 0.11	8.5 ± 0.5	9.3 ± 1.4	–
	V	4.54 ± 0.04	7.8 ± 0.3	11.1 ± 0.9	–
	VI[a]	4.28 ± 0.02	7.4 ± 0.2	10.5 ± 0.7	−17 ± 5
	VII	4.24 ± 0.04	7.0 ± 0.2	9.1 ± 0.6	–
	VIII	2.19 ± 0.03	2.0 ± 0.2	−0.6 ± 0.6	–
	VIII[a]	2.20 ± 0.04	1.9 ± 0.3	−1.0 ± 1.2	5 ± 11
$b \rightarrow c$	I (Ref. 1)	−3.89 ± 0.05	−3.4 ± 0.3[b]	1.7 ± 0.8	–
	II	−4.40 ± 0.07	−4.2 ± 0.4	0.6 ± 1.0	–
	II[a]	−4.38 ± 0.06	−3.6 ± 0.8	2.7 ± 2.8	−14 ± 18
	III[a]	−3.93 ± 0.02	−2.7 ± 0.3	4.3 ± 1.2	−8 ± 7
	IV (Ref. 2)	6.10 ± 0.06	10.3 ± 0.3	13.9 ± 0.8	–
	V	4.99 ± 0.05	9.2 ± 0.3	14.2 ± 1.0	–
	VI[a]	4.38 ± 0.03	8.3 ± 0.3	13.0 ± 1.0	−19 ± 7
	VII	4.63 ± 0.03	7.7 ± 0.2	10.3 ± 0.5	–
	VIII	1.82 ± 0.03	1.9 ± 0.2	0.3 ± 0.5	–
	VIII[a]	1.82 ± 0.03	2.0 ± 0.3	0.5 ± 1.0	−2 ± 10

[a] Calculated from eqn. (4). [b] The sign of $\Delta H°$ given in Ref. 1 is in error.

Table 2. Values of $\Delta H°$ and $\Delta S°$ in the gas phase at 298.15 K for the isomerization reactions studied in this work together with some literature data.

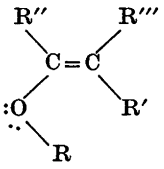
Reaction	Compound	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
$a \rightarrow b$	I (Ref. 1)	11.6 ± 0.4	19.5 ± 0.9
	II[a]	10.9 ± 0.9	19.4 ± 2.5
	III[b]	4.6 ± 0.4	4.0 ± 1.2
	IV (Ref. 2)	0.5 ± 0.4	−1.6 ± 0.8
	V	0.6 ± 0.4	−0.6 ± 1.1
	VI[b]	1.0 ± 0.4	−0.3 ± 0.6
	VII	0.7 ± 0.4	0.3 ± 0.8
	VIII[c]	1.8 ± 0.4	0.9 ± 0.8
	XII	9.5 ± 0.4	19.6 ± 0.9
$a \rightarrow c$	I (Ref. 1)	9.0 ± 0.3	22.1 ± 0.7
	II[b]	8.5 ± 0.7	24.0 ± 2.1
	III[b]	2.6 ± 0.4	8.7 ± 1.2
	IV (Ref. 2)	10.3 ± 0.6	11.8 ± 1.5
	V	9.1 ± 0.4	12.7 ± 1.0
	VI[b]	9.2 ± 0.4	12.5 ± 0.9
	VII	7.7 ± 0.4	9.7 ± 0.8
	VIII[c]	3.6 ± 0.4	1.2 ± 0.8
$b \rightarrow c$	I (Ref. 1)	−2.6 ± 0.4	2.7 ± 1.0
	II[b]	−2.7 ± 0.9	3.7 ± 2.9
	III[b]	−1.9 ± 0.5	5.0 ± 1.3
	IV (Ref. 2)	9.9 ± 0.4	13.4 ± 0.9
	V	8.5 ± 0.4	13.3 ± 1.1
	VI[b]	8.3 ± 0.4	13.0 ± 1.1
	VII	7.1 ± 0.4	9.7 ± 0.7
	VIII[b]	1.9 ± 0.4	0.3 ± 0.7

[a] The values of $\Delta H°$(g) and $\Delta S°$(g) are based on the mean of the corresponding liquid-phase values given in Table 1. [b] Based on the corresponding liquid-phase values obtained from eqn. (4). [c] Based on the corresponding liquid-phase values obtained for a linear least-squares treatment of $\Delta G°$ *vs.* T.

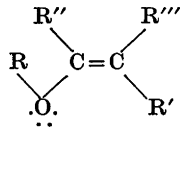
The use of this equation requires that $\Delta H°$ is a linear function of T ($\Delta C_p° = \text{constant}$). The values of the thermodynamic parameters of isomerization in the liquid phase at 298.15 K are shown in Table 1. The values of $\Delta H°$(g) and $\Delta S°$(g) at 298.15 K (Table 2) were evaluated from the liquid-phase data and the normal boiling temperatures.[1]

DISCUSSION

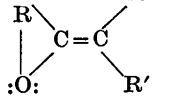
Rotation of the alkoxy group of alkoxyolefins about the C(sp^2) – O bond leads to the following rotamers:



s-*cis* (planar)



s-*trans* (planar)



gauche (nonplanar)

If R′ = H and R is not too bulky, the s-*cis* rotamer is known to be the most stable rotameric form, but if R is bulky or R′ is larger than hydrogen, the relative stability of the other rotamers increases (see, for example, Ref. 6 and the references cited therein). In the present compounds, R″ is always an alkyl group and it seems likely that the s-*trans* rotamer is unfavored owing to considerable crowding between R and R″ in this spatial arrangement. Thus it is evident that if both R′ and R″ are alkyl groups or if R″ is an alkyl group and the alkoxy group is sufficiently bulky, the latter is forced to adopt the *gauche* configuration. The bulkiest alkoxy group in the present compounds is the Et_2CHO group the steric requirements of which

should be approximately equal to those of a *t*-BuO group (the Taft's E_s values [7] are 0.00, −1.54, and −1.98 for Me, *t*-Bu, and Et_2CH, respectively), and it might be expected that the Et_2CHO group should exist mainly in the *gauche* configuration irrespective of the bulkiness of the R′ and R″ groups. If a reaction involves a change in the spatial arrangement of the alkoxy group, the change is reflected in the values of the thermodynamic functions of isomerization. Thus it is convenient to divide the reactions studied into the following groups:

Group A. Reactions involving a formal transfer of a methyl group to the *β trans* position (R‴) with no (significant) change in the spatial orientation of the alkoxy group. Reactions Ib→Ic to IIIb→IIIc and IVa→IVb to VIIIa→VIIIb belong to this group.

Group B. Reactions involving a formal transfer of a methyl group to the *β cis* position (R′). The reactions may or may not involve a change in the arrangement of the alkoxy group: if R is bulky, such as Et_2CH, the *gauche* structure should be predominant in both the reactant and the reaction product, but if R is a small group, such as Me, the orientation of the alkoxy group changes from s-*cis* to *gauche*. This group involves the reactions Ia→Ic to VIIIa→VIIIc.

Group C. Reactions in which two methyl groups are formally transferred to the *β* carbon. The reactions may or may not involve a change in the orientation of the alkoxy group; see Group B. Reactions IXa→IXb to XIIa→XIIb belong to this group.

Reactions of Group A. In the following examples the values of $\Delta H°$(g) and $\Delta S°$(g) for formally similar isomerization reactions are given for R = Me, Et and Et_2CH. The Δ term is used to show the increments in $\Delta H°$ and $\Delta S°$ on going from R = Me to R = Et_2CH.

Reaction	$\Delta H°$(g)/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}
(Series A1)		
Ib→Ic	−2.6	2.7
IIb→IIc	−2.7	3.7
IIIb→IIIc	−1.9	5.0
	Δ = +0.7	Δ = +2.3
(Series A2)		
IVa→IVb	0.5	−1.6
Va→Vb	0.6	−0.6
VIIIa→VIIIb	1.8	0.9
	Δ = +1.3	Δ = +2.5

Thus, (i) in each series, the values of $\Delta H°$ increase by about 1 kJ mol^{-1} and those of $\Delta S°$ by about 2.4 J K^{-1} mol^{-1} on going from R = Me to R = Et_2CH; (ii) the values of $\Delta H°$ are, on average, about 3.4 kJ mol^{-1} more *negative* and those of $\Delta S°$ about 4.2 J K^{-1} mol^{-1} more *positive* in Series A1 than those in Series A2.

The greater exothermicity of the reactions of Series A1 may in part be due to the fact that the reactions involve replacement of an i-Pr group by an Et group whereby the destabilization between R and R″ decreases by about 0.8 kJ mol^{-1}.[2] However, since the difference in total strain between the *c* and *b* structures of Series A1 is likely to be at least equal to that between the *b* and *a* structures of Series A2,[2] it is evident that the *trans* methyl group (R‴) in the *c* isomers of Series A1 stabilizes the double bond more effectively than does the corresponding group in the *b* isomers of Series A2. Accordingly, the double-bond character of the ethylenic linkage in the *c* isomers of Series A1 is higher than that in the *b* isomers of Series A2; this is reasonable if the alkoxy group in the *c* isomer of Series A1 exists in the *gauche* configuration where conjugation of the lone-pair electrons of the oxygen atom with the *π*-electrons of the double bond should be at a minimum.

The positive change in $\Delta H°$ with increasing size of the R group in Series A2 is surprising: from ^{1}H and ^{13}C NMR spectra of alkyl vinyl ethers it has been concluded that conjugation in the vinyloxy group decreases with increasing bulkiness of the alkyl group,[8–10] and hence the double-bond character of the ethylenic linkage and the stabilizing effect of the *β* methyl group should increase (*i.e.* $\Delta H°$ should become more negative) in the same sequence. Thus one might be tempted to draw the conclusion that the Et_2CH group does not adopt the *gauche* configuration if R′ = H, but this is not supported by the data shown below.

Reactions of Group B

Reaction	$\Delta H°$(g)/kJ mol^{-1}	$\Delta S°$(g)/J K^{-1} mol^{-1}
(Series B1)		
Ia→Ic	9.0	22.1
IIa→IIc	8.5	24.0
IIIa→IIIc	2.6	8.7
	Δ = −6.4	$\Delta \cong$ −13

(Series B2)		
IVa→IVc	10.3	11.8
Va→Vc	9.1	12.7
VIIIa→VIIIc	3.6	1.2
	$\Delta = -6.7$	$\Delta \simeq -11$

Thus, (i) the apparent stabilizing effect of the β methyl group in position R′ increases by about 6.5 kJ mol^{-1} in each series on going from R = Me to R = Et_2CH; (ii) the entropy of the *c* isomer (relative to that of the *a* isomer) decreases by about 12 J K^{-1} mol^{-1} in the same sequence; (iii) on average, the values of $\Delta H°$ are about 1 kJ mol^{-1} *less* positive and those of $\Delta S°$ about 10 J K^{-1} mol^{-1} *more* positive in Series B1 than the corresponding values in Series B2.

The changes of $\Delta H°$ and $\Delta S°$ with the bulkiness of the alkoxy group are satisfactorily explained if the configurations of the alkoxy groups are taken as proposed above, *i.e. gauche* for *c* (independent of the size of R), and s-*cis* for *a* in case R = Me, but *gauche* if R = Et_2CH. If we consider the reaction IVa→IVc, the *gauche* structure in IVc gives rise to two enantiomeric isomers with the R group either below or above the plane formed by the group C=C−O, thus increasing the values of $\Delta S°$ by the entropy of mixing, R ln 2$\simeq$5.8 J K^{-1} mol^{-1}. Thus the "chemical" entropy change in IVa→IVc is 11.8 − 5.8 = 6.0 J K^{-1} mol^{-1}. The positive entropy change might be ascribed to increased rotational freedom of the methyl group (in MeO) in the *gauche* structure of IVc. On the other hand, the spatial orientation of the Et_2CHO group in VIIIa→VIIIc remains essentially unchanged and hence the entropy change is small. The enthalpy change in IVa→IVc includes the energy required for the s-*cis* → *gauche* reorientation of the methoxy group while this energy is not included in the enthalpy change for VIIIa→VIIIc, since the configuration of the Et_2CHO remains unchanged in the reaction.

As stated above, the values of $\Delta S°$(g) for the reactions of Series A1 are about 4 J K^{-1} mol^{-1} more positive than those for Series A2, and correspondingly, those for Series B1 about 10 J K^{-1} mol^{-1} more positive than those for Series B2. Obviously, the high values of $\Delta S°$ in Series B1 are due to the fact that in the *a* isomer the rotational freedom of the i-Pr group is restricted by the *cis* methyl group; restricted rotation decreases the entropy of the *a* isomer thus leading to the exceptionally high entropy change in *a*→*c*.[11]

Reactions of Group C

Reaction	$\Delta H°$(g)/kJ mol^{-1}	$\Delta S°$(g)/J K^{-1} mol^{-1}
IXa→IXb	9.5	17.9
Xa→Xb	8.8	18.8
XIa→XIb	5.4	12.5
	$\Delta = -4.1$	$\Delta = -5.4$

The Δ terms are somewhat smaller than might be expected on the basis of the values of the corresponding Δ terms presented above.

It is of interest to consider the effect of R on the values of $\Delta H°$ and $\Delta S°$ for the interconversion of geometric isomers:

Reaction	$\Delta H°$(g)/kJ mol^{-1}	$\Delta S°$(g)/J K^{-1} mol^{-1}
IVb→IVc	9.9	13.4
Vb→Vc	8.5	13.3
VIIIb→VIIIc	1.9	0.3
	$\Delta = -8.0$	$\Delta = -13$

As mentioned above, it is likely that in IVb→IVc the methoxy group is transferred from the s-*cis* to the *gauche* configuration, but in VIIIb→VIIIc the spatial arrangement of the Et_2CHO group remains unchanged. Does this mean that the difference (8 kJ mol^{-1}) in the values of $\Delta H°$(g) for these reactions should represent the energy required for the s-*cis*→*gauche* reorientation of the methoxy group? Obviously not exactly, since from what has been stated above it appears that the double bond of IVc is more effectively (by several kJ) stabilized by the β methyl group than that of IVb, while the double bonds of VIIIb and VIIIc should be equally stabilized by the β methyl group. Hence the enthalpy change in IVb→IVc is several kJ less positive than it would be if there were in the reaction no change in the stabilizing effect of the β methyl group. Accordingly, the difference in the values of $\Delta H°$ for the two reactions in question is several kJ less than the energy required for the s-*cis*→*gauche* reorientation of the methoxy group. Hence a more reasonable value for this process might be around 12 kJ mol^{-1}. In a previous study,[6] the enthalpy change for the s-*cis*→s-

trans reorientation of the methoxy group in methyl 1-propenyl ether was deduced to be about 4 kJ mol^{-1}. For comparison, the SCF calculations of Cadioli and Pincelli [12] suggest that the enthalpies of the s-*trans* and *gauche* rotamers of methyl vinyl ether should be about 8 and 26 kJ mol^{-1}, respectively, higher than the enthalpy of the s-*cis* rotamer.

In many of the reactions studied in this work (especially in VIa→VIc and XIIa→XIIb), considerable changes in the standard heat capacity were observed. An attempt to explain the differences in heat capacity between isomeric vinyl ethers has been published previously.[4]

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Sterically Hindered Benzoic Acid Derivatives

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The base-catalysed hydrolysis of 2,4,6-tribromobenzoyl- and 2,4,6-triiodobenzoyl chloride in 65 % dioxane/water at 5 °C was investigated. The rate constants found were $k_2 = 0.15$ l mol^{-1} min^{-1} and $k_2 = 0.03$ l mol^{-1} min^{-1}, respectively. The behaviour of 2,4,6-triiodobenzoic acid in concentrated sulfuric acid was examined. Simple protonation was shown to occur, corresponding to an *i*-factor of 2. 4-Nitrophenyl 2,4,6-tribromo- and 4-nitrophenyl 2,4,6-triiodobenzoate were hydrolyzed in 56 % dioxane/water at 30 °C and the constants were determined to be $k_2 = 0.469$ l mol^{-1} min^{-1} and 0.070 l mol^{-1} min^{-1}, respectively.

During a search for stable aroyl thiocyanates [1] our attention was focused on 2,4,6-tribromo- and 2,4,6-triiodobenzoic acid. We have shown that the corresponding acid chlorides react smoothly with 1,2,3,4-thiatriazole-5-thiolate in aqueous acetone at 0 °C to yield 5-aroylthio-1,2,3,4-thiatriazole.[1]

However, according to the literature 2,4,6-tribromobenzoyl chloride is a very unreactive acid chloride, surviving boiling aqueous sodium hydroxide for an extended period of time.[2] This observation is surprising since in the same paper a good yield of the methyl ester was obtained by boiling a methanolic solution of the acid chloride. Furthermore, the solvolysis in methanol at 25 °C has been found to proceed with $k = 1.86 \times 10^{-4}$ min^{-1}.[3] From the data in Ref. 2 it appears that the rate of hydrolysis is zero order in 2,4,6-tribromobenzoyl chloride concentration. Since this reaction was carried out heterogeneously we are inclined to believe that these measurements reflect the velocity of solution of the acid chloride in water or aqueous base rather than the rate of hydrolysis.

Solvolysis experiments have now been carried out in mixtures of water and dioxane where the reaction could be made homogeneous. The rate of basic hydrolysis was determined by quenching the reaction with excess hydrochloric acid at various time intervals followed by titrimetric determination of the acid in excess. This method is justified if the rates of neutral or acid-catalyzed solvolysis are negligible compared to the base-catalyzed reaction. This was true both for 2,4,6-triiodobenzoyl and 2,4,6-tribromobenzoyl chloride, whereas, *e.g.*, 4-nitro-2,6-dimethylbenzoyl chloride reacts so fast in basic as well as neutral and acidic media, that this method was not justified.[4] Another limitation of this method is that the reactions studied should be slow enough to allow concentration determinations early in the reaction. In this work the latter restriction could be overcome by running the reaction at 5 °C.

We found that both above-mentioned acid chlorides upon base catalyzed hydrolysis in 65 % (V/V) dioxane at 5±1 °C displayed second order kinetics. The values found were $k_2 = 0.15$ l mol^{-1} min^{-1} for 2,4,6-tribromobenzoyl chloride and $k_2 = 0.03$ l mol^{-1} min^{-1} for 2,4,6-triiodobenzoyl chloride. As would be expected for steric reasons the rate constant of 2,4,6-tribromobenzoylchloride has a higher value than the one of 2,4,6-triiodobenzoyl chloride.

Having established the S_N2 reactivity of these compounds we turned to investigate the possible S_N1 reactivity. Formation of 2,4,6-triiodobenzoylium ion from any derivative of this species would result in steric relief. Furthermore the inductive effect of the iodine atoms, which presumably would retard the development of a positive charge on the carbonyl carbon in the transition state, is likely to be small (according

to Pauling the electronegativity of I is 2.5 which is the same as that of C). Mesomeric effects of the iodine atoms would tend to stabilize the developing charge in the transition state.

To establish whether 2,4,6-triiodobenzoylium ion is formed from the parent acid in concentrated sulfuric acid, we have carried out cryoscopic measurements in this solvent.[5] Within the experimental uncertainty van't Hoff's *i*-factor never exceeded 2, even after 47 h at room temperature, meaning that only simple protonation occurs. Chemical evidence substantiated the physical measurements. When the solutions of 2,4,6-triiodobenzoic acid in concentrated sulfuric acid were poured into cold methanol, no trace of the methyl ester could be detected in the product, which always consisted of the pure acid.

Esters of 2,4,6-trihalobenzoic acid are readily accessible from the reaction of the acid chlorides and alkoxide or phenoxide ions. These esters are stable under neutral and acid conditions. Under basic conditions, however, they are hydrolyzed, alkyl esters slowly but aryl esters easily. If another alcohol is present transesterification occurs. If, *e.g.*, methyl 2,4,6-triiodobenzoate is dissolved in ethanol containing ethoxide ions, ethyl 2,4,6-triiodobenzoate appears in the reaction mixture (60 % after 24 h at room temperature), or if the 2-octyl ester is recrystallized from methanol containing traces of strong base, the ester is partly transformed to the methyl ester.

The hydrolysis at 30 °C of 4-nitrophenyl 2,4,6-triiodobenzoate and 4-nitrophenyl 2,4,6-tribromobenzoate in 56 % (V/V) dioxane with hydroxide ions obeys second order kinetics.[6] The rate determination were run under pseudo-first-order conditions. The rate was followed by continuous UV recording of the absorption at 406 nm, which is λ_{max} for the 4-nitrophenoxide ion in 56 % dioxane. In both cases the reaction mixtures exhibit isosbestic points, 4-nitrophenyl 2,4,6-tribromobenzoate at 238 nm and 316 nm, and 4-nitrophenyl 2,4,6-triiodobenzoate at 300 nm. From a plot of the observed pseudo-first-order rate constants *versus* hydroxide ion concentration at 30 °C the second-order rate constant was evaluated to be $k_2 = 0.469$ l mol^{-1} min^{-1} for 4-nitrophenyl 2,4,6-tribromobenzoate (initial concentration $= 0.945 \times 10^{-4}$ mol l^{-1}) and $k_2 = 0.070$ l mol^{-1} min^{-1} for 4-nitrophenyl 2,4,6-triiodobenzoate (initial concentration 0.084×10^{-4} mol l^{-1}). Using the same technique, we found that in 1 N hydrochloric acid and in neutral solution no hydrolysis has occurred even after 4 days.

EXPERIMENTAL

Hydrolyses of acid chlorides (neutral and acid conditions). Approximately 5×10^{-2} M solutions of 2,4,6-tribromobenzoyl or 2,4,6-triiodobenzoyl chloride in 70 % (V/V) dioxane were left for 120 min at room temperature. Titration with 0.1 M sodium hydroxide showed no base consumption. In the case of the iodo compound even after 22 h at room temperature the base consumption was negligible. Similar results were found for the reaction in acid media. A solution of 4-nitro-2,6-dimethylbenzoyl chloride (5.26×10^{-4} M in 65 % dioxane) released hydrochloric acid so fast that the titration procedure could not be used. This was also the case in acid solution.

Hydrolyses of acid chlorides (basic conditions). About 4 mmol of the acid chloride was dissolved in 65 ml of dioxane. 20 ml of a 0.5000 N sodium hydroxide solution was added, and the solution was made up to 100 ml with water. The temperature of the reaction mixture was kept at 5 °C. The rate was followed by adding 5 or 10 ml aliquots of the solution to 20 ml of 0.1000 N hydrochloric acid. Back titration determined the consumption of sodium hydroxide in the hydrolysis. By means of these data, the concentration of base and acid chloride was determined. Second-order rate constant was evaluated from the plot of $\ln[(B_0-x)/(A_0-x)]$ against time. B_0 and A_0 are initial base and acid chloride concentrations, respectively.

Cryoscopic technique. Cryoscopic measurements were carried out as described by Newman *et al.*,[5] using the same type of freezing-point cell. An ordinary Beckmann mercury thermometer was used, and stirring was effected by means of a magnetic stirrer.

4-Nitrophenyl 2,4,6-trihalobenzoate. To a solution of 5 mmol of 2,4,6-trihalobenzoyl chloride in 100 ml of acetonitrile was added a solution of 8 mmol (1.43 g) of sodium 4-nitrophenolate in 100 ml of the same solvent. The reaction mixture was refluxed for 1 h. After filtration and evaporation of the solvent, the crude product was dissolved in diethyl ether. The solution was washed several times with water, dried over sodium sulfate, and evaporated. Recrystallization from ether gave the pure product.

4-Nitrophenyl 2,4,6-tribromobenzoate. Yield: 75 %. M.p.: 132–133 °C. (Found: C 32.62; H 1.40; N 2.87; Br 49.75. Calc. for $C_{13}H_6NO_4Br_3$: C 32.52; H 1.25; N 2.92; Br 49.97). UV: $\lambda_{max} = 265$ nm, $\varepsilon = 3.2 \times 10^3$ (60 % dioxane/water).

4-Nitrophenyl 2,4,6-triiodobenzoate. Yield: 71 %. M.p.: 153.5–154.5 °C. (Found: C 25.05; H 1.01; N 2.17; I 61.20. Calc. for $C_{13}H_6NO_4I_3$: C 25.15; H 0.97; H 2.25; I 61.35). UV: $\lambda_{max}=239$ nm, $\varepsilon=2.3\times10^4$. $\lambda_{max}=264$ nm (shoulder), $\varepsilon=1.1\times10^4$ (60 % dioxane/water).

Basic hydrolysis of 4-nitrophenyl 2,4,6-trihalobenzoate in 56 % (V/V) dioxane. Procedure for kinetic runs. Stock solutions of the ester (approx. 1×10^{-4} M) and sodium hydroxide were prepared in 56 % (V/V) dioxane. Twenty ml of the ester stock solution was mixed with various amounts of the sodium hydroxide and the volume was made up to 25 ml. The solution was then approx. 0.8×10^{-4} M in ester and 0.2–0.02 M in sodium hydroxide. The rate of hydrolysis was followed at 30 °C by continuous measurements of the absorbance at 406 nm. Plots of log $(A_\infty-A)$ *vs.* time were always linear. From the slope of these curves, pseudo-first-order rate constants were determined. The second-order rate constant was obtained from the slope of a plot of the observed pseudo-first-order constant *vs.* hydroxide concentrations. The apparatus used was a UNICAM SP 1800 UV-VIS Spectrophotometer.

Trans-esterification of methyl 2,4,6-triiodobenzoate. A 5 % solution of methyl 2,4,6-triiodobenzoate in ethanol containing 0.2 N ethoxide ion was allowed to stand for 24 h. NMR showed that about 60 % of the methyl ester was converted to the ethyl ester.

Trans-esterification of 4-nitrophenyl 2,4,6-triiodobenzoate. When a 5 % solution of this ester in ethanol containing 0.1 N KOH was allowed to stand for 24 h, NMR analysis showed complete conversion to the ethyl ester.

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A Study of ^{13}CH Coupling Constants in Pentopyranoses and Some of their Derivatives

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Proton decoupled and undecoupled ^{13}C NMR spectra were measured on the D-pentopyranoses and a number of their derivatives. The direct coupling constants between the anomeric carbon atoms and protons $^1J(^{13}CH1)$ were found to be *ca.* 10 Hz lower when H1 was axial than when it was equatorial. The $^1J(^{13}CH1)$ values may therefore be used for the determination of conformational equilibria. Chemical shifts and $^1J(^{13}CH1)$ values of the other carbon atoms in the pyranose rings were also measured.

Directly bonded ^{13}CH coupling constants were reported previously for a number of hexopyranoses and it was found that the $^1J(^{13}CH1)$ value is consistently *ca.* 10 Hz lower when H1 is axial than when it is equatorial.[1,2] These coupling constants may therefore be used for the assignment of anomeric structure.

It was of interest to see whether pentopyranoses and their derivatives have similar $^1J(^{13}CH1)$ values and, especially, whether these constants can be used as a supplement to proton-proton coupling constants for the determination of conformational equilibria. We have therefore measured proton decoupled and undecoupled ^{13}C NMR spectra of a number of pentopyranoses and of some of their derivatives; their chemical shifts and coupling constants are shown in Table 1.

Some of the chemical shifts shown in Table 1 differ slightly from those reported previously. This is probably due to the use of internal references in the present work whereas many previous workers used external references. The assignments shown in Table 1 were in some cases confirmed by selective proton decoupling.[3] This method works well with compounds which have reasonable well resolved proton spectra, such as acetylated sugars. In other cases assignments could be made from the proton undecoupled ^{13}C spectra since C2 in most cases gives a less complicated signal than C3. This is probably due to the fact that $^3J(CH1)$ values are considerably larger than $^2J(CH)$ values [4] and C2 has only one proton (H4) three bonds away, whereas C3 has three protons (H1 and H5) in this position.

The $^1J(^{13}CH1)$ values of the D-xylopyranoses (α- and *β-1a*) and of the methyl D-xylosides (α and *β-1b*) were found to be 158 – 160 Hz for the β-anomers and 170 Hz for the α-anomers. Slightly higher values were found for the acetylated methyl xylosides (α- and *β-1c*), in agreement with previous results.[1] All these xylopyranose derivatives are almost completely in the $^4C_1$ conformation [5–7] and thus the β-anomers have an axial H1 and the α-anomers an equatorial H1. The $^{13}C1H1$ coupling constants are therefore in agreement with those found in the hexose-series.[1]

Spectra of the 2,4-phenylboronates derived from (α- and *β-1b*) were also measured. These two compounds must necessarily adopt the $^1C_4$ conformation and this is seen clearly from the $^1J(^{13}CH1)$ values (Table 1).

Tetra-*O*-acetyl-α- and β-D-xylopyranose (α- and *β-1d*) have $^1J(^{13}CH1)$ values of 177 and 166 Hz, respectively. These values are very close to those of the homomorphous α- and β-D-glucopyranose pentaacetates [2] indicating that *α-1d* and *β-1d* are almost completely in the $^4C_1$ conformation in chloroform solution. If *β-1d* adopted the $^1C_4$ conformation it would be similar to penta-*O*-acetyl-α-D-idopyranose which has $^1J(^{13}CH1)$ 175 Hz (Table 2). In acetone-d_6

Table 1. Chemical shifts (ppm) and observed first-order coupling constants (± 1 Hz) of pentopyranoses and some derivatives in D_2O or in deuteriochloroform solution.

Compound	C1 $^1J_{CH1}$	C2 $^1J_{CH2}$	C3 $^1J_{CH3}$	C4 $^1J_{CH4}$	C5 $^1J_{CH5}$	OMe $^1J_{CH}$	Conformational equilibrium % ± 10 % $^4C_1$	$^1C_4$	Ref.
α-*1a*	93.08 170	72.38 147.5	73.75 145	70.28 147	61.81 142.5 151		100		10,11,12,13
β-*1a*	97.51 160.5	74.96 147.5	76.74 145	70.12 147	66.01 142.5 150.0		>90		10,11,12,13
α-*1b*	100.29 170	72.29 146	74.20 145	70.31 146	61.90 145 148	56.01 143		100	10,11,12,14
α-*1b* phenylboronate [16] in DMSO-d_6	98.50 159	70.34 [a] 154	65.17 [a] 148	68.21 [a] 151	64.65 145	56.17 143		100	
β-*1b*	104.82 159	73.88 144	76.72 144	70.12 147	66.00 142 150	57.82 142	100		10,11,12,14
β-*1b* phenylboronate [16] in $CDCl_3$	100.2 171	68.9 [a] 156	64.9 154	66.3 [a] 155	58.6 145	55.7 143		100	
β-*1b* phenylboronate [16] in DMSO-d_6	99.93 170	68.92 154	64.20 150	67.69 157	58.24 145	53.33 142		100	
α-*1c*	96.40 171.0	70.46 153	69.10 155 [b]	68.77 155 [b]	57.66 147.5 [b]	54.73 142	100		15,[c]
β-*1c*	100.95 161.0	70.21 153	70.99 152	68.32 153	61.30 143 151	55.84 144	100		[c]
α-*1d*	88.94 177	69.20 153	69.20 153	68.84 152	60.52 148 [b]		100		
β-*1d*	91.72 166	69.33 155	70.75 156	68.10 153	62.53 146 151		100		
α-*2a*	97.64 160.0	72.83	73.34	69.34	67.14			>90	10,11,12,13
β-*2a*	93.44 168.0	69.53	69.53	69.34	63.30 142.5 150		20	80	10,11,12,13
α-*2b*	104.75 160	71.61 148	73.29 143	69.15 146	66.95 140 150	57.82 145		>90	10,11,14

Table 1. Continued.

β-2b	100.74	69.80	69.80	69.15	63.39	56.07			10,11,14
	169	145 [b]	145 [b]	145	142 149	143	10	90	
α-2c	101.91	69.28	70.38	67.92	63.24	56.61			[c]
	159	153	148	152	140 150	143		100	
β-2c	97.62	68.43	69.28	67.20	60.25	55.44			[c]
	171	153	152.5	152	144.5 151.5	143		100	
α-2d	92.16	68.24	69.86	67.26	63.82				
	168	156	149	150	147.5		20	80	
β-2d	90.40	67.27	68.70	66.94	62.85				
	176	154 [b]	153	154 [b]	145 152			100	
α-3a	94.92	70.96	71.41	68.40	63.88				12,13
	167	146	146	147.5	147.5		75	25	
α-3b	101.97	70.44	71.61	67.72	63.25	55.88			
	169	145	142	147	144 150	143	90	10	
α-3c	98.41	69.26	68.23	66.57	59.42	54.93			
	170.5	156	153	154	147 149	143.5	100		[c]
α-3d	90.66	68.24	68.24	66.55	61.94				[c]
	175	155	155	154	146 151		100		
β-3d	90.0								
	169.5						60	40	
β-4a	94.73	71.92	69.69	68.18	68.83				10,12,13,14
	164	146	175	146	147.5		70	30	
β-4a with 2N $CaCl_2$	94.76	70.89	69.82	67.34	63.53				
	168	146	146 [b]	146 [b]	147		10	90	
α-4b	100.41	69.18	70.41	67.40	60.78	56.65			
	167.5	145	146	146.5	146	144	30	70	
β-4b	103.07	71.0	68.6	68.6	63.9	57.0			10,12,14
	166	146	146	146	147	143	50	50	
β-4b with 2 N $CaCl_2$	101.87	70.15	68.50	66.76	63.56	56.58			
	170	148	147.5	148	146	144	10	90	
α-4c	97.49	67.52	67.39	66.09	57.91	56.22			[c]
	166	146.5	146.5	148	147	143	50	50	

Table 1. Continued.

β-4c	99.37	68.31	65.97	66.88	61.09	55.70			*c*
	168	153	149	151	145 151	143	30	70	
α-4d	88.71	67.14	65.58	66.49	59.27				*c*
	173	154	148	147	149		70	30	
β-4d in $CDCl_3$	90.68	67.13	66.03	66.03	62.45				
	174.5	152	152 [b]	152 [b]	~150 [b]		10	90	
β-4d in acetone-d_6	91.14	67.48	66.38	66.38	62.61				
	174	152	152 [b]	152 [b]	150 [b]		10	90	

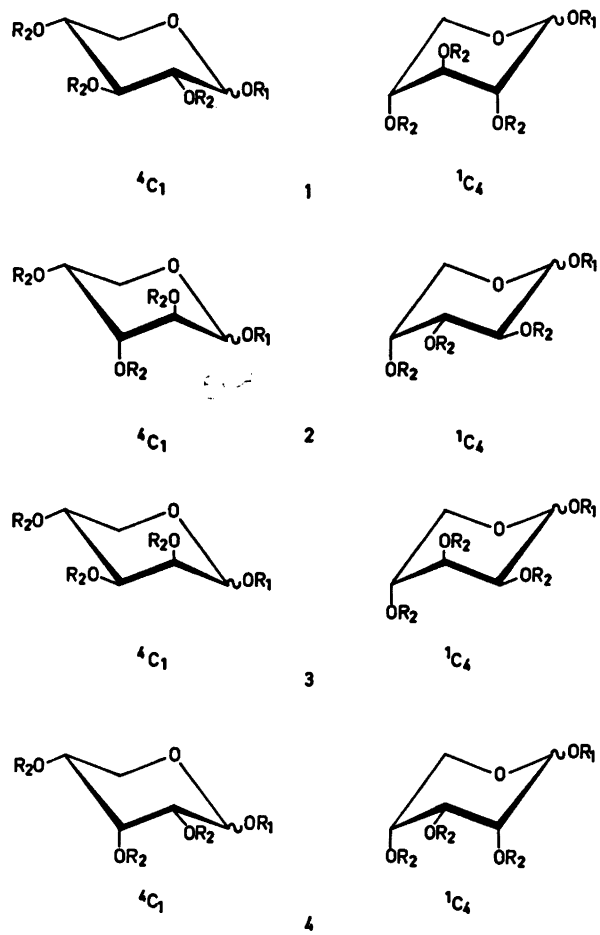
[a] Assignment may be reversed. [b] Measured from center of broad multiplet. [c] Assigment confirmed by selective proton decoupling.

solution (*α-1d*) is reported to be more than 98 % in the $^4C_1$ conformation whereas (*β-1d*) is only 72 % in this conformation.[8]

The D-arabinopyranoses (α- and *β-2a*) and the methyl D-arabinosides (α- and *β-2b*) have ^{13}C1H1 coupling constants of 160 Hz for the α-anomers and 168 – 169 Hz for the β-anomers in aqueous solution. Comparison of these values with those described above and previously [1] show that the four compounds are more than 90 % in the $^1C_4$ form. This has also been shown for the α-series from proton spectra whereas the conformation of *β-2a* was uncertain.[5,6] The acetylated methyl D-arabinopyranosides (α- and *β-2c*) adopt the same conformation in chloroform. The 168 Hz found for tetra-*O*-acetyl-α-D-arabinopyranose (*α-2d*) may be compared to the values found for acetylated hexoses such as penta-*O*-acetyl-*β*-D-galactopyranose with an axial H1 (J 167 Hz) and penta-*O*-acetyl-α-D-altropyranose with an equatorial H1 (J 175.5 Hz) (Table 2). This would indicate that *ca.* 20 % of *α-2d* is in the $^4C_1$ form, in agreement with results found from proton spectra in acetone.[8] The β-anomer (*β-2d*) has $^1J(^{13}CH1)$ 176 Hz, very close to that of acetylated α-D-hexopyranoses (Table 2).[1] It must therefore have an equatorial H1 and be almost exclusively in the $^1C_4$ form.[8]

The $^1J(^{13}CH1)$ value of 167 Hz for α-D-lyxopyranose (*α-3a*) is 2 – 3 Hz too low for a conformation with an equatorial H1; (*α-3a*) must therefore be a 3:1 mixture of $^4C_1$ and $^1C_4$ form. Lemieux and Stevens [5] found from proton spectra that equal amounts of the two conformers were present. Methyl α-D-lyxopyranoside (*α-3b*) and its triacetate (*α-3c*) have coupling constants corresponding to higher proportions of $^4C_1$ form. A solution of (*α-3c*) in acetone has been found to contain 83 % $^4C_1$ form.[8] Tetra-*O*-acetyl-α-D-lyxopyranose (*α-3d*) has $^1J(^{13}CH1)$ 175 Hz which, compared to 176 Hz for α-D-mannopyranose pentaacetate,[2] shows that it is completely in the $^4C_1$ conformation. The β-anomer (*β-3d*) with J 169.5 Hz must be a *ca.* 4:6 mixture of $^1C_4$ and $^4C_1$ forms. Using acetone as solvent Durette and Horton [8] found 60 % $^1C_4$ for *β-3d* and 70 % $^4C_1$ for *α-3d*.

An aqueous solution of β-D-ribopyranose (*β-4a*) has $^1J(^{13}CH1)$ 164 Hz, *i.e.* 3 Hz higher than that of β-D-allopyranose (Table 2). It is therefore reasonable to assume that *β-4a* is a mixture of *ca.* 30 % $^1C_4$ and 70 % $^4C_1$ form in agreement with the results obtained from ^{1}H NMR.[5,7] Methyl β-D-ribopyranoside with J 166 Hz is approximately a 1:1 mixture of the two chair forms whereas the α-anomer (*α-4b*) contains *ca.* 70 % $^1C_4$ form. The coupling constant of the acetylated methyl α-D-ribopyranoside (*α-4c*) in chloroform corresponds to a 1:1 mixture of the chair forms whereas the β-anomer (*β-4c*)



a) $R_1 = R_2 = H$
b) $R_1 = CH_3, R_2 = H$
c) $R_1 = CH_3, R_2 = CH_3CO$
d) $R_1 = R_2 = CH_3CO$

has 70 % $^1C_4$ form. These values agree fairly well with those found in acetone by proton spectroscopy.[8]

When calcium chloride was added to aqueous solutions of β-D-ribopyranose (*β-4a*), or methyl β-D-ribopyranoside (*β-4b*), the $^1J(^{13}CH1)$ values increased by 4 Hz corresponding to a conformational change towards *ca.* 90 % $^1C_4$ form. This is in complete agreement with the results found by Angyal and Davies from proton spectra.[9]

Tetra-*O*-acetyl-β-D-ribopyranose (*β-4d*) has $^1J(^{13}CH1)$ 174.5 Hz and must therefore largely adopt the $^1C_4$ conformation. Penta-*O*-acetyl β-D-allopyranose, with an axial H1, has $^1J(^{13}CH1)$ 10 Hz lower wheras penta-*O*-acetyl-α-D-talopyranose, with an equatorial H1, has *J* 177 Hz (Table 2).

Durette and Horton found that *β-4d* contains approximately equal amounts of the two chair form in acetone solution.[8] An undecoupled ^{13}C NMR spectrum of *β-4d* in acetone-d_6 gave $^1J(^{13}CH1)$ 174 Hz, indicating that *ca.* 80 % of *β-4d* is in the $^1C_4$ form. The difference between the results obtained from proton spectra and from ^{13}C spectra could be explained by assuming that the chair form is distorted. This would probably not affect the $^{13}CH1$ coupling constant,

Table 2. Chemical shifts (ppm) and observed first-order coupling constants (± 1 Hz) of hexopyranoses in D_2O or in deuteriochloroform solution.

Compound	C1 $^1J_{CH1}$	C2 $^1J_{CH2}$	C3 $^1J_{CH3}$	C4 $^1J_{CH4}$	C5 $^1J_{CH5}$	C6 $^1J_{CH6}$	Ref.
Penta-*O*-acetyl-α-D-idopyranose	90.36 175	65.90 [a] 152 [b]	66.15 [a] 152 [b]	65.90 152 [b]	66.15 152 [b]	61.75 149	
Penta-*O*-acetyl-β-D-galactopyranose	91.79 167	67.84 156	70.56 147	66.80 153	71.46 143	60.98 150	
Penta-*O*-acetyl-α-D-altropyranose	90.23 175.5	68.16 [a] 155	66.41 [a] 155 [b]	66.41 155 [b]	64.41 148	62.14 149	
β-D-Allopyranose	94.28 161	72.15 142	72.02 148	67.69 145	74.41 150 [b]	62.10 145	12,13
Penta-*O*-acetyl-β-D-allopyranose	90.14 165	68.24 155 [b]	68.24 155 [b]	65.83 145	71.16 148	61.94 149	
Penta-*O*-acetyl-α-D-talopyranose	91.44 177	65.19 [a] 151 [b]	66.29 [a] 154	65.32 [a] 151 [b]	68.83 145	61.48 149	

but the proton-proton coupling constants would be different from those expected for an ideal chair and might therefore not give correct information about the conformational equilibrium.

The two protons at C5 in a pentopyranose are in a position similar to that of H1 with regard to the electrons at the ring-oxygen, and it might be expected that $^1J[^{13}CH(5ax)]$ should be smaller than the corresponding value for H5eq. This was actually found in many cases as seen from Table 1. The couplings between C5 and H5ax were *ca.* 142 Hz whereas the corresponding values for H5eq were found to be *ca.* 150 Hz in free sugars and methyl glycosides. Acetylated pyranoses gave slightly higher values. In compounds which contain appreciable amounts of both chair forms only an average value for $^1J(^{13}CH5)$ could be measured. It should, however, be remembered that because of the complexity of the undecoupled ^{13}C NMR spectra the C5H5 coupling constants cannot always be measured accurately on a first-order basis.

In hexopyranoses and derivatives thereof H5 is usually axially oriented and consequently a $^1J(^{13}CH5)$ value of *ca.* 145 Hz would be expected. This was observed in many cases, but in some cases a higher value (*ca.* 150 Hz) was found,[1] possibly because a wrong signal was assigned to C5. Some of the previous assignments in the hexose series were therefore reassigned using selective proton decoupling.[3] This gave the following revised results (*cf.* Table 1 in Ref. 1): methyl tetra-*O*-acetyl-α-D-glucopyranoside; C2 70.4; C3 69.7; C4 68.2; C5 66.8 ppm. [$^1J(^{13}CH5)$ 145 Hz]; methyl tetra-*O*-acetyl-α-D-galactopyranoside; C2 and C3 67.6; C4 67.0; C5 65.7 ppm [$^1J(^{13}CH5)$ 143 Hz]; methyl tetra-*O*-acetyl-α-D-mannopyranoside; C2 69.1; C3 68.8; C4 65.8; C5 68.0 ppm. [$^1J(^{13}CH5)$ 148 Hz]; methyl tetra-*O*-acetyl-β-D-glucopyranoside; C2 70.9; C3 72.5; C4 68.1; C5 71.4 ppm. [$^1J(^{13}CH5)$ 140 Hz].

EXPERIMENTAL

Spectra were obtained as previously described.[1] Unacetylated compounds were measured in D_2O, acetylated compounds in deuteriochloroform unless otherwise stated. The solutions were *ca.* 20 %. Free sugars were measured as mutarotated solutions.

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Syntheses and Conformations of Monomeric and Dimeric Cyclic Dimethylketals

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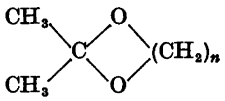
The syntheses of dimeric cyclic ketals of ring size 12 – 22 are described.

The monomeric 9 – 12 membered cyclic ketals were unstable and could not be isolated unless substituted with *gem*-dimethyl groups in conformationally stabilizing positions.

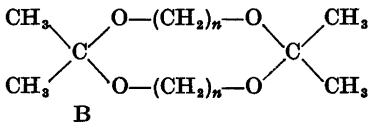
In contrast to the corresponding formals, long chain polymers were not formed, probably because of the *gem*-dimethyl-effect of the 2,2-dimethyl-1,3-dioxa group.

The 12-membered cyclic ketal was shown by low temperature NMR to take a square conformation. Three-bond bridged rectangular conformations were assumed for the dimeric cyclic ketals, with the 1,3-dioxa group +*gauche*+*gauche* across corners.

Cyclic compounds with *gem*-dimethyl groups in the 2-position of 1,3-dioxa systems, A and B, were of interest for conformational studies. We



A



B

were also interested in the effect exercized by *gem*-dimethyl groups in other positions of cyclic ketals on their conformations and synthetic yield.

For the preparation of most of the cyclic ketals the following method was used: a mixture of a diol and an acid ion exchange resin in benzene was boiled with equivalent amounts of dimethoxypropane, and an azeotropic mixture of methanol and benzene was distilled out.

The yields and the melting or boiling points of the monomeric and dimeric cyclic ketals prepared, and analytical data of new compounds are shown in Table 1. From propane-1,3 and butane-1,4 diol only the monomeric cyclic ketals were formed by this method, as expected because of the general tendency for formation of 6- and 7-membered rings. The dimeric cyclic ketal of butanediol, a 14-membered ring, was therefore synthesized from but-2-yne-1,4-diol by the same ketalization method. Formation of the 7-membered ring is then excluded because of the triple bond. The mixture of oligomers of the acetylenic cyclic ketal was hydrogenated and the saturated 14-membered ring isolated by distillation. This compound has now also been synthesized by oligomerisation of 2,2-dimethyl-1,3-dioxepan.[1] The monomeric and dimeric cyclic ketals of pentane-1,5-diol have been described earlier.[2]

We did not succeed in preparing the 9-, 10- and 11-membered cyclic ketals by the general method, nor was it possible to prepare them from the dimeric compounds by sublimation or by pyrolysis with p-toluenesulfonic acid. The 12-membered ring monomer from nonane-1,9-diol was most probably formed, but hydrolyzed immediately. However, with *gem*-dimethyl groups in the 6- and 9-positions even the 11-membered cyclic ketal was stable. With *gem*-dimethyl groups in the 6- and 10-positions the 12-membered ring was unstable, and only the dimeric compound was isolated.

The dimeric cyclic ketal of propane-1,3-diol was obtained in a small yield by oligomerization of 2,2-dimethyl-1,3-dioxane with boron trifluoride.

Table 1.

Diol	Homologue	[a]	M.p. (b.p.) °C/mmHg	Yield %	Analytical data Formula	Calc. C	H	Mol. wt.	Found C	H	Mol.wt. osm.
Propane-1,3-diol	Monomer	6	124/760	~35							
Propane-1,3-diol	Dimer [b]	12	115	2	$C_{12}H_{24}O_4$	62.04	10.41	232	62.77	10.02	231
2,2-Dimethylpropane-1,3-diol	Monomer	6	35/15	~80							
Butane-1,4-diol	Monomer	7	137/760	~30							
But-2-yne-1,4-diol (Hydrogenated ketal)	Dimer	14	145	3							
Hexane-1,6-diol	Dimer	18	106	34	$C_{18}H_{36}O_4$	63.31	11.47	316	68.19	11.28	316
Heptane-1,7-diol	Dimer	20	99	5	$C_{20}H_{40}O_4$	69.72	11.70	344	69.97	11.55	343
Octane-1,8-diol	Dimer	22	106	30	$C_{22}H_{44}O_4$	70.92	11.90	372	71.16	11.80	372
3,3,6 6-Tetramethyloctane-1,8-diol [14]	Monomer	11	~106/14	~20	$C_{15}H_{30}O_2$			242			265
3,3,6,6-Tetramethyloctane-1,8-diol	Dimer	22	135	8	$C_{30}H_{60}O_4$	74.32	12.48	484	74.52	12.40	478
3,3,7,7-Tetramethylnonane-1,9-diol [15]	Dimer	24	70	8	$C_{32}H_{64}O_4$	74.94	12.58	512	74.38	12.44	517

[a] Number of ring atoms of ketal. [b] Prepared by dimerization of the monomer.

Table 2.

Cyclic ketal	Number of ring atoms	Dipole moment in benzene (D)	ΔH_m (kcal/mol)	ΔS_m (e.u.)	Infrared spectrum in CCl_4-solution as compared with solid
2,2,6,6-Tetramethyl-1,3-dioxan	6	1.7	2.6	10.4	Some small differences
2,2-Dimethyldioxepan	7		2.9	12.6	Identical
2,2,6,6,9,9-Hexamethyl-1,3-dioxacycloundecane	11				
2,2,8,8-Tetramethyl-1,3,7,9-tetraoxacyclododecane	12		5.6	14.6	Identical
2,2,9,9-Tetramethyl-1,3,8,10-tetraoxacyclotetradecane	14	0	7.3	17.8	Only intensity differences
2,2,11,11-Tetramethyl-1,3,10,12-tetraoxacyclo-octadecane	18	0.6	8.4	22.5	Fairly similar
2,2,12,12-Tetramethyl-1,3,11,13-tetraoxacycloeicosane	20	0	10.9	29.5	Crystal conf. + others in solution
2,2,13,13-Tetramethyl-1,3,12,14-tetraoxacyclodocosane	22	0	14.8	39.5	Different
2,2,6,6,9,9,13,13,17,17,20,20-Dodecamethyl-1,3,12,14-tetra-oxacyclodocosane	22	0	13.7	33.7	Fairly similar
2,2,6,6,10,10,14,14,18,18,22,22-Dodecamethyl-1,3,13,15-tetra-oxacyclotetracosane	24	0	9.5	27.7	Different

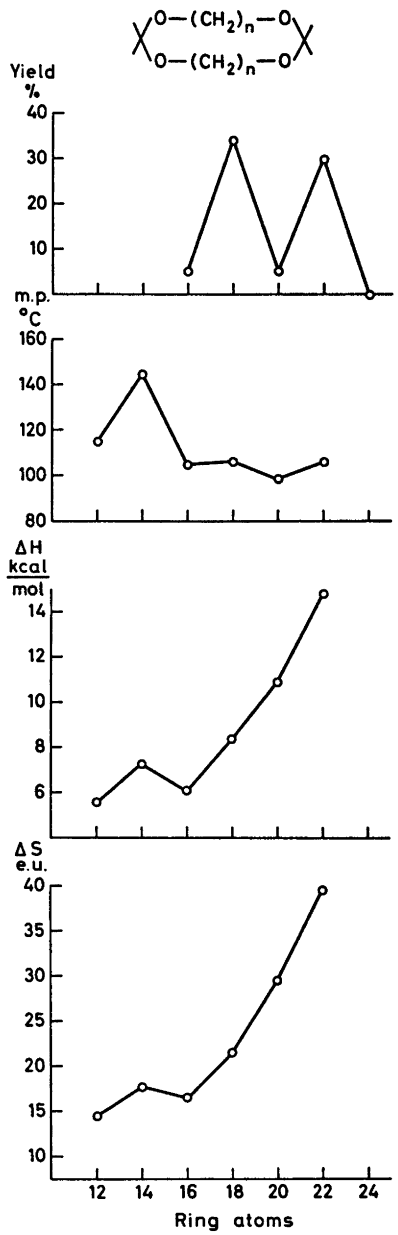


Fig. 1. Yields, melting points, enthalpies and entropies of fusion of dimeric cyclic dimethyl ketals.

The dimeric cyclic dimethyl ketals of butane-, pentane-,[2] hexane-, heptane- and octane-α,ω-diols are crystalline, stable compounds. Solid polymers were not formed, to the contrary of what is described for the corresponding formals.[2]

The physical properties of the cyclic ketals were investigated by IR and NMR-spectroscopy, dipole moments and differential calorimetry. The results are given in Table 2.

For the unsubstituted dimeric ketals, the values for the melting point and enthalpy and entropy of fusion are compared graphically in Fig. 1. Also given are the yields for those compounds that are synthesized by the same method and are therefore comparable.

DISCUSSION

The 2,2-dimethyl-1,3-dioxepan had identical IR-spectra in the solid phase (below −37 °C) and as liquid; this indicates a single low-energy conformation, the same in both phases. For 1,1,-dimethylcycloheptane the lowest-energy conformation is found by calculations [4,5] to be a twist-chair with isoclinal methyl groups. Most probably this is also the conformation for 2,2-dimethyl-1,3-dioxepan (Fig. 2). The NMR-spectra of this compound at −130 °C indicate the expected equivalence of the methyl groups.

For cycloundecane Dale [6] has found by semi-quantitative calculations a low-energy triangular conformation which can accommodate *gem*-dimethyl groups in the 1 and 4 positions. Also on the third corner of this conformation, position 8, *gem*-dimethyl groups may be accommodated without steric hindrance. These corners are the substitution positions for 1,3-dioxa-2,2,6,6,9,9-hexamethylcycloundecane as shown in Fig. 3 and the conformation therefore possible for this compound. However, low-temperature spectra at −130 °C did not indicate equivalence of the methyls in the dioxa group and could not prove this assumption.

A 5,5,9,9-tetramethyl-substituted 12-membered cyclic ketal does not suit any of the

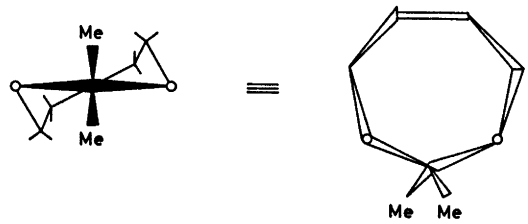


Fig. 2. Conformation of 2,2-dimethyl-1,3-dioxepan.

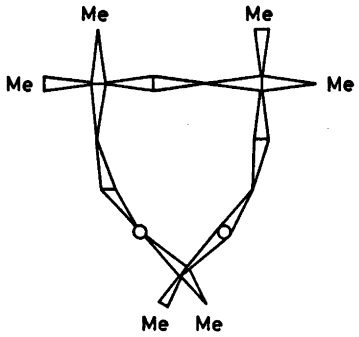


Fig. 3. Conformation of 1,3-dioxa-2,2,6,6,9,9-hexamethylcycloundecane.

calculated low-energy conformations,[6] and so it may be understood why it is not formed. On the other hand, for the monomeric 12-membered cyclic ketal from unsubstituted nonane-1,9-diol there should exist a low-energy square conformation, but we did not succeed in isolating this molecule.

The 1,3-dioxa-group is known to prefer a +*gauche*+*gauche* conformation across a "corner", as found by NMR in 1,3,7,9-tetraoxacyclododecane[7] and by X-ray structure analysis in 1,3,8,10-tetraoxacyclotetradecane.[8] For the dimeric ketals, with *gem*-dimethyl groups in position 2 this tendency should be even more pronounced because the *gem*-dimethyl groups in large rings generally prefer corner positions because of their steric requirements.[9] All the dimeric cyclic ketals should therefore prefer to have the oxygens across corners. This is clearly demonstrated for the new compound 2,2,8,8-tetramethyl-1,3,7,9-tetraoxacyclododecane. The IR spectra were identical in the solid phase and in solution (Fig. 4), and this indicates high conformational stability. The NMR spectra (Fig. 5) show a single peak for the methyl groups which persists by lowering the temperature to −100 °C, as expected when the methyl groups are on corners of the square conformation, Fig. 6A. For the β-hydrogens, also on corners of the square conformation and therefore equivalent, no broadening occurs in the low temperature spectrum, only the small changes in the coupling expected because of the splitting

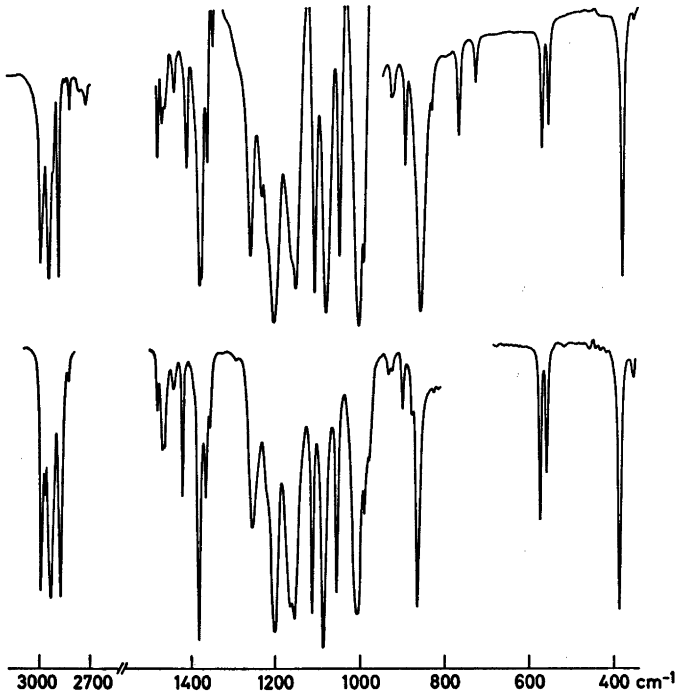


Fig. 4. Infrared spectra of 2,2,8,8-tetramethyl-1,3,7,9-tetraoxacyclododecane as solid in KI (top) and in solution in CCl_4 (bottom).

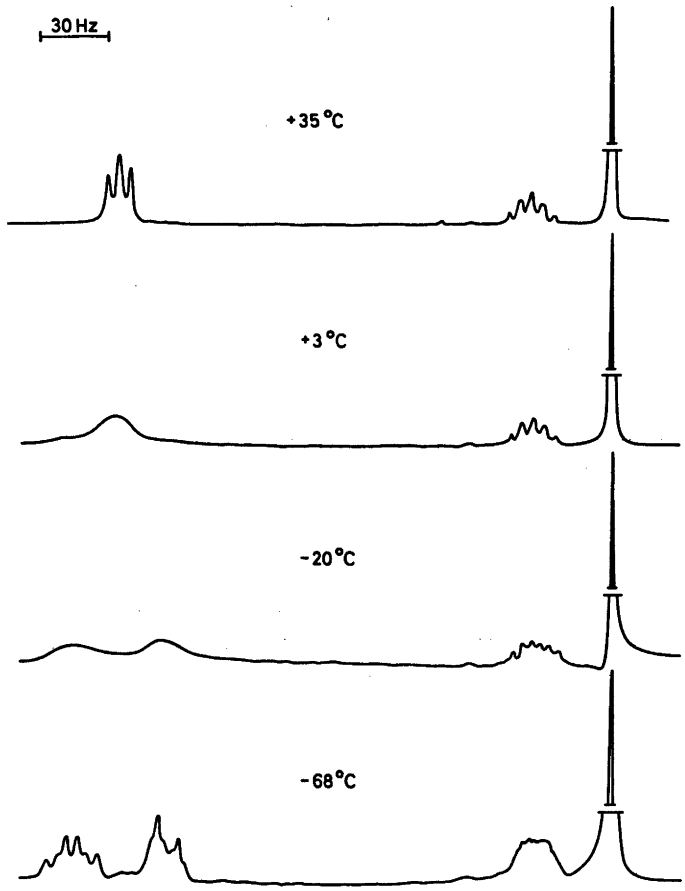


Fig. 5. ^{1}H NMR spectra at 100 MHz of 2,2,8,8-tetramethyl-1,3,7,9-tetraoxacyclododecane at various temperatures in $CHFCl_2$ (top curve in $CDCl_3$).

of the α-hydrogens. There is also a striking resemblance in coupling pattern between this low temperature NMR-spectrum and that of the dimer of 1,3-dioxane, which we have earlier found [7] to take the same square conformation. Notable is the high coalescence temperatures in these two twelve membered rings, −52 °C for the diformal and −5 °C for the diketal.

The tendency for C−C-bond, α-β to the 1,3-dioxa group, to be *gauche*, as pointed out by Dale [10] and found in all known crystal structures, will tend to give the dimeric cyclic ketals compact conformations, with three-bond "bridges" and close van der Waals contact between the parallel chains. This is shown in Fig. 6B for 2,2,12,12-tetramethyl-1,3,11,13-tetraoxacycloeicosane. This is the same three-bond bridge as in the above mentioned 12-membered and 14-membered ring diformals and the 12-membered ring dimeric ketal. The same is also found by low temperature ^{13}C NMR of 2,2,10,10-tetramethyl-1,3,9,11-tetraoxacyclohexadecane [11] and recently by crystal structure determination of 1,3,9,11-tetraoxacyclohexadecane.[12] The fact that our new 20-membered ring, 2,2,12,12-tetramethyl-1,3,11,13-tetraoxacycloeicosane does not show the tendency to absorb solvent molecules, as we have found earlier in 20-membered rings when four-bond bridge open conformations are imposed [13] is an indication of the compact conformation also for the 20-membered cyclic diketal.

The dimeric, large ring ketals therefore all seem to prefer the rectangular conformation

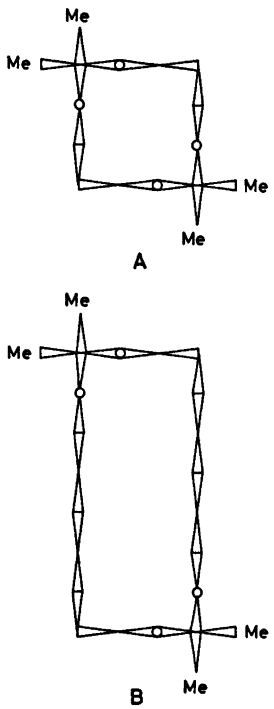


Fig. 6. Conformation of A: 2,2,8,8-tetramethyl-1,3,7,9-tetraoxacyclododecane and B: 2,2,12,12-tetramethyl-1,3,11,13-tetraoxacycloeicosane.

in the crystal with the dioxa groups across opposite corners and three-bond "bridges" between the parallel chains. The 12-, 16- and 20-membered rings will thereby leave the diamond lattice, and this results in lower stability which shows up in a lower yield and also in slightly lower melting points than for the 14-, 18- and 22-membered rings.

EXPERIMENTAL

All the monomeric and dimeric cyclic ketals except 2,2,8,8-tetramethyl-1,3,7,9-tetraoxacyclododecane were synthesized in the following way:

A mixture of the diol (0.25 mol), benzene (300 ml) and Amberlite IR-120 resin in its acid form was heated and moisture distilled out together with some benzene. 2,2-Dimethoxypropane (0.27 mol) was added dropwise and the methanol formed during the reaction was distilled out as an azeotrop with benzene, b.p. 59 °C. Distillation was continued slowly until b.p. 80 °C (7 – 10 h). The catalyst was filtered off and the filtrate stirred with sodium hydrogen carbonate (5 g) for 2 h. After filtration the benzene was evaporated and the residue dissolved or extracted with pentane. By cooling of the pentane solution the dimeric ketals crystallized out and was recrystallized from pentane. An exception was the tetraoxadodecamethylcyclotetracosan which was isolated by vacuum distillation (b.p. 190 – 200 °C/0.1 mmHg) and recrystallized from methanol.

The stable monomeric cyclic ketals were isolated by vacuum distillation of the pentane solution after precipitation of the dimers. The distillation residues contained higher homologues of the cyclic ketals and some open-chain reaction products.

Butane-1,4-diol gave only the monomeric cyclic ketal by this procedure, besides some tetrahydrofuran. Recrystallized but-2-yne-1,4-diol gave, however, a mixture of cyclic ketals with triple bonds which were hydrogenated with palladium catalyst in benzene solution at 2.5 kg/cm² for 20 h. After vacuum distillation the saturated dimeric cyclic ketal, a 14-membered ring, was isolated from a fraction boiling at 115 °C/0.2 mmHg. The main yield was higher-boiling cyclic ketals. Some monomeric cyclic ketal was formed during the hydrogenation.

The 2,2,8,8-tetramethyl-1,3,7,9-tetraoxacyclododecane was synthesized from 2,2-dimethyl-1,3-dioxan by adding dry, gaseous boron trifluoride (1 %). After 2 days at room temperature the mixture was refluxed for one hour, neutralized with gaseous ammonia and distilled in vacuum. Crystals in a fraction boiling at 100 – 110 °C/18 mmHg was separated from accompanying liquid by absorption on filter paper. Sublimation gave the clean 2,2,8,8-tetramethyl-1,3,7,9-tetraoxacyclododecane.

Determination of dipole moments. Dielectric constants were measured at 20 °C in a Weilheim Dipolmeter DM 01.

Infrared spectra. These were recorded in a Perkin-Elmer Grating Infrared Spectrophotometer 457.

Calorimetric measurements. A Perkin-Elmer Differential Scanning Calorimeter IB was used down to a temperature of – 90 °C.

^1H NMR spectroscopy. The NMR spectra were recorded with a Varian HA 100 15 D instrument.

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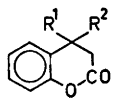
Short Communications

Thermal Instability of Substituted Ethyl 3,4-Dihydro-3-coumarincarboxylates during Gas Chromatographic Analysis*

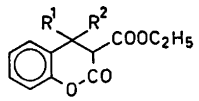
BILL GUSTAFSSON, ULF WENNSTRÖM and GUST.-AD. HOLMBERG

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The action of isopropylmagnesium bromide on ethyl 4-methyl-3-coumarincarboxylate has recently been studied in this laboratory.[1] When the primary reaction product still containing magnesium had been decomposed with aqueous hydrochloric acid and the organic phase was analysed by gas chromatography, both 4-isopropyl-4-methyl-3,4-dihydrocoumarin [I; $R^1=CH_3$, $R^2=CH(CH_3)_2$] and its 3-carbethoxy derivative [II; $R^1=CH_3$, $R^2=CH(CH_3)_2$] were detected. This fact was interpreted as a result of hydrolysis that partly transformed the single reaction product, ethyl 4-isopropyl-4-methyl-3,4-dihydrocoumarin-3-carboxylate, into 4-isopropyl-4-methyl-3,4-dihydrocoumarin during treatment with the aqueous acid. This is, however, not correct. Ethyl 3,4-dihydro-3-coumarincarboxylates, substituted in position 4 are thermally instable and decompose at temperatures above 150 °C. Therefore, the formation of 4-isopropyl-4-methyl-3,4-dihydrocoumarin occurs during the gas chromatographic analysis. Carbon dioxide and ethene are probably formed as by-products.



I



II

The examinations that led to these results were carried out with two columns both of stainless steel (3 mm × 1.5 m), one with 1 % the other with 5 % SE-30 on Chromosorb W as stationary phases. The initial temperature of the column oven was 150 °C and its linear programme 10 °C/min. Nitrogen (about 25 ml/min) was used as the carrier gas. 4-Aryl and 4-alkyl derivatives of ethyl 3,4-dihydro-3-coumarincarboxylate were used as substrates. The identities of the compounds to which the peaks in the chromatograms are due were usually established by mass spectra taken by a GLC/MS combination instrument. A glass column filled with 1 % OV-17 was used in this instrument. The chromatograms obtained with this column were very similar to those obtained with the steel columns but showed, if anything, larger proportions of 3,4-dihydrocoumarins as a consequence of the longer retention times. This result reveals that decomposition is not catalysed by the metal in the steel columns.

Ethyl 4-propyl-3,4-dihydro-3-coumarincarboxylate (II; $R^1=C_3H_7$, R = H) and ethyl 4-isopropyl-3,4-dihydro-3-coumarincarboxylate (II; $R^1=CH(CH_3)_2$, R = H) decompose partly to the corresponding 3,4-dihydrocoumarins when the analyses were performed according to the temperature programme given. The decomposition is, however, totally suppressed if the analyses are carried out isothermally at 140 °C which is the lowest practicable temperature.

The 4-phenyl, 4-*o*-tolyl, 4-mesityl, 4-*o*-*t*-butylphenyl, and 4-*o*-methoxyphenyl derivatives of ethyl 3,4-dihydro-3-coumarincarboxylate (II; $R^1=C_6H_5$, *o*-CH_3-C_6H_4, 2,4,6-$(CH_3)_3C_6H_2$, *o*-*t*-C_4H_9-C_6H_4, and *o*-CH_3O-C_6H_4, respectively, R = H) decompose to the corresponding 3,4-dihydrocoumarins when the analyses are performed according to the temperature programme given. In these cases, isothermal analysis at lower temperature is impracticable.

Unlike these substances, ethyl 3,4-dihydro-3-coumarincarboxylate (II; $R^1=R^2=H$) and ethyl 4-methyl-3,4-dihydro-3-coumarincarboxylate (II; $R^1=CH_3$, R = H) can be analysed by means of the programme. The stability of ethyl 4,4-dimethyl-3,4-dihydro-3-coumarincarboxylate (II; $R^1=R^2=CH_3$) has previously been established.[1]

The results reveal that ethyl 3,4-dihydro-3-coumarincarboxylates are thermally instable. Only those with rather small groups in position 4 permit gas chromatographic analysis using the programme given above.

Preparation of the substrates. All monosubstituted ethyl 3,4-dihydro-3-coumarincarboxylates were prepared from ethyl 3-coumarincarboxylate and the appropriate alkyl- or

* Correction to the article mentioned in Ref. 1.

arylmagnesium bromides using the previously published method [2] and were recrystallised from ethanol.

The syntheses of the 4-phenyl and 4-methoxyphenyl derivatives have already been reported.[2,3]

The 4-propyl derivative: reaction time 15 min. M.p. 45–46 °C. (Found: C 68.63; H 6.93. Calc. for $C_{15}H_{18}O_4$: C 68.69; H. 6.92.)

The 4-isopropylderivative: reaction time 15 min. M.p. 81.5–82.5 °C. (Found: C 68.67; H 7.00. Calc. for $C_{15}H_{18}O_4$: C 68.69; H 6.92.)

The 4-*o*-tolyl derivative: reaction time 15 min. M.p. 145.5–146.5 °C. (Found: C 73.51; H 5.80. Calc. for $C_{19}H_{18}O_4$: C 73.53; H 5.85.)

The 4-mesityl derivative: reaction time 20 min. M.p. 123.5–125.5 °C. (Found: C 74.32; H 6.53. Calc. for $C_{21}H_{22}O_4$: C 74.52; H 6.56.)

The 4-*o*-*t*-butylphenyl derivative: the ratio of ester to Grignard reagent was increased to 1:5; reaction time 48 h. M.p. 106–107 °C. (Found: C 75.21; H 6.79. Calc. for $C_{22}H_{24}O_4$: C 74.98; H 6.86.)

The 4-methyl derivative: reaction time 15 min. The compound did not crystallise. After careful evaporation of the solvent, the remaining oil gave an excellent NMR spectrum that showed that the reaction product was ethyl 4-methyl-3,4-dihydro-3-coumarincarboxylate.

The unsubstituted ethyl 3,4-dihydro-3-coumarincarboxylate was prepared by esterification of 3,4-dihydro-3-coumarincarboxylic acid in ethanol in the presence of dry hydrogen chloride. The reaction product did not crystallise but after careful evaporation of the solvent gave an excellent NMR spectrum that showed that it was ethyl 3,4-dihydro-3-coumarincarboxylate.

3,4-Dihydro-3-coumarincarboxylic acid was prepared from 3-coumarincarboxylic acid [4] by reduction with sodium amalgam according to the method used in the synthesis of *N*-methyl-3,4-dihydroxyphenylalanin.[5] The melting point, 141–142 °C, agrees with the value given in the literature.[6]

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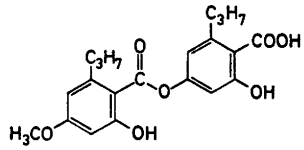
Received January 22, 1975.

5α,8α-Peroxyergosteryl Divaricatinate from *Haematomma ventosum*

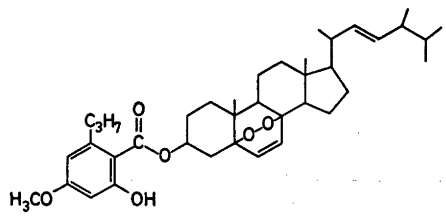
TORGER BRUUN and ANNE-MARGRETHE MOTZFELDT

Institutt for organisk kjemi, Universitetet i Trondheim, Norges tekniske høgskole, N-7034 Trondheim-NTH, Norway

The crystalline material from *Haematomma ventosum* (L.) Mass. (for details, see Experimental) gave a mass spectrum which exhibited two important fragmentations. One corresponded to the elimination of divaricatinic acid, to give a fragment of composition as ergosterol peroxide which had lost one molecule of water. The other fragmentation corresponded to a further loss of O_2. The observed light absorption of the isolated material, *cf.* Table 1, was very similar to that of methyl divaricatinate,



1



2

which may be obtained from divaricatic acid (*1*), a depside which has been isolated from *H. ventosum*.[1] The correctness of the idea that the isolated substance would be peroxyergosteryl divaricatinate (*2*) was shown by partial synthesis. The synthetic and natural materials showed identical UV, IR, and mass spectra, no depression of m.p. on admixture, and a similarly low optical activity.

Whilst peroxyergosterol has been isolated from natural sources on several occasions, so far mainly from lichens,[2,3] this appears to be the first time an ester of it has been reported.

Experimental. IR spectra in KBr were recorded on a Perkin-Elmer Model 257 spectrometer, UV spectra on a Hitachi 124 spectrometer,

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The syntheses of the 4-phenyl and 4-methoxyphenyl derivatives have already been reported.[2,3]

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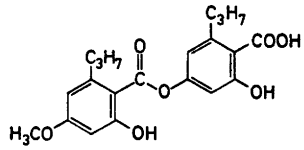
Received January 22, 1975.

5α,8α-Peroxyergosteryl Divaricatinate from *Haematomma ventosum*

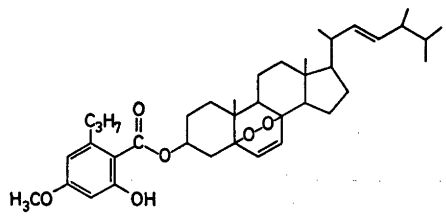
TORGER BRUUN and ANNE-MARGRETHE MOTZFELDT

Institutt for organisk kjemi, Universitetet i Trondheim, Norges tekniske høgskole, N-7034 Trondheim-NTH, Norway

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Experimental. IR spectra in KBr were recorded on a Perkin-Elmer Model 257 spectrometer, UV spectra on a Hitachi 124 spectrometer,

Table 1. UV light absorption of isolated and synthetic peroxyergosteryl divaricatinate and of methyl divaricatinate.

Peroxyergosteryl divaricatinate				Methyl divaricatinate	
Isolated Max.	ε	Synthetic Max.	ε	Max.	ε
302	6 000	301	5 500	303	5 000
264	14 500	263	14 000	263	15 500

mass spectra on an AEI MS902 instrument, and ^{1}H NMR spectra on a Varian A-60A spectrometer.

The material (4.48 kg) was collected in the Oppdal region some 140 km south of Trondheim. It was extracted for 24 h with ether in a glass Soxhlet apparatus. The neutral part (20.3 g) of the material was obtained by extracting the acidic substances with alkali. It was chromatographed on alumina (600 g). The material eluted with ether and ether-methanol 9:1 (3.7 g) was rechromatographed on silica gel, 400 g, retention volume 700 ml. After 800 mg had been eluted with benzene (700 ml) and benzene-ether 99:1 (2100 ml), 162 mg were eluted with the first 700 ml of benzene-ether 49:1. It slowly deposited *crystals*, which, recrystallised from acetone, melted at 171 – 172 °C, $[\alpha]_D$ + 1° (*c* 1.00, 1 dm tube, $CHCl_3$). M^+ at *m/e* 620 (11 %), found 620.4073, calculated for $C_{39}H_{56}O_6$ 620.4077; *m/e* 410 (10 %), $C_{28}H_{42}O_2$; *m/e* 378 (100 %), $C_{28}H_{42}$. The IR spectrum had bands at 3500 – 3000 cm^{-1} (OH) and at 1643 cm^{-1} (C=O). UV spectrum, see Table 1. The ^{1}H NMR spectrum had a singlet at δ 3.80 (OCH_3) and a multiplet at 5.23 (olefinic proton) as the two most interesting features.

Ergosteryl peroxide was prepared according to Windaus and Brunken,[4] m.p. 176 – 177 °C, reported 178 °C.

Divaricatinic acid chloride was prepared with oxalyl chloride according to Adams and Ulich.[5] Divaricatinic acid (250 mg) in dry benzene (10 ml) was cooled in ice-water and oxalyl chloride (1.5 ml) was added. After 40 min at room temperature benzene and excess oxalyl chloride were removed in a vacuum whilst nitrogen was passed through.

Peroxyergosteryl divaricatinate. Peroxyergosterol (0.5 g) in dry benzene (10 ml) and redistilled dry pyridine (5 ml) was added dropwise to crude divaricatinic acid chloride. After standing for 18 h the mixture was heated for 1 h on the water bath. The cooled mixture was diluted with ether and extracted with 2 N sulfuric acid, sodium carbonate and water. The residue (0.66 g) was chromatographed on silica gel (60 g). Elution with benzene-ether 99:1 gave a substance which was crystallised from acetone to give long, colourless needles (30 mg), m.p. 169 – 170 °C, $[\alpha]_D$ + 5° (*c* 2.00, 1 dm tube, $CHCl_3$).

Acknowledgements. The mass spectra were due to Mrs. T. Sigmond and her staff. The work was supported by a grant from Norges Almenvitenskapelige Forskningsråd.

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Received January 16, 1975.

Correction to "Tobacco Chemistry. 22"*

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Research Department, Swedish Tobacco Co., S-104 62 Stockholm 17, Sweden

The configurational designation of one of the title compounds, (1′*S*,6′*S*)-4-(2′,2′,6′-trimethyl-6′-vinylcyclohexyl)-2-butanone, is incorrect and its name should read (1′*S*,6′*R*)-4-(2′,2′,6′-trimethyl-6′-vinylcyclohexyl)-2-butanone. The structure and stereochemistry of the compound, and the formula given, corresponding to the latter name, are reproduced correctly.

Received February 5, 1975.

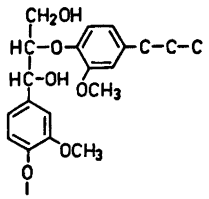
* *Acta Chem. Scand. B 28* (1974) 18.

New Synthetic Routes to Lignin Model Compounds of the Arylglycerol-β-aryl Ether Type

KNUT LUNDQUIST and STIG REMMERTH

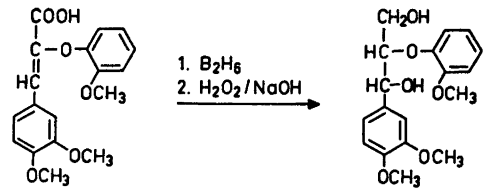
Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, S-402 20 Göteborg 5, Sweden

The arylglycerol-β-aryl ether type of structure (*1*) has long been considered to constitute the most prominent substructure in lignin.[1] Lignin model compounds of type *1* are therefore useful in research concerning lignin. Syntheses of such compounds have been described by several authors.[2]



1

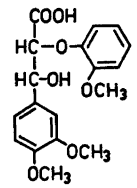
We wish to report two new synthetic routes to model compounds for structure *1*. One of these involves hydroboration of an α-aryloxycinnamic acid as the key step. α-Aryloxycinnamic acid *2* was prepared by a Claisen reaction (*cf.* Refs. 2b and 3) from veratraldehyde and the methyl ester of 2-methoxyphenoxyacetic acid, as well as by a Perkin reaction (*cf.* Ref. 4) from veratraldehyde and 2-methoxyphenoxyacetic acid. The Perkin reaction provides the simplest route to acid *2*, but gives only poor yields. A higher yield (20 %) was obtained by a Claisen reaction using sodium hydride as condensing agent and toluene as solvent. We intend to further investigate methods for the preparation of acids of type *2*. The product obtained on hydroboration of compound *2* (Fig. 1) was identified as the *erythro* form [5] of compound *3* (yields were about 40 %). Since hydroboration is known to proceed by *cis* addition, we assume that the starting material has the *trans* configuration. Additional compounds present in the reaction mixtures were guaiacol, 3,4-dimethoxycinnamyl alcohol, 3-(3,4-dimethoxyphenyl)-1,2-propanediol, and 3-(3,4-dimethoxyphenyl)-1-propanol. The formation of the by-products can be explained in the following way. The organoborane derivative, formed on reduction by and addition of diborane to acid *2*, undergoes an elimination reaction yielding guaiacol and 3,4-dimethoxycinnamyl alcohol; the additional two by-products result from subsequent reactions of the 3,4-dimethoxycinnamyl alcohol formed (*cf.*



2 *3*

Fig. 1. Preparation of lignin model compound *3* by hydroboration of α-aryloxycinnamic acid *2*.

Ref. 6). Brown and co-workers [6] have studied reactions of the types assumed to be involved in the formation of the above-mentioned by-products. Their results suggest that it should be possible to increase the yield of compound *3* considerably by optimizing the reaction conditions.



4

Acid *4* was obtained as a by-product in the preparation of acid *2* by the Claisen reaction. In accord with earlier observations in similar syntheses,[2b] decreased reaction temperature favored the formation of β-hydroxy acid (*i.e.* in our case acid *4*). We have obtained acid *4* in 25 % yield; the product was a mixture of the two possible diastereomers. These were partially separated by chromatography on silica gel. The configuration of the isomers was determined by reduction to compound *3* and examination of the product by spectral comparisons with the known [5] diastereomers of this compound.

It was found that acid *4* can be alternatively prepared from veratraldehyde and lithium α-lithio(2-methoxyphenoxy)acetate (yield 60 %). Preparation of acids of type *4* by this method and subsequent reduction to compounds of type *3* represents a second new route to lignin model compounds of type *1*. Syntheses of such model compounds from acids of type *4*, obtained by the Claisen reaction, have been carried out by Freudenberg and Müller.[2b] It is noteworthy that in the preparation of acid *4* by the Claisen reaction, the *threo* form dominated in the product, while more *erythro* form than *threo* form was present in the product obtained using lithium α-lithiocarboxylate.

Experimental. NMR spectra were recorded on a Varian A-60 instrument with TMS as internal standard. *Mass spectra* were taken using an

AEI MS 902 instrument and this equipment was also used for accurate mass measurements of molecular ions.

Thin layer chromatography (TLC) was performed with silica gel, using benzene-dioxan-acetic acid (90:25:4)[7] as eluent. Spots were made visible by spraying with formalin-H_2SO_4 (1:9) and subsequent heating. R_F values: *4* (*threo* form), 0.06; *4* (*erythro* form), 0.11; 3-(3,4-dimethoxyphenyl)-1,2-propanediol, 0.13; *3* (*erythro* and *threo* forms), 0.28; *2*, 0.35; 3-(3,4-dimethoxyphenyl)-1-propanol, 0.36; 3,4-dimethoxycinnamyl alcohol, 0.38; guaiacol, 0.56.

Preparation of α-(2-methoxyphenoxy)-3,4-dimethoxycinnamic acid (2) by the Perkin reaction. 2-Methoxyphenoxyacetic acid (10.0 g), veratraldehyde (9.2 g), NaH (2.7 g of a 50 % dispersion in mineral oil), and acetic anhydride (20 ml) were heated at 150 °C for 10 h. The reaction mixture was cooled and some water was added to decompose excess acetic anhydride. The mixture was then dissolved in 150 ml ether. The ether solution was extracted with 5 % aqueous Na_2CO_3 ($100+2\times50$ ml). Acidification of the extract with 2 M HCl gave a precipitate, which, according to TLC, consisted essentially of compound *2* and 3,4-dimethoxycinnamic acid. The latter compound was removed from the precipitate by repeated extraction with boiling water and, finally, with a small amount of acetone at room temperature. A crystalline residue (m.p. 201 °C) consisting of compound *2* (TLC) weighing 1.5 g (8 %) was obtained. Recrystallization from acetone raised the m.p. to 202 °C. Accurate mass measurements of the molecular ion gave *m/e* 330.1073. Calc. for $C_{18}H_{18}O_6$: *m/e* 330.1103. NMR spectrum (δ units, 60 °C; solvent, $CDCl_3$): 3.72 (3 H, s; OCH_3), 3.87 (3 H, s; OCH_3), 3.93 (3 H, s; OCH_3), 6.6—7.6 (8 H, m; aromatic protons and the vinyl proton); 9.32 (1 H, s; COOH). The IR spectrum showed a strong band at 1690 cm^{-1} (C=O).

Hydroboration of α-(2-methoxyphenoxy)-3,4-dimethoxycinnamic acid (2). Ten ml of a solution of B_2H_6 in tetrahydrofuran[8] ($\approx$0.8 M) were added to a solution of compound *2* (990 mg, 3 mmol) in tetrahydrofuran (N_2 atmosphere). After an initial evolution of hydrogen, the reaction mixture turned yellow. The yellow colour disappeared after about 30 min (separate experiments indicated that the disappearance of the yellow colour was associated with the consumption of starting material). After 3 h, the excess of B_2H_6 was decomposed by addition of water (4 ml). To oxidize C—B bonds the reaction mixture was treated with 6 ml 3 M NaOH and, in portions, with a total of 0.6 ml 30 % H_2O_2. The mixture was stirred vigourously at 30—40 °C for 1 h. After addition of 30 ml H_2O, the reaction mixture was extracted with chloroform ($60+2\times30$ ml). The extract was dried over Na_2SO_4 and solvent removed by film evaporation. The residue (0.92 g) was dissolved in 20 ml ether and kept in a refrigerator for some days. Crystals precipitated (0.28 g) which were identified as the *erythro* form of 2-(2-methoxyphenoxy)-1-(3,4-dimethoxyphenyl)-1,3-propandiol (*3*) (m.p. 99 °C, lit.[5] 97.5—98.5 °C). Column chromatography [20 g SiO_2; eluent, benzene-ethyl acetate (1:1)] gave 90 mg of the same compound (eluate 120—240 ml). Total yield: 37 %. Additional fractions from the column consisted of guaiacol (eluate 25—40 ml; identified by IR and TLC), 3,4-dimethoxycinnamyl alcohol (eluate 60—70 ml, 106 mg; identified by TLC, IR, NMR, and m.p. (75—76 °C, lit.[9] 79—80 °C), 3-(3,4-dimethoxyphenyl)-1-propanol (eluate 90—120 ml, 42 mg; identified by TLC and IR), and a mixture of the latter two compounds (eluate 70—90 ml, 87 mg; NMR). Continued elution with ethyl acetate gave 3-(3,4-dimethoxyphenyl)-1,2-propanediol (identified by TLC and IR). This compound and guaiacol are left in part in the aqueous layer and the yields of these compounds have therefore not been determined.

Preparation of acid 4, using lithium α-lithiocarboxylate. The method described by Moersch and Burkett[10] was used (work-up procedure B). 2-Methoxyphenoxyacetic acid (9.1 g) and veratraldehyde (8.3 g) were used as starting materials. As indicated by TLC the crude product (16 g) consisted mainly of a mixture of the two possible diastereomers of acid *4*. The two isomers were separated by chromatography on silica gel (210 g) with, successively, dichloromethane and mixtures of dichloromethane and ethyl acetate (9:1, 4:1, 7:3, 1:1) as eluent. The *erythro* form was eluted somewhat more rapidly than the *threo* form. Fractions of the *erythro* form (2.8 g, impure crystals), the *threo* form (1.3 g, oil), and a mixture of the two isomers (6.3 g, oil) were obtained. Total yield: 60 %. The fraction consisting of the *erythro* form of acid *4* was heated briefly with 30 ml benzene and the solid filtered off the following day. A product (2.5 g) melting at 119—121 °C was obtained. Recrystallization from benzene raised the m.p. to 121—122 °C. Accurate mass measurement of the molecular ion showed that the elemental composition was $C_{18}H_{20}O_7$ which is in accord with structure *4*. NMR spectrum (δ units; chloroform-*d* solution treated with D_2O): 3.83 (9 H, s; OCH_3), 4.63 (1 H, d, $J=5.2$ Hz; $-CO-CH<$), 5.16 (1 H, d, $J=5.2$ Hz; $Ar-CH<$), 6.6—7.2 (7 H, multiplet; aromatic protons). The IR spectrum showed a strong band at 1725 cm^{-1} (C=O). The *erythro* configuration was demonstrated by reduction with B_2H_6 and identification of the product as the *erythro* form of compound *3*. Structural proof for the *threo* form of acid *4* was obtained with the same methods as those used for the *erythro* form. NMR spectrum (δ units; conditions as above): 3.80 (9 H, s; OCH_3), 4.63 (1 H, d, $J=3.4$ Hz; $-CO-CH<$), 5.24 (1 H, d, $J=3.4$ Hz; $Ar-CH<$), 6.6—7.2 (7 H, aromatic protons). The IR spectrum showed a strong band at 1750 cm^{-1}.

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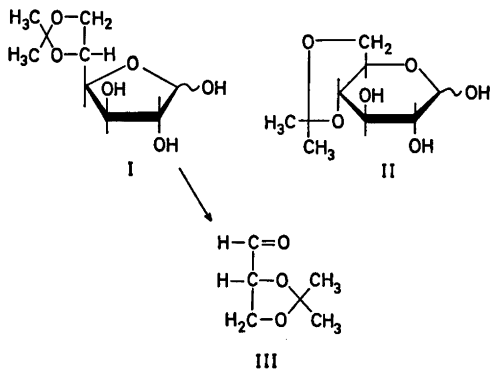
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5,6-*O*-Isopropylidene-D-glucofuranose

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Treatment of D-galactose with acetone containing 15 – 20 % *N*,*N*-dimethylformamide and anhydrous cupric sulfate at reflux temperature, was found in a previous work [1] to give among other products 5,6-*O*-isopropylidene-D-galactofuranose in 16 % yield. A claim that the corresponding D-glucose derivative is formed by partial acid hydrolysis of 1,2:5,6-di-*O*-isopropylidene-3-*O*-vinyl-α-D-glucofuranose [2] is questionable, and the product obtained has later been suggested to be 1,2-*O*-isopropylidene-α-D-glucofuranose, based on the way of preparation and the given melting point.[3] An investigation of the possibility to prepare 5,6-*O*-isopropylidene-D-glucofuranose by treatment of D-glucose with anhydrous cupric sulfate in acetone-*N*,*N*-dimethylformamide was therefore undertaken, and is the subject of the present report.



Refluxing of D-glucose for 24 h with these reagents, led to the formation of several products, of which two exhibited chromatographic mobility indicating the presence of one *O*-isopropylidene group. These compounds were isolated by column chromatogtaphy on silica gel. The slowest moving compound was indistinguishable from 4,6-*O*-isopropylidene-D-glucopyranose (II)[3,4] whereas the fastest moving, obtained in 14 % yield, was identified as 5,6-*O*-isopropylidene-D-glucofuranose (I). The identification was based on the facts that oxidation with periodate gave 2,3-*O*-isopropylidene-D-glyceraldehyde (III), and the mass spectrum (Fig. 1a) was almost identical with that of 5,6-*O*-isopropylidene-D-galactofuranose [1] (Fig. 1b). A peak of high intensity in the mass spectrum at *m/e* 101 is strongly indicative of the

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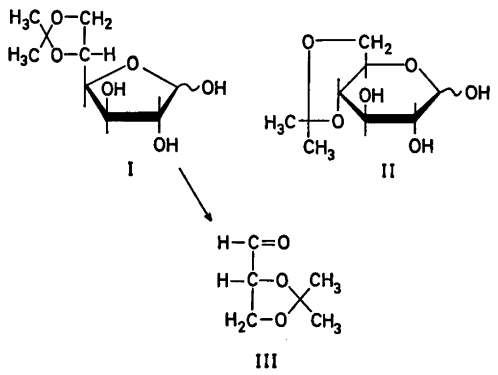
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Refluxing of D-glucose for 24 h with these reagents, led to the formation of several products, of which two exhibited chromatographic mobility indicating the presence of one *O*-isopropylidene group. These compounds were isolated by column chromatogtaphy on silica gel. The slowest moving compound was indistinguishable from 4,6-*O*-isopropylidene-D-glucopyranose (II)[3,4] whereas the fastest moving, obtained in 14 % yield, was identified as 5,6-*O*-isopropylidene-D-glucofuranose (I). The identification was based on the facts that oxidation with periodate gave 2,3-*O*-isopropylidene-D-glyceraldehyde (III), and the mass spectrum (Fig. 1a) was almost identical with that of 5,6-*O*-isopropylidene-D-galactofuranose [1] (Fig. 1b). A peak of high intensity in the mass spectrum at *m/e* 101 is strongly indicative of the

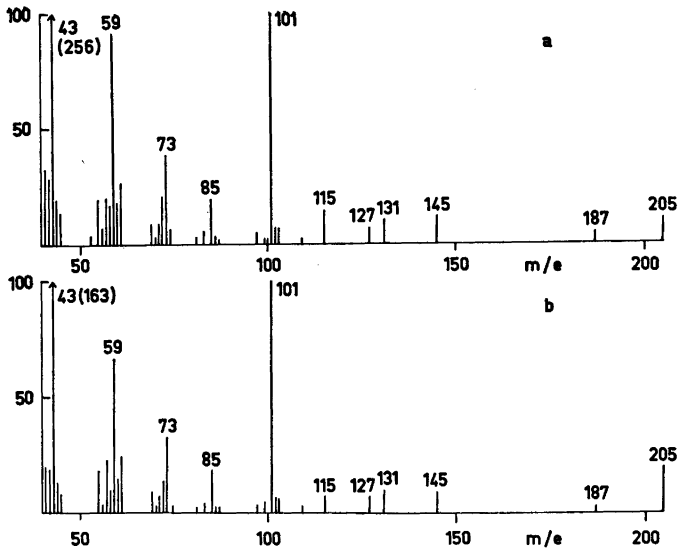


Fig. 1. Mass spectrum of (a) 5,6-*O*-isopropylidene-D-glucofuranose (I) and (b) 5,6-*O*-isopropylidene-D-galactofuranose.

presence of a 5,6-*O*-isopropylidene group,[5] the fragment results from cleavage of the C4 – C5 bond with the charge located at the C5 – C6 containing fragment. Other important peaks observed are those at *m/e* 205 ($M^+ - CH_3$), 187 (205 – H_2O), 145 (205 – AcOH), 127 (205 – H_2O – AcOH), 59 (Me_2COH^+), and 43 ($MeCO^+$), all of which are expected fragments of a mono-*O*-isopropylidene hexose.[5] The possibility of configurational isomerization of the parent hexose under the conditions employed in preparation of the mono-*O*-isopropylidene derivative is not likely, and it was shown that the 5,6-*O*-isopropylidene hexose (II) was a derivative of glucose by conversion to 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (IV).

The melting point of the obtained 5,6-*O*-isopropylidene-D-glucofuranose (I), 124 – 125 °C, is clearly different from that found for the compound formed by acid hydrolysis of 1,2:5,6-di-*O*-isopropylidene-3-*O*-vinyl-α-D-glucofuranose,[2] supporting the assumption [3] that the latter was the 1,2-*O*-isopropylidene derivative.

The present result is an additional example of the previously observed applicability of cupric sulfate-acetone, with or without *N*,*N*-dimethylformamide, in prepraration of otherwise inaccessible reducing mono-*O*-isopropylidene aldoses,[1,4,6] and also of the promoting effect of elevated temperatures on the formation of *O*-isopropylidene derivatives in furanose form.[1,5,7]

Experimental. Thin layer chromatography (TLC) was performed on Silica gel G in (A) benzene-ethanol 5:1 (v/v), (B) benzene-ethanol 3:1, and (C) chloroform-methanol 8:1. Paper electrophoresis was run in borate buffer, pH 10, on Whatman No. 1 paper. As spray reagent was applied diphenylamine-aniline-phosphoric acid.[8] Mass spectra were recorded on an AEI MS 902 mass spectrometer at an ionizing potential of 70 eV.

Reaction of D-*glucose with cupric sulfate-acetone-*N,N-*dimethylformamide.* D-Glucose (1 g) in *N*,*N*-dimethylformamide (30 ml) was poured into a stirred, refluxing mixture of acetone (125 ml) and anhydrous cupric sulfate (3 g). Stirring at reflux temperature was continued for 24 h. After cooling of the reaction mixture, benzene (100 ml) was added and the solution filtered, and subsequently stirred with solid sodium hydrogencarbonate for 30 min. The solution was filtered, concentrated to 3 ml under reduced pressure and then subjected to chromatography on a column (3 × 24 cm) of Silica gel H in solvent C. Three fractions were obtained; fraction 1 contained products with chromatographic mobilities indicating the presence of two *O*-isopropylidene groups, fraction 2 and fraction 3 contained mono-*O*-isopropylidene derivatives, both fractions were chromatographically homogeneous (TLC, solvents A and C).

*5,6-*O-*Isopropylidene-*D-*glucofuranose (I)* Evaporation of the solvent from fraction 2 gave a crystalline residue (171 mg, 14 %) which was recrystallized from ethyl acetate, m.p. 124 – 125 °C, $[\alpha]_D^{20}$ + 9° (c 2, water, 24 h). (Found: C 49.48; H 7.69. Calc. for $C_9H_{16}O_6$: C 49.09; H 7.27). The mass spectrum is shown in Fig. 1.

4,6-O-Isopropylidene-D-glucopyranose (II). Removal of the solvent from fraction 3 gave 4,6-*O*-isopropylidene-D-glucopyranose (II, 111 mg), it was recrystallized from ethyl acetate-acetone, m.p. 163–167 °C, $[\alpha]_D^{20}$ −5° (*c* 2, water, 48 h) (Lit.[3] m.p. 169.5–170.5 °C, $[\alpha]_D$ −7.3°). The product was chromatographically (TLC, solvents A and C) indistinguishable from authentic II.[4]

Periodate oxidation of 5,6-O-isopropylidene-D-glucofuranose (I). 5,6-*O*-Isopropylidene-D-glucofuranose (I, 40 mg) was dissolved in water (3 ml), and sodium periodate (200 mg) in water (3 ml) was added. Sodium hydrogencarbonate was added at intervals to neutralize the acid formed under the reaction. After 2 h, barium acetate (0.5 M) was added until the precipitation was complete, the precipitate was removed by centrifugation and the solvent concentrated under diminished pressure. The residue, still containing small amounts of water, was extracted by chloroform, the chloroform solution dried by sodium sulfate and the solvent was evaporated to give a chromatographically homogeneous syrup (11 mg), indistinguishable (TLC, solvent A) from 2,3-*O*-isopropylidene-D-glyceraldehyde (III),[9] the IR spectrum ($CHCl_3$) was identical with that of the authentic sample, stored a few days after preparation; both samples showed only a very weak carbonyl absorption at 1730 cm^{-1}, $[\alpha]_D$ +45° (*c* 0.5, benzene) (Lit.[9] +64.9°, *c* 5.75, benzene, freshly prepared III).

Hydrolysis of the oxidation product with 30 % aqueous acetic acid at 40 °C for 2 h and evaporation of the solvents gave a chromatographically (TLC, solvent B) and electrophoretically (M_{Glc} 0.75) homogeneous syrup, indistinguishable from authentic D-glyceraldehyde.

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (IV). 5,6-*O*-Isopropylidene-D-glucofuranose (I, 15 mg) was treated with acetone (2 ml) containing concentrated sulfuric acid (0.02 ml) for 2 h. The solution was neutralized with solid sodium hydrogencarbonate, filtered, and the solvent removed. The crystalline residue, indistinguishable from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (IV) by TLC (solvent A), was recrystallized from heptane-ethyl acetate, m.p. 106–109 °C (Lit.[10] 110–111 °C).

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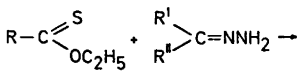
Preparation and Characterisation of N^2,N^4-Dialkylidene Hydrazidines

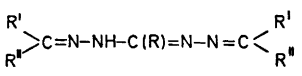
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The reactions between hydrazones and aliphatic thioacid-*O*-esters have been investigated. Substituted benzophenone hydrazones form N^2,N^4-dialkylidene hydrazidines with high yields, while the main product from the reactions with aliphatic hydrazones are the corresponding azines. ^{1}H NMR, IR, MS, and in two cases ^{13}C NMR spectra have been recorded.

In the literature [1–5] it has been established that *N*-substituted thiohydrazides are stable, while the aliphatic *N*-unsubstituted thiohydrazides are unstable, but the aromatic ones stable.[2,3] Attempts to prepare the benzylidene derivative of a thiohydrazide by reaction of dithioesters with benzalhydrazone has been reported as unsuccessful.[2] Mayer and Orgis [6] demonstrated the efficiency of *O*-ethyl thioformate in the preparation of thioformamide and later Walter [4] showed that *O*-ethyl thioformate could be used in the preparation of *N*-substituted thioformhydrazides. The purpose of this investigation was to try to prepare the alkylidene derivatives of thioformhydrazides by the reaction of *O*-ethyl thioformate with hydrazones. It turned out, however, that equimolar amounts of hydrazone and *O*-ethyl thioformate gave N^2,N^4-dialkylidene formohydrazide hydrazones (I) as the main product under conditions suitable for thioformylation of hydrazines.





I (a-g)

Several hydrazidines have been reported as unstable intermediates in formazane formation [7–11] but no dialkylidene derivatives have been reported in the literature. Therefore, a closer investigation of the chemical and physical properties of I was carried out.

O-Ethyl thioformate and an *N*-unsubstituted benzophenone hydrazone in benzene or ethanol solution react at room temperature under instantaneous evolution of hydrogen sulfide. Reasonable yields of the hydrazidine derivatives (I) can be isolated after stirring for few hours (Table 1).

Mixtures of products are obtained if the reactions are carried out under cooling. It is clear from the IR and ^{1}H NMR spectra of these mixtures that one of the components is the expected hydrazidine derivate. One of the other components has spectroscopical properties that might fit a thioformylated hydrazone. Attempts to isolate the components by preparative layer chromatography gave no thioformylated hydrazone.

The reaction was carried out with hydrazones of aliphatic, aromatic, and mixed aliphatic-aromatic ketones. With aliphatic hydrazones the corresponding azine was the main product and no hydrazidine was isolated. This is in accordance with the wellknown instability of aliphatic hydrazones and with results of other reactions with hydrazones.[12,13]

Mixtures of the corresponding azine and the hydrazidine (I) were formed when acetophenone hydrazone or dibenzylketone hydrazone were reacted with *O*-ethyl thioformate. In the latter case the two compounds were separated by PLC.

Table 1. N^2,N^4-Dialkylidene-hydrazidines, R'R''CNNHC(R)NNCR'R''.

Compound	R	R'	R''	Yield %	M.p. °C	Formula	Analyses (C, H, N)
Ia	H	C_6H_5	C_6H_5	77	187–188	$C_{27}H_{22}N_4$	Found: 80.45; 5.55; 13.89 Calc: 80.55; 5.51; 13.92
Ib	H	*p*-$CH_3C_6H_4$	*p*-$CH_3C_6H_4$	62	140–141	$C_{31}H_{30}N_4$	Found: 81.15; 6.71; 12.20 Calc: 81.19; 6.59; 12.22
Ic	H	*o*-ClC_6H_4	C_6H_5	80	164–165	$C_{27}H_{20}N_4Cl_2$	Found: 68.65; 4.31; 11.82 Calc: 68.79; 4.28; 11.87
Id	H	*p*-ClC_6H_4	C_6H_5	67	178–180	$C_{27}H_{20}N_4Cl_2$	Found: 68.65; — 11.79 Calc: 68.79; 4.28; 11.87
Ie	H	$C_6H_5CH_2$	$C_6H_5CH_2$	15	82–83	$C_{31}H_{30}N_4$	Found: 80.80; 7.05; 11.93 Calc: 81.19; 6.59; 12.22
If	CH_3	C_6H_5	C_6H_5	73	147–148	$C_{28}H_{24}N_4$	Found: 80.55; 5.84; 13.46 Calc: 80.74; 5.81; 13.47
Ig	CH_3	C_6H_5	CH_3	22	96–98	$C_{18}H_{20}N_4$	Found: 73.16; 6.81; 18.86 Calc: 73.94; 6.90; 19.16

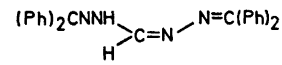
To investigate if thioacylated hydrazone of other aliphatic thioacids could be formed, some experiments were carried out with *O*-ethyl thioacetate. The hydrazidine derivative was the main product and no thioacylated hydrazone was isolated.

It has been stated in the literature [6] that it is necessary to use pure *O*-ethyl thioformate to obtain a reasonable yield in the thioformylation reactions. It was found in this investigation that *O*-ethyl thioformate triple-distilled under nitrogen, was not pure.[14] The best yields were obtained from freshly prepared *O*-ethyl thioformate distilled once under nitrogen.

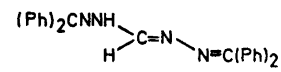
Some chemical properties of compound Ia were investigated. Thus methylation with methyl iodide and dimethyl sulfate and acetylation with acetic anhydride were attempted, but no methylated or acetylated product was isolated. From an attempted hydrolysis in excess 1 M NaOH the starting materials were recovered after 12 h reflux. Hydrolysis in excess 1 M HCl gave benzophenone and benzophenone azine, identified by m.p., IR and MS. A quantitative experiment using excess 1.000 M HCl and titration with 1.000 M NaOH showed that one equivalent of HCl was consumed, which is in accordance with the proposed formula for Ia.

The structure of I was based on spectroscopic evidence. The ^1H NMR spectra of compounds Ia–e taken in deuteriochloroform showed CH signals as doublets in the region δ 7.62–7.80 and NH signals as broad doublets in the region δ 9.0–9.5. By shaking with deuterium oxide the signals at $\delta \sim 9$ disappeared and the doublets at $\delta \sim 7.7$ collapsed. This proves that the CH and NH protons are placed at atoms which are neighbours, as in structure I. The same was shown by spin decoupling experiments. The phenyl protons gave complex signals in the region δ 6.9–7.8. For compounds If and Ig the NH and CH_3 signals were singlets in accordance with the hydrazidine structure.

^{13}C NMR spectra were recorded for compounds Ia and Ib. These support the structure I, showing four quaternary carbon atoms and three C=N carbon atoms one of which is attached to hydrogen for compound Ia. The ^{13}C NMR spectra give no indication as to whether the compounds obtained are mixtures of *Z* and *E* forms or only one of the isomers.



Z



E

The infrared spectra exhibited NH stretch absorptions in the region 3295–3382 cm^{-1}, and several bands in the region 3085–3020

cm^{-1}. (the aromatic protons and the hydrogen attached to CN double bond). In the region 1638 – 1580 cm^{-1} two to three strong and broad absorption-bands were found which possibly arise from C=N-stretching and the aromatic skeletal vibrations. This region seems to be in accordance with that quoted in Bellamy.[15] Some other characteristic absorptions are given in the experimental part.

The mass spectra all gave a molecular ion corresponding to the actual molecular weight (variable intensity 0.2 – 26 % of base peak). The fragmentation pattern is in accordance with structure I.

EXPERIMENTAL

Microanalyses were carried out in the microanalysis department of Chemical Laboratory II, the H. C. Ørsted Institute. ^1H NMR spectra were obtained on a Varian A-60 or a JEOL JNM-MH 60/II instrument with TMS as internal reference. IR spectra were recorded on a Perkin-Elmer model 225 grating spectrograph. Mass spectra were taken on a Finnigan 1015 S/L or an AEI-902 instrument operating at 70 eV. ^{13}C NMR spectra were recorded on a Brucker WH 90 instrument. Melting points were taken on a Büchi melting point apparatus and are uncorrected.

O-Ethyl thioformate was prepared according to the literature.[16]

O-Ethyl thioacetate. This compound was prepared from ethyl acetoimidate hydrochloride and hydrogen sulfide according to the literature.[17] The acetoimidate hydrochloride was prepared following the method described in *Organic Syntheses* [18] with small modifications. Thus after the calculated amount of HCl-gas had been added to the solution the resulting oily mass was evaporated to dryness on a rotavapor and further dried *in vacuo* for 1 h. The resulting crystalline mass was used without further purification.

2-Chlorobenzophenone hydrazone was prepared from 2-chlorobenzophenone (0.03 mol 8.65 g) and excess hydrazine (8.7 g 95 %) in 25 ml abs. ethanol. The mixture was refluxed for 8 h, stirred overnight at room temperature, cooled and filtered. The precipitate was recrystallized from ethanol. Yield 74 %, m.p. 126 °C. (Found: 67.60; H 4.49; N 12.18. Calc. for $C_{13}H_{11}N_2Cl$: C 67.68; H 4.81; N 12.14).

The other hydrazones were prepared in a similar way, their physical constants were found in accordance with literature values.[19–23]

N^2,N^4-*Bis(diphenylmethylene)-formohydrazide hydrazone. (Ia).* Benzophenone hydrazone (19.6 g, 0.1 mol) was dissolved in 70 ml ethanol (abs.). *O*-Ethyl thioformate (9.0 g, 0.1 mol) was added dropwise over a period of 10 min at room temperature. The mixture was stirred until the evolution of hydrogen sulfide ceased. The precipitate was filtered off and the mother-liquor concentrated to give additional crystals. The compound was recrystallized from abs. ethanol.

(Ia) is insoluble in water, pentane, ether and soluble in benzene and chloroform.

IR(KBr, in cm^{-1}): 3310m, 1610s, 1583s, 1554m, 1491m, 1488m, 1443s, 1392m, 1118m.

^1H *NMR* ($CDCl_3$): δ 7.25 – 7.67 (20 H, m); 7.80 (1 H, d); 9.40 (1 H, d); J_{CH-NH} = 9.5 Hz.

^{13}C *NMR:* δ 162,3, 150.0, 138.1, 137.1, 135.6, 132.5, 129.8, 129.7, 129.4, 129.1, 128.7, 128.3, 127.9, 127.1,

MS m/e, (% of base peak); 402(38)M^+, 401(29), 325(15), 22(3) 207(5), 195(35), 180(100), 165(31), 104(17), and 77(67).

N^2,N^4-*Bis-(4,4'-dimethyldiphenylmethylene)-formohydrazide hydrazone (Ib)* was prepared from equimolar amounts (0.1 mol) of 4,4'-dimethylbenzophenone hydrazone and *O*-ethyl thioformate in 70 ml ethanol as described for Ia. The mixture was stirred overnight and subsequently evaporated to dryness. The residual crystals were recrystallized from abs. ethanol.

IR(KBr, in cm^{-1}); 3300w, 1610sh, 1599s, 1582s, 1508m, 1240m, 1122w, 1110w.

^1H *NMR*($CDCl_3$): δ 6.9 – 7.6 (16 H, m); 2.36 (s) 2.50 (s) 3 H, intensity 3/1; 7.76 (1 H, d); 9.40 (1 H, d); J_{CH-NH} = 9.5 Hz.

^{13}C *NMR:* δ 149.6, 139.5, 139.0, 138.8, 138.1, 135.7, 132.8, 130.3, 129.6, 128.9, 128.6, 127.1, 21.3.

MS m/e (% of base peak): 458(20)M^+, 457(21), 367(4), 223(30), 208(77), 194(20), 179(32), 178(29), 165(13), 118(26), 91(100).

N^2,N^4-*Di(2-chlorophenylphenylmethylene)-formohydrazide hydrazone (Ic).* Prepared from 2-chlorobenzophenone hydrazone and *O*-ethyl thioformate as described for Ib.

IR(KBr, in cm^{-1}); 3320w, 1610s, 1585s, 1565m, 1442m, 1392m, 1124m, 1060w.

^1H *NMR*($CDCl_3$): δ 7.6 – 7.67(18 H, m); 7.80 (1 H, d); 9.21 (1 H, d) J_{CH-NH} = 9.5 Hz.

MS m/e (% of base peak); 472(0.2), 471(0.2), 470(0.2), 435(2.5), 229(5), 214(18), 195(52), 180(4), 165(38), 138(7), 111(16), 104(13), 77(100).

N^2,N^4-*Di(4-chlorophenylphenylmethylene)-formohydrazide hydrazone (Id).* Prepared from 4-chlorobenzophenone hydrazone and *O*-ethyl thioformate as described for Ib.

IR(KBr, in cm^{-1}); 3305m, 1600s, 1580s, 1552m, 1484s, 1445m, 1395m, 1118m, 1085m, 998m.

^1H *NMR*($CDCl_3$): δ 7.17 – 7.67 (18 H, m); 7.78 (1 H, d); 9.47 (1 H, d).

MS m/e (% of base peak): 472(10), 471(26), 470(26), 469(40), 408(17), 393(10), 359(10), 258(10), 231(21), 230(28), 229(54), 216(44), 215(19), 214(100), 199(10), 195(7), 180(25), 165(39), 119(21), 111(23), 105(17), 104(19), 91(21), 77(86).

N^2,N^4-*Bis(dibenzylmethylene)-formohydrazide hydrazone (Ie).* The procedure described for Ib yielded a crystalline mass which was a mixture of (Ie) and dibenzylketone azine. The two compounds were separated on silica gel plates using $CHCl_3$ as eluent. The azine was identified by m.p. and IR, superimposable with IR of an authentic sample.

IR(KBr, in cm^{-1}): 3382m 1638s, 1620s, 1599m, 1491s, 1451s, 1425m, 1340m, 1218m, 1091m, 1072m.

^1H *NMR* ($CDCl_3$): δ the CH_2 protons occurred as 4 singlets of equal intensity at 3.35, 3.41, 3.59 and 3.83; 6.9–7.4 (20H,m); 7.62 (1H,d); 9.05 (1H,d); $J_{CH-NH}=9.0$ Hz.

MS m/e (% of base peak): 458(0.7)M^+ 416(6), 326(3), 325(8), 235(2), 206(6), 130(10), 117(11), 116(10), 115(7), 103(5), 92(31), 91(100), 77(8).

N^2,N^4-*Bis(diphenylmethylene)-acetohydrazide hydrazone (If).* Benzophenone hydrazone (7.45 g, 0.038 mol) was dispersed in 30 ml of abs. ethanol. *O*-Ethyl thioacetate (2 g, 0.019 mol) in 1 ml abs. ethanol was added dropwise at room temperature. The mixture was refluxed for 6 h. Standing for three days at room temperature and an additional few hours in the freezer caused precipitation of If, which was recrystallized from ethanol. The residue which could be isolated from the mother liquor was a mixture of unreacted hydrazone and If according to the NMR spectrum.

IR(KBr, in cm^{-1}); 3295w, 1599s, 1591s, 1572s, 1548s, 1440m, 1410m, 1125m, 1038m, 1028m, 1022m.

^1H *NMR* ($CDCl_3$): δ 2.33(3 H, s); 7.0–7.67 (20 H, m); 9.88(1 H, s).

MS m/e (% of base peak): 416(4)M^+, 339(3), 211(1), 196(4), 195(4), 180(87), 165(27), 152(5), 104(16), 78(14), 77(100).

N^2,N^4-*Di-(methylphenylmethylene)-acetohydrazide hydrazone (Ig).* Acetophenone hydrazone (6.7 g, 0.05 mol) was dissolved in 20 ml abs. ethanol. *O*-Ethyl thioacetate (5.2 g, 0.05 mol) was added dropwise over a period of 5 min. After standing overnight at room temperature with stirring, crystals were formed. These were filtered off and identified as acetophenone azine by m.p. and IR spectrum.

The mother liquor was evaporated to dryness and from the formed crystalline mixture Ig was isolated by separation on silica gel plates with $CHCl_3$ as eluent.

IR(KBr, in cm^{-1}): 3332w, 1608m, 1578m, 1558m, 1492m, 1418m, 1282m, 1142m, 1045m, 1040m.

^1H *NMR*($CDCl_3$): δ 2.23, 2.33, 2.40, 2.47, 2.52 (9 H, singlets) 7.17–7.57 and 7.63–7.87 (10 H, m); 9.50–9.84 (1 H, s).

MS m/e (% of base peak); 292(15)M^+, 277(40), 236(33), 221(44), 215(8), 180(6), 176(8), 159(13), 134(13), 133(15), 199(19), 118(100), 104(17), 103(17), 92(6), 91(8), 77(75).

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The Reaction between 2,4,6-Triarylpyrylium Salts and Sodium Nitrite*

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The reaction between a series of 2,4,6-triarylpyrylium salts and sodium nitrite in various solvents has been examined. When the reaction was carried out in acetonitrile the main reaction led to 3,5-diaroyl-4-arylisoxazoles. If the reaction was carried out in alcoholic solvents, complex mixtures were formed. The main product in most of these cases consisted of 2,4,6-triaryl-2,6-dialkoxy-5-nitro-5,6-dihydro-2*H*-pyrans. Besides this, various by-products were isolated and characterized. The mechanism and scope of the reactions are discussed.

In continuation of our previous studies of the reactions of pyrylium salts,[2] this examination of the reaction between 2,4,6-triarylpyrylium salts and sodium nitrite (nitrite-nitrous acid) was instigated.

Rather complex reaction mixtures resulted in most cases; a variety of products could be isolated, and the product distribution was found to be highly dependent on solvent as well as other variables, *e.g.*, the pyrylium counter ion and the oxygen concentration.

A number of the types of product isolated would be very hard to synthesize by known methods. Thus we directed our studies towards making the reactions potentially useful for synthetic purposes, as well as towards understanding the mechanisms involved. Our study was met with a certain degree of success, since we were able to find conditions where one or the other of the main products dominated. Furthermore a mechanistic scheme that explains the main reactions was derived.

Most of the detailed studies employed 2,4,6-triphenylpyrylium tetrachloroferrate as substrate. The product distribution from this substrate in a typical experiment is illustrated by Chart 1.

* For a preliminary account of part of this work, see Ref. 1.

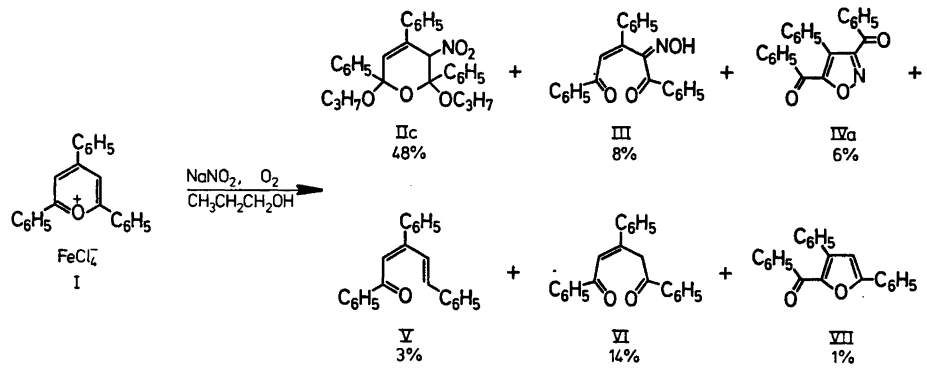


Chart 1.

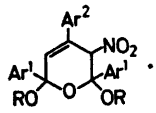
RESULTS

In this section the synthetically useful results are discussed, whereas some results which are especially useful in our attempt to unravel the mechanistic picture will be presented in the discussion section.

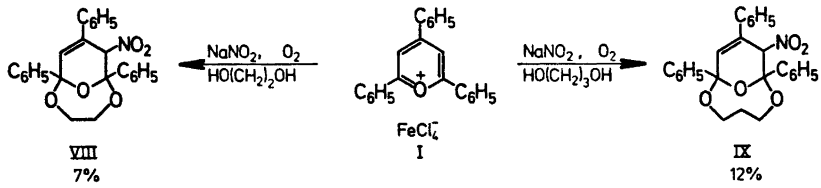
a. The formation of dihydropyrans (II) using monovalent alcohols as the solvent. A series of experiments were performed with 2,4,6-triphenylpyrylium tetrachloroferrate (I) as substrate and a monoalcohol both as solvent and one of the reagents. The reaction vessels were open, and thus oxygen from the air was present. The results are summarized in Table 1.

b. The reaction between glycols and 2,4,6-triphenylpyrylium tetrachloroferrate. In this series of experiments it was found that ethylene glycol and 1,3-propanediol gave the bicyclic dihydropyrans VIII and IX, respectively (Chart 2), whereas 1,4-butanediol did not lead to a dihydropyran in observable amounts. In this case the only isolated product consisted of IVa (Table 2). It should be noted that the reactions with monoalcohols and glycols as a rule were discontinued before all pyrylium ion was consumed, since the dihydropyrans were slowly decomposed under the reaction conditions.

From the results it appears that the formation of dihydropyrans is a very general reaction. However, steric crowding was observed to be a limiting factor. By using secondary or tertiary butyl alcohol as solvents and reagents, we were not able to isolate any dihydropyran, but only compound IVa (Table 2). Furthermore, it should be noted that the so-called pseudobase VI was

Table 1. Dihydropyrans IIa–r,  .

	Ar^1	Ar^2	R	Yield (%)
a	C_6H_5	C_6H_5	CH_3	27
b	C_6H_5	C_6H_5	CH_3CH_2	46
c	C_6H_5	C_6H_5	$CH_3CH_2CH_2$	48
d	C_6H_5	C_6H_5	$CH_3CH_2CH_2CH_2$	71
e	C_6H_5	C_6H_5	$CH_3CH_2CH_2CH_2CH_2$	48
f	C_6H_5	C_6H_5	$(CH_3)_2CH$	43
g	C_6H_5	C_6H_5	$(CH_3)_3CCH_2$	21
h	C_6H_5	C_6H_5	$(-)$-$CH_3CH_2CH(CH_3)CH_2$	66
i	C_6H_5	C_6H_5	$C_6H_5CH_2$	24
j	C_6H_5	p-CH_3-C_6H_4	$CH_3CH_2CH_2$	80
k	C_6H_5	m-NO_2-C_6H_4	$CH_3CH_2CH_2$	19
l	C_6H_5	p-Pr^i-C_6H_4	$CH_3CH_2CH_2$	91
m	p-Br-C_6H_4	C_6H_5	$CH_3CH_2CH_2$	40
n	p-CH_3-C_6H_4	C_6H_5	$CH_3CH_2CH_2$	89
o	p-Cl-C_6H_4	C_6H_5	$CH_3CH_2CH_2$	60
p	p-CH_3-C_6H_4	p-CH_3-C_6H_4	$CH_3CH_2CH_2$	50
q	p-Cl-C_6H_4	p-Cl-C_6H_4	$CH_3CH_2CH_2$	47
r	C_6H_5	p-Br-C_6H_4	CH_3CH_2	57



VIII 7% I IX 12%

Chart 2.

formed in all experiments using alcohols as solvents.

c. The influence of oxygen, and the pyrylium counter ion, on the dihydropyran formation. The 2,4,6-triphenylpyrylium salts were also in this study chosen as model substrates, and as the alcohol was used 1-propanol. In an experiment with 2,4,6-triphenylpyrylium tetrachloroferrate, where oxygen was carefully excluded, we observed a decrease in the yield of dihydropyran IIc (from ≈48 % to <7 %). Concurrently the yield of oxime (III) increased (from 8 % to 20 %) as did that of pseudobase VI (from 14 % to 60 %). The influence of the counter ion was adequately demonstrated by a comparison with 2,4,6-triphenylpyrylium tetrafluoroborate, which only gave 11 % of dihydropyran IIc, while the yield of oxime (III) increased to 61 %.

d. Dihydropyrans from other 2,4,6-triarylpyrylium derivatives. The reaction in which the dihydropyrans were generated was found to be rather independent of substituents in the aryl groups (Table 1), although there is undoubtedly a limit to this.

In all of the above experiments, some minor products were formed, but as a rule it was not tried to isolate them quantitatively. However, in Chart 1 such a quantitative experiment is depicted.

e. Formation of isoxazoles (IV), and the oxime (III). The isoxazoles (IV) were formed to some extent in all our experiments, and it was attempted to find conditions, whereby the yield of this product could be increased to the point where it became of synthetic usefulness. The reaction between 2,4,6-triphenylpyrylium ion and nitrite ion was therefore examined in a series of solvents (Table 2). From the table it emerges that the best yield of isoxazoles is obtained with acetonitrile as solvent under refluxing conditions. Furthermore, as can be seen in Table 2, this reaction is also general for 2,4,6-triarylpyrylium salts. It was found that a tetrafluoroborate counter ion gave better yields than when the tetrachloroferrate was employed. In the former case, it was furthermore observed, that the benzoate of the oxime, X, was formed (Chart 3). If oxygen was excluded, an almost quantitative yield of the oxime III was ob-

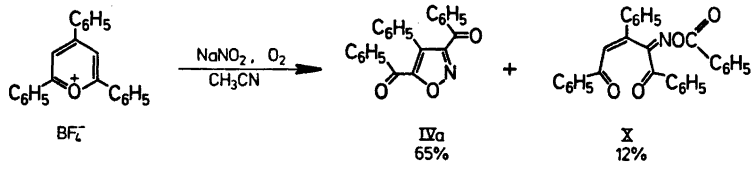
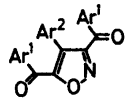


Chart 3.

Table 2. Isoxazoles IVa–d, 

	Solvent	Ar^1	Ar^2	Counter ion	Yield (%)
a	H_2O/CH_3COCH_3 (5/95)	C_6H_5	C_6H_5	$FeCl_4^-$	44
a	$CH_3CH_2CHOHCH_3$	C_6H_5	C_6H_5	$FeCl_4^-$	29
a	$(CH_3)_3COH$	C_6H_5	C_6H_5	$FeCl_4^-$	25
a	$CH_3CH_2COH(CH_3)_2$	C_6H_5	C_6H_5	$FeCl_4^-$	15
a	$HO(CH_2)_4OH$	C_6H_5	C_6H_5	$FeCl_4^-$	23
a	CH_3CN	C_6H_5	C_6H_5	BF_4^-	65
b	CH_3CN	C_6H_5	*m*-Cl-C_6H_4	BF_4^-	33
c	CH_3CN	C_6H_5	*p*-NO_2-C_6H_4	BF_4^-	26
d	CH_3CN	*p*-CH_3-C_6H_4	C_6H_5	BF_4^-	39

Table 3. Nuclear magnetic resonance spectra of compounds IIa–r, VIII and IX.[a]

Compound	Aromatic	H[b]	H[b]	Alkoxy
IIa	2.12–2.88 (15 H)	3.35	4.20	6.76, 6.84 ($-CH_3$, 6 H)
IIb	2.03–2.71 (15 H)	3.25	4.14	6.05–6.92 ($-OCH_2-$, 4 H) 8.85, 8.87 ($-CH_3$, 6 H)
IIc	2.02–2.70 (15 H)	3.25	4.12	6.16–7.10 ($-OCH_2-$, 4 H) 8.08–8.87 ($-CH_2CH_3$, 4 H) 8.93–9.37 ($-CH_3$, 6 H)
IId	2.04–2.69 (15 H)	3.25	4.11	6.12–6.93 ($-OCH_2-$, 4 H) 8.28–8.85 ($-OCH_2CH_2CH_2-$, 8 H) 8.93–9.37 ($-CH_3$, 6 H)
IIe	2.07–2.74 (15 H)	3.28	4.15	6.16–7.15 ($-OCH_2-$, 4 H) 8.17–9.00 ($-OCH_2CH_2CH_2CH_2$, 12 H) 9.00–9.33 ($-CH_3$, 6 H)
IIf[c]	1.97–2.71 (15 H)	3.15	4.04	5.45–6.35 ($-OCH<$, 2 H) 8.71, 8.80, 8.89, 9.38 ($-CH_3$, 12 H)
IIg	2.05–2.69 (15 H)	3.19	4.15	6.47–7.30 ($-OCH_2-$, 4 H) 9.12, 9.14 ($-CH_3$, 18 H)
IIh	2.00–2.94 (15 H)	3.23	4.08	6.22–7.18 ($-OCH_2-$, 4 H) 8.83–9.50 ($-CH(CH_3)CH_2CH_3$, 18 H)
IIi	1.81–2.81 (25 H)	3.00	3.92	5.10–5.83 ($-OCH_2-$, 4 H)
IIj	2.03–2.90 (14 H)	3.30	4.13	6.15–7.08 ($-OCH_2-$, 4 H) 8.18–8.78 ($-CH_2CH_3$, 4 H) 8.93–9.35 ($-CH_3$, 6 H)
IIk	1.45–2.54 (14 H)	3.00	4.03	6.22–7.02 ($-OCH_2-$, 4 H) 8.15–8.72 ($-CH_2CH_3$, 4 H) 8.93–9.33 ($-CH_3$, 6 H)
III[d]	2.05–2.95 (14 H)	3.28	4.14	6.23–7.44 ($-OCH_2-$, $(CH_3)_2CH-C_6H_4-$, 5 H) 8.10–8.82 ($-CH_2CH_3$, 4 H) 8.95–9.37 ($-CH_3$, 6 H)
IIm	2.14–2.55 (13 H)	3.21	4.05	6.15–7.07 ($-OCH_2-$, 4 H) 8.17–8.62 ($-CH_2CH_3$, 4 H) 8.93–9.33 ($-CH_3$, 6 H)
IIn	2.20–2.87 (13 H)	3.28	4.15	6.28–7.08 ($-OCH_2-$, 4 H) 8.20–8.77 ($-CH_2CH_3$, 4 H) 8.95–9.37 ($-CH_3$, 6 H)
IIo	2.17–2.80 (13 H)	3.36	4.18	6.23–7.20 ($-OCH_2-$, 4 H) 8.08–8.83 ($-CH_2CH_3$, 4 H) 8.93–9.37 ($-CH_3$, 6 H)
IIp	2.20–3.03 (12 H)	3.35	4.18	6.31–7.16 ($-OCH_2-$, 4 H) 8.10–8.82 ($-CH_2CH_3$, 4 H) 8.95–9.38 ($-CH_3$, 6 H)
IIq	2.17–2.73 (12 H)	3.38	4.25	6.18–7.06 ($-OCH_2-$, 4 H) 8.12–8.78 ($-CH_2CH_3$, 4 H) 8.92–9.38 ($-CH_3$, 6 H)
IIr	2.12–2.93 (14 H)	3.35	4.25	6.15–6.96 ($-OCH_2-$, 4 H) 8.86, 8.88 (CH_3, 6 H)
VIII	1.75–2.55 (15 H)	3.36	4.15	5.67–6.47 ($-OCH_2CH_2O-$, 4 H)
IX	1.69–2.69 (15 H)	3.43	4.02	5.82–6.42 ($-OCH_2CH_2CH_2O-$, 4 H) 7.57–9.22 ($-CH_2-$, 2 H)

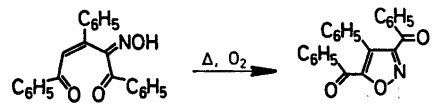
[a] The spectra were recorded at 60 MHz in $CDCl_3$ with TMS as internal reference. Chemical shifts are in τ-values. Relative intensities are given in parenthesis. [b] Singlets. The integrals of all signals correspond to one proton. [c] $J(CH-CH_3)=6$ Hz for all four methyl groups. [d] $J(CH-CH_3)=7$ Hz.

tained. No attempts to maximize the other by-products (Chart 1) were undertaken.

STRUCTURE ELUCIDATION

a. The dihydropyrans (II). The structure of the product, IIr, from 2,6-diphenyl-4-(4-bromophenyl)pyrylium tetrachloroferrate, sodium nitrite and ethanol was unambiguously determined by X-ray crystallography.[1] The molecule has the following geometrical features: 1, Five of the atoms of the dihydropyran ring are coplanar, whereas the carbon atom in the 6-position is 0.59 Å out of this plane. 2, The phenyl groups in the 2- and 6-positions, and the nitro group are on the same side of the dihydropyran ring, and thus the ethoxy groups are *cis* to each other. The structures of the analogues of IIr were determined on the basis of their elemental analysis (Table 7), and by comparison of their NMR spectra (Table 3) and their IR and UV spectra (Table 4) with those of compound IIr.

b. The isoxazoles (IV). The elemental analysis (Table 7) and the mass spectrum of compound IVa, *i.e.*, the product from 2,4,6-triphenylpyrylium tetrafluoroborate and sodium nitrite in acetonitrile, established its formula as $C_{23}H_{15}NO_3$. However, although its spectra (NMR, only aromatic protons; IR, strong absorption at 1670 cm^{-1}) (Tables 5 and 6) agree with our structural assignment, they do not permit its identification. We thus particularly base the assigned structure on its formation from the oxime (III), which by oxidation with air yielded compound IVa (Chart 4).



III IVa

Chart 4.

c. The oxime (III). This compound was identified as 4-oximino-1,3,5-triphenyl-2-pentene-1,5-dione on the basis of its elemental analysis (Table 7) and its spectra. In the NMR spectrum (Table 5) were found signals due to the aromatic protons and at very low field a singlet (-2.20 τ), which disappeared upon

Table 4. Characteristic infrared and ultraviolet absorptions of compounds IIa–r, VIII and IX.

Compound	IR[a] cm^{-1}	UV (96 % ethanol)					
		λ_{max} nm	log ε	λ_{max} nm	log ε	λ_{max} nm	log ε
IIa	1555	209	4.47	251	4.16		
IIb	1555	210	4.29	249	4.10		
IIc	1555	212	4.27	248	4.10		
IId	1555	211	4.23	248	4.05		
IIe	1560	214	4.25	248	4.14		
IIf	1555	209	4.30	247	4.06		
IIg	1560	210	4.34	246	4.11		
IIh	1560						
IIi	1560						
IIj	1555	210	4.42	254	4.23		
IIk	1560	211	4.32	248	4.29		
III	1560	211	4.31	254	4.16		
IIm	1555	210	4.31	228	4.31	242[sh]	4.15
IIn	1555	216	4.33	248	4.20		
IIo	1560	212[sh]	4.33	223	4.36	248	4.20
IIp	1555	220	4.34	256	4.25		
IIq	1555	226	4.36	258	4.28		
IIr	1550	211	4.11	259	3.98	298[sh]	3.21
VIII	1560	212	4.22	249	4.10		
IX	1555	210	4.27	244	4.09		

[a] The spectra of compounds IIh, i were recorded in $CHCl_3$, all other compounds in KBr.

Table 5. Nuclear magnetic resonance spectra of compounds III, IVa–d, X and XI[a].

Compound	Aromatic	Other
III	1.78–2.75 (15 H)	2.17 (alkene, 1 H), –2.20 (OH, 1 H)[b]
IVa	1.61–2.64	
IVb	1.80–2.87	
IVc	1.68–2.72	
IVd	1.97–2.93 (13 H)	7.60 (methyl, 6 H)
X	1.38–2.75	
XI	1.93–2.98 (16 H)	1.82 (methine, 1 H)[b]

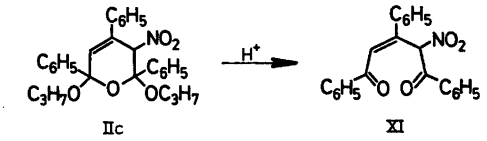
[a] The spectra were recorded at 60 MHz with TMS as internal reference. Chemical shifts are in τ-values. Relative intensities are given in parenthesis. The spectrum of compound III was recorded in DMSO-d_6, those of the other compounds in $CDCl_3$.
[b] These singlets disappeared after shaking with D_2O.

Table 6. Characteristic infrared and ultraviolet absorptions of compounds III, IVa–d, X and XI.

Compound	IR in KBr, cm^{-1}	UV (96 % ethanol)							
		λ_{max} nm	log ε	λ_{max} nm	log ε	λ_{max} nm	log ε	λ_{max} nm	log ε
III	3290(OH), 1680, 1660(C=O)	209	4.43	264	4.46	310	4.32		
IVa	1670(C=O)	208	4.44	263	4.41	287[sh]	4.10		
IVb	1680(C=O)	208	4.43	264	4.30	289[sh]	4.04		
IVc	1670(C=O), 1515(–NO_2)	207	4.38	270	4.41				
IVd	1670(C=O)	208	4.37	274	4.11				
X	1760, 1660, 1650(C=O)	206	4.34	237	4.28	264	4.28	311[sh]	4.16
XI	1705, 1645(C=O), 1570(–NO_2)	206	4.40	256	4.24	284[sh]	4.11	343[sh]	3.72

shaking with D_2O. This singlet is believed to be due to the oxime proton; the signal due to the vinyl proton is found at 2.17 τ, and is unchanged upon shaking with D_2O. Its IR spectrum (Table 6) showed two carbonyl absorptions, and a broad band at 3300 cm^{-1} from the oxime function.

d. Compound XI (4-nitro-1,3,5-triphenyl-2-pentene-1,5-dione). The assignments of structure to this compound is based on its generation from the corresponding dihydropyran, IIc, by mild acid hydrolysis (Chart 5).



IIc XI

Chart 5.

Furthermore, its spectroscopic features support the proposed structure (Tables 5 and 6).

e. 4-O-Benzoyloximino-1,3,5-triphenyl-2-pentene-1,5-dione (X). This compound is assigned its structure on the basis of its IR and NMR spectra (Tables 5 and 6). Furthermore, its molecular weight was confirmed by mass spectrometry. 1,3,5-Triphenyl-2-pentene-1,5-dione (VI),[3] 1,3,5-triphenyl-2,4-pentadiene-1-one (V) [4] and 2-benzoyl-3,5-diphenylfuran (VII) [5] were identical (m.p.'s and IR spectra) to the respective authentic samples.

DISCUSSION

The reactions between 2,4,6-triarylpyrylium salts and nitrite ion represent a complicated sequence of reactions, and consequently no complete understanding of the reaction pathways leading to each individual product could

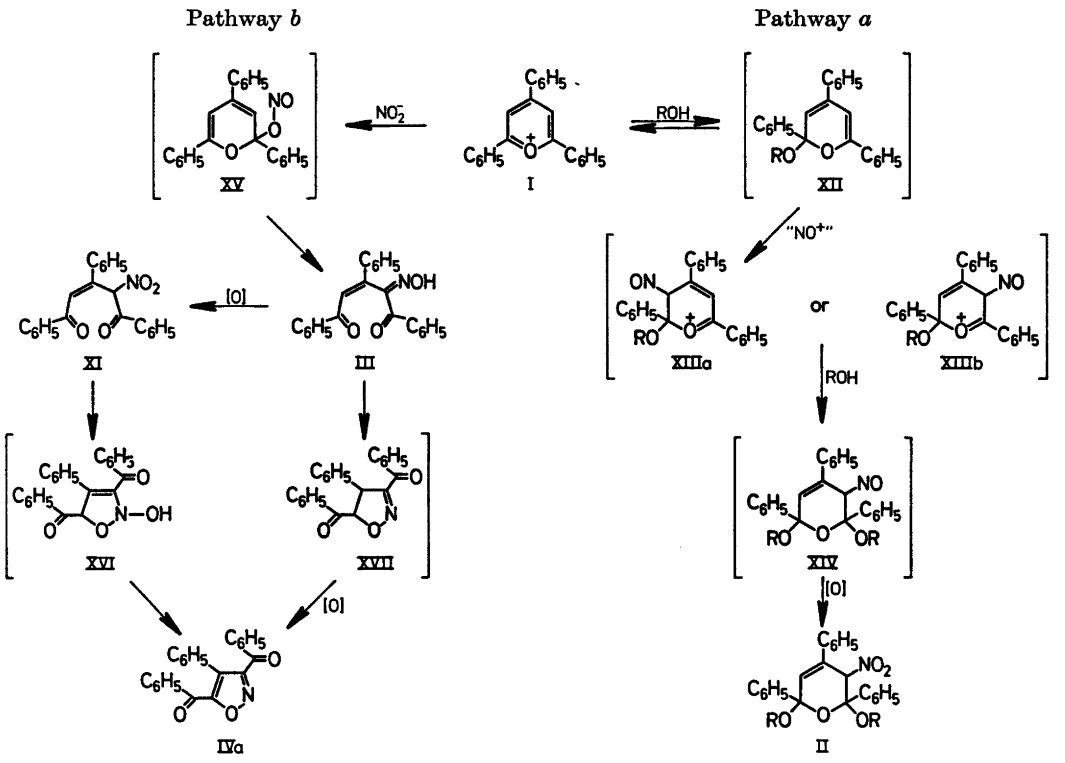


Chart 6.

be obtained. However, in order to obtain some understanding of the reaction mechanisms some further experiments have been performed, again using the 2,4,6-triphenylpyrylium ion as substrate.

We believe that the following represents the main pattern of events.

There are two mutually independent types of nucleophilic attack on the pyrylium ion (Chart 6), namely by alcohol (Pathway *a*) and by nitrite (Pathway *b*). After the initial steps, the reactions presumably proceed like shown in the scheme. This assumption is based on: (1) the pH-dependency of the dihydropyran formation, (2) the isolation of intermediates.

(1) The formation of IIc from I and 1-propanol was investigated with either tetrachloroferrate or tetrafluoroborate as counter ions. It turns out that tetrachloroferrates solvolyze slowly in protic solvents as seen from pH measurements (eqn. 1), whereas tetrafluoroborates do not under the experimental conditions employed.

$$FeCl_4^- + ROH \rightarrow \text{"}[FeCl_3(OR)]^-\text{"} + HCl, \textit{ etc.} \quad (1)$$

By using the tetrachloroferrate salt of I, a yield of 48 % of IIc was obtained, whereas with the tetrafluoroborate only 11 % of IIc could be obtained; concurrently, the yield of the oxime III increased from 8 % to 61 %. In each experiment the reaction vessels were open to the air.

The influence of pH was corroborated by doing an experiment with the tetrafluoroborate salt with added phosphoric acid, whereby the yield of dihydropyran (IIc) could be increased to 37 %, and the yield of oxime (III) was 34 %. This clearly illustrates the influence of pH, but it is not disproved that the tetrachloroferrate does not have some specific effect, like *e.g.*, increasing the oxidation potential.

(2) That two independent pathways to the main products (II and IV) exist was further

consolidated by isolating and examining possible intermediates. Thus the oxime (III) could be generated almost quantitatively when I,BF_4^- and sodium nitrite was allowed to react in boiling acetonitrile with exclusion of air. If boiling of the reaction mixture was continued in the presence of air the oxime III was converted to isoxazole IVa in the same yield as could be obtained from I,BF_4^- and nitrite if air was present from the beginning. This clearly demonstrates that III is the precursor for IVa in acetonitrile, and probably also in other solvents (note that III could also be isolated from reaction mixtures with propanol as solvent as shown in Chart 1). Furthermore, by following the reaction in acetonitrile with air present, by thin layer chromatography, it was observed that the oxime concentration increased in the beginning of the reaction whereafter it slowly disappeared in favor of isoxazole (IVa). Furthermore, a number of attempts were undertaken to convert the oxime III to dihydropyran (IIc), but in all cases the only product observed was IVa.

The nitro compound XI could also have been a possible intermediate for both isoxazole and dihydropyran formation. It was only detected in the reaction between $I,FeCl_4^-$ and sodium nitrite in 2-propanol and could be isolated in 38 % yield, but its formation is probably due to partial degradation of the dihydropyran, IIf. Compound XI was most conveniently prepared from the dihydropyran, IIc, by acid hydrolysis. All attempts to convert it back to dihydropyran, IIc, failed; instead the isoxazole, IVa, was formed.

In conclusion we postulate that the dihydropyran is formed from XII by attack of nitrous acid or maybe NO^+ to give XIII, which reacts with the alcohol to give intermediate XIV which in turn in oxidized to the dihydropyran (II). The isoxazole is assumed to be formed *via* initial attack of nitrite on I to give intermediate XV which in turn rearranges to III. The reaction of III to give the product IV may go *via* either of the two pathways shown.

Most probably the main reaction goes *via* intermediate XVII, since refluxing of the nitro compound, XI, gave IVa and a 20 % yield of the furan (VII) (Chart 1). This contrasted with results from reacting I,BF_4^- and sodium nitrite in acetonitrile, which only gave compounds IVa and X.

The furan (VII) could be isolated in very low yield from $I,FeCl_4^-$ and sodium nitrite in propanol, but since it can also be generated from the pseudo base (VI) (Chart 1) by mild oxidations,[5] VI may be the progenitor.

The pentadienone (V) (Chart 1) is most likely formed directly from I. It was shown to be the main product upon reduction of 2,4,6-triphenylpyrylium tetrafluoroborate with sodium borohydride, or phenylsilane.[5]

EXPERIMENTAL

Microanalysis was carried out in the microanalysis department of this laboratory. Melting points (uncorrected) were determined on a Reichert melting point microscope or a Büchi melting point apparatus. Infrared spectra were recorded on a Perkin Elmer model 337 grating infrared spectrophotometer. Ultraviolet spectra were recorded on a Perkin Elmer model 137 UV spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian A60A spectrometer. Mass spectra were obtained with an A.E.I. MS-902 instrument, using direct sample insertion into the ion source. The electron energy was maintained at 70 eV. Preparative layer chromatography (PLC) was performed on 20 × 100 cm plates with a 2.5 mm thick layer of silica gel (Merck $PF_{254+366}$). The plates were developed 2 – 3 times with benzene or benzene-petroleum ether (1:1). The fractions were isolated by continuous extraction with chloroform in a Soxhlet apparatus.

Pyrylium salts. The tetrachloroferrates were prepared according to Ref. 6 and the tetrafluoroborates according to Ref. 7.

Reaction of pyrylium salts with sodium nitrite in different alcohols. In all preparations except with the very involatile glycols the following procedure was used: *e.g.* 2,4,6-triphenylpyrylium tetrachloroferrate (5.00 g, 9.9 mmol) and sodium nitrite (1.37 g, 19.8 mmol) were suspended in propanol (100 ml) and the mixture was stirred at room temperature for 64 h. After this, 2.26 g of unreacted pyrylium salt was removed by filtration and the reaction mixture was evaporated to dryness and extracted with benzene. After evaporation of the benzene, the resulting oil was separated by PLC (eluent: benzene/petroleum ether 1:1) into: (1) 2,4,6-triphenyl-2,6-dipropoxy-5-nitro-5,6-dihydro-2*H*-pyran (IIc) (1.27 g ~48 %),* (2) 3,5-dibenzoyl-4-

* This compound was contaminated with about 3 % of 1,3,5-triphenyl-2,4-pentadiene-1-one (V) as measured by NMR-spectroscopy. Direct isolation of compound V was only possible when the reaction was run in methanol. In that case compound V was obtained in 4 % yield.

Table 7. Melting points and elemental analyses of compounds IIa–r, III, IVa–d and VIII–XI.

Compound[a]	M.p. °C	Formula	Analyses							
			% C calc.	found	% H calc.	found	% N calc.	found	% halogen calc.	found
IIa[c]	164–165	$C_{25}H_{23}NO_5$	71.93	71.85	5.55	5.60	3.36	3.29		
IIb[d]	158–159	$C_{27}H_{27}NO_5$	72.79	72.70	6.11	6.33	3.14	3.20		
IIc[c]	142–143	$C_{29}H_{31}NO_5$	73.55	73.44	6.60	6.78	2.96	3.00		
IId[c]	114–115	$C_{31}H_{35}NO_5$	74.23	74.10	7.03	7.05	2.79	2.76		
IIe[c]	71–73	$C_{33}H_{39}NO_5$	74.83	74.60	7.42	7.44	2.64	2.61		
IIf[c]	163–164	$C_{29}H_{31}NO_5$	73.55	73.80	6.60	6.73	2.96	2.90		
IIg[c]	152–155	$C_{33}H_{39}NO_5$	74.83	74.70	7.42	7.45	2.64	2.46		
IIh	[b]	$C_{33}H_{39}NO_5$								
IIi	[b]	$C_{37}H_{30}NO_5$								
IIj[c]	124–125	$C_{30}H_{33}NO_5$	73.90	73.70	6.82	6.84	2.87	2.70		
IIk[c]	162–164	$C_{39}H_{30}N_2O_7$	67.17	67.12	5.83	5.97	5.40	5.52		
III[f]	117–119	$C_{32}H_{37}NO_5$	74.54	74.43	7.23	7.30	2.72	2.66		
IIm[f]	167–168	$C_{29}H_{29}Br_2NO_5$	55.17	55.18	4.63	4.73	2.22	2.15	25.31	25.58
IIn[f]	149–151	$C_{31}H_{35}NO_5$	74.23	74.32	7.03	7.10	2.79	2.76		
IIo[d]	146–148	$C_{29}H_{29}Cl_2NO_5$	64.21	64.29	5.39	5.42	2.58	2.58	13.07	12.96
IIp[d]	143–145	$C_{32}H_{37}NO_5$	74.54	74.56	7.23	7.34	2.72	2.73		
IIq[f]	159–160	$C_{29}H_{28}Cl_3NO_5$	60.38	60.26	4.89	4.91	2.43	2.39	18.44	18.43
IIr[c]	125–127	$C_{27}H_{26}BrNO_5$	61.83	61.75	5.00	5.11	2.67	2.58	15.24	15.39
III[e]	156–158	$C_{23}H_{17}NO_3$	77.73	78.00	4.82	5.00	3.94	3.93		
IVa[c]	122–123	$C_{23}H_{15}NO_3$	78.17	78.02	4.28	4.29	3.96	3.97		
IVb[c]	126–127	$C_{23}H_{14}ClNO_3$	71.22	71.45	3.64	3.87	3.61	3.34	9.14	9.25
IVc[c]	113–114	$C_{23}H_{14}N_2O_5$	69.34	69.20	3.54	3.73	7.03	6.98		
IVd[c]	118–119	$C_{25}H_{19}NO_3$	78.72	78.90	5.02	5.18	3.67	3.68		
VIII[c]	158–159	$C_{25}H_{21}NO_5$	72.28	72.82	5.10	5.20	3.37	3.37		
IX[e]	—	$C_{26}H_{23}NO_5$	72.71	74.20	5.40	5.86	3.26	2.97		
X[c]	153–154	$C_{30}H_{21}NO_4$	78.41	78.12	4.61	4.77	3.05	2.96		
IX[e]	126[d]	$C_{23}H_{17}NO_4$	74.38	74.50	4.61	4.73	3.77	3.75		

[a] Recrystallization solvents; [c] Methanol, [d] Ethanol, [e] Benzene/petroleum ether and [f] ethanol/benzene. [b] Compounds IIh,i were isolated as oils.

phenylisoxazole (IVa) (0.12 g ~6 %), (3) 2-benzoyl-3,5-diphenylfuran (VII) (0.017 g ~1%), (4) 1,3,5-triphenyl-1,5-pentenedione (VI) (0.25 g ~14 %), and (5) 4-oximino-1,3,5-triphenyl-2-pentene-1,5-dione (III) (0.16 g ~8 %).

The experiment with 2,4,6-triphenylpyrylium tetrafluoroborate and phosphoric acid was carried out as described above; pyrylium salt (1.00 g ~2.52 mmol), 90 % phosphoric acid (0.14 g ~1.26 mmol) and sodium nitrite (0.35 g ~5.04 mmol) were suspended in propanol (20 ml). PLC gave the dihydropyran IIc (0.44 g ~37 %) and the oxime III (0.30 g ~34 %).

In those preparations where glycols were used, a slightly different procedure was employed: After stirring for five days at room temperature, 200 ml of water was added, and the mixture was extracted three times with chloroform. After evaporation, the residue was extracted with benzene. The resulting oil was separated as above.

Reaction of pyrylium salts with sodium nitrite in acetonitrile. Compounds IVa-d were all prepared according to the following procedure: *e.g.* 2,4,6-triphenylpyrylium tetrafluoroborate (1.50 g, 3.8 mmol) was dissolved in acetonitrile (30 ml) and sodium nitrite (0.79 g, 11.4 mmol) was added. The mixture was refluxed for 20 h * and evaporated to dryness. The resulting oil was extracted with chloroform and the crude material from evaporation of the chloroform was recrystallized from methanol to give 0.79 g ~59 % of 3,5-dibenzoyl-4-phenylisoxazole (IVa). By the method of PLC (eluent: benzene) a further product was found. Separation of the residue from the chloroform extraction (the same amount of starting materials were used) gave (1) 3,5-dibenzoyl-4-phenylisoxazole (0.87 g ~65 %) and (2) a compound believed to be the benzoate of 4-oximino-1,3,5-triphenyl-2-pentene-1,5-dione (X) (0.22 g ~12 %).

Preparation of 4-oximino-1,3,5-triphenyl-2-pentene-1,5-dione (III). This compound was prepared in the same way as compounds IVa – d except that the reaction mixture was very carefully protected against air. The crude product from the chloroform extraction was recrystallized from benzene/petroleum ether to give an almost quantitative yield of III. Protection against air was not found necessary during the work up.

Preparation of 4-nitro-1,3,5-triphenyl-2-pentene-1,5-dione (XI). Compound IIc (0.50 g) was dissolved in chloroform (5 ml). To this solution was added concentrated hydrochloric acid (1 ml) and the resulting two phase system was stirred vigorously for 10 min. Water and additional chloroform was added and the organic phase was separated and dried over anhydrous sodium sulfate. After evaporation of the chloroform, the residue was recrystallized from benzene/petroleum ether to give an almost quantitative yield of compound XI.

Thermolysis of compound XI. Compound XI (0.40 g) was dissolved in acetonitrile (20 ml) and the solution was refluxed for 2 h. After evaporation of the solvent the residue was separated by PLC (eluent: benzene) into (1) 3,5-dibenzoyl-4-phenylisoxazole (IVa) (0.20 g ~52 %) and (2) 2-benzoyl-3,5-diphenylfuran (VII) (0.07 g ~20 %).

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* However, in the preparation of IVd it was necessary to reflux for 7 days.

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Enzymic Decarboxylation of *S*-Adenosyl-L-methionine in Rat Liver: Possible Interaction of Putrescine with the Prosthetic Group

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S-Adenosyl-L-methionine decarboxylase (EC 4.1.1.50) has been purified more than 1000-fold from rat liver. The molecular weight of the decarboxylase was calculated to be 68 000. No evidence was obtained indicating that pyridoxal phosphate acts as the prosthetic group of the enzyme. On the other hand, the decarboxylase apparently contains some carbonyl group(s) participating in the catalysis as supported by the inhibition of *S*-adenosyl-L-methionine decarboxylation in the presence of $NaBH_4$, phenylhydrazine or NaCN. Putrescine, the specific activator of mammalian *S*-adenosyl-L-methionine decarboxylase, might interact, directly or indirectly, with the carbonyl group(s) of the enzyme as suggested by the protection of the decarboxylase activity against borohydride reduction by the diamine.

Two soluble proteins are needed for the synthesis of spermidine from putrescine and *S*-adenosyl-L-methionine (Ado-Met) both in *prokaryotic* and *eukaryotic* cells. The first (*S*-adenosyl-L-methionine decarboxylase, Ado-Met decarboxylase, EC 4.1.1.50) catalyses the decarboxylation of Ado-Met and the second (spermidine synthase, EC 2.5.1.16) transfers the propylamino moiety from decarboxylated Ado-Met to putrescine.[1–4] The decarboxylation of Ado-Met apparently is the rate limiting reaction in the synthesis of spermidine in *eukaryotic* organisms.[3,4] Ado-Met decarboxylase from *Escherichia coli*, which has been purified to homogeneity, is specifically activated by magnesium,[1] and the enzyme contains, instead of pyridoxal phosphate, one or two mol of covalently bound pyruvate per one mol of enzyme, probably functioning as the prosthetic group.[5] Mammalian Ado-Met decarboxylase, unlike the *prokaryotic* enzyme, does not require magnesium ions, but is strongly and specifically activated by putrescine.[6,7] Ado-Met decarboxylase from rat prostate [6,7] and from rat liver [8] is inactivated by 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate treatment, which inhibits some enzymes requiring pyridoxal phosphate.[9] Moreover, a prior treatment of the prostatic Ado-Met decarboxylase with pyridoxal phosphate, but not with free pyridoxal, was shown to reverse the inhibitory action of the drug.[6,7] It has also been reported [8] that mammalian Ado-Met decarboxylase loses a part of its activity during dialysis against dilute buffers, but the full activity can be restored by the addition of pyridoxal phosphate in the incubation mixture.

The present communication reports an attempt to explore the nature of the prosthetic group of mammalian Ado-Met decarboxylase and to elucidate the mechanism of the activation of the decarboxylase by putrescine. No evidence was obtained to indicate that pyridoxal phosphate would function as the prosthetic group of Ado-Met decarboxylase from rat liver. On the other hand, the results show that the specific activation of mammalian Ado-Met decarboxylase by putrescine may involve an interaction of the amine with some carbonyl group(s) of the decarboxylase, may be with its prosthetic group.

MATERIAL AND METHODS

D,L-Methionine-1-^{14}C was purchased from the New England Nuclear Corporation (sp.act. 5.13 – 5.65 mC/mmol) or from the Radiochemical Centre, Amersham, Bucks., UK (sp.act. 55

mC/mmol), L-canaline, *p*-hydroxymercuribenzoate (HgBzOH), and *N*-ethylmaleimide (MalNEt) were supplied by Sigma. Methyl glyoxal bis(guanylhydrazone) (MGBG) was a product of Aldrich Chemical Company, and pyridoxal phosphate (PLP) was obtained from E. Merck AG, Darmstadt, Germany. NSD 1055 (4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate) was a gift to Dr. A. Raina from Smith and Nephew Research, Ltd. Dithiothreitol, putrescine, spermidine, and spermine were purchased from Calbiochem. Other chemicals were of analytical grade.

Radioactive and unlabelled Ado-Met was synthesized as described earlier.[10] The incubation conditions for the assay of Ado-Met decarboxylase activity have been described elsewhere.[4]

Partial purification of Ado-Met decarboxylase from rat liver. Female rats of the Wistar strain, weighing 250 – 350 g, received 10 mg MGBG per rat intraperitoneally at 24 and 12 h before sacrifice. MGBG has been shown to be a specific inhibitor of putrescine activated Ado-Met decaboxylase *in vitro*.[11] Using the principles of the method described earlier,[4] which included ammonium sulfate fractionation (0.35 – 0.60), chromatography on DEAE cellulose and on hydroxylapatite followed by four gel filtrations on Sephadex G-150, Sephahex G-100, Sephadex G-100, and Sephadex G-75, it was possible to purify Ado-Met decarboxylase over 1000-fold. The final enzyme preparation catalyzed a release of more than 1100 nmol of CO_2 from Ado-Met per mg protein per 30 min under standard assay conditions. The enzyme, however, contained some impurities as revealed by polyacrylamide gel electrophoresis.

Determination of the molecular weight. The sedimentation coefficient for Ado-Met decarboxylase was calculated from sucrose density gradient centrifugation experiments[12] using bovine liver catalase (Sigma) and yeast alcohol dehydrogenase (Boehringer, Mannheim) as markers. The molecular Stokes radius and the diffusion coefficient for the decarboxylase were calculated as described by Ackers and Steere[13] using Blue Dextran 2000, potassium dichromate, yeast alcohol dehydrogenase and bovine albumin (Armour Pharmaceutical Company) for the calibration of Sephadex G-150 column. The partial specific volume of the decarboxylase was assumed to be 0.74 ml/g.

Isoelectric focusing was performed as described earlier.[14] Protein was measured by the method of Lowry *et al.*[15]

RESULTS AND DISCUSSION

Table 1 summarizes some properties of highly purified Ado-Met decarboxylase from rat liver. These results are in good agreement with results obtained earlier with crude enzyme preparations from rat ventral prostate[16] and from rat liver.[4]

Consistent with earlier reports.[7,17] the addition of 5 mM dithiothreitol to the incubation mixture reverses the complete inhibition of Ado-Met decarboxylase by 1 mM HgBzOH or 0.3 mM $HgCl_2$. Furthermore, a pretreatment of the decarboxylase with 5 mM MalNEt for 1 h in ice resulted in 94 % inhibition which was not reversed by exposure to 5 mM dithiothreitol.

The activity of purified rat liver Ado-Met decarboxylase was stimulated 6-fold by 2.5 mM putrescine and nearly 2-fold by 5 mM 1,3-diaminopropane under standard assay conditions. Spermidine (10 mM) did not change the enzyme activity whereas Mg^{2+} (10 mM) was somewhat inhibitory. From various metals tested Zn^{2+} (1 mM) and Co^{2+} (1 mM) almost totally inhibited the decarboxylase activity in tris-HCl buffer, pH 7.4, but most of the activity could be restored by 5 mM dithiothreitol or 1 mM EDTA in the incubation mixture.

Common inhibitors of enzymes requiring pyridoxal phosphate had only slight inhibitory effect on the activity of purified rat liver Ado-Met decarboxylase when assayed in the presence

Table 1. Some properties of partially purified Ado-Met decarboxylase from rat liver.

K_m for Ado-Met (in the presence of 2.5 mM putrescine)	0.020 mM
K_m for Ado-Met (in the absence of putrescine)	0.350 mM
K_m for putrescine as the activator (in the presence of 0.2 mM Ado-Met)	0.010 mM
pH optimum	7.0 – 7.5
Isoelectric point	5.3
Stokes radius	3.03 nm
Sedimentation coefficient	5.1 S
Diffusion coefficient	7.03 F
M.W.	68 000

Table 2. Inhibition of rat liver Ado-Met decarboxylase by inhibitors of enzymes requiring pyridoxal phosphate. The incubations were performed by adding neutralized solutions of inhibitors to the standard incubation mixture.

Inhibitor	Concentration (mM)	Relative activity − putrescine	+putrescine
None	–	100	100
Isonicotinic acid hydrazine	10	82	84
Semicarbazide	10	52	96
Canaline	5	97	103
NSD 1055	5	0	0

Table 3. Partially purified rat liver Ado-Met decarboxylase preparations (Sephadex G-100 liberating 603 nmol of CO_2 from Ado-Met in standard incubation conditions: protein concentration varied between 0.03 and 0.1 mg per ml) were exposed to 10 mM $NaBH_4$ for 10 min at 37 °C. After the reduction the enzyme preparations were diluted with 25 mM potassium phosphate, pH 7.4, for subsequent assays.

Prior incubation in the presence of	Activity without $NaBH_4$ reduction nmol CO_2 liberated in 30 min (%)	Activity after $NaBH_4$ reduction
Experiment 1		
No additions	5.07 (100)	0.55 (11)
Putrescine (5 mM)	6.81 (100)	4.56 (67)
Spermidine (10 mM)	5.60 (100)	0.44 (8)
Spermine (10 mM)	6.10 (100)	0.12 (2)
Magnesium (20 mM)	6.22 (100)	2.48 (40)
1,3-Diaminopropane (5 mM)	6.73 (100)	2.89 (43)
Experiment 2		
No additions	3.16 (100)	0.19 (6)
Putrescine (5 mM)	2.85 (100)	2.13 (75)
Ado-Met (0.4 mM)	0.91 (100)	0.10 (11)
Putrescine (5 mM)+ Ado-Met (0.4 mM)	1.56 (100)	0.21 (14)
Ado-Met (0.4 mM)+ putrescine (5 mM)	0.92 (100)	0.11 (12)
Experiment 3		
No additions	3.31 (100)	0.13 (4)
Putrescine (3 mM)	4.95 (100)	4.05 (82)
Ado-Met (0.3 mM)	2.45 (100)	0.09 (4)
Ado-Met (0.3 mM)+ putrescine (3 mM)	4.35 (100)	0.99 (23)

of putrescine (Table 2). Semicarbazide (10 mM), however, clearly inhibited the decarboxylation of Ado-Met when assayed in the absence of putrescine. Furthermore, a prior treatment of the enzyme with 10 mM semicarbazide for 10 min at 37 °C totally inactivated the enzyme (not tabulated). Five mM NSD 1055 in the incubation resulted in a complete inhibition of Ado-Met decarboxylase activity. In contrast to those experiments with Ado-Met decarboxylase from rat ventral prostate,[6,7] the activity could not be restored by a prior incubation of the enzyme with 1 mM PLP. A treatment of the decarboxylase with imidazolium and cysteine in citrate buffer, pH 7.2, which is known to cleave the covalent bond between PLP and the apoenzyme in rabbit muscle phosphorylase b,[18] reduced the activity of Ado-Met decarboxylase

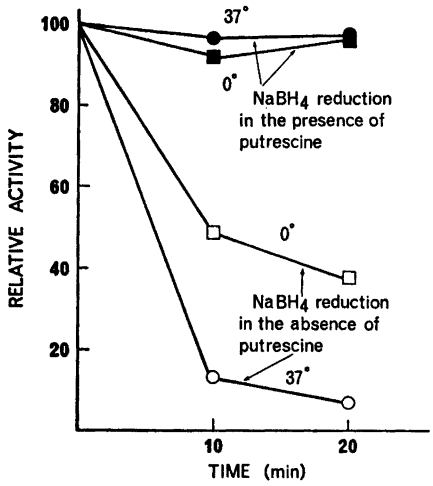


Fig. 1. A prior incubation of Ado-Met decarboxylase (Sephadex G-150 fraction, 0.075 mg protein per ml, decarboxylating 110 nmol of Ado-Met in standard assay conditions) with 5 mM putrescine before reduction with $NaBH_4$ protects the enzyme activity from the reductant. After incubating for 10 min at 37 °C with $NaBH_4$, the enzyme preparations were diluted with 25 mM potassium phosphate for the assay of enzyme activity.

from rat liver by less than 10 %. This inhibition could not be reversed by an inclusion of 1 mM PLP in the incubation mixture.

Carbonyl group reagents, sodium borohydride (10 mM), sodium cyanide (5 mM) or phenylhydrazine (1 mM) readily abolished the activity of rat liver Ado-Met decarboxylase. However, when the decarboxylase was incubated in the presence of 5 mM putrescine, prior to the exposure to $NaBH_4$, the inactivation of the enzyme was prevented as demonstrated in Fig. 1. As shown in Table 3, a prior incubation of the decarboxylase with magnesium or 1,3-diaminopropane also protected the enzyme activity from $NaBH_4$ reduction. On the other hand, when the enzyme was preincubated with the substrate, Ado-Met, the activity of the decarboxylase was completely inhibited by $NaBH_4$ reduction. Furthermore, the addition of the substrate together with putrescine to the enzyme mixture, prior to the treatment with $NaBH_4$, abolished the protective effect of putrescine against the reduction (Table 3).

Covalently bound pyruvate almost certainly functions as the prosthetic group of Ado-Met decarboxylase from *E. coli*.[5] Based mainly on experiments with NSD 1055, which inhibits the decarboxylation of Ado-Met by crude enzyme preparations from the cytosol fraction from rat ventral prostate [6,7] and from rat liver,[8] it has been suggested that PLP might act as the prosthetic group of mammalian Ado-Met decarboxylase. This suggestion has not been supported by experiments using Ado-Met decarboxylase from rat brain.[19]

In the absence of direct evidence it would be premature to conclude that mammalian Ado-Met decarboxylase contains carbonyl group(s) that functions as the prosthetic group. Nevertheless it is possible that this group(s) could be a part of the active center of the decarboxylase as shown by Recsei and Snell [20] for histidine decarboxylase from *Lactobacillus* 30a.

The mechanism of the specific activation of *eukaryotic* Ado-Met decarboxylase by putrescine still remains to be elaborated, but the present data suggest that putrescine might interact with the prosthetic group (carbonyl group) of the enzyme. This interaction may or may not involve a conformational change of the enzyme.

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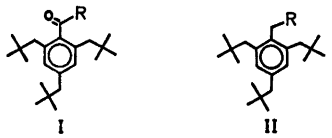
1,3,5-Trineopentylbenzene. VIII.* Restricted Rotation of Alkyl and Acyl Substituents

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The barriers to internal rotation of 2-acyl and 2-alkyl substituents in the 1,3,5-trineopentylbenzene system have been determined by means of computer simulation of ^{1}H NMR exchange-broadened spectra. An increase in barrier height with increasing branching at the C-2 carbon in the acyl or alkyl substituent has been found in both series. A discussion of the possible transition-state conformations for the slowly rotating substituent is presented, as well as the syntheses of the novel compounds utilized in this study.

In a previous investigation [1] of acylation reactions of 1,3,5-trineopentylbenzene under Friedel-Crafts conditions 2-acetyl, 2-propanoyl-, 2-(2-methylpropanoyl)- and 2-benzoyl-1,3,5-trineopentylbenzene (Ia – c) and (Ie) were synthesized. The present work deals with the reduction of these compounds to form 2-ethyl-, 2-propyl-, 2-(2-methylpropyl)- and 2-benzyl-1,3,5-trineopentylbenzene (IIa – c) and (IIe), and with the syntheses of 2-(2,2-dimethylpropanoyl)-1,3,5-trineopentylbenzene (Id) and 1,2,3,5-tetraneopentylbenzene (IId) by lithium-organic reactions. One main purpose of this investigation was the determination of the barriers to internal rotation ($\Delta G_C^{\ddagger}$, the free energy of activation at the NMR coalescence temperature) for the restricted rotation of the 2-alkyl and 2-acyl groups of the above-mentioned compounds. A discussion of the heights of these barrier and of the conformations of the compounds is presented.



I II

Fig. 1. a: R = methyl, b: R = ethyl, c: R = isopropyl, d: R = *tert*-butyl, e: R = phenyl.

RESULTS AND DISCUSSION

A. Syntheses. In order to carry out a Wolff-Kishner reduction, several attempts were made to convert Ia to a hydrazone. Treatment of Ia with 85 % hydrazine hydrate under reflux in ethanol, butanol, or 1,2-ethanediol did not yield the desired product. The IR spectra of the worked-up products from the syntheses were identical with that of Ia.

A Huang-Minlon synthesis was carried out with Ia according to a method described by Nagata and Itazaki.[2] This method (designated A in their publication) was reported to be successful for sterically hindered 11-ketosteroids. A method described by Martinson [3] for reduction of 1,3,5-tris(2,2-dimethylpropanoyl)-benzene under Huang-Minlon conditions was also applied to Ia. Both methods were unsuccessful, and again the IR spectra of the worked-up products from the syntheses were identical with that of Ia.

Several attempts were made to carry out a Clemmensen reduction of Ia according to a method described by Martin,[4] but the yield of 2-ethyl-1,3,5-trineopentylbenzene was never higher than 5 %. Preparation of amalgamated

* Part VII: see Ref. 1.

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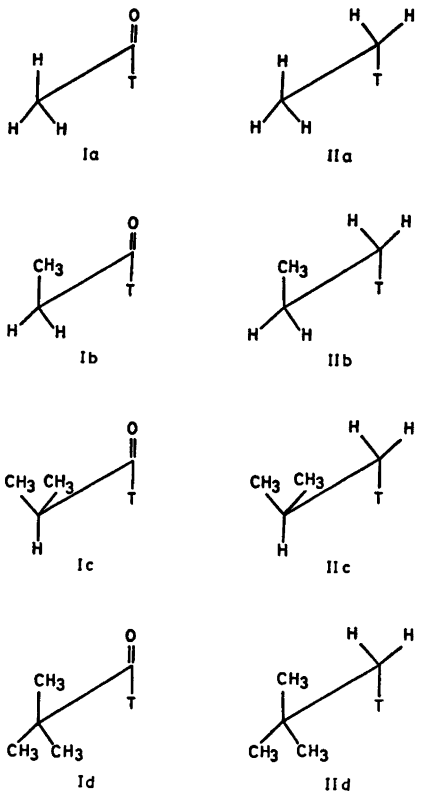


Fig. 2. Suggested transition state conformations of the 2-acyl and 2-alkyl groups of compounds Ia – d and IIa – d. T = 2,4,6-trineopentylphenyl.

zinc dust, as described by Caesar,[5] prior to the Clemmensen reduction, made no improvement.

The preferred method for synthesis of IIa – c and IIe included reduction with lithium tetrahydridoaluminate in diethyl ether to the corresponding alcohols, conversion to chlorides by sulfinyl chloride in hexane and reduction with lithium in liquid ammonia to yield the alkyl derivatives. The overall yields for the three-step reactions were 76 – 84 % after recrystallization. It was observed that 2-(1-chloroethyl)-1,3,5-trineopentylbenzene was unstable. On standing overnight at room temperature in hexane this compound was converted to 2,4,6-trineopentylstyrene (III). This product was synthesized by treating 2-(1-hydroxyethyl)-1,3,5-trineopentylbenzene with iodine and red phosphorus in cyclohexane. In order to avoid such elimination of hydrogen chloride, the chloroalkyl-1,3,5-trineopentylbenzenes were reduced with lithium in liquid ammonia immediately after they had been synthesized.

When 2-chloromethyl-1,3,5-trineopentylbenzene was treated with *tert*-butyllithium in pentane, 1,2,3,5-tetraneopentylbenzene (IId) was formed, in addition to 2-methyl-1,3,5-trineopentylbenzene and 1,2-bis(2,4,6-trineopentylphenyl)ethane. The yields of the three products were 21, 17 and 28 %, respectively.

2-(2,2-Dimethylpropanoyl)-1,3,5-trineopentylbenzene (Id) was synthesized by treating 2-iodo-1,3,5-trineopentylbenzene with *tert*-butyllithium in pentane, under a nitrogen atmosphere, to form 2-lithio-1,3,5-trineopentylbenzene, and then adding freshly distilled 2,2-dimethylpropanoyl chloride. In this way Id was formed, in addition to 2-[1-(2,2-dimethylpropanoyloxy)-2,2-dimethylpropyl]-1,3,5-trineopentylbenzene (IV). The yields of Id and IV were 42 and 33 %, respectively.

B. ^1H NMR spectra. It is well known that in unhindered aromatic ketones there is resonance energy stabilization due to overlap between the π electrons of the carbonyl group and the π electrons of the aromatic ring. This will lead to a preferred conformation in which the carbonyl group and the aromatic ring are coplanar. In sterically hindered aromatic ketones, deviations from coplanarity has been found.[6,7]

In 2,2,3-trimethyl-5,7-dineopentyl-1-indanone [8] the carbonyl group is locked, and the methylene protons of the 7-neopentyl group are in the deshielding zone of the carbonyl group appearing at 3.09 ppm.

In 2-(2,2-dimethylpropanoyl)-1,3,5-trineopentylbenzene (Id), on the other hand, the rotation about the $sp^2 - sp^2$ carbon-carbon bond is sterically restricted at ordinary probe temperature and even at 190 °C. The methylene protons *ortho* to the 2,2-dimethylpropanoyl group appear as an AB pattern centered at 2.31 ppm. The upfield shift of these protons (0.78 ppm with

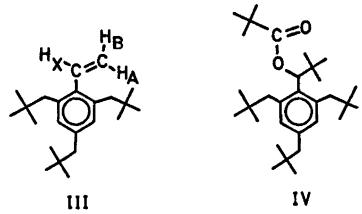


Fig. 3.

respect to the methylene protons of the 7-neopentyl group of 2,2,3-trimethyl-5,7-dineopentyl-1-indanone) shows that they are in the shielding zone of the carbonyl group. The oxygen atom and the *tert*-butyl group of the 2,2-dimethylpropanoyl group are thus situated on different sides of the plane of the aromatic ring.

In 2-(2-methylpropanoyl)-1,3,5-trineopentylbenzene (Ic) and 2-propanoyl-1,3,5-trineopentylbenzene (Ib), no frozen conformation could be detected at ordinary probe temperature, but the methylene protons *ortho* to the acyl groups appear at the same chemical shift (2.30 ppm), which is nearly equal to that found for the *ortho* methylene protons of Id. This indicates that the preferred conformations of these three compounds (Ib – d) must be very similar. The most strain-free conformation of Id is that in which the plane defined by the C-1 – C-2 bond of the acyl group and the bond between the acyl group and the aromatic ring is perpendicular to the plane of the latter. This conformation is most probably also the preferred conformation of compounds Ib and Ic, a conclusion based on the chemical shifts mentioned above.

In 2-acetyl-1,3,5-trineopentylbenzene (Ia) the interaction between the acyl group and the *ortho* neopentyl groups is decreased (compared to Ib – d), the *ortho* methylene protons spend more time in the deshielding zone of the carbonyl group, and appear at 2.38 ppm, which is 0.07 – 0.08 ppm downfield from those of Ib – d. It can be mentioned that in the preferred conformation of 2,4,6-trimethylacetophenone, the dihedral angle between the phenyl and acetyl planes has been reported to be about 45° in the liquid phase.[6] This value was obtained by matching the experimental and theoretical shielding values. In the same publication it was also reported that 2-methylacetophenone has a preferred conformation in which the acetyl group is coplanar with the phenyl group, with the oxygen atom close to the 2-methyl group. This shows that the "effective size" of the carbonyl oxygen is small compared to that of the methyl group of the acetyl group in their interactions with *ortho* alkyl substituents.

2-Benzoyl-1,3,5-trineopentylbenzene (Ie) shows sterically restricted rotation about the benzoyl-trineopentylphenyl sp^2-sp^2 carbon-carbon bond at room temperature, and the methylene protons *ortho* to the benzoyl group appear as an AB pattern centered at 2.27 ppm. The small upfield shift of these protons (0.04 ppm compared to those of Id) may be explained by shielding effect of the adjacent phenyl group, and the preferred conformation of this compound is most probably that in which the plane defined by the carbon-oxygen bond and the carbonyl-trineopentylphenyl bond is perpendicular to the plane of the substituted phenyl group, as in Id.

In 1,3,5-trineopentylbenzene,[3] the methylene protons appear at 2.43 ppm, which is also found for the *para* methylene protons of Ia – d. It is interesting to note that the *ortho* methylene protons which are situated in the shielding zone of the carbonyl group, as in Id, are shifted 0.12 ppm upfield (compared to 2.43 ppm), while *ortho* methylene protons which are situated in the deshielding zone of the carbonyl group, as in 2,2,3-trimethyl-5,7-dineopentyl-1-indanone, are shifted 0.66 ppm downfield (compared to 2.43 ppm).

In propylbenzene, isobutylbenzene and neopentylbenzene the terminal hydrogens appear at 0.90 ppm (CCl_4).[9] The effects of the interaction between the 2-alkyl groups and the *ortho* neopentyl groups are unclear, but in the NMR spectra of compounds IIb – d the terminal hydrogens of the 2-alkyl group appear at 1.03, 0.86 and 0.83 ppm, respectively. The terminal methyl groups thus seem to spend more time in the shielding zone of the aromatic ring with increasing methyl substitution at the β-carbon of the 2-alkyl group.

In the present study, 2-acyl and 2-alkyl derivatives of 1,3,5-trineopentylbenzene were found to give rise to AB patterns for the methylene protons in the 1- and 3-positions at sufficiently low temperatures. At the same temperatures the 5-methylene protons appeared as singlets. The restricted rotation of the acyl or alkyl substituent in the 2-position gives rise to the nonequivalence of the 1- and 3-methylene proton signals. The 1- and 3-methylene groups are enantiotopic due to the symmetry of the molecule, but the methylene protons of the 1- or 3-methylene groups are diastereotopic. These groups would thus appear as only one AB spectrum for each compound, which is consistent with the observed NMR spectral behavior.

Table 1. NMR parameters and $\Delta G_C^{\ddagger}$ values for the restricted rotation of acyl or alkyl groups in 2-substituted 1,3,5-trineopentylbenzenes.

Compound	t_C °C	J_{AB} Hz	$\Delta\nu_{AB}$ Hz	$\Delta G_C^{\ddagger}$ kJ/mol	Solvent
Ia[a]	−47	13.2	28.4	46.5	$CHCl_2F$
Ib[a]	−29	13.2	30.7	51.1	$CHCl_2F$
Ic[a]	−11	12.9	28.0	54.4	$CHCl_2F$
Id[b]	>+190	12.8	17.0	>96	$CDBr_3$
Ie[a]	+24	13.4	22.7	63.2	$CHCl_2F$
IIa[a]	−56	13.2	46.9	43.6	$CHCl_2F$
IIb[a]	−53	12.9	44.7	44.0	$CHCl_2F$
IIc[a]	−15	13.3	55.1	52.3	$CHCl_2F$
IId[b]	+82	13.6	42.5	73.3	$CDBr_3$
IIe[a]	−54	13.0	41.2	44.8	$CHCl_2F$

[a] Determined at low temperature on 100 Hz sweep width (100 MHz). [b] Parameters obtained from 60 MHz spectra.

Complete line-shape investigations are expected to be difficult, especially for compounds Ib and IIb, because the AB spectra were overlapped by the methylene signals of the 5-neopentyl group. Therefore a complete line-shape analysis was only performed on one compound (II d), and for all the other compounds the free energy of activation ($\Delta G_C^{\ddagger}$) was determined at some temperature near coalescence. The accuracy in such determinations of $\Delta G_C^{\ddagger}$ is estimated to be better than ± 1.3 kJ/mol.

The entropies of activation for the compounds studied are assumed to be close to zero. Support for this assumption is that the $\Delta S^{\ddagger}$ value for the restricted rotation of the 2-neopentyl group in IId was found to be -13.0 ± 8.8 J/(mol K) ($\Delta G^{\ddagger}_{298} = 73.7 \pm 0.8$ kJ/mol and $\Delta H^{\ddagger} = 69.9 \pm 2.9$ kJ/mol), and that $\Delta S^{\ddagger}$ values of zero within ± 13 J/(mol K) have been deduced from data for a large series of dihalo and/or methyl substituted 1,3,5-trineopentylbenzenes.[10]

In Table 1 we have summarized the $\Delta G^{\ddagger}$ values for the restricted rotation of an ethyl, propyl, 2-methylpropyl, neopentyl and a benzyl group, as well as for an acetyl, propanoyl, 2-methylpropanoyl and a benzoyl group, all as substituents in a nuclear position of 1,3,5-trineopentylbenzene. The rotational barrier of a 2,2-dimethylpropanoyl group could not be determined due to instrumental limitations. The lower limit of this barrier was estimated to be 96 kJ/mol.

In the following discussion of barrier heights, we assume that the initial state is represented by a conformation in which the plane defined by the C-1−C-2 bond of the 2-acyl or 2-alkyl group and the bond attaching the substituent to the trineopentylphenyl residue is perpendicular to the ring plane of the latter moiety, whereas the transition state is assumed to be the conformation in which the C-1−C-2 bond of these groups is in the plane of the aromatic ring.

Although the concept of size of alkyl or acyl groups is poorly defined in their steric interactions with the neopentyl groups, we found that the $\Delta G^{\ddagger}$ values increased in the series ethyl ≃ propyl < 2-methylpropyl < 2,2-dimethylpropyl, and, similarly, acetyl < propanoyl < 2-methylpropanoyl ≪ 2,2-dimethylpropanoyl (Table 1).

The barriers to internal rotation of the acyl groups were greater than those of the alkyl groups with the same number of carbon atoms. This may be surprising due to an expected resonance energy stabilization of the transition state in the presence of an acyl group, possibly of the order of 33 kJ/mol, as deduced from the magnitude of the rotational barrier in benzaldehyde (^{1}H NMR studies).[11] In addition, the greater bond angle of an acyl group (120°) compared to that of an alkyl group (109.5°) may give rise to smaller non-bonded interactions in the transition state between the alkyl substituent of the 2-acyl group and the 1- or 3-neopentyl group than between the substituent on the benzylic carbon of the 2-alkyl group (which has the same number of carbon atoms as the acyl group) and the 1- or 3-neopentyl group. On the contrary, in the rotational transition state of the acyl derivatives Ia−d, there may also be non-bonded interactions between the carbonyl group and an *ortho* neopentyl group. These interactions are assumed to be greater than those between the benzylic 2-methylene group and an *ortho* neopentyl group in the alkyl derivatives IIa−d.

The increased branching at the C-2 carbon atom in the 2-acyl and 2-alkyl substituents is thus covariant with an increase in the $\Delta G_C^{\ddagger}$ values in both series. Intuitively, the non-bonded interactions between the α-hydrogens of the acyl substituent and an *ortho* neopentyl group will be at a minimum in the transition

state of Ia if one of the α-hydrogens eclipses the carbonyl oxygen, and of Ib if the methyl group eclipses the carbonyl oxygen. Similarly, the interactions in the transition state would be most favorable in IIa and IIb, if the 2-alkyl group of IIa is in one of the staggered conformations, and if this group of IIb is in the staggered conformation in which the methyl group is between the α-methylene hydrogens (see Fig. 2).

Within the estimated limits of error the $\Delta G_C^{\ddagger}$ values for the internal rotation in IIa and IIb are equal, which is most probably explained by the very similar interactions in the transition state (as well as in the ground state). The difference in $\Delta G_C^{\ddagger}$ between Ia and IIa is the same as the difference between Ic and IIc within the limits of error (2.9 and 2.1 kJ/mol, respectively, see Table 1). The difference in $\Delta G_C^{\ddagger}$ between Ib and IIb is greater (7.1 kJ/mol), which may be caused by interactions between the eclipsing methyl group and carbonyl oxygen of the 2-acyl group in the transition state of Ib (see Fig. 2). The higher barrier for the 2-methylpropyl (compound IIc) and the 2-methylpropanoyl group (compound Ic) with respect to the corresponding groups in Ia, Ib, IIa and IIb may be explained in terms of their conformations in the transition state. If the methyl groups and the α-methylene hydrogens of the 2-methylpropyl group of IIc are in the eclipsed conformation, and if the methyl groups and the carbonyl oxygen of the 2-methylpropanoyl group of Ic are in the conformation in which the carbonyl oxygen is between the methyl groups (see Fig. 2) there will be interactions between the methine protons of the 2-alkyl and 2-acyl groups, respectively, and one of the *ortho* neopentyl groups in both the compounds, These interactions will be greater than the corresponding methylene-neopentyl interactions in the most favored conformations of the transition states of Ia, Ib, IIa and IIb. All other conformations of the 2-methylpropyl and 2-methylpropanoyl groups in the transition state will probably be less favorable due to the interactions between the methyl groups and the *ortho* neopentyl groups. Only in the 2,2-dimethylpropyl and 2,2-dimethylpropanoyl compounds (IId and Id) does a methyl group necessarily have to approach an *ortho* neopentyl group, thus resulting in particularly high $\Delta G_C^{\ddagger}$ values. The difference in $\Delta G_C^{\ddagger}$ (> 22 kJ mol) between Id and IId may be explained in the following way. In the transition state for the rotation of a 2-(2,2-dimethylpropyl) group of IId, we assume that there are bond angle deformations (in the plane of the benzene ring) at the substituent-carrying aromatic carbon atom as well as at the 2-benzylic carbon atom to minimize the steric interactions between one *ortho* neopentyl group and the passing *tert*-butyl group. In I d a similar deformation would lead to an increased interaction between the carbonyl group and the other neopentyl group, which would raise the barrier of this compound.

The $\Delta G_C^{\ddagger}$ value for the rotation of the benzyl group (*i.e.* compound IId) is close to that of an ethyl or a propyl group in the alkyl series, while the $\Delta G_C^{\ddagger}$ value of the benzoyl group (*i.e.* compound Ie) falls between that of a 2-methylpropanoyl and a 2,2-dimethylpropanoyl group in the acyl series. The difference in $\Delta G^{\ddagger}$ values between Ie and IIe is 18.4 kJ/mol. This great difference may be explained by the preferred coplanarity within the unsubstituted benzoyl group in the ground state of Ie, causing an increased steric interaction if the coplanarity still exists in the transition state, or an increased energy because of decreased conjugation, if the coplanarity is not retained in the transition state.

EXPERIMENTAL

Measurements. Gas chromatographic (GLC) analyses were carried out on a Perkin-Elmer 900 gas chromatograph fitted with a flame ionization detector. The inner diameter of the columns used was 2 mm and the length 2 m. The stationary phase was 3 % of SE-30 silicon gum rubber on Gaschrom Q 100–200 mesh. The areas of the peaks on the gas chromatograms were measured by triangulation.

The IR spectra were recorded on a Beckman IR 9 spectrophotometer using potassium bromide pellets or a potassium bromide cell. The absorption maxima are reported in cm^{-1} and the intensities are characterized as weak (w), medium (m), strong (s), or very strong (vs).

The NMR spectra were recorded on either a Varian A 60 or a Varian XL-100-15 spectrometer. Solutions, about 10 % by weight in carbon tetrachloride, were used for the purpose of identifying the compounds (Varian A 60), whereas solutions as dilute as possible (< 1 mol %) in the solvents dichlorofluoromethane (compounds Ia–c. Ie, IIa–c and IIe) and deuteriobromoform (compounds Id and IId) were used in the variable-temperature studies

(Varian XL-100-15). In the latter studies a small amount of dichloromethane was added as a standard of resolution. The chemical shifts are reported in ppm downfield from tetramethylsilane (probe temperature, 35 °C). The multiplicities of the peaks are reported as singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). Temperatures were measured by means of a copper constantan thermocouple which was fixed near the radiofrequency coil. The accuracy in these values is believed to be at least ±2 °C. The scale expansion and sweep rate for all spectra were 2 Hz/cm and 0.1 Hz/s, respectively.

In the line-shape studies of the 1- and 3-methylene proton signals of IId, spectra were run at a few temperatures where exchange broadening was of importance, and were visually fitted to theoretical ones made by means of a computer program, described elsewhere.[12] A UNIVAC 1108 computer equipped with a Calcomp plotter was utilized in the calculations. The activation parameters were calculated from the Eyring equation [13] and the reported errors were obtained by means of a least-squares treatment of the ln $1/\tau T$ *vs.* $1/T$ plot data.

For the other compounds, $\Delta G_C^{\ddagger}$ was calculated by means of the Eyring equation [13] from iterated rate constants, which were found by visually fitting spectra, obtained near the temperature of coalescence, to theoretical ones made by means of the computer program.[12] The estimated errors in the determined rate constants for the slowly rotating 2-substituent give errors in $\Delta G_C^{\ddagger}$ of ±0.8 kJ/mol, and the errors in the temperature (±2 °C) give error in $\Delta G_C^{\ddagger}$ that are less than ±0.5 kJ/mol. The total error is thus estimated to be less than ±1.3 kJ/mol.

The mass spectra (MS) were determined (at the Department of Medical Biochemistry, University of Göteborg) on an AEI 902 mass spectrometer with the following conditions: electron energy 70 eV, accelerating voltage 8 kV and emission 100 μA. The intensities of the peaks are reported in parentheses as percentages of the base peak. Only the most abundant peaks are reported, together with the parent peaks and the isotope peaks corresponding to the latter.

The melting points were determined on a Kofler micro hot stage.

Materials. 1,3,5-Trineopentylbenzene was prepared as described by Martinson and Márton.[14] The acyl derivatives of 1,3,5-trineopentylbenzene were prepared as described by Dahlberg, Martinson and Olsson.[1] 2-Chloromethyl-1,3,5-trineopentylbenzene and 2-iodo-1,3,5-trineopentylbenzene were prepared as described by Olsson.[15] All other chemicals were of reagent grade and were used without further purification unless stated otherwise.

2-(1-Hydroxyethyl)-1,3,5-trineopentylbenzene. In a two-necked flask equipped with a magnetic stirring bar, a dropping funnel and a reflux condenser, 6.9 g (182.0 mmol) of lithium tetrahydridoaluminate was covered with 50 ml of diethyl ether. 2-Acetyl-1,3,5-trineopentylbenzene (6.0 g, 18.2 mmol), dissolved in 20 ml of diethyl ether, was added dropwise and the stirred reaction mixture was then refluxed. At regular time intervals small aliquots were withdrawn and analysed by GLC. After 15 h, 2-(1-hydroxyethyl)-1,3,5-trineopentylbenzene had been formed in 99 % yield (GLC). The flask was then cooled with ice water, and 6.9 ml of water, 6.9 ml of 10 % sodium hydroxide solution and 20.7 ml of water were added slowly in that order. The mixture was filtered and the precipitate was washed several times with diethyl ether. The organic and aqueous layers were separated, and the organic layer was dried over magnesium sulfate. After evaporation of the diethyl ether, the product was purified by recrystallization from ethanol (water was cautiously added to the hot solution until turbidity appeared). The yield was 5.5 g (91 %) of white crystals. *M.p.* 97–98 °C. *IR:* 3559vs, 2955vs, 2905m, 2865s, 1605m, 1570m, 1478s, 1468w, 1450w, 1425w, 1394s, 1365vs, 1338m, 1280m, 1240s, 1232s, 1200s, 1165w, 1145m, 1129m, 1093s, 1074s, 1016m, 970w, 935w, 916w, 895w, 884s, 874w, 793m, 755w, 723m, 640w, 593m, 488w. *MS:* 29(38), 39(10), 41(54), 43(15), 55(6), 57(100), 73(5), 117(5), 130(6), 131(6), 147(6), 148(7), 149(7), 164(33), 220(15), 317(6), 332(0.016), 333(0.004). *NMR:* 0.92 (s, 9 H, *tert*-butyl), 0.96(s, 18 H, *tert*-butyl), 1.40(d, 3 H, methyl, $J = 7$ Hz), 1.48(s, 1 H, hydroxylic), 2.38(s, 2 H, methylene), 2.76(q, 4 H, methylene, $J = 13.6$ Hz, $\Delta\nu_{AB} = 13.0$ Hz), 5.35(q, 1 H, methine, $J = 7$ Hz), 6.72(s, 2 H, aromatic).

2-(1-Chloroethyl)-1,3,5-trineopentylbenzene. In a flask equipped with a magnetic stirring bar and a dropping funnel, 3.0 g (9.0 mmol) of 2-(1-hydroxyethyl)-1,3,5-trineopentylbenzene was dissolved in 30 ml of hexane, and 2.0 ml (3.2 g, 27.0 mmol) of freshly distilled sulfinyl chloride was added dropwise. The mixture was stirred overnight at room temperature, and the reaction was then found to be complete according to GLC. The solvent and the excess of sulfinyl chloride were evaporated. The yield was 3.2 g (100 %) of an oil which was used immediately in the next step. *NMR:* 0.93(s, 9 H, *tert*-butyl), 1.01(s, 18 H, *tert*-butyl), 1.82(d, 3 H, methyl, $J = 7$ Hz), 2.39(s, 2 H, methylene), 2.77(s, broad, 4 H, methylene), 5.57(q, 1 H, methine, $J = 7$ Hz), 6.80(s, 2 H, aromatic).

2-Ethyl-1,3,5-trineopentylbenzene (IIa). A two-necked flask equipped with a magnetic stirring bar, a dropping funnel and a gas inlet tube was cooled to −50 °C, and 50 ml of ammonia was condensed in the flask. Lithium (0.62 g, 90.0 mmol) was dissolved in the ammonia, and a solution of 3.2 g (9.0 mmol) of 2-(1-chloroethyl)-1,3,5-trineopentylbenzene in 10 ml of hexane was added dropwise. The reaction mixture was stirred at −50 °C, and after 2 h the excess of lithium was destroyed with water and the mixture was neutralised with 6 M hydrochloric acid. The aqueous layer was ex-

tracted several times with hexane and the combined hexane extracts were dried over magnesium sulfate. After evaporation of the solvent, the product was decolorized by adsorption chromatography (alumina, hexane). Recrystallization from ethanol-water gave 2.6 g (91 %) of white crystals, which according to GLC were more than 99.9 % pure. *M.p.* 39 – 40 °C. *IR*: 2947vs, 2906m, 2870s, 2742w, 2712w, 1609w, 1575w, 1480vs, 1467w, 1435w, 1394s, 1366vs, 1330w, 1284w, 1235s, 1204m, 1163w, 1082w, 1058s, 1041w, 965w, 935w, 916w, 882vs, 794m, 756w, 733w, 651w, 626m, 560m, 553m, 475w. *MS:* 29(29), 41(25), 43(20), 57(67), 69(20), 71(18), 145(7), 147(20), 148(42), 203(31), 204(57), 205(9), 231(7), 259(100), 260(73), 261(13), 301(30), 302(9), 316(96.2), 317(24.5), 318(3.1). *NMR*: 0.92(s, 27 H, *tert*-butyl), 0.97(t, 3 H, methyl, $J = 7$ Hz), 2.36(s, 2 H, methylene), 2.53(s, 4 H, methylene), 2.74(q, 2 H, methylene, $J = 7$ Hz), 6.65(s, 2 H, aromatic).

2-Propyl-1,3,5-trineopentylbenzene (IIb) was prepared from 2-propanoyl-1,3,5-trineopentylbenzene according to the method used above for the three-step synthesis of 2-ethyl-1,3,5-trineopentylbenzene from 2-acetyl-1,3,5-trineopentylbenzene. 2-Propyl-1,3,5-trineopentylbenzene was separated from unreacted starting materials on a column of silica gel. With hexane only 2-propyl-1,3,5-trineopentylbenzene was eluted. The overall yield was 80 % of an oil. *IR:* 3044w, 2947vs, 2900m, 2962s, 2740w, 2712w, 1609m, 1575w, 1477vs, 1465s, 1425w, 1390s, 1377w, 1361vs, 1328w, 1275w, 1233s, 1200m, 1162w, 1092m, 1040w, 1030w, 973m, 933w, 913w, 881s, 818w, 804w, 748m, 645w, 567w, 551w, 412s. *MS:* 29(17), 41(31), 43(29), 55(10), 57(67), 69(38), 71(33), 120(10), 161(10), 162(27), 176(8), 203(8), 217(29), 218(42), 219(8), 272(65), 273(50), 274(10), 315(23), 330(100), 331(26.2), 332(3.51). *NMR*: 0.91(s, 27 H, *tert*-butyl), 1.03(t, 3 H, methyl, $J = 7$ Hz), 1.0 – 1.7(m, 2 H, methylene), 2.35(s, 2 H, methylene), 2.53(s, 4 H, methylene), 2.69(t, 2 H, methylene, $J = 7$ Hz), 6.64(s, 2 H, aromatic).

2-(2-Methylpropyl)-1,3,5-trineopentylbenzene (IIc) was prepared from 2-(2-methylpropanoyl)-1,3,5-trineopentylbenzene according to the method used above for the three-step synthesis of 2-ethyl-1,3,5-trineopentylbenzene from 2-acetyl-1,3,5-trineopentylbenzene. 2-(2-Methylpropyl)-1,3,5-trineopentylbenzene was purified as described for 2-propyl-1,3,5-trineopentylbenzene. After recrystallization the overall yield was 76 % of white crystals. *M.p.* 36 – 37 °C. *IR:* 2964vs, 2908m, 2866s, 2718w, 1609m, 1574w, 1478vs, 1467m, 1426w, 1391s, 1383w, 1364vs, 1332w, 1318w, 1286w, 1280w, 1236s, 1202m, 1163m, 1100m, 1040w, 1030w, 933w, 920w, 913w, 883s, 876s, 854w, 872w, 799m, 749s, 734w, 698w, 648w, 615w, 566w, 551w, 484w, 422w. *MS*: 29(25), 41(43), 43(48), 57(100), 69(50), 71(64), 119(8), 120(9), 133(16), 159(10), 173(11), 175(11), 176(27), 189(18), 231(30), 232(39), 245(43), 246(9), 287(75), 288(50), 289(10), 301(81), 302(20), 329(30), 330(9), 344(84.8), 345(23.6), 346(3.2). *NMR*: 0.86(d, 6 H, methyl, $J = 7$ Hz), 0.92(s, 27 H, *tert*-butyl), 1.1 – 2.0(m, 1 H, methine), 2.36(s, 2 H, methylene), 2.55 (s. 4 H, methylene), 2.61(d, 2 H, methylene, $J = 7$ Hz), 6.67(s, 2 H, aromatic).

2-Benzyl-1,3,5-trineopentylbenzene (IIe) was prepared from 2-benzoyl-1,3,5-trineopentylbenzene according to the method used for the three-step synthesis of 2-ethyl-1,3,5-trineopentylbenzene from 2-acetyl-1,3,5-trineopentylbenzene. 2-Benzyl-1,3,5-trineopentylbenzene was purified as described for 2-propyl-1,3,5-trineopentylbenzene. After recrystallization the overall yield was 79 % of white crystals. *M.p.* 80 – 81 °C. *IR*: 3080w, 3063w, 3025w, 2960vs, 2905m, 2865s, 2748w, 2718w, 1942w, 1804w, 1604s, 1575m, 1495s, 1480s, 1463s, 1451s,1425m, 1391s, 1362vs, 1330m, 1280m, 1235s, 1202m, 1191w, 1166w, 1075m, 1032s, 1002w, 922m, 899w, 873s, 823w, 749w, 724vs, 697s, 653w, 637w, 611m, 548w, 460s. *MS*: 29(16), 41(27), 43(38), 57(68), 71(100), 91(19), 193(9), 209(9), 210(51), 211(9), 266(51), 267(11), 321(14), 322(32), 363(10), 378(54.9), 379(17.0), 380(2.5). *NMR:* 0.93(s, 27 H, *tert*-butyl), 2.41(s, 2 H, methylene), 2.45(s, 4 H, methylene), 4.11(s, 2 H, methylene), 6.73(s, 2 H, aromatic), 6.8 – 7.3(m, 5 H, aromatic).

1,2,3,5-Tetraneopentylbenzene (IId). A solution of 60 mmol of *tert*-butyllithium in 30 ml of pentane, under an argon atmopshere, was cooled with ice and 1.0 g (3.0 mmol) of 2-chloromethyl-1,3,5-trineopentylbenzene was added in small portions. The reaction mixture was then stirred at room temperature. A GLC analysis of the mixture after 2 h showed that all 2-chloromethyl-1,3,5-trineopentylbenzene had been consumed and that three products had been formed. The excess of *tert*-butyllithium was destroyed with water. The aqueous layer was neutralized with 6 M hydrochloric acid and extracted several times with pentane. The combined pentane extracts were dried over magnesium sulfate. The products were separated on a column of silica gel with pentane as eluent. The three products were identified as 1,2-bis(2,4,6-trineopentylphenyl)ethane, 2-methyl-1,3,5-trineopentylbenzene and 1,2,3,5-tetraneopentylbenzene. The yields of the compounds were 0.25 g (28 %), 0.15 g (17 %) and 0.22 g (21 %), respectively, of white crystals.

1,2-Bis(2,4,6-trineopentylphenyl)ethane: *M.p.* 213 – 214 °C. *IR:* 2960vs, 2908m, 2868m, 2748w, 2715w, 1608m, 1572w, 1477vs, 1465w, 1435w, 1392s, 1365vs, 1330w, 1280w, 1236s, 1202m, 1160m, 1125w, 930w, 913w, 881s, 791m, 751w, 682m broad, 633w, 557w, 532w, 445m broad. *MS:* 29(17), 41(7), 43(13), 57(29), 69(8), 71(14), 245(12), 301(100), 302(14), 602(8.6), 603(4.2), 604(1.0). *NMR*: The chemical-shift values were identical with those previously reported.[16] 2-Methyl-1,3,5-trineopentylbenzene: The physical properties were identical with those previously reported.[15]

1,2,3,5-Tetraneopentylbenzene: *M.p.* 78–79 °C. *IR*: 2958vs, 2904m, 2862s, 2742w, 2712w, 1606w, 1570w, 1475vs, 1424w, 1392s, 1364vs, 1316w, 1282w, 1234s, 1204m, 1165w, 1153w, 1031w, 935w, 915w, 882w, 876m, 853w, 813m, 751w, 742w, 679s, 643w, 440vs. *MS*: 29(14), 41(22), 43(22), 57(64), 69(17), 71(28), 133(8), 189(11), 190(8), 245(39), 246(17), 301(100), 302(39), 343(9), 358(16.1), 359(4.6), 360(0.6). *NMR:* 0.83(s, 9 H, *tert*-butyl), 0.86(s, 18 H, *tert*-butyl) 0.92(s, 9 H, *tert*-butyl), 2.37(s, 2 H, methylene), 2.64(q, 4 H, methylene, $J = 13.6$ Hz, $\Delta\nu_{AB} = 42.5$ Hz), 2.78(s, 2 H, methylene), 6.68(s, 2 H, aromatic).

2-(2,2-Dimethylpropanoyl)-1,3,5-trineopentylbenzene (Id). To a solution of 2 mmol of *tert*-butyllithium in 10 ml of pentane, under a nitrogen atmosphere, 0.2 g (0.5 mmol) of 2-iodo-1,3,5-trineopentylbenzene was added. The mixture was stirred for 10 min at room temperature and then cooled with ice before 1 ml (0.98 g, 8.1 mmol) of freshly distilled 2,2-dimethylpropanoyl chloride was added dropwise. After 1 h the excess of acyl chloride was destroyed with water. The aqueous layer was extracted several times with hexane, and the combined organic layers were dried over magnesium sulfate. A GLC analysis showed that 1,3,5-trineopentylbenzene and two other products had been formed. The products were separated on a column of silica gel. With hexane 1,3,5-trineopentylbenzene and unreacted 2-iodo-1,3,5-trineopentylbenzene were eluted. The remaining two products were separated by a gradient elution with hexane and benzene. One of the two products was identified as 2-(2,2-dimethylpropanoyl)-1,3,5-trineopentylbenzene and the other as 2-[1-(2,2-dimethylpropanoyloxy)-2,2-dimethylpropyl]-1,3,5-trineopentylbenzene. The yields were 75 mg (42 %) and 73 mg (33 %,) respectively, of crystalline products.

2-(2,2-Dimethylpropanoyl)-1,3,5-trineopentylbenzene: *IR*: 2955vs, 2863m, 1695vs, 1608m, 1568w, 1480vs, 1469w, 1445w, 1394m, 1368vs, 1289w, 1237s, 1202m, 1132m, 1095w, 1034w, 955vs, 833m, 870s, 791m, 752w, 643w, 580w. *MS*: 29(19), 41(26), 43(29), 57(67), 71(17), 83(25), 85(17), 202(5), 259(6), 260(10), 315(100), 316(67), 357(25), 358(9), 372(1.1), 373(0.3), 374(0.05). The mass of the molecule was determined by high-resolution mass spectrometry, and the value 372.339 ± 0.002 u was found. The value [17] for $C_{26}H_{44}O$ is 372.339 u. *NMR*: 0.84(s, 18 H, *tert*-butyl), 0.93(s, 9 H, *tert*-butyl), 1.05(s, 9 H, *tert*-butyl), 2.31(q, 4 H, methylene, $J = 13$ Hz, $\Delta\nu_{AB} = 14$ Hz), 2.43(s, 2 H, methylene), 6.76(s, 2 H, aromatic).

2-[1-(2,2-Dimethylpropanoyloxy)-2,2-dimethylpropyl]-1,3,5-trineopentylbenzene (IV): *IR*: 2965vs, 2920w, 2880m, 1727vs, 1607m, 1565w, 1480vs, 1469m, 1397s, 1365s, 1283s, 1240s, 1205m, 1147vs, 1140vs, 1125m, 1045w, 1033s, 980m, 957s, 935w, 918w, 912w, 885m, 831m, 784w, 769w, 755w, 685w, 651w, 575m. *MS*: 29(9), 41(18), 43(18), 57(100), 71(15), 85(45), 299(9), 315(18), 316(5), 317(29), 318(7), 401(45), 458(4.36), 459(1.5), 460(0.3). The mass of the molecule was determined by high-resolution mass spectrometry and the value 458.413 ± 0.002 u. The value [17] for $C_{31}H_{54}O_2$ is 458.412 u. *NMR:* 0.71(s, 9 H, *tert*-butyl), 0.87(s, 9 H, *tert*-butyl), 0.92(s, 18 H, *tert*-butyl), 1.33(s, 9 H, *tert*-butyl), 2.42(s, 2 H, methylene), 2.80(q, 2 H, methylene, $J = 13.5$ Hz, $\Delta\nu_{AB} = 49.5$ Hz), 2.93(q, 2 H, methylene, $J = 13.5$ Hz, $\Delta\nu_{AB} = 52.0$ Hz), 6.67(d, 1 H, aromatic, $J = 2$ Hz), 6.82(s, 1 H, methine), 6.91(d, 1 H, aromatic. $J = 2$ Hz).

Clemmensen reduction of 2-acetyl-1,3,5-trineopentylbenzene. In a three-necked flask equipped with a mechanical stirrer, a reflux condenser and a dropping funnel, 5.5 g (84.1 mmol) of granulated zinc and 0.5 g (1.8 mmol) of mercury(II) chloride were covered with 10 ml of 6 M hydrochloric acid. The mixture was refluxed for 5 min. The aqueous solution was then decanted, and 20 ml of 6 M hydrochloric acid and 2 ml of acetic acid were added. The stirred mixture was then refluxed and a solution of 3.0 g (9.0 mmol) of 2-acetyl-1,3,5-trineopentylbenzene in 10 ml of toluene was added. At the end of each of the first 10 h of the reaction time, 1 ml of conc. hydrochloric acid was added. After 46 h the flask was cooled and the organic and aqueous layers were separated. The aqueous layer was extracted with toluene and the combined organic layers were dried over magnesium sulfate. The solvent was evaporated and 2-ethyl-1,3,5-trineopentylbenzene was separated from unreacted 2-acetyl-1,3,5-trineopentylbenzene on a column of silica gel. With hexane only 2-ethyl-1,3,5-trineopentylbenzene was eluted. Recrystallization of this compound from ethanol-water gave 0.15 g (5 %) of white crystals.

2,4,6-Trineopentylstyrene (III). Purified [18] red phosphorus (0.5 g, 4.0 mmol P) was covered with a solution of 0.2 g (0.6 mmol) of 2-(1-hydroxyethyl-1,3,5-trineopentylbenzene in 50 ml of cyclohexane. The mixture was refluxed, and 0.5 g (2.0 mmol) of iodine dissolved in cyclohexane was added. After the addition of the iodine the reaction mixture was refluxed for 14 h, and the reaction was then found to be complete according to GLC. The flask was cooled, and a solution of 1 g of sodium sulfite in 10 ml of water was added. The organic and aqueous layers were separated and the aqueous layer was extracted with cyclohexane. The combined organic layers were dried over magnesium sulfate. After evaporation of the solvent the product was decolorized by adsorption chromatography (alumina, hexane). The yield was 0.16 g (85 %) of an oily product which was identified as 2,4,6-trineopentylstyrene. *IR*: 3080m, 3050w, 2950vs, 2905m, 2862s, 2740w, 2712w, 1847w, 1631w, 1605m, 1563w, 1477vs, 1465m, 1425w, 1407w, 1392s, 1362vs, 1330w, 1228w, 1234s, 1200m, 1162w, 1035w, 995m, 920s, 879m, 792w, 749w, 733w, 675w, 618w, 541w. *MS*: 26(6), 27(5), 29(19), 39(9), 41(37),

43(27), 55(9), 56(7), 57(100), 71(29), 128(10), 129(13), 131(16), 143(9), 144(7), 145(46), 146(49), 187(25), 201(59), 202(51), 203(9), 257(68), 258(32), 299(19), 314(44.6), 315(11.3), 316(1.4). The mass of the molecule was determined by high-resolution mass spectrometry and the value 314.296 ± 0.003 u was found. The value [17] for $C_{23}H_{38}$ is 314.297 u. *NMR*: 0.88(s, 18 H, *tert*-butyl), 0.93(s, 9 H, *tert*-butyl), 2.42(s, 2 H, methylene), 2.62(s, 4 H, methylene), 5.02(q, H_A, methylene), 5.45(q, H_B, methylene), 6.74(q, H_X, methine), 6.76(s, 2 H, aromatic). $J_{AX} = 18$ Hz, $J_{BX} = 11$ Hz, $J_{AB} = 3$ Hz.

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Treatment of Birch Xylan with Chlorine in Aqueous Solution

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The depolymerization of xylan by chlorine in aqueous solution resulted in the formation of xylonic acid and reducing xylose end groups. Glyceric, threonic and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid moieties were present in minor amounts. Some of the reducing galacturonic acid moieties glycosidically linked to potentially reducing xylose end groups were split off during the chlorine treatment.

Chlorination is an important stage in the production of bleached wood pulps. Kinetic studies and a review of older publications were presented by Fredricks, Lindgren and Theander.[1] The formation of acid groups in the cellulose was studied recently [2] but no studies seem to have been published on the reactions of hemicellulose. To fill this gap we now report on reactions which occur when xylan is treated with chlorine in aqueous solution.

EXPERIMENTAL

The xylan sample was the same as that used in a study of the galacturonic acid end groups in birch xylan.[3] The sample (34.8 g) was treated with 725 ml of an aqueous solution containing 5.12 g Cl_2/l for 8 h at 27.7 °C. During the entire treatment, which was carried out in darkness, 3.7 g Cl_2 were consumed. The pH decreased from 1.8 to 1.5. The excess chlorine was destroyed with sulfurous acid and ethanol was added to precipitate the xylan. After centrifugation the precipitate was washed first with 50 % ethanol and then with water on an ultrafilter [Amicon UM-2]. Finally, the xylan was washed successively with 70 % ethanol, absolute ethanol and ethyl ether and dried under vacuum. The loss in yield during the treatment was 23 %.

The xylan sample was hydrolyzed and after removal of hydrochloric acid the organic material was separated into a non-electrolyte fraction, a fraction containing non-volatile monocarboxylic acids and a dicarboxylic acid fraction.[4]

The carbonyl end groups in the xylan samples were determined after reduction to alditols by potassium borohydride.[5] The xylan sample (7.5 g) was stirred in 350 ml 0.01 M sodium hydroxide, containing 25 g sodium chloride per litre, for 24 h under nitrogen to split lactones. After neutralization with hydrochloric acid 3.9 g of potassium borohydride were added. The suspension was stirred for 75 h. The xylan was precipitated with acetic acid and ethanol and after centrifugation the sample was washed with ethanol and ether and dried. In the experiment with the untreated xylan the loss in yield was 6.8 % whereas with the chlorinated sample the corresponding value was 14.3 %. The reduced sample was hydrolyzed and the hydrolyzate was separated into three fractions.[4]

The non-volatile acids present in the monocarboxylic acid fraction were separated by anion exchange chromatography in 0.08 M sodium acetate.[6] The composition of the eluate was recorded with a Waters' R4 differential refractometer. The acids present in the individual fractions were tentatively identified by anion exchange chromatography in sodium acetate and acetic acid media using an automatic three-channel analyzer.[7] When several acids were present the fractions were rechromatographed on a preparative scale with 1.0 or 0.5 M acetic acid, 0.02 M sodium acetate and 0.15 M sodium tetraborate as eluents.

The aldonic (including glycolic) acids as well as 2,4-dihydroxybutanoic [8] and galacturonic acids were identified by combined gas chromatography-mass spectrometry. The other uronic acids were identified by their D_v values, color reactions and hydrolysis products. 3,4-Dihydroxybutanoic acid was identified by its D_v values and by gas chromatography whereas the deoxypentonic acids which were present in trace amounts were identified by gas chromatography only.

All weights of the individual acids are reported per 100 g xylan. Most figures were obtained by weighing the isolated acids. With species present in very small amounts estimates were made

Table 1. Relative carbohydrate composition, % by weight.

	Untreated xylan	Chlorinated xylan	Chlorinated reduced xylan	Solution after chlorination
Rhamnose	1.52	1.19	0.46	3.73
threo-2-Pentulose	0.18	0.34	0.14	0.41
Lyxose	0.04	0.02	0.06	0.07
Arabinose	0.04	trace	trace	0.42
Xylose	97.1	98.1	99.3	89.5
Mannose	0.47	0.13	trace	1.93
Galactose	0.30	trace	trace	2.21
Glucose	0.29	0.20	trace	1.00

from the areas recorded on the chromatograms.

The sugars and alditols were determined by partition chromatography in aqueous ethanol on both an anion exchanger in the sulfate form and a cation exchanger in the lithium form.[9]

RESULTS AND DISCUSSION

A study of the carbohydrate composition of the isolated xylan showed that minor amounts of polysaccharides containing rhamnose, mannose, galactose, and glucose and trace amounts of arabinose were present (Table 1). Their contents decreased during treatment with chlorine and these constituents were enriched in the non-electrolyte fraction, isolated from the solution after removal of the soluble acids by means of an anion exchanger. This fraction which contained mainly oligomeric sugars was obtained in a yield of 4.6 % calculated on the xylan.

The number of xylitol end groups in the xylan after reduction with potassium borohydride was 0.82 mmol per 100 g whereas after chlorination it was 2.12 (Table 2). Evidently, the number of reducing xylose end groups increased markedly during the treatment but still remained low compared to that expected from the DP of the xylan. These observations which at first seemed puzzling are explained by the finding that in the untreated xylan a large portion of the potentially reducing xylose end groups were, at C-1, linked glycosidically to a reducing terminal galacturonic acid moiety.[3] The amount of galacturonic acid in the hydrolyzate from the untreated xylan was significantly higher than that obtained after chlorine treatment (Table 3), demonstrating that galacturonic acid groups are in part split off during this treatment. As previously shown the reducing galacturonic acid end groups which constitute only a fraction of the total number of the terminal galacturonic acid moieties are reduced to galactonic acid end groups by borohydride.[3] While galacturonic acid suffers severe degradation during acid hydrolysis, galactonic acid can be recovered almost quantitatively.[10] If the loss in galacturonic acid groups is due to a loss of terminal reducing galacturonic acid moieties, the amount of galactonic acid end groups in the reduced sample should be much less after treatment with chlorine. The results given in Table 2 show that the number of galactonic acid end groups in the reduced starting material was 2.68 mmol per 100 g whereas in the chlorinated (and subsequently reduced) xylan it was 1.09. A large portion of reducing galacturonic acid end groups disappeared during the chlorination but an appreciable amount remained.

Table 2. End groups in reduced xylan, mmol per 100 g.

	Before chlorination	After chlorination
Xylitol	0.82	2.12
Lyxitol	0.136	0.08
Threitol	0.103	–
Erythritol	0.026	–
Xylonic acid	0.121	4.69
Lyxonic acid	0.87	0.32
Threonic acid	0.50	0.38
Glyceric acid	0.83	1.13
Galactonic acid	2.68	1.09
Total amount	6.09	9.81

Table 3. Monocarboxylic acids isolated from the hydrolyzates of xylan. The weights refer to 100 g xylan.

Acids	Untreated xylan mg	Untreated reduced xylan mg	Chlorinated xylan mg	Chlorinated reduced xylan mg
Higher uronic	–	62	58	130
O-(4-*O*-Methyl-α-D-glucopyranosyl-uronic acid)-(1→2)-*O*-β-D-xylo-pyranosyl-(1→4)-D-xylose	340	166	322	198
O-(α-D-Glucopyranosyluronic acid)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose	140	100	68	94
Unknown uronic (D_v 5.6, 0.5 M HAc)	–	–	14	60
Unknown uronic (D_v 8.6, 0.5 M HAc)	160	140	216	182
2-*O*-(4-*O*-Methyl-α-D-glucopyranosyl-uronic acid)-D-xylose	5886	5344	6336	6064
2-*O*-(4-*O*-Methyl-α-D-glucopyranosyl-uronic acid)-D-xylitol	–	2	–	22
2-*O*-(α-D-Glucopyranosyluronic acid)-D-xylose	410	140	654	274
4-*O*-Methylglucuronic	1502	1322	2000	1316
Glucuronic	24	16	40	10
Dianhydrides of 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose (D_v=26; 0.5 M HAc)	188	246	188	168
(D_v=45; 0.5 M HAc)	220	144	262	212
(D_v=51; 0.5 M HAc)	156	146	140	138
2-*O*-(α-D-Galatopyranosyluronic acid)-L-rhamnose	30	18	30	trace
Galacturonic	628	248	522	16
Galactonic	–	526	–	214
Xylonic	38	20	1590	778
3-Hydroxypropionic	24	trace	trace	78
Lyxonic	106	144	54	53
Threonic	40	68	112	51
Glyceric	92	88	186	120
Glycolic	–	–	trace	36
2-Deoxytetronic	4	12	trace	8
3-Deoxytetronic	–	–	62	trace
2-Deoxy-*threo*-pentonic	–	1	trace	–
3-Deoxy-*erythro*-pentonic	–	4	trace	–

The number of lyxitol end groups in the reduced xylan samples decreased during treatment with chlorine (Table 2). These groups are derived from terminal lyxose and *threo*-2-pentulose moieties formed by Lobry de Bruyn-Alberda van Ekenstein transformations of xylose end groups in the alkaline solution used for the isolation of the xylan. In addition to xylitol and lyxitol end groups, small amounts of threitol and erythritol end groups were observed in the starting material (after reduction). Only traces were recorded in the chlorinated and reduced xylan. The results permit the conclusion that small amounts of threose and erythrose end groups were present in the original hemicellulose but disappeared during the chlorine treatment. Oxidation of terminal reducing units gives rise to the corresponding aldonic acid end groups but as shown below these terminal groups can be further attacked.

Among the aldonic acids which are derived from the end groups, lyxonic acid was present in larger amounts before the treatment with chlorine. Lyxonic acid end groups are formed by oxidation of xylan by oxygen in alkaline medium [11] and their presence can be ascribed

Table 4. Viscosity of the xylan samples in copper ethylenediamine solution.

	Untreated xylan	Untreated reduced xylan	Chlorinated xylan	Chlorinated reduced xylan
Viscosity $[\eta]$, cm^3/g	100	98	56	62
DP, calculated [12] from $[\eta]$	212	208	119	131

to a partial oxidation of xylose end groups during the procedure used for the isolation of the xylan.

As can be seen from Table 3 very large amounts of xylonic acid end groups were formed during the chlorine treatment. This reaction was expected since the formation of terminal gluconic acid moieties is one of the more important cellulose reactions during the treatment with chlorine.[2] No mannonic acid was formed in the cellulose and analogously no formation of lyxonic acid was observed in the experiments with xylan. The decrease in lyxonic acid content can be ascribed to a degradation to threonic and glyceric acid end groups (*cf.* Appendix I) and to the loss of low molecular weight xylan.

The number of xylonic acid groups was much lower in the sample reduced with potassium borohydride. The chlorinated xylan was kept in alkaline solution to split lactones and other possible ester linkages before addition of borohydride. This means that no reduction of the xylonic acid end groups should occur. The decrease is probably due to an appreciable amount (14.3 %) of the xylan being lost and the dissolved fraction having a low molecular weight (*cf.* Table 4).

Similarly, the amounts of threonic and glyceric acids in the chlorine treated sample were much higher before the reduction. The results given in the table permit the conclusion that threonic and glyceric acid end groups were formed during the treatment with chlorine. Their presence before the chlorine treatment is in accordance with the observation that not only lyxonic acid but also threonic and glyceric acids are formed as end groups during the isolation of xylan.

The determination of terminal moieties consisting of aldonic acids or alditols in the reduced xylan samples are listed in Table 2. If it is assumed that these and only these end groups are present in the potentially reducing end, the total amounts would be 6.09 mmol/100 g before and 9.81 mmol/100 g after the treatment with chlorine. The values correspond to a $(DP)_n$ of 109 and 68, respectively. In these calculations it was assumed that every tenth xylose unit contained a 4-*O*-methylglucuronic acid moiety. A calculation of the DP from the viscosity [12] gave the values 208 and 131.

Two diastereomeric 3-deoxypentonic acids (which cannot be present as end groups) were found in the hydrolyzate of cellulose.[2] When the chlorinated sample was treated with potassium borohydride before the hydrolysis these acids were absent. These results together with the observation that 3-deoxytetronic acid was present in the hydrolyzate from chlorinated xylan but was not detected when the sample was reduced (see Table 3), strongly indicate that these 3-deoxyaldonic acids are derived from oxidized units in the polysaccharide chains, or more specifically from sugar moieties containing a keto group. Most likely the keto groups are present at C-2, *i.e.* the oxidized moieties are hexosulose in cellulose and pentosulose in xylan.

A small amount of glyoxylic acid was found in similar experiments with cellulose.[2] It was concluded that the bond between carbon atoms 2 and 3 in a non-terminal glucose moiety was broken and carboxylic acid groups were formed in these positions. After treatment of birch xylan with chlorine no glyoxylic acid was found. One explanation would be that the chlorine treatment of the birch xylan was carried out

Table 5. Volume distribution coefficients (D_v) of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid in ion exchange chromatography on Dowex 1-X8.

Medium	°C	D_v
0.3 M Sodium acetate, 2 M acetic acid	30	4.8 (12.5)
5.0 M Acetic acid	30	22.2 (19.0)
0.2 M Magnesium acetate, pH 7.0	70	6.7 (10.5)
0.3 M Magnesium acetate, pH 7.0	70	3.0 (4.1)

The D_v values given within parenthesis refer to galactaric acid.

Table 6. Oxidation of model compounds.

Added acid	mg	Amounts of acids after chlorination, mg				
		Xylonic	Threonic	Glyceric	Glycolic	Carbonic
Xylonic	25.6	15.4	5.6	1.1	1.1	n.d.[a]
Threonic	33.2	–	25.6	3.6	2.1	n.d.
Glyceric	196.5	–	–	68.6	80.1	58.5
Glycolic	182.9	–	–	–	171.6	7.7

[a] n.d. = not determined

at a lower pH (1.8) than that (2.5) used in the experiments with cellulose (*cf.* Ref. 13).

The uronic acids present in the hydrolyzates before chlorine treatment were the same as found in previous investigations.[14] The amount of the predominant 4-*O*-methylglucuronic acid and of the corresponding biouronic and higher uronic acids must, considering the errors involved in the hydrolysis procedure, be considered to be about the same as after the treatment with chlorine.

An interesting observation is that the dicarboxylic acid fraction contained an appreciable amount (0.331 mmol per 100 g xylan) of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid. This acid has not been studied previously and the volume distribution coefficients (D_v) found on ion exchange chromatography are given in Table 5. The retention of the trimethylsilyl derivative (ester) relative to that of cellobiitol on OV-1 at 250 °C was 0.614 (*cf.* Ref. 8). The identity was established by comparison with the data obtained with a sample prepared by hypochlorite oxidation of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. The presence of this acid shows that terminal xylose units containing a 4-*O*-methylglucuronic acid substituent are oxidized to the corresponding xylonic acid moiety. No appreciable amounts of other dicarboxylic acids were present.

Appendix I. Oxidation of glycolic, glyceric, threonic, and xylonic acids with chlorine.

Glycolic, glyceric, threonic, and xylonic acids were in separate experiments treated for 48 h at 28 °C with 200 cm³ of an aqueous solution containing 5.7 g Cl_2 per litre. The excess chlorine was destroyed with sulfurous acid and the solutions kept at pH 8.5 for 4 h at room temperature. The monocarboxylic acid fraction was isolated and analyzed. Dicarboxylic acids and sugars were not present in appreciable amounts. The results given in Table 6 show that glycolic acid was very stable. Only about 4 % were converted into carbon dioxide. Glyceric acid was severely attacked. The glycolic and carbonic acids produced account for 95 % of the loss. Threonic acid gave rise to glyceric and glycolic acids but the decomposition was less severe than that of glyceric and xylonic acids. Threonic, glyceric, and glycolic acids were produced from xylonic acid.

It is worth mentioning that the conditions used in the model experiments were more severe than those used in the experiments with xylan. However, the results lend support to

the conclusion that the aldonic acid end groups in xylan are oxidized during treatment of xylan with chlorine and converted into terminal moieties consisting of lower aldonic acids.

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Reactions of Diapocarotenals with *N*-Bromosuccinimide and Synthetic Applications

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The allylic alcohols 8,8′-diapo-20-hydroxycarotene-8,8′-dial (*4*), 8,8′-diapo-20,20′-dihydroxycarotene-8,8′-dial (*5*), and 12,12′-diapo-20-hydroxycaroten-12,12′-dial (*12*) are described for the first time. Their preparation from the corresponding diapocarotenals crocetindial (*1*) and 12,12′-diapo-12,12′-dial (*10*) by *N*-bromosuccinimide (NBS) treatment *via* the corresponding bromides 8,8′-diapo-20-bromocarotene-8,8′-dial (*2*), 8,8′-diapo-20,20′-dibromocarotene-8,8′-dial (*3*), and 12,12′-diapo-20-bromocarotene-12,12′-dial (*11*) has been investigated.

The acetates 8,8′-diapo-20-acetoxycarotene-8,8′-dial (*6*), 8,8′-diapo-20,20′-diacetoxycarotene-8,8′-dial (*7*), and 12,12′-diapo-20-acetoxycaroten-12,12′-dial (*13*) represent useful intermediates for the synthesis of cross-conjugated carotenals.

On the basis of previous experience with NBS-treatment of carotenoids, the reaction of NBS with crocetindial was expected to give either the acetylenic analogue or to result in allylic subsitution.[1–3] Preliminary studies revealed that such treatment resulted in allylic substitution in the 20-position, providing a useful intermediate in the synthesis of the cross-conjugated carotenals of the rhodopinal series.[4,5]

The aim of the present project was to study in more detail the reaction of NBS with diapocarotenals, and to characterize further the primary products and derivatives thereof, suitable as intermediates in the synthesis of more stable C_{40} cross-conjugated carotenals.

RESULTS AND DISCUSSION

Treatment of crocetindial (*1*) with NBS in ethanol-free chloroform gave the rather unstable bromide *2*, judged by chromatographic data, electronic and mass spectra. As a minor product was obtained the unstable dibromide *3*, directly transferred to and identified as the diol *5*. Proof of dominant (if not exclusive) bromine substitution in 20,20′-position rather than in the 19,19′-methyl groups comes from the ^1H NMR spectra of the diol *5* and the diacetate *7*. One signal only at δ 1.85 (d_5-pyridine) and δ 1.93 ($CDCl_3$), respectively, was observed for the methyl groups, integrating for 6 protons.

In mixture with the dibromide *3* was presumably some 8,8′-diapo-20,20′-dibromocarotene 8,8′-dial (*3b*), judged by the loss of Br_2 from the molecular ion on electron impact. On subsequent derivatization was isolated an additional unreactive product (*3c*) of the same polarity as *3*, but with more hypsochromically displaced electronic spectrum, and for which no mass spectrum could be obtained.

Hydrolysis of the bromides *2* and *3* was accomplished by treatment with silver acetate or deactivated alumina. In small scale test reactions the silver acetate catalyzed hydrolysis afforded some of the acetates *6* and *7*, but the main batch gave the alcohols *4* and *5* and practically no acetates. Alkaline hydrolysis of the monobromide *2* gave unsatisfactory results. Thus treatment of *2* with 10 % KOH in methanol and ether (1:1) gave the methyl ether *8* as the only product. Moist ether or dioxane, or a two-face system with THF-ether (1:1) and 10 % aqueous K_2CO_3 did not seem to attack the bromide *2*, *cf.* Ref. 2, whereas treatment with dioxane – 10 % aqueous KOH (1:1) and dioxane – 10 % aqueous K_2CO_3 (4:1) gave con-

version to the alcohol *4*, albeit with low pigment recovery.

In preparative work conversion of the relatively unstable bromide to the alcohol was generally effected without isolation of the bromide, also because the alcohol was more readily separated from the starting material *1*.

Presence of acetic acid during the NBS-reaction [2] gave predominantly bromide and only trace amounts of the acetate *6*. Acetylation of the alcohols *4* and *5* with acetic anhydride in pyridine provided the acetates *6* and *7* in quantitative yield. Oxidation of the mono-ol *4* to the trial *9* was quantitatively effected with activated MnO_2,[6] while oxidation with NiO_2 [7,8] and DDQ [9,10] gave less satisfactory results.

On the basis of the reaction between crocetindial (*1*) and NBS which almost exclusively gave bromination in the central methyl group, one might expect that the carbonyl groups have some directive influence. If the C_{10}-dial *10* exhibits the same influence from the carbonyl groups, this compound should not be brominated. However, it suffered bromination, but much slower than crocetindial (*1*). Allylic bromination by NBS is considered to be a radical reaction.[11,12] It has been shown that addition of a radical initiator like benzoyl peroxyde [13] or azodiisobutyronitrile [14] promotes the reaction in cases where the normal conditions give unsatisfactory results. Addition of small amounts of benzoyl peroxide gave slightly higher yield of the bromide *11* (from 6 to 11 % at 50 °C and with four times excess of NBS), while addition of azodiisobutyronitrile did not increase the yield of *11*.

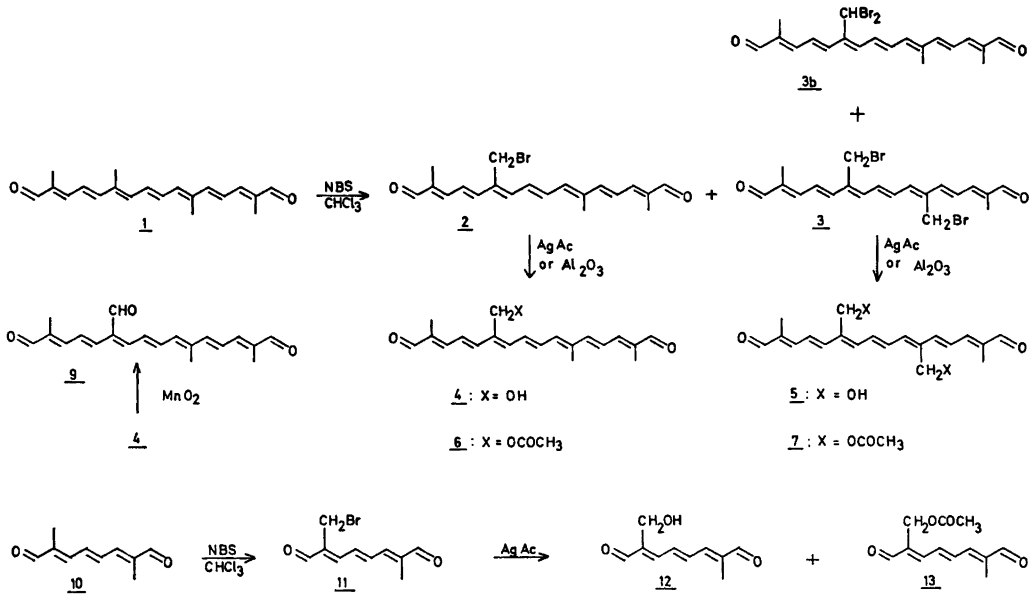
The bromide obtained (*11*) seemed to be much more resistent towards hydrolysis, than *2* and *3*, but hydrolysis to *12* could be effected with silver acetate in a mixture of ether-THF-water.

The acetates *6*, *7*, and *13* are considered useful intermediates for the preparation of apocarotenals by Wittig condensation of the aldehydes with appropriate phosphoranes, followed by hydrolysis of the acetate and allylic oxidation, in principle as for the oxidation of *4* to *9*. Thus the preparation of stable cross-conjugated C_{40}-carotenals like renierapurpurin-20-al from the acetate *6* has been carried out.[15]

The stereochemistry of the new compounds described (*4* – *9*) will be discussed separately.[16] For convenience only *trans*-configuration of the polyene chain is given in Scheme 1.

EXPERIMENTAL

Materials and methods. These were as described elsewhere.[17] Relative R_F-values on TLC



Scheme 1.

(silica gel G) were as follows: *1* > *2* = *8* > *3* = *6* > *7* > *4* > *5* and *10* > *11* > *13* > *12*.

Yields were calculated on the basis of extinction coefficients: *E* (1 %, 1 cm = 3 200 for C_{10}-dial (*10*) and derivatives, *E* (1 %, 1 cm) = 3 300 for crocetindial (*1*), *E* (1 %, 1 cm = 3 000 for derivatives of *1* in cases where crystallization was not effected and individual determination of extinction coefficients not determined. Due to the ready *trans-cis* isomerization extinction coefficient for crystalline and dissolved materials differ and calculation of yields are approximate.

NBS-reaction with crocetindial (*1*). Introductory experiments: Crocetindial (*1*, 1 equiv. in ethanol-free chloroform was added to a suspension of NBS (1 equiv.) in ethanol-free chloroform and the mixture stirred at 50 °C for 4 h. Chromatography on silica gel G (25 % acetone in petroleum ether = 25 % APE) gave in order of decreasing R_F-values recovered crocetindial (*1*, 15 – 25 %), 8,8′-diapo-20-bromocarotene-8,8′-dial (*2*, 70 – 80 %), 8,8′-diapo-20,20′-dibromocarotene-8,8′-dial (*3*) in mixture with tentatively other dibromo products (5 – 10 %) and 8,8′-diapo-20-hydroxycarotene-8,8′-dial (*4*, 4 – 6 %). Impure *3* had λ_{max} (acetone) *441* and 465 nm (most intense maximum is italicized), *m/e* (210 °C) 456, 454, and 452 (M, 1.5, 3.2, and 1.7 %, respectively), 375 and 373 (M – Br, both 3.5 %), 294 (M_{3b} – Br_2, 2.4 %) and 83 (100 %).

The reaction was tested with addition from very little to large excess of acetic acid. Judged by TLC and mass spectra, some, but very little, of the monoacetate *6* was obtained in mixture with the tentatively dibrominated material, in addition to the monobromo derivative *2*, the hydrolysed product *4*, and unreacted *1*.

Uncoloured reaction products were not searched for.

Substitution of bromides 2 and 3. The monobromide (*2*) was treated with ether – 10 % KOH in methanol (1:1) at room temperature for 2½ h. This gave 8,8′-diapo-20-methoxy-8,8′-dial (*8*); 43 % after purification by TLC.

The following reactions were carried out without isolation of the bromides *2* and *3* from the NBS product mixture above: The product mixture in THF – ether – 10 % aqueous K_2CO_3 (1:1:1), stirred at room temperature for 24 h, did not give any conversion of *2* to *4*. The pigment recovery was 91 % before chromatography. Treatment with 5 % KOH in dioxane – water (1:1) gave after 4 min 13 % pigment recovery. TLC showed low yield of the alcohol *4*. The NBS product mixture in dioxane-10 % aqueous K_2CO_3 (4:1) kept at room temperature for 24 h gave 27 % pigment recovery before chromatography. TLC on kieselgel G gave the bromide *2* (2.1 % of starting crocetindial) and the alcohol *4* (6.8 %) in addition to recovered crocetindial (*1*) and minor products not further investigated.

The NBS product mixture in benzene stirred with neutral or basic alumina (grade III) for 5 min gave satisfactory conversion of *2* to the alcohol *4* (see below).

The NBS product mixture in THF-ether (1:1), treated with an aqueous suspension of silver acetate gave conversion of the bromides *2* and *3* to the corresponding alcohols *4* and *5* in mixture with their acetates *5*-monoacetate, *6*, and *7* (see below).

The fraction containing the dibromide *3* treated as above with silver acetate for 15 h gave after TLC on silica gel G (35 % APE) mixed alcohols and acetates in addition to tentatively unreacted material. The tentatively unreacted material (*3c*) exhibited λ_{max} (acetone) 357, *434*, and 459 nm. The mass spectrum could not be obtained (temperatures from 140 to 210 °C).

NBS-reaction of crocetindial (1) followed by hydrolysis. From the above experiments the following two procedures were developed:

(*a*) Crocetindial (*1*, 400 mg) in ethanol-free chloroform (70 ml) was added to a suspension of NBS (240 mg) in ethanol-free chloroform (20 ml) and the mixture stirred at 50 °C. The reaction, which was repeated several times and monitored by TLC, gave after 4 h 70 – 80 % conversion to bromides and 95 – 100 % pigment recovery. The mixture was diluted with benzene (500 ml), neutral Al_2O_3 grade III [18] (100 g) added and the mixture stirred for 5 min. The Al_2O_3 was removed by filtration and washed with benzene and methanol. The solvents were evaporated, and the pigments chromatographed on a silica gel column, eluted with APE. This gave recovered crocetindial (*1*, 87.8 mg, *E* (1 %, 1 cm) = 3 360 in acetone) and 8,8′diapo-20-hydroxycarotene-8,8′-dial (*4*, 85.0 mg, 20 %).

(*b*) Crocetindial (*1*, 2.40 g) was treated as above with NBS (1.44 g). The reaction mixture was filtered, the chloroform removed, and the residue dissolved in THF-ether (1:1, 1 l). Due to solubility problems, the pigment was first partly dissolved in hot THF, and then diluted with ether. Some undissolved pigment remained. By addition of water (200 ml) a two-face system was obtained. Silver acetate (420 mg) was added, and the suspension stirred until all bromide was transferred to alcohol (*ca.* 6 h). Co-chromatography test showed that only the alcohols *4* and *5* were formed, and practically none of the corresponding acetates *6* and *7*. The suspension was filtered, washed with water and dried (Na_2SO_4). Column chromatography on silica gel eluted with benzene/chloroform/acetone gave recovered crocetindial (*2*, 483 mg, 20 %); 8,8′-diapo-20-hydroxycarotene-8,8′-dial (*4*, 1.116 g, 44 %) and 8,8′-diapo-20,20′-dihydroxycarotene-8,8′-dial (*5*, 71.2 mg, 2.7 %).

8,8′-Diapo-20-bromocarotene-8,8′-dial (*2*) had λ_{max} (acetone) *440* and 465.5, *m/e* (190 °C) 376 and 374 (M, 32 and 34 %), 295 (M – Br, 55 %) and 80 (100 %).

8,8′-Diapo-20,20′-dibromocarotene-8,8′-dial (*3*)

was tentatively obtained in mixture with other products and could not be further investigated (see above under Substitution of bromides *2* and *3*).

8,8'-Diapo-20-hydroxycarotene-8,8'-dial (*4*). *4* from procedure (a) above was crystallized from acetone/hexane as a *cis-trans* mixture (14.3 mg); m.p. 178.5–179 °C; λ_{max} (acetone) *434.5* (*E* (1 %, 1 cm) = 2980) and 460 nm; λ_{max} (KBr) 3410 (–OH), 1069, 1010 (prim. OH) cm^{-1}; δ ($CDCl_3$) 1.91 s (6 H, H-19,19'), 2.22 s (3 H, H-20'), 4.43 and 4.53 (2 H, $-CH_2OH$), 6.3–7.2 (10 olefinic H), 9.50 and 9.53 (2 aldehyde H); *m/e* (140 °C), 312 (M, 100 %), 296 (M–16, 1.8 %), 294 (M–18, 1.1 %) and 281 (M–31, 2.2 %).

8,8'-Diapo-20,20'-dihydroxycarotene-8,8'-dial (*5*) from procedure (b) above was rechromatographed on a silica gel column (10 % acetone in chloroform), and showed δ (d_5-pyridine) 1.85 s (6 H, H-19, 19'), no signals due to CH_3–20,20', 4.80 and 4.86 (4 H, H-20,20'), 9.76, 9.80 and 9.82 (2 H, H-8,8'). *5* was crystallized from methanol; yield 8.5 mg; m.p. 190.5–192.5 °C; λ_{max} (acetone) *432.5* (*E* (1 %, 1 cm) = 2410) and 457 nm, λ_{max} (methanol) 265.5, 328 and *433.5* nm; ν_{max} (KBr) 3437 and 3395 (OH), 1021 and 1010 (prim. OH); *m/e* (200 °C) 328 (M, 100 %), 312 (M–16, 4.5 %), 310 (M–18, 3.2 %), and 299 (M–31, 3.1 %).

8,8'-Diapo-20-acetoxycarotene-8,8'-dial (*6*). 8,8'-Diapo-20-hydroxycarotene-8,8'-dial (*4*, 42.5 mg) treated at room temperature with acetic anhydride (1.0 ml) in dry pyridine (10 ml) for 12 h gave *6*. The product was purified on a silica gel column eluted with APE; yield 41.3 mg (88 %). Crystallization from APE gave 13-*cis*-*6* (13.6 mg); m.p. 166–167 °C; λ_{max} (acetone *432.5* (*E* (1 %, 1 cm) = 2840) and 458 nm; λ_{max} (hexane) 261, 312, 324, 401, *423.5* and 449.5 nm, % D_B/D_{II} = 0.40;[6] ν_{max} (KBr) 1732 cm^{-1} (–CO–O–); δ ($CDCl_3$) 1.92 s (6 H, H-19,19'), 2.06 s (3 H, H-20'), 2.22 s (3 H, $-OCOCH_3$), 4.91 s (2 H, $-CH_2OAc$), 6.4–7.15 (10 olefinic H), 9.52 and 9.54 (2 aldehydic H); *m/e* (140 °C) 354 (M, 100 %) and 295 (M–59, 7.9 %).

8,8'-Diapo-20,20'-diacetoxycarotene-8,8'-dial (*7*). 8,8'-Diapo-20,20'-dihydroxycarotene-8,8'-dial (*5*, 30.6 mg) treated at room temperature with acetic anhydride (0.5 ml) in dry pyridine (5 ml) for 17 h gave *7*. The product was purified on a silica gel column eluted with benzene/chloroform; yield 22.1 mg (72 %). ^1H NMR ($CDCl_3$) before crystallization showed two peaks for the acetate $-CH_3$; δ($CDCl_3$) 2.09 and 2.13. Crystallization from chloroform/acetone gave *trans*-*7*, yield 10.2 mg; m.p. 208–208.5 °C; λ_{max} (acetone) *434* (*E* (1 %, 1 cm) = 2780) and 459 nm, λ_{max} (hexane) 260, 308, 319, *427* and 452 nm; ν_{max} (KBr) 1729 cm^{-1} (CO–O); δ ($CDCl_3$) 1.93 s (6 H, H-19,19'), 2.09 s (6 H, $-OCOCH_3$), 5.04 s (4 H, H-20,20'), 6.5–7.1 (10 olefinic H) and 9.60 s (2 H, H-8,8'); *m/e* (210 °C) 412 (M, 77 %), 353 (M–59, 6.8 %), 292 (M–120, 14 %), and 95 (100 %).

8,8'-Diapo-20-methoxycarotene-8,8'-dial (*8*) had λ_{max} (acetone) *436* and 461 nm, *m/e* 326 (M, 100 %), 295 (M–31, 3.5 %) and 381 (M–45, 3.0 %).

8,8'-Diapo-carotene-8,8'-20-trial (*9*). 8,8'-Diapo-20-hydroxycarotene-8,8'-dial (*2*, 44.8 mg) in acetone (20 ml) was added activated MnO_2 (1 g) and stirred for 1½ h. The mixture was filtered and the solvent removed. TLC on silica gel G (25 % APE) gave *12* yield 38.5 mg (87 %). Crystallization from chloroform/acetone yielded *cis*-*12* (7.9 mg); m.p. 179–183 °; λ_{max} (acetone) *443.5* (*E* (1 %, 1 cm) = 2390) and 462 nm, λ_{max} (hexane) 260, 269, 311.5, 322, *431.5*, and 455.5 nm; ν_{max} (KBr) 1666 and 1608 (conj. C=O) and 909 cm^{-1}; δ ($CDCl_3$) 1.93 s (6 H, H-19,19'), 2.11 s (3 H, H-20'), 6.5–7.7 (10 olefinic H), 9.52 s (2 H, H-8,8'), 9.82 (1 H, H-20); *m/e* (180 °C) 310 (M, 100 %), and 281 (M–29, 5.7 %).

NBS-reaction with 12,12'-diapo-12,12'-dial (*10*). *10* was treated with NBS as crocetindial (*1*) above. Optimal yield of the monobromide *11* was obtained after 11 h at 50 °C with 4 times excess of NBS. The reaction was tested with and without addition of benzoylperoxide (a spatula tip), and gave slightly higher yield with this admixture (8 % against 13 % under the conditions above). Thus from *10* (50.5 mg) treated under the optimum conditions was isolated after chromatography on silica gel 60 PF (22 % APE) the following five zones, ranged in order of decreasing R_F-values: product A (traces); λ_{max} (ether) 278, *m/e* (95 °C) 326, 324 and 322 (M, 0.4, 0.7, and 0.3 %, respectively), 299, 297, and 295 (M–29, 0.7, 1.1, and 0.7 %), 245 and 243 (M–Br, 52 and 54 %), and 91 (100 %), *10* (26.7 mg, 53 % of starting material), 12,12'-diapo-20-bromocarotene-12,12'-dial (*11*, 5.3 mg, 11 % of starting material), product B (traces); λ_{max} ($CHCl_3$) *331.5* and 347.5 nm, *m/e* (95 °C) 245 and 243 (9.6 and 10.0 %), 164 (245, 243–Br, 100 %), and product C (1.8 mg, 4 % of starting material); λ_{max} ($CHCl_3$) *342* and (353) nm, *m/e* (145 °C) 242 and 240 (8.8 and 8.1 %), 162 (100 %), no evident molecular ion.

12,12'-Diapo-20-bromocaroten-12,12'-dial (*11*) exhibited λ_{max} (ether) *328* and 341; λ_{max} ($CHCl_3$) *335* and 347.5; δ ($CDCl_3$) 1.19 s ($-CH_3$, 3 H), 4.29 s ($-CH_2Br$, 2 H), 7.0–7.2 (4 olefinic H), 9.57 and 9.60 ($-CHO$, 2 H); *m/e* (95 °C) 244 and 242 (M, 19 %) and 163 (M–Br, 100 %).

12,12'-Diapo-20-hydroxycaroten-12,12'-dial (*12*) and *12,12'-diapo-20-acetoxycaroten-12,12'-dial* (*13*). *11* (5.1 mg) was dissolved in THF-ether (1:1, 10 ml). Water (3 ml) and silver acetate (130 mg) were added and the suspension stirred for 22 h at room temperature. This gave after chromatography on silica gel 60 PF (22 % APE) recovered *11* (0.5 mg, 10 %), *13* (0.8 mg, 16 %); λ_{max} ($CHCl_3$) *329* and 344, *m/e* (95 °C) 222 (M, 30 %), 193 (M–29, 3.2 %), 162 (M–60, 100 %), 133 (M–60–29, 78 %), and *12* (1.3 mg, 25 %), λ_{max} ($CHCl_3$) *329.5* and 344, *m/e* (95 °C) 180

(M, 100 %), 162 (M−18, 35 %), 151 (M−29, 40 %), and 133 (M−18−29, 57 %).

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Syntheses and Conformational Properties of *gem*-Dimethyl Substituted Cyclohexadecanes

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1,1-Dimethyl-, 1,1,9,9-tetramethyl- and 1,1,4,4-tetramethylcyclohexadecane have been prepared. The first two are shown by IR spectroscopy to have the same [4444] diamond lattice conformation in melt and solution as in the crystal. The latter is a conformer mixture in melt and solution.

Only one diamond-lattice-type conformation is possible for cyclohexadecane; it has a "square" projection and D_{2d} symmetry (Fig. 1), and has been designated [1] as [4444], each digit representing the number of CC-bonds in one "side". An infrared spectrum simple enough (few and well resolved sharp lines) to correspond to a single conformation of this high symmetry is, however, only observed [2,3] below a solid-solid transition point at -2 °C. In the expanded lattice of the higher solid phase, in the melt, and in solution, the infrared spectrum (Fig. 2) has a complexity (broad and new absorption regions) indicative of a conformer mixture. Simple calculations show [1] that among other quadrangular conformations those of lowest enthalpy are, in increasing order, [3535], [3445], [3454], and [2545] (Fig. 1). The expected higher entropy of these non-diamond-lattice-type conformations may of course increase their importance at higher temperatures.

Geminal dimethyl substituents can be ac-

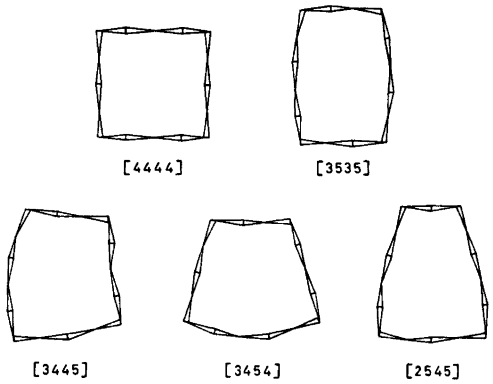


Fig. 1. Conformations of lowest calculated energy for cyclohexadecane.[1]

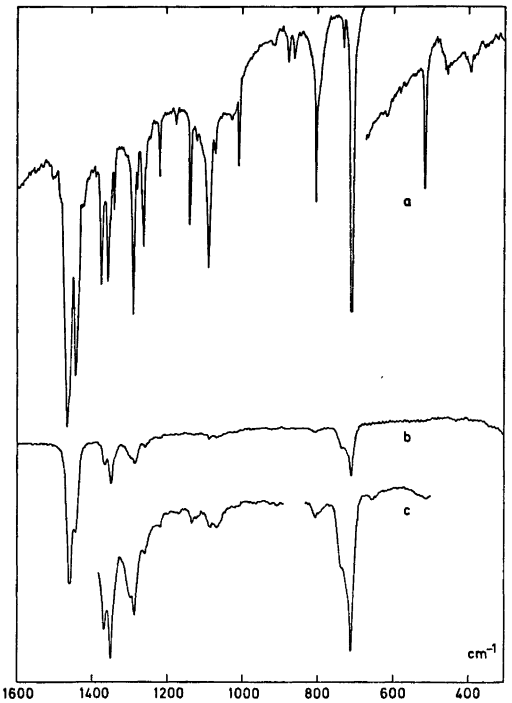


Fig. 2. Infrared spectra of cyclohexadecane in the solid state at -80 °C (a), in the solid state at 40 °C (b), and in CS_2-solution (c).

Table 1. Melting points and first-order transitions for cyclohexadecanes.

	M.p. °C	Tr.p. °C	ΔH_m[a]	ΔH_{tr}[a]	ΔS_m[b]	ΔS_{tr}[b]	ΔS_{total}[b]
Cyclohexadecane	59	10 −2	1.0	0.3 4.5	3.1	1.0 16.7	20.8
1,1-Dimethyl-cyclohexadecane	17	−52 −57	3.4	0.01 0.3	11.7	0.04 1.5	13.2
1,1,9,9-Tetramethyl cyclohexadecane	91		6.0		16.6		16.6
1,1,4,4-Tetramethyl-cyclohexadecane	30		6.0		19.8		19.8

[a] In kcal/mol. [b] In cal/mol deg.

commodated only in corner positions on any of these conformations.[4] Two, three or four such dimethyl groups in a given substitution pattern may therefore disfavour certain and favour other conformations. Thus, in 1,1,5,5,9,9,13,13-octamethylcyclohexadecane the substitution should fit all corner positions on the [4444] conformation and virtually exclude any other conformation. Although its attempted synthesis failed in the last step, the reduction of the intermediate 3,3,7,7,11,11,15,15-octamethylcyclohexadecane-1,9-dione, already the dione itself showed conformational homogeneity.[3,5] It is therefore extremely likely that this would be true also for the hydrocarbon.

We now present a study of three other cyclohexadecane derivatives, carrying one or two *gem*-dimethyl groups, obtained by full hydrogenation of acetylenic precursors reported earlier.[6,7] Since low-temperature NMR spectroscopy did not yield any information, the method used has been to compare IR spectra of solution, liquid and crystalline state in order to decide whether the solution and the liquid contains only the crystal conformer or a mixture of conformers. In addition, scanning calorimetry was used to check whether the whole melting process takes place at the melting point or is split into several steps, as for cyclohexadecane itself which has two solid-solid transition points (Table 1).

1,1-Dimethylcyclohexadecane. Substitution at a single corner is possible in one or more ways on any of the five conformations shown in Fig. 1, so that the conformational possibilities are even more numerous than for cyclohexadecane

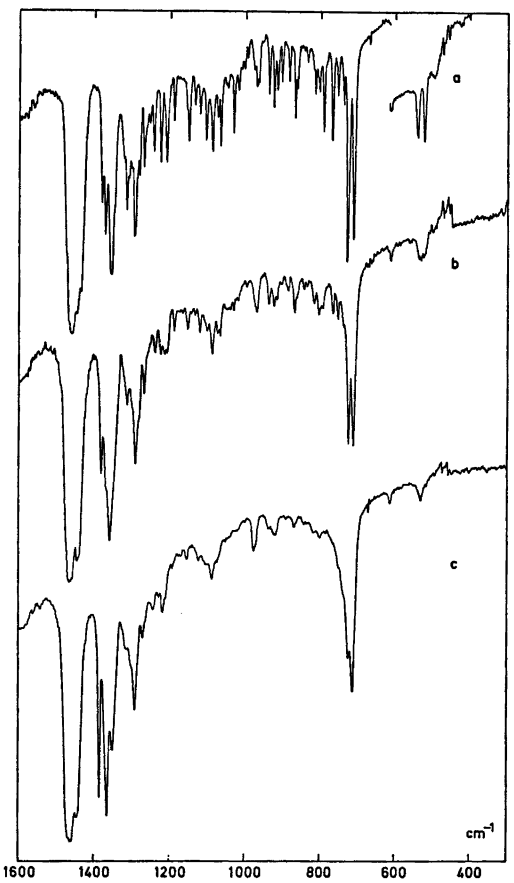


Fig. 3. Infrared spectra of 1,1-dimethylcyclohexadecane in the solid state at liquid N_2 temp. (a), in the solid state at −20 °C (b), and in the melt at 40 °C (c).

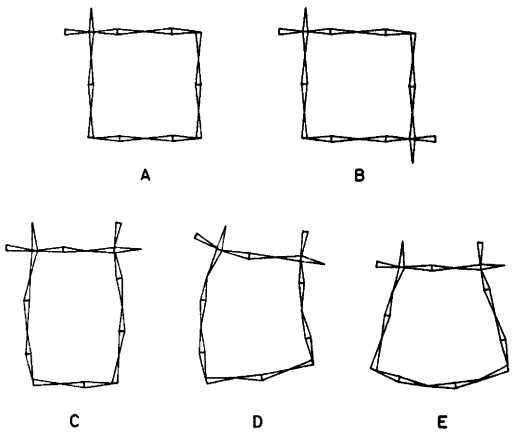


Fig. 4. Proposed conformations for 1,1-dimethylcyclohexadecane (A), for 1,1,9,9-tetramethylcyclohexadecane (B), and for 1,1,4,4-tetramethylcyclohexadecane (C, D, and E).

itself. Nevertheless, the infrared spectrum (Fig. 3) is similar in melt and solid, and the entropy of melting (Table 1) is too high to correspond to the melting of an expanded crystal lattice containing a mixture of conformers as in the case of cyclohexadecane. Transition points are in fact observed at -52 and -57 °C, but involve very small entropy changes, and the infrared spectrum of the low-temperature phase is different only in having sharper bands as generally expected for IR spectra of strongly cooled samples.

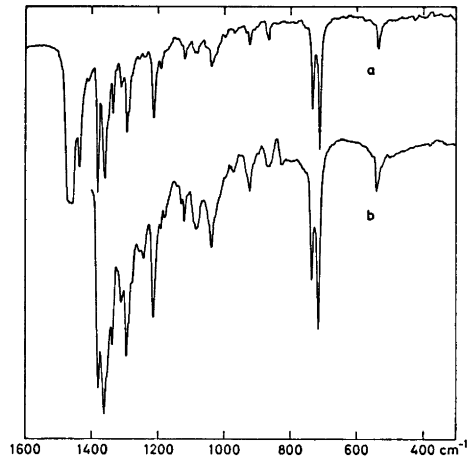


Fig. 5. Infrared spectra of 1,1,9,9-tetramethylcyclohexadecane in the solid state at 40 °C (a) and in CS_2-solution (b).

We conclude that the low-temperature crystal conformer is also present in the high-temperature solid and at least predominant in the liquid. It is extremely likely that it has the [4444] ring conformation (A, Fig. 4). There are two reasons why *gem*-dimethyl substitution at one corner should disfavour relatively the other ring conformations: Firstly, the ring valency angle at the substituted corner must be closer to the tetrahedral value of the diamond lattice, whereas at unbranched carbon atoms this angle is 112°. Secondly, the presence of the methyl substituents increases the torsional barriers in the adjoining CC bonds and fixes the torsional minimum at the diamond lattice dihedral angle of 60° instead of 65 – 70° without substitution.[1] Both changes must disturb the energy balance; the energy of that one conformation [4444] which is close to the diamond lattice is lowered, and the energy of all others are raised.

1,1,9,9-Tetramethylcyclohexadecane. This diametric substitution pattern fits only the [4444], the [3434] and the [3445] conformations. The sharpness of the IR-bands and identity of solid and liquid spectra (Fig. 5), the absence of a solid-solid transition point and a reasonable value of the entropy of melting (Table 1), suggest a single identical conformer in all phases. The spectral simplicity suggests a high symmetry, hence the [4444] ring conformation (B, Fig. 4) is again most likely.* The final identification has come from the crystal structure of the solid determined by Groth,[8] who finds in fact this conformation.

Thus, also for this molecule, the effect of *gem*-dimethyl substitution is to further favour that one of the three conformations which is of diamond-lattice type, and to disfavour the others, presumably to an even greater extent than in the 1,1-dimethyl derivative.

1,1,4,4-Tetramethylcyclohexadecane. This substitution does not fit the lowest-energy [4444]

* An observed broadening of the methyl line in the ^1H NMR spectrum in various solvent systems below -60 °C might in itself have indicated a lower symmetry, but the broadening at the lowest attainable temperature (-150 °C) was much too large to correspond to chemical shift differences, hence is ascribed to slow molecular tumbling of this large molecule as compared with the small spherical TMS molecule, for which a sharp line was still observed.

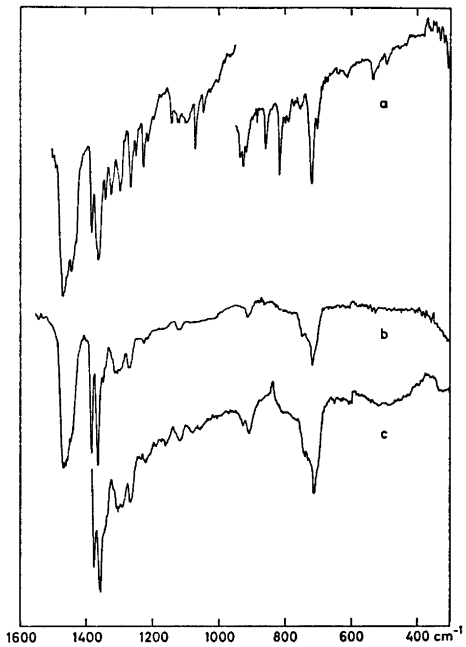


Fig. 6. Infrared spectra of 1,1,4,4-tetramethylcyclohexadecane in the solid state at −80 °C (a), in the melt at 40 °C (b), and in CS_2-solution (c).

conformation, but only the [3535], the [3445] and the [3454] conformations, of which the first has the lowest energy. The infrared spectrum (Fig. 6) of the solid is now much simpler than that of the liquid, so that conformational selection by the crystal lattice is clearly indicated. This is also borne out by the relatively high entropy of melting (Table 1). We propose that the crystal conformer has the rectangular [3535] ring skeleton (C, Fig. 4) of lowest calculated energy.

The coexistence of other conformers in solution and liquid may either mean that the [3445] and [3454] conformations are not so much higher in energy as the calculations suggest,[1] or that the change of preferred valency angles and torsional angles at the substituted corners largely eliminate the energy differences. Thus, the [3535] conformation needs dihedral angles substantially larger than 60° at the *gauche*-bonds terminating the three-bond "sides",[1] and *gem*-dimethyl substitution at both ends will prevent these from opening up. The alternative [3445] and [3454] conformations (D and E, Fig. 4) may be less affected by the substitution. We propose that these are the additional conformers in the liquid.

EXPERIMENTAL

1,1-Dimethylcyclohexadecane. To 13,13-dimethylcyclohexadeca-1,8-diyne [7] (0.2 g) dissolved in cyclohexane (50 ml) was added 5 % palladium on charcoal (0.1 g) and the solution hydrogenated at 3.5 atm. (40 h). The catalyst was filtered off, the solvent evaporated. Preparative gas chromatography, (column: 150 cm × 6 mm, 10 % OV 17 on chromosorb WAW DMCS,) gave *1,1-dimethylcyclohexadecane*, m.p. 17 °C. Mol.w. 252 (mass spectrometry). (Found: C 85.79; H 14.21. Calc. for $C_{18}H_{36}$: C 85.63; H 14.37).

1,1,9,9-Tetramethylcyclohexadecane. 5,5,13,13-Tetramethylcyclohexadeca-1,8-diyne [6] (0.23 g) was dissolved in cyclohexane (70 ml) and hydrogenated with palladium on charcoal (0.1 g) as described above. After evaporation of solvent recrystallization from ethanol gave *1,1,9,9-tetramethylcyclohexadecane*, m.p. 91 °C. Mol.w. 280 (mass spectrometry). (Found: C 85.46; H 14.54. Calc. for $C_{20}H_{40}$: C 85.63; H 14.37).

1,1,4,4-Tetramethylcyclohexadecane. 10,10,13,-13-Tetramethylcyclohexadeca-1,5-diyne [6] (0.8 g) was dissolved in cyclohexane (150 ml) and hydrogenated with palladium on charcoal (0.35 g) at 4.2 atm. (20 h). Repeated hydrogenation with fresh catalyst was necessary. The catalyst was filtered off, the solvent evaporated, the residue dissolved in pentane and filtered through alumina. Recrystallization from ethanol gave *1,1,4,4-tetramethylcyclohexadecane*, m.p. 31 °C. Mol.w. 280 (mass spectrometry). (Found: C 85.56; H 14.48. Calc. for $C_{20}H_{40}$: C 85.63; H 14.37).

Calorimetric data. A Perkin-Elmer Differential Scanning Calorimeter 1 B was used down to a temperature of −90 °C.

Infrared spectra. These were recorded with a Perkin-Elmer model 225 spectrometer. Solutions in CS_2 and CCl_4 were measured using 1 mm KBr cells. The low temperature spectra were obtained with a VLT-2 cell from RIIC, cooled with dry ice. The sample was contained between two pressed KBr or KJ discs.

NMR-spectroscopy. The NMR spectra were recorded with a Varian HA 100 15 D instrument. Solvent systems used for low-temperature studies were: CS_2, $CHFCl_2$, and a 2:1 mixture of $CHFCl_2$ and CF_2Cl_2.

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Solvent Effects in ^1H NMR Spectra of Pyrazoles

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The ^1H NMR spectra of nine 4-substituted 1,3,5-trimethylpyrazoles have been recorded in CCl_4/C_6H_6 solutions with varying mol fractions of benzene. The substituent influence on the chemical shifts and the aromatic solvent induced shifts are discussed, and the data for the 1-CH_3 hydrogens are successfully correlated with Hammett's σ-values.

A change in solvent from a non-aromatic one (such as cyclohexane, deuteriochloroform or carbon tetrachloride) to an aromatic one, generally causes solvent induced shifts. These aromatic solvent induced shifts, ASIS, defined by $\Delta = \delta_{\text{non-aromatic}} - \delta_{\text{aromatic}}$, measure the difference between the effect of the solvent on a given proton group and the protons of the internal references. These ASIS values have proven very helpful in determining the position of a given proton relative to polar sites (*cf.* reviews by Ronayne and Williams [1] and by Laszlo [2]). Elguero and coworkers [3–5] discovered that for 1-methyl substituted pyrazole derivatives, the chemical shifts are very sensitive to solvent effects, and that ring protons and methyl group protons in positions 1 and 5 are much more sensitive to solvent change (CCl_4 or $CDCl_3$ to C_6H_6) than ring protons and methyl group protons in positions 3 and 4. The following average solvent shifts were calculated $[\delta(CDCl_3) - \delta(C_6H_6)]$: 1-$CH_3$, 0.51 ppm; 3-$CH_3$, −0.02 ppm; 5-$CH_3$, 0.52 ppm. Some of the pyrazole solvent shifts of Elguero and coworkers [3–5] relevant to the present work are found in Table 1. In investigations of solvent shifts for pyrroles, indoles, and some of their methyl derivatives, Ronayne and Williams [6,7] discovered aromatic solvent induced shifts characteristic in magnitude and sign and thus useful in structure elucidation. In these π-excessive heteroaromatics, the benzene molecules are assumed to solvate the positive end of the pyrrole and indole dipoles, giving shifts diagnostic for 2- *versus* 3-substitution. The 3-H and 3-CH_3 resonances have ASIS between −0.54 and 0.05 ppm, whereas the 2-H and 2-CH_3 resonances have ASIS values between 0.03 and 0.45 ppm. The N−CH_3 resonances have values of ASIS between 0.65 and 0.80 ppm.

Table 1. ASIS $[\delta(CDCl_3) - \delta(C_6H_6)]$ or $[\delta(CCl_4) - \delta(C_6H_6)]$, in ppm) for some pyrazole derivatives (1-CH_3-3-R_3-4-R_4-5-R_5-pyrazole).[3–5]

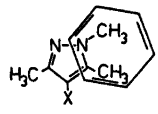
Compound			ASIS			
R_3	R_4	R_5	1-CH_3	R_3	R_4	R_5
H	H	H		−0.24	0.06	0.48[a]
H	H	H		−0.04	0.19	0.59[b]
CH_3	H	H	0.60	−0.01	0.11	0.50[b]
CH_3	Br	H	0.73	0.00		[b]
CH_3	Cl	H	0.78	−0.07		[b]
H	CH_3	H	0.50	−0.10	0.14	0.56[b]
H	H	CH_3	0.55		0.13	0.55[b]
H	Cl	CH_3	0.78			0.68[b]
CH_3	H	CH_3	0.49	−0.07	0.05	0.49[b]
H	Br	CH_3	0.78			0.68[b]

[a] Inert solvent: CCl_4. [b] Inert solvent: $CDCl_3$.

The discussion of the interaction between the polar solute and the aromatic solvent and the solvent shifts is based on the large magnetic anisotropy of the benzene ring. Ledaal [8] suggests that the dipole axis of the solute is located along the six-fold symmetry axis of the benzene ring. Ronayne and Williams [6] propose a model in which the benzene solvent molecules solvate each electron-deficient site of the solute, with the benzene molecule as far as possible away from the negative end of the local dipole with

its orientation determined by local dipole-induced dipole interactions. The interactions may occur in transient 1:1 collision complexes. Engler and Laszlo [9] have stressed the inadequacy of a fixed 1:1 complex as a model for these weak interactions. They introduce a general solvation model and express solvent shifts as products of a site factor for the proton groups of the solute and a solvent parameter.

With the simple 1:1 complex model, the interaction between the benzene solvent molecule and the pyrazole solute molecule is described by I, in analogy with the complex between pyrroles and indoles and aromatic solvents.[6,7] The dipole moment of the pyrazole ring is directed from N_1 towards N_2 and nearly parallel with the N–N bond.[10] It is then clear that ring protons and methyl group protons in positions 1 and 5 are in the strongly shielding region of the benzene ring. The benzene molecule

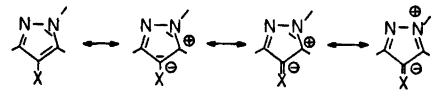


I

in I may be regarded as a symbol for the time-averaged solvent cloud around the pyrazole molecule.

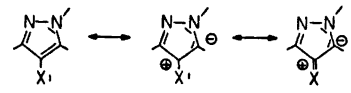
Even though several ^{1}H NMR studies of pyrazoles have appeared in the literature (see, *e.g.*, Refs. 3–5, 11–23), the spectrum of 1,3,4,5-tetramethylpyrazoles seems to be the only one determined for a 1,3,5-trimethyl-4-X-substituted pyrazole in CCl_4 solution. With 1,5- and 1,3-dimethylpyrazole as model compounds, Habraken *et al.*[21] assigned the signal at δ 2.04 to the 3-CH_3 protons and the signal at δ 1.98 to the 5-CH_3 protons, *i.e.* the 5-CH_3 protons absorb at higher field than the 3-CH_3 protons. For some 1-CH_3-4-X-substituted pyrazoles they discovered that in $CDCl_3$ solution, the 1-CH_3 protons are shifted downfield by electron-attracting substituents in position four, and similar displacements are found for the 3- and 5-proton peaks.

There is a strong resonance interaction between the 1-nitrogen atom in the pyrazole ring and –M substituents in position 4 (see II). This has been demonstrated in the slow hydrolysis of pyrazole-4-carboxylic esters,[24] in the



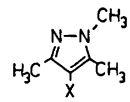
II

low frequency of the C=O stretching vibration of 4-acylpyrazoles,[25] and in the chemical shifts of the ring carbon atoms in ^{13}C investigations (Refs. 26–28).



III

To study the influence of –M (*cf.* II) and +M (*cf.* III) substituents on the aromatic solvent induced shifts, a number of 4-substituted 1,3,5-trimethylpyrazoles were synthesized and investigated (IVa-i).



IV

a. X=NH_2 f. X=I
b. X=CH_3 g. X=$CO_2C_2H_5$
c. X=H h. X=NO_2
d. X=Cl i. X=NO
e. X=Br

PREPARATIONS AND ^{1}H NMR SPECTRA

The compounds were synthesized by reported procedures: IVa,[29] IVb,[30] IVc,[30] IVd,[31] IVe,[32] IVf,[30] IVg,[33] IVh,[30] IVi.[34] The pyrazoles were distilled or recrystallized immediately before use.

The spectra were recorded on a Varian A-60 spectrometer in solutions (1 %, w/v) with increasing mol fraction of benzene: 0.000, 0.071, 0.287, 0.426, 0.673, 0.789, 0.928, 1.000. The spectra were recorded at normal probe temperature, *ca.* 310 K, using TMS as an internal standard. The chemical shifts were determined with the aid of sidebands generated by a Hewlett-Packard Model 200 oscillator and measured by a HP Model 3734 A frequency counter with scale expansion. The accuracy in the chemical shifts is presumed to be *ca.* 0.3 Hz. CCl_4 was chosen as the inert solvent for solubility reasons.

DISCUSSION

Signal assignments. The chemical shifts and solvent shift values for the 4-substituted 1,3,5-trimethylpyrazoles investigated are summarized

Table 2. Chemical shifts (δ-values) and ASIS (in ppm) for some 4-substituted 1,3,5-trimethylpyrazoles (IV).[a]

X	δ(1-CH_3)	ASIS	δ(3-CH_3)	ASIS	δ(5-CH_3)	ASIS	δ(5-CH_3) − δ(3-CH_3)
NH_2	3.567[a]		2.010[a]		2.073[a]		0.063
	3.217[b]	0.350	2.152[b]	−0.142	1.657[b]	0.416	
CH_3	3.573		2.017		2.067		0.050
	3.235	0.338	2.215	−0.198	1.638	0.429	
H	3.565		2.083		2.163		0.080
	3.158	0.407	2.283	−0.200	1.713	0.450	
Cl	3.645		2.103		2.177		0.074
	3.055	0.590	2.238	−0.135	1.638	0.539	
Br	3.675		2.100		2.193		0.093
	3.040	0.635	2.258	−0.158	1.625	0.568	
I	3.685		2.115		2.240		0.125
	3.042	0.643	2.267	−0.152	1.670	0.570	
$CO_2CH_2CH_3$	3.658		2.285		2.448		0.163
	3.005	0.653	2.627	−0.342	2.107	0.341	
NO_2	3.717		2.408		2.573		0.165
	2.775	0.942	2.450	−0.042	1.812	0.761	
NO	3.731		2.252		2.783		0.531
	2.775	0.956	2.373	−0.121	2.055	0.728	

[a] Refers to CCl_4 solution. [b] C_6H_6 solution.

in Table 2. The problem of assigning signals to protons 3 and 5 in the proton magnetic resonance spectrum of 1-methylpyrazole has been previously studied by means of coupling constants,[12] homo- and heteronuclear decoupling,[5] and deuterated pyrazole derivatives.[22] It has been found that in $CDCl_3$ and CCl_4 solution, H_5 in 1-methylpyrazole absorbs at higher field than H_3.

The signal assignments for the methyl hydrogens in positions 3 and 5 in the pyrazole series investigated in the present work were made by recording the spectra in CCl_4/C_6H_6 solutions with increasing benzene content. In accordance with the aromatic solvent shifts discussed above, the *C*-methyl resonance signal most affected by the solvent change is ascribed to the methyl hydrogens in position 5. The solvent dependence of the *C*-methyl hydrogens for three 1,3,5-trimethylpyrazoles is shown in Fig. 1. For all pyrazole derivatives the *C*-methyl resonance signal at lowest field (in CCl_4 solution) is most sensitive to solvent change, and is consequently ascribed to the methyl group in position 5. It is noticeable that in 1-methylpyrazole, H_3 absorbs at lower field than H_5. Habraken *et al.*[21] made the reverse assignments (*cf.* above) for the 3-CH_3 and 5-CH_3 protons in the tetramethylpyrazole. However, these assignments were based on the ^{1}H NMR spectra of 1,5- and 1,3-dimethylpyrazole, where the 3-CH_3 protons absorb at lower field than the 5-CH_3 protons (0.03 ppm).

A change in solvent from CCl_4 to $CDCl_3$ causes downfield shifts for the ^{1}H NMR signals of 1-methylpyrazole (for H_3 0.20 ppm, for H_5 0.11 ppm).[22] These solvents shifts can be understood as a consequence of hydrogen bonding between $CDCl_3$ and the pyridine nitrogen atom in the pyrazole ring ($Cl_3C-D\cdots N_2-N_1$) resulting in decreased electron density more pronounced in position 3 than in position 5. This is reflected in the shift changes given above. With the signal assignments made with the aid of ASIS for 1,3,5-trimethylpyrazole, the same order in downfield shifts is obtained for methyl groups 3 and 5 (0.09 and 0.01 ppm for a solvent change $CCl_4 \rightarrow CDCl_3$). This is further proof for the signal assignments made above.

The substituent dependence of the chemical shifts. The influence of substituents on chemical shifts of ring protons has been studied in benzenes and heterocycles. Linear correlations have sometimes been attempted between proton chemical shifts and substituent constants based on reactivity data.[35]

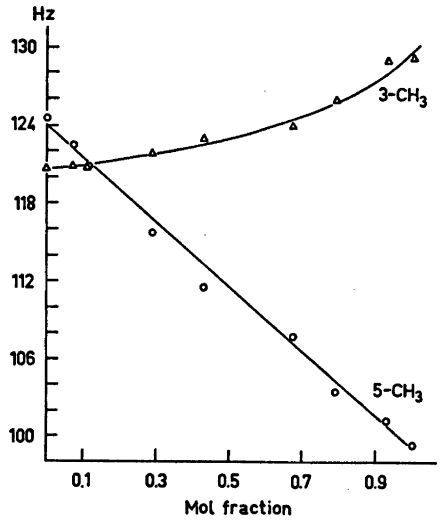


Fig. 1a. Chemical shifts (in Hz from TMS) for the 3-CH_3 and 5-CH_3 hydrogens in compound IVa *versus* mol fraction of benzene.

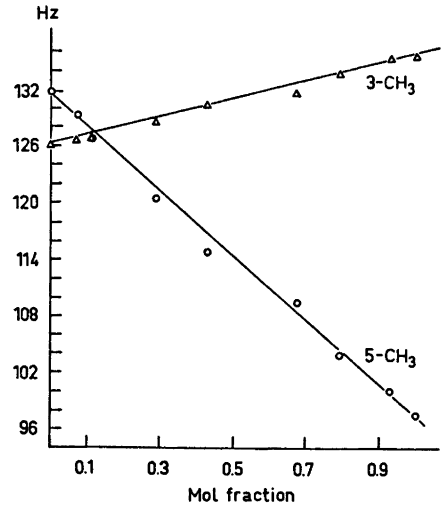


Fig. 1b. Chemical shifts (in Hz from TMS) for the 3-CH_3 and the 5-CH_3 hydrogens in compound IVe *versus* mol fraction of benzene.

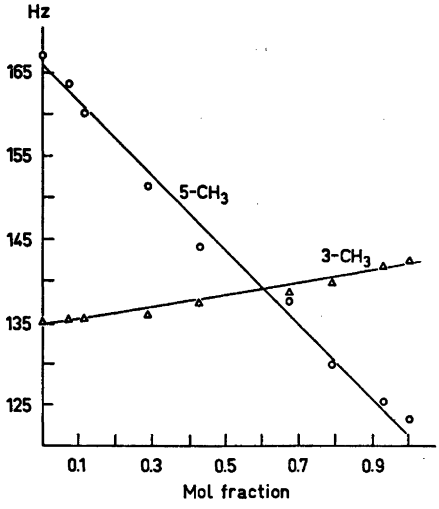


Fig. 1c. Chemical shifts (in Hz from TMS) for the 3-CH_3 and the 5-CH_3 hydrogens in compound IVh *versus* mol fraction of benzene.

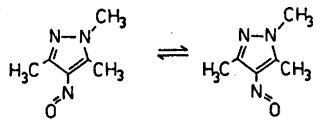
In the system investigated here, the methyl groups are connected to the substituent *via* the conjugated pyrazole system. The influence of substituents on chemical shifts of such methyl protons has been investigated for 2-CH_3-5-R-substituted thiophenes,[36] methoxy protons in substituted anisoles,[37] and substituted toluenes.[38]

Table 2 shows that a strong −M substituent in position 4 in the pyrazole ring causes a considerable downfield shift for the 1-CH_3 protons (IVc: δ(1-CH_3) = 3.565 ppm; IVh: δ(1-CH_3) = 3.717 ppm; CCl_4 solution). This can be understood as the result of the strong resonance interaction between positions 1 and 4 in the pyrazole ring (II). The inductive and magnetic anisotropy effects of the substituent on δ(1-CH_3) are assumed to be small. For stronger −M substituents in position 4 there is an increased contribution from the dipolar structures, resulting in reduced shielding (*via* inductive effects) and downfield shifts for the 1-CH_3 protons.

The chemical shifts of the 1-CH_3 protons are not susceptible to a +M substituent such as the amino group (IVc: δ(1-CH_3) = 3.565 ppm; IVa: δ(1-CH_3) = 3.567 ppm; CCl_4 solution). As is shown by III, a +M substituent, introduced in position 4, is conjugatively electron-donating to position 5 in the pyrazole ring, whereas only its inductive effect operates to positions 3 and 1. The change in electron density at the 1-CH_3 protons on introducing the amino group is consequently negligible and the chemical shift of the 1-CH_3 protons is not affected.

The difference in chemical shifts between methyl groups 5 and 3 (CCl_4 solution) is determined not only by resonance effects but also by field and anisotropy effects in a rather complex way. Table 2 shows that the shift difference increases with increasing −M capacity of the 4 substituents (0.08 ppm for IVc, 0.165 ppm for IVh). This is ascribed to a decrease in the π-electron density in position 5 (II) resulting in downfield shifts for 5-CH_3 protons. Position 3, on the other hand, is unaffected by this resonance effect. The inductive effect of the substituent is assumed to be the same in the two positions.

The large shift difference for the nitroso compound is ascribed to the anisotropy of the nitroso group. Positions along the N−O bond direction are deshielded and positions perpendicular to the bond are shielded.[39] For 1,3,5-trimethyl-4-nitrosopyrazole two planar conformations are possible (Va and Vb). It has been found that in CD_2Cl_2 solution at 240 K,



Va Vb

the conformer ratio Va/Vb is 4/1.[40] With an assumed dominance of conformer Va in CCl_4 solution at probe temperature, at which temperature the rotation of the nitroso group is fast on the NMR time scale, methyl group 5 is in the deshielded region and methyl group 3 in the shielded region. Thus the large shift difference is obtained.

The aromatic solvent induced shifts. The ASIS values are summarized in Table 2 and Fig. 4. Table 2 shows the ranges of the ASIS for the three methyl group protons: for 1-CH_3 0.338 − 0.956 ppm, for 3-CH_3 −0.342 to −0.042 ppm and for 5-CH_3 0.341 − 0.728 ppm. As discussed above, the signal assignments for the *C*-methyl protons were made by means of ASIS. For the 1-CH_3 and 5-CH_3 protons, the ASIS increases with increasing −M capacity of the substituent. This can be understood as the result of an increased order in the solvent cage around the partially positive 1-nitrogen atom and ring carbon atom 5 of the pyrazole ring (*cf.* II) as

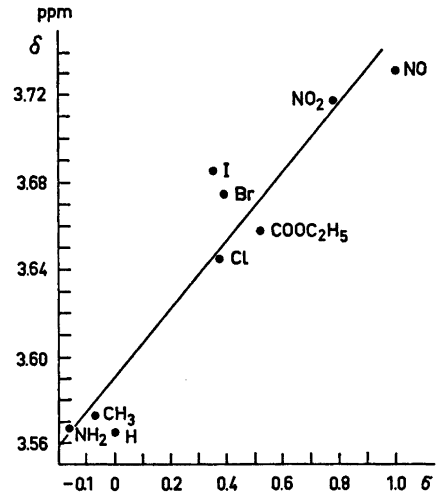


Fig. 2. Chemical shifts of the 1-CH_3 hydrogens (in ppm, from TMS, CCl_4 solution) *versus* Hammett's σ-values. The σ-values are taken from Jaffé.[49]

the benzene molecules interact with positive centers and avoid the negative parts. ASIS of the 3-CH_3 protons depend on the substituent in a complicated way. For the nitroso compound, it is possible that the isomer ratio Va/Vb may change from CCl_4 to C_6H_6 and that this change affects the ASIS.

The solvent shifts obtained for different positions in pyrroles, indoles,[6,7] and pyrazoles all emphasize the potential possibility of this technique for structure elucidation among azoles and diazoles.

Hammett correlations. In the pyrazole series, the Hammett equation has been used to relate reactivities in side-chains in positions 1 to σ_p values for the substituent in position 4. Good linear relations have been obtained between σ_p values and log *k* for the hydrolysis of 1-acylpyrazoles,[41] the ethanolysis of 1,1′-thiocarbonylbispyrazoles,[42] and the rotation of the dimethylamino group in 1-(*N*,*N*-dimethylthiocarbamoyl)-pyrazoles.[43] Discussions of the Hammett equation applied to heterocyclic compounds have been given by Jaffé [44] and by Gronowitz.[45]

A linear correlation is obtained for the trimethylpyrazoles between the chemical shifts (CCl_4 solution) of the 1-CH_3 protons and Hammet's σ-values, with a correlation coefficient of

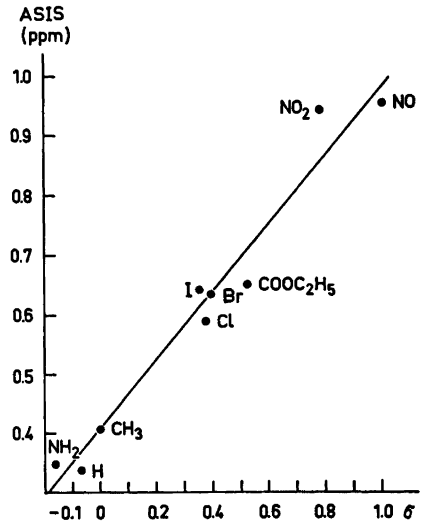


Fig. 3. Aromatic solvent induced shifts (ASIS) for the 1-CH_3 hydrogens *versus* Hammett's σ-values.

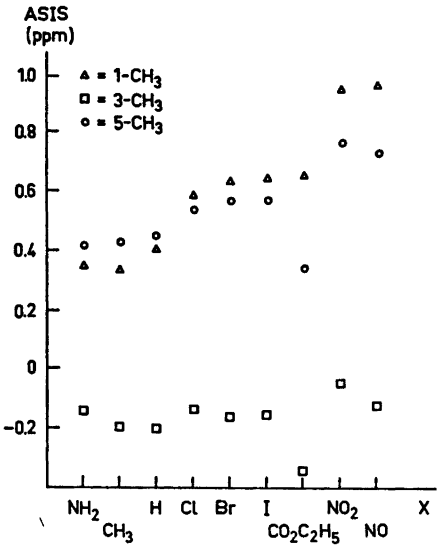


Fig. 4. Aromatic solvent induced shifts (ASIS) for the methyl groups in some 1,3,5-trimethyl-4-X-pyrazoles.

0.95 and a ϱ-value of 0.159 (Fig. 2). A −M substituent in position 4 is regarded to be *para*-related to position 1, a +M substituent, on the other hand *meta*-related (*cf.* II and III). Habraken *et al.*[46] showed that the nitroso group in 3,5-dimethyl-4-nitrosopyrazole is a more powerful electron attractor than the nitro group. Lüttke *et al.*[47] have reached the same conclusion in a study of nitroso- and nitrodimethylamine. For a *para* nitroso group, Habraken reports a σ_p^--value of 1.46, computed from pK_a data of pyrazoles. In this work, a σ_p-value of 1.0 is used for the nitroso group (calculated by subtracting the difference $\sigma_p^- - \sigma_p$ for the nitro group from σ_p^- for the nitroso group).

A survey of about 100 Hammett correlations of ^{1}H NMR data, made by Yokoyama *et al.*,[48] shows that most shift correlations give unsatisfactory correlation coefficients, *e.g.* $< 0.90 - 0.95$. Furthermore, the ϱ-values vary stronly between different series of compounds. From a study of 4-substituted 1-methylpyrazoles, a ϱ-value of 0.48 can be calculated ($r = 0.91$).[21] This refers to $CDCl_3$ solution, however, and ϱ-values are known to vary with the solvent.[48] For the methyl protons in $RC^6H^4CH_2-H$ a ϱ-value of 0.2 is given ($CDCl_3$ solution).[38] The methoxy methyl protons in *para*-substituted anisoles correlate with ϱ_p parameters with a ϱ-value of 0.19 (CCl_4 solution).[37] A positive ϱ-value means that the downfield shift increases when σ increases. The correlation coefficients for the systems above are unsatisfactory and this make a comparison of the different ϱ values uncertain.

An excellent linear correlation is obtained between the ASIS for the 1-CH_3 protons and Hammett's σ-values (Fig. 3) with a correlation coefficient of 0,98 and a ϱ-value of 0,57. On the basis of ASIS measurements on the methyl group in *para*-substituted toluenes, a ϱ-value of 0.41 can be calculated ($r = 0.97$).[38] ASIS of pyrazole derivatives seem to be somewhat more substituent dependent than in the benzene series.

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On the Synthesis of 2*H*-Benzo[*b*]thiete 1,1-Dioxide

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The synthesis of 2*H*-benzo[*b*]thiete 1,1-dioxide, first described by Dittmer and Davis, has been improved. An intermediate, *trans*-2,5-dibromo-7-thiabicyclo[4.2.0]-1(6)-octene 7,7-dioxide, has been obtained in crystalline form, and from it the title compound has been prepared in 15 % yield with silver oxide in boiling xylene. A new by-product in the cycloaddition of sulfene to 1-(*N*-pyrrolidino)-cyclohexene has been identified as 2-methanesulfonyl 1-(*N*-pyrrolidino)-7-thiabicyclo[4.2.0]octane 7,7-dioxide using ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.

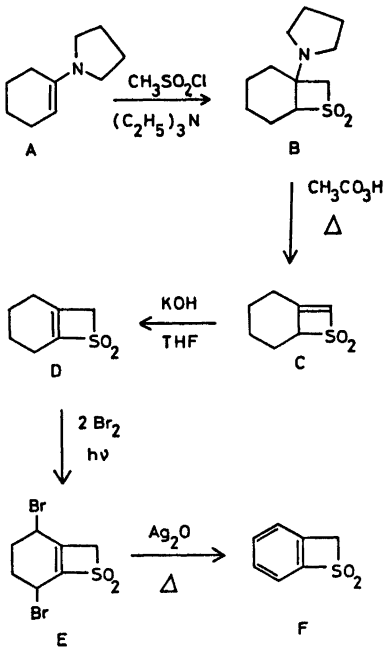


Fig. 1. Synthetic route to 2*H*-benzo[*b*]thiete 1,1-dioxide.

In a recent electrochemical study of some cyclic sulfones,[1] the four-membered ring compound 2*H*-benzo[*b*]thiete 1,1-dioxide was included. It was first synthesized by Dittmer and Davis [2] as shown in Fig. 1. The present paper describes a reinvestigation of the synthesis. The original route has been followed, but some modifications have made it possible to increase the yields in some of the steps and at the same time make them technically easier. Thus, the entire preparation can be performed without chromatographic separation.

A different synthetic route, the key step of which is a Diels-Alder reaction between 3-bromothiete 1,1-dioxide and butadiene, has recently been described by Nelsen and Dittmer.[3] The overall yield, starting from thietane, is 10 – 20 %.

In our work, the starting material, enamine A (for brevity, the designations A, B *etc.* in Fig. 1 will be used) was prepared from cyclohexanone and pyrrolidine.[4] The cycloaddition of A to sulfene, generated *in situ*, was carried out according to Dittmer and Davis.[5] In addition to B, a minor amount of another, less soluble product was obtained, which was characterized as 2-methanesulfonyl 1-(*N*-pyrrolidino)-7-thiabicyclo[4.2.0]octane 7,7-dioxide. A possible route to this by-product, G, is shown in Fig. 2.

Borowitz has reported [6] that the four-membered ring adduct of sulfene to 1-*N*-morpholinocyclohexene can be thermally rearranged to a 2:1 mixture of 6-methanesulfonyl and 2-methanesulfonyl 1-*N*-morpholinocyclohexene. Although the temperature required for this, 128 – 137 °C, is much higher than the one

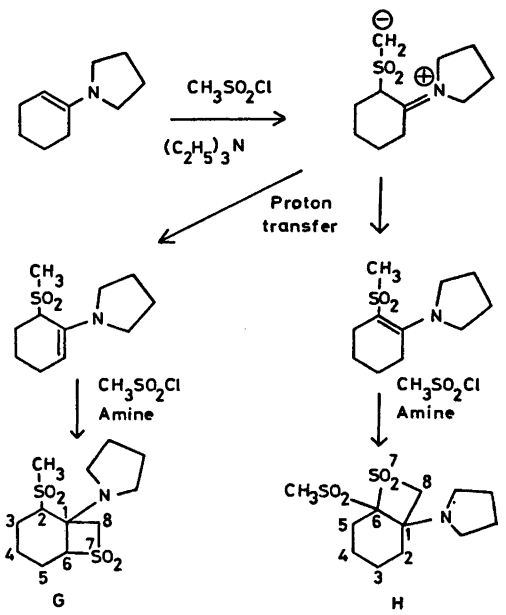


Fig. 2. Isomeric 2+1 adducts of sulfene to 1-(*N*-pyrrolidino)-cyclohexene.

in the present synthesis, the finding suggests that methanesulfonyl enamines can be formed as minor products even at low temperature, perhaps *via* proton transfer to or within a zwitterionic intermediate. Addition of a second molecule of sulfene to the 6-methanesulfonyl isomer would yield the by-product observed in this work. The 2-methanesulfonyl isomer is expected to be less reactive both for steric and electronic reasons, and structure G rather than H (see Fig. 2) is therefore preferred.

An unambigous distinction between G and H was provided by ^{13}C NMR spectroscopy. The spectrum of the by-product was kindly recorded and interpreted by Prof. Dr. W. v. Philipsborn at the Organisch-Chemisches Institut der Universität Zürich, using a Varian XL-100 instrument. The chemical shift and multiplicity data are given in the Experimental part. In the off-resonance decoupled spectrum, there is one singlet for carbon atom 1 but none for carbon atom 6. There are three resonances at rather high frequency, which must be assigned to three carbon atoms α to the sulfonyl groups; atoms 2, 6, and 8. None of these is a quaternary atom. On the other hand, the low-frequency part of the spectrum exhibits three but not four CH_2 resonances for the six-membered ring. The above constitutes three independent pieces of evidence against structure H.

In the Cope elimination step, B→C, it was found to be advantageous to omit the neutralization with sodium carbonate [5] after the oxidation with peracetic acid. In runs with neutralization, a brown, tarry material was formed, rendering the work-up difficult. C, an α,β-unsaturated sulfone, is a good Michael acceptor, and conditions favouring nucleophilic attack should be avoided.

The rearrangement of the *exo* sulfone C to the *endo* isomer D was performed with powdered potassium hydroxide in tetrahydrofuran as described by Dittmer and Davis.[3] In trials with solid lithium hydroxide in tetrahydrofuran and 1,8-bis(*N*,*N*-dimethylamino)naphthalene [7] in benzene, no trace of rearrangement was seen.

The dibromination of D was first made with *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide but, as also found by Dittmer and Davis,[3] the reaction required at least two days at 60 °C. If, instead, the allylic bromination is carried out photochemically using elemental bromine, the time required for reaction was 7 h at room temperature.

The crude dibrominated product is an almost colourless oil. It was fortuitously discovered that half of it can be obtained in crystalline form from a benzene-ether solution. The solid contains benzene of crystallization, which is lost upon drying. Thin-layer chromatography of the noncrystalline part of the oil showed it to contain at least two components, since two spots of roughly equal size were obtained. The slower moving one of these matched the single spot given by the crystalline material. It is tempting to assume that the oil is a mixture of *cis* and *trans* isomers, but, as has been kindly pointed out by Dr. H.-D. Becker at this Department, it could contain structurally different isomers if allylic rearrangement takes place during the bromination.

The crystalline material was first thought to be the *cis* isomer for the following reasons. Allylic bromination of a cyclohexene system should take place (pseudo)axially to allow maximum resonance stabilization of the intermediate radical. As illustrated in Fig. 3, the intermediate monobrominated compound would

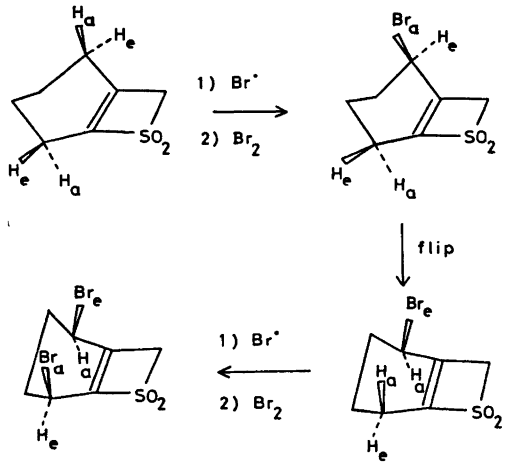


Fig. 3. Formation of *cis* isomer of compound E.

then flip to bring the bromine atom into a (pseudo) equatorial position. If the second bromine atom were also introduced (pseudo)-axially, the *cis* compound would result. Another argument for the *cis* assignment is that in the subsequent elimination with silver oxide, a higher yield of the final product F is obtained from the crystalline material than from the isomer mixture. In accordance with well-known rules for eliminations of this kind, a trans-diaxial arrangement of a bromine and a hydrogen atom should favour the reaction, and this arrangement is present in the *cis* isomer.

In collaboration with the Department of Inorganic Chemistry at the Chalmers University of Technology and University of Göteborg, a single-crystal X-ray investigation of the solid isomer was undertaken.[8] The configuration was, in fact, shown to be *trans*. It must therefore be concluded that the arguments presented above have no predictive power. The difference between a pseudoaxial and a pseudoequatorial substituent apparently is not large enough in this system to decide the steric course of the photobromination.

The 60 MHz ^{1}H NMR spectrum of the isomer mixture was found to be quite similar to that of the pure, crystalline isomer, and since an attempted preparative layer chromatographic separation failed, further attempts at characterizing the minor component (*cis*?) of the oil were abandoned.

The final conversion E→F gave only a 3 % yield when carried out as originally described.[2] After much experimentation, it was found that silver oxide in refluxing xylene brings about the desired transformation in a yield of 15 % starting with the pure crystalline isomer of E.

The oil containing two isomers of E also yields the product F upon treatment with silver oxide, but the purification in this case is much more difficult since the reaction mixture does not crystallize, and the yield is lower, around 5 %.

A reagent which caused complete reaction of E within a few minutes at room temperature was prepared by dissolving silver phosphate in polyphosphoric acid. However, no trace of F was obtained. Apparently, silver oxide furnishes the best balance between nucleophilic/basic and eletrophilic/acidic conditions among the reagents yet tried.

EXPERIMENTAL

1-(N-pyrrolidino)-cyclohexene (A). This compound was prepared in 97.5 % yield on a 3 mol scale using the standard method.[4]

1-(N-Pyrrolidino)-7-thiabicyclo[4.2.0]octane 7,7-dioxide (B) and its *2-methanesulfonyl derivative* (G). Of the above enamine, 442 g (2.93 mol) and 510 ml (3.7 mol) of triethylamine, purified by distillation from phthalic anhydride,[9] were dissolved in 1.5 l of absolute ether. Methanesulfonyl chloride, 375 g (3.27 mol) was added dropwise with stirring and cooling in a Dry Ice-acetone bath to keep the temperature between −10 °C and 0 °C. The reaction mixture was left for 8 h at room temperature and filtered. The triethylamine hydrochloride was washed with ether and the combined filtrate and washings evaporated *in vacuo* below 40 °C. The remainder was dissolved in 500 g of ice and 400 ml of conc. hydrochloric acid. The solution was washed with ether which removed some cyclohexanone resulting from hydrolysis of unreacted enamine, and an excess of aqueous ammonia was added. Cooling to 5 °C gave a semi-crystalline mass. The supernatant water solution was decanted and the crystals were washed with ice-cold water and dissolved in 1 l of ether and 500 ml of benzene. An insoluble rest was filtered off and investigated separately; see below. The clear filtrate was washed with water and evaporated below 40 °C to yield 570 g of product B (85 % yield). Upon being stored at 5 °C, the material slowly crystallized.

The ether-insoluble by-product was recrystallized from a mixture of equal volumes of chloroform and ethanol to yield 18.2 g of colourless crystals, m.p. 195−197 °C with

decomposition; 60 MHz ^{1}H NMR ($CDCl_3$) δ 1.3–2.6 (multiplet, 10 H), 2.8–3.1 (multiplet, 4 H), 3.0 (singlet, 3 H), 3.6–3.9 (multiplet, 1 H), 4.4–4.8 (multiplet, 1 H), 4.5 (singlet, 2 H). The 25.2 MHz ^{13}C NMR spectrum ($CDCl_3$), recorded using proton noise decoupling, as well as off-resonance decoupling with irradiation at the TMS signal to determine multiplicities, had the following peaks: (δ-value in ppm rel. to TMS, rel. signal height, multiplicity using s for singlet, d for doublet, t for triplet, and q for quartet), 75.6, 94, d, C(2) or C(6); 72.0, 70, t, C(8); 69.0, 74, d, C(6) or C(2); 50.5, 64, s, C(1); 50.0, 168, t+t, 2 NCH_2; 40.3, 56, q, CH_3; 23.8, 178, t+t+t; 21.4, 56, t; 20.5, 56, t. The resonance at 23.8 ppm could be resolved into a *two*-carbon (t+t) and a *one*-carbon (t) signal using $Pr(fod)_3$ as paramagnetic shift reagent. Thus the 12 carbon atoms are definitely accounted for. The two-carbon (t+t) signal (23.8 ppm) is assigned to the β carbons of the pyrrolidine ring whereas the residual resonances at 23.8, 21.4, and 20.5 ppm originate from the three CH_2 groups in the six-membered ring.

The mass spectrum, determined on an LKB 9000 instrument using 70 eV electrons for ionization, had the following peaks: *m/e* 307 (parent ion, 16.1 %), 228 ($P-CH_3SO_2$, 32.3 %), 201 (18.2 %), 200 (11.9 %), 164 ($P-CH_3SO_2-SO_2$, 100 %), 150 ($P-CH_3SO_2-CH_2SO_2$, 51.6 %), 149 (37.1 %), 136 (35.5 %), 122 (38.7 %), 110 (19.4 %), 96 (22.6 %), 79 (17.7 %), 77 (21.0 %).

7-Thiabicyclo[4.2.0]-1(8)-octene 7,7-dioxide (C). The oxidation of compound B to the corresponding amine oxide was performed according to Ref. 5. Peracetic acid had been prepared the previous day by mixing 2 volumes of glacial acetic acid, 1 volume of 50 % hydrogen peroxide, and 0.03 volume of conc. sulfuric acid. Just before use, enough sodium acetate was added to neutralize the sulfuric acid present.

Contrary to the directions in Ref. 5, the reaction mixture was evaporated *in vacuo* at 60 °C without prior treatment with sodium carbonate. The remaining oil was dissolved in the minimum amount of water and extracted three times with dichloromethane. The combined organic phases were washed with 10 % hydrochloric acid followed by saturated sodium chloride solution and dried over anhydrous magnesium sulfate. Evaporation and trituration with ether caused the *exo* sulfone C to crystallize. The yield in this step varied between 75 and 90 % (Ref. 5; 57 %), 1 mol batches being run.

7-Thiabicyclo[4.2.0]-1(6)-octene 7,7-dioxide (D). The rearrangement to *endo* sulfone D was performed with potassium hydroxide in tetrahydrofuran according to Ref. 2. It was found impossible to reproduce the 90 % yield reported. In this work, 70 % was obtained.

2,5-Dibromo-7-thiabicyclo[4.2.0]-1(6)-octene 7,7-dioxide (E). Instead of using *N*-bromosuccinimide,[2] a photochemical bromination was performed. A solution of 15.8 g (0.1 mol) of *endo* sulfone D and 32.0 g (0.2 mol) of bromine in 500 ml of ethanol-free chloroform was irradiated in a Rayonet photochemical reactor, using 350 nm lamps. A quartz vessel equipped with an internal cooling tube and a nitrogen bubbler was used, although glass most probably works equally well. The reaction required 7 h and could be easily followed by the evolution of hydrogen bromide. At the end of this period, the bromine colour had completely disappeared. Evaporation of the solution *in vacuo* gave 34.0 g of an almost colourless oil (theoretical yield, based on dibromination). This was dissolved in the minimum quantity of a boiling ether–benzene mixture, 2:1 by volume. Cooling to 5 °C gave large, transparent crystals which were collected and washed. The yield was 17.6 g (42 %), 60 MHz ^{1}NMR ($CDCl_3$) δ 2.0–2.8 (multiplet, 4 H), 4.45–4.65 (multiplet, 2 H), 4.8–5.0 (multiplet, 2 H), 7.35 (singlet, 6 H). The crystals have been shown by X-ray diffraction [8] to be the *trans* isomer of E, and also to contain benzene of crystallization in a molar ratio 1:1 (compare the NMR data). Upon exposure to the atmosphere for a few days or more rapidly *in vacuo*, this benzene disappeared with efflorescence, resulting in a 20 % weight loss. The melting point of the dry material was 107–108 °C. Upon TLC analysis of the mother liquor (silica gel/dichloromethane), two components having R_F values 0.27 and 0.38 were found. The crystalline material gave one spot, R_F 0.27.

2H-Benzo[b]thiete 1,1-dioxide (F). Of the dried, solid isomer E, 7.9 g (25 mmol) was refluxed for 10 h under stirring with 20 g (86 mmol) of silver oxide and 50 ml of xylene. Filtration and evaporation *in vacuo* gave 2.0 g of a yellow oil. Addition of a few ml of ether, cooling and scratching gave crystals which were collected and recrystallized from 50 ml of ether. Colourless needles were obtained, 0.75 g, m.p. 127–127.5 °C, lit.[2] 126–128 °C. An impurity, revealed in the ^{1}H NMR spectrum, was left behind by sublimation *in vacuo* (80 °C, 27 Pa). The recovery was 0.6 g (15 %), m.p. 127–127.5 °C, 60 MHz ^{1}H NMR ($CDCl_3$), δ 5.1 (singlet, 2 H), 7.55 (singlet, 4 H) in agreement with the literature data.[2]

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Structural Studies of the Extracellular Polysaccharide Produced by the Diatom *Chaetoceros curvisetus* Cleve

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Methylation studies and partial acid hydrolysis have been carried out on the extracellular polysaccharide produced by the diatom *Chaetoceros curvisetus*. The polysaccharide contains the sugars galactose, rhamnose, and fucose and is partially sulfated. The presence of fucose in both furanose and pyranose forms within one molecule is reported for the first time. The fucofuranose is present as end groups, 1,2-linked and as branch points. Fucopyranose is mainly present as branch points in the polysaccharide, some being 1,3-linked, and a small fraction as end groups. The main part of rhamnose is 1,2-linked and galactose is mainly 1,3-linked.

Several diatoms are known to produce extracellular polysaccharide.[1] The production of extracellular polysaccharides produced by species of the *Chaetoceros* family, has been studied [2,3] and three members of this family were shown to excrete significant amounts of polysaccharide into the surrounding medium when being in the stationary growth phase. The three species were *C. affinis* (clone CH1), *C. curvisetus* (CH24), and *C. decipiens* (CH40).

These polysaccharides were all sulfated and contained the sugars galactose, rhamnose, and fucose in different proportions. Structural studies on the polysaccharide produced by *C. affinis* [4] showed that this polysaccharide was highly branched and had a complex structure. Methylation studies showed that the polysaccharide produced by *C. decipiens* had a very similar structure,[5] while the polysaccharide produced by *C. curvisetus* was different. The present paper describes structural studies of this polysaccharide.

RESULTS AND DISCUSSION

The cultivation of the diatom *C. curvisetus* and isolation of the extracellular polysaccharide was performed as for *C. affinis*.[2,6]

The polysaccharide (CH24) had $[\alpha]_D = -68°$, a carbohydrate content of 80 %, $-SO_3Na$ was 6.9 % determined by the method of Antonopoulos.[7] Potentiometric titration gave an equivalent weight of 950, which, if the only anion present is sulfate, was calculated to correspond to approximately 10 % $-SO_3Na$.

Subjected to free boundary electrophoresis at pH 2 and pH 7, the polysaccharide moved as a single, anionic compound, with approximately the same mobility at both pH values, also indicating that the anion is sulfate (or another strong acid).

Complete acid hydrolysis of the polysaccharide and analysis by GLC of the derived alditol acetates [6,12] showed the presence of rhamnose, fucose, and galactose in the proportions 0.3 to 3.5 to 1.0. No other sugars were detected. The configuration of the sugars has not been determined due to shortage of material.

Preliminary methylation experiments, combined with analysis of the derived partially methylated alditol acetates by GLC-MS,[4] indicated that some of the fucose present as end groups was in the furanose form. It is well known that end groups in furanose form are readily released from the rest of the polysaccharide under weak acidic conditions. In the extracellular polysaccharide produced by *C. affinis* (CH1) rhamnopyranose is responsible for the main part of the non reducing end groups.[4] CH1 and CH24 polysaccharides were

Table 1. Composition of polysaccharide before and after partial hydrolysis.

	Carbohydrate content (mg)	Sugar ratio Rhamnose	Fucose	Galactose
CH24	19	0.3	3.5	1.0
1 h	10	0.3	2.3	1.0
3 h	7.5	0.2	1.6	1.0

both subjected to weak acid hydrolysis (see Experimental). The hydrolysates of both polysaccharides were dialysed, and the dialysates tested for carbohydrate.[8] The dialysate from CH1 polysaccharide contained no carbohydrate, while that from CH24 polysaccharide contained considerable amounts. When analysed by TLC, and GLC after conversion to alditol acetates, no other monosaccharide than fucose was found in the dialysate.

The starting material and the products after 1 and 3 h of hydrolysis, were all analysed for sugar ratios by GLC and for carbohydrate contents by the phenol-sulfuric acid method,[8] using as standards solutions containing the sugars in appropriate ratios (Table 1). It can clearly be seen from Table 1 that fucose is lost during this hydrolysis, and the fact that it is readily released indicates that it is present in the furanose form.

The three polysaccharide samples were subjected to methylation,[9,10] hydrolysed and converted into the partially methylated alditol acetates. These were all analysed by combined GLC-MS (col. OV-225).[4] The identifications were thus confirmed both by their retention times (T) and mass-spectra (Table 2). The compounds with T-values 0.56, 0.96 and 1.33, gave a MS-fragmentation pattern different from those derived from partially methylated alditol acetates originally present in pyranose form. The compound with T = 0.56 gave as the main fragments *m/e* 59, 117, and 175 (Fig. 1), which previously has been shown to derive from the alditol acetate corresponding to 2,3,5-tri-*O*-methylfucose.[11] That with T = 0.96 gave as main fragments *m/e* 59, 175, and 189 (Fig. 1). These can only be obtained from the alditol acetate corresponding to 3,5-di-*O*-methylfucose. The compound with T = 1.33 gave as the main fragment *m/e* = 59 (Fig. 1), indicating that the compound is the alditol acetate corresponding to 5-*O*-methylfucose.

The presence of these three methylated products shows that fucose in the furanose form is not only present as end groups, but also as part of the chain and as branch points. The presence of these forms in a polysaccharide

Table 2. GLC analysis of the methylated alditol acetates.

Retention times [a] (T)	Column OV – 225	Weights calculated from GLC (mg) Start	1 h	3 h
2,3,4-Tri-*O*-methylrhamnose	0.47	0.2	0.2	0.5
2,3,5-Tri-*O*-methylfucose	0.56	2.9	1.2	0.6
2,3,4-Tri-*O*-methylfucose	0.60	tr	0.2	0.1
3,4-Di-*O*-methylrhamnose	0.88	1.0	0.8	0.5
3,5-Di-*O*-methylfucose	0.96	4.0	1.6	1.0
2,4-Di-*O*-methylfucose	1.04	1.8	1.3	0.8
2,3,4,6-Tetra-*O*-methylgalactose	1.15	0.3	0.8	0.5
5-*O*-Methylfucose	1.33	1.8	0.3	0.2
2-*O*-Methylfucose	1.41	1.1	0.2	0.2
4-*O*-Methylfucose	1.71	2.9	1.3	1.1
2,4,6-Tri-*O*-methylgalactose	1.92	1.5	1.5	1.5
4,6-Di-*O*-methylgalactose	2.95	1.3	0.5	0.5

[a] Relative to 2,3,4,6-tetra-*O*-methyl-D-glucitol 1,5-diacetate.

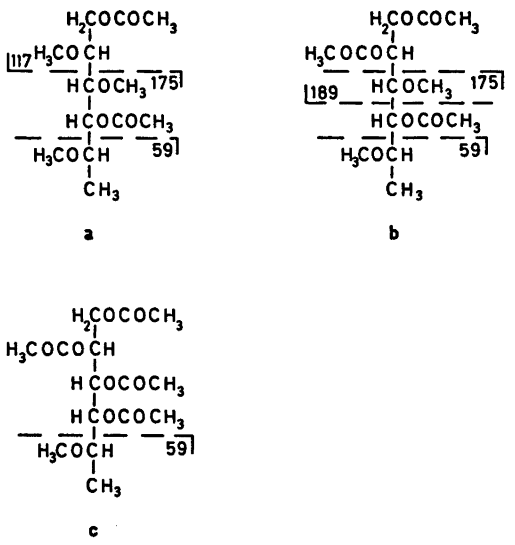


Fig. 1. Alditol acetates corresponding to partially methylated fucose. Primary fragments obtained by mass-spectrometry. a. 2,3,5-tri-*O*-methylfucose; b. 3,5-di-*O*-methylfucose; c. 5-*O*-methylfucose.

has not been reported previously.

Table 2 also shows the presence of fucose in pyranose forms, both as end groups, within the chain, and as branch points, the main part as the latter. The rhamnose is mainly present as 1,2-linked pyranose units, while the galactose is mainly found as 1,3-linked; a smaller part as branch points, then being 1,3- and 1,2-linked, both in pyranose forms. The mass spectra of these compounds corresponded to those published by Björndal *et al.*[11] When subjected to weak acid hydrolysis, only fucose was lost (Table 1). The approximate weights of the alditol acetates obtained from the methylated original polysaccharide and the methylated residual polysaccharides after 1 and 3 h of hydrolyses were estimated from the GLC result by determining the area under the peaks. This will not give a correct estimate of the amounts of methylated products obtained from the polysaccharides, but may be used for comparing the composition of the three samples.

The major loss of fucose is from the part present as furanose. Fucopyranose is also lost; the main change occurring in the fucopyranose present as branch points. This shows that some of the fucose residues lost were linked to fucopyranose residues being branch points; the main part has probably been linked to fucose in the 2-position, as the weight of 4-*O*-methylfucose has dropped most. 2-*O*-Methylfucose can be derived from fucose present both in the pyranose and furanose form. There is a considerable loss of 2-*O*-methylfucose during the first period of the hydrolysis (Table 2). This indicates that some of the fucose present as 2-*O*-methylfucose in the methylated starting material is present as furanose in the polysaccharide.

The ratio between rhamnose present as end groups to that present as part of the chain increases from 0.2:1 to 1:1 as hydrolysis time increases. From this it can be deduced that some of the fucose lost was linked to rhamnose at C-2, which then gives rise to end groups when the fucose residues disappear. The changes in the amounts of the different methylated galactose derivatives are relatively small. The main being in 4,6-di-*O*-methylgalactose, indicating that some half-estersulfate and/or fucose attached to galactose is released during hydrolysis.

The results above show that the extracellular polysaccharide produced by the diatom *C. curvisetus* is a highly branched polysaccharide of a complex structure. Fucose is present both in furanose and pyranose forms, the former being responsible for the main part of the end groups. Fucofuranose is also present as part of chains and as branch points. Fucopyranose appears to be responsible for the main part of the branch points of the polysaccharide. Some of the fucose appears to be linked to rhamnose at C-2. Rhamnose and galactose are responsible for a small part of the end groups. Galactose is mainly present at the inner part of the molecule.

EXPERIMENTAL

Weak acid hydrolysis. The polysaccharide was dissolved (1 %) in 0.01 N sulfuric acid. The mixture was heated at 100 °C and aliquots withdrawn after 1 and 3 h. The hydrolysates were, after cooling, neutralized with sodium hydrogencarbonate. The hydrolysates were freeze dried after dialysis.

Carbohydrate contents and sugar ratios were measured after all the above-mentioned steps. Other experimental details were as previously described.[4]

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Studies on the Fluorophore Forming Reactions of Various Catecholamines and Tetrahydroisoquinolines with Glyoxylic Acid

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Strongly fluorescent 2-carboxymethyl-3,4-dihydro-7-hydroxyisoquinolin-6-ones are formed in high yields when catecholamines are reacted with glyoxylic acid. Formation of the fluorophores has been found to take place in two steps; *i.e. via* virtually non-fluorescent tetrahydroisoquinoline-1-carboxylic acids, which react to give the fluorophores in a subsequent, rapid reaction with glyoxylic acid. The rates of reaction (pseudo first-order) with glyoxylic acid for 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, and 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid show that introduction of a carboxyl group at either C_1 or C_3 in a tetrahydroisoquinoline highly facilitates the reaction with glyoxylic acid. This behaviour is discussed in terms of a mechanism involving both intramolecular acid catalysis by the C_1 or C_3-carboxyl groups during dehydration of the carbinolamine intermediate, and facilitation of the prototropic shifts of the resulting Schiff's base by decarboxylation.

The versatility of glyoxylic acid (GA) as a new histochemical reagent for fluorescence visualization of different biogenic amines, is now becoming well established.[1] Knowledge of the reaction mechanisms underlying the fluorophore-forming reaction is of great importance, not only as a basis for the specificity of the histochemical GA method, but also because such knowledge may offer possibilities both of increasing the yield of the fluorophores as well as favouring reaction with a single, or a group of compounds, by merely changing the reaction conditions in the proper direction. Therefore, a detailed investigation of the reaction of GA with representative biogenic amines has been undertaken. The reaction mechanism for formation of fluorophores from tryptamine after reaction with GA has recently been elucidated [2] and found to involve an initial Pictet-Spengler reaction between GA and tryptamine, followed by a second reaction with GA by the initially formed 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (I), yielding a fluorescent 2-carboxymethyl-3,4-dihydro-β-carbolinium compound. Moreover, the great difference in rate of reaction with GA as observed for I and 1,2,3,4-tetrahydro-β-carboline, suggests that one of the important features of the -1-COOH group in I may be to act as an intramolecular acid catalyst, and it was proposed that also catecholamines should react with GA in a similar way.

In this paper we report the results from a study of the fluorophore forming reactions of dopamine, noradrenaline, and some derivatives of 1,2,3,4-tetrahydroisoquinoline with GA and analogous carbonyl compounds. Some kinetic measurements have also been performed as an attempt to establish the role of the C_1- or C_3-carboxyl group as intramolecularly operating catalysts.

EXPERIMENTAL

Instrumentation. Infra red spectra (in KBr) were recorded on a Perkin-Elmer 157G infra red spectrophotometer and NMR-spectra on a Varian T-60 instrument. Chemical shifts in D_2O are usually given together with the shift for the DOH-signal on an arbitrary scale,

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while in other solvents the chemical shifts refer to TMS as an internal standard. Mass spectral analyses were performed on a LKB 9000 instrument, usually operated at 70 eV. Kinetic measurements were performed on a Zeiss DMR 21 ultra violet spectrophotometer, equipped with a HETO ultra-thermostat (Birkerød, Denmark), set at 30.0 °C.

Kinetic measurements. The kinetic measurements were performed by rapidly adding a small volume (usually 100 μl) of the appropriate amine ($\sim 10^{-3}$ M stock solution) to a thermostated cuvette, containing 0.1 M glyoxylic acid (3.0 ml). After stirring for a few seconds by blowing air through the solution, the time *versus* extinction curve was recorded at an appropriate wavelength. The rates were usually followed to completion and the pseudo-first-order rate constants were calculated in the conventional manner. The method of Guggenheim,[3] was, however, used to calculate constants from the slower reactions. In all cases, excellent first order kinetics was observed, and the rate constants obtained from triplicate runs were usually reproducible within ± 5 % of the mean value.

Thin layer chromatography (TLC) was performed on Merck Fertigplatten F_{254}, silica gel and cellulose, and the solvent systems used were ethanol – 2 M HOAc, 1:1, saturated with boric acid; ethanol – HOAc, 1:1; butanol – HOAc – H_2O, 4:1:5.

Materials. All solvents and other commercially available reagents were of *p.a.* quality.

Ethyl glyoxylate was prepared according to Ref. 4.

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, VIII, was prepared according to Ref. 5. Mass spectrum M+ = 209.

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline, X, was prepared by reacting dopamine-HBr (0.23 g) and formaldehyde (0.2 ml, 33 % solution) in ethanol (5 ml) for 20 h at room temperature. After precipitation with diethyl ether/$CHCl_3$, 0.2 g X, m.p. 260 °C (decomp.) was obtained. NMR (D_2O): δ 3.09 (m, 2 H), 3.60 (m, 2 H), 4.90 (DOH), and 6.84 (m, 2 H).

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, VII. Dopamine-HBr (0.24 g) was dissolved in a small volume of water, whereafter glyoxylic acid monohydrate (0.1 g) dissolved in 1 ml of water was added dropwise with stirring during 15 min. pH was then adjusted to ~ 5 and the zwitterionic form of VII precipitated; IR: $\nu_{C=O}$ 1640 cm^{-1}. Yield: 0.17 g. Recrystallization from ethanolic hydrogen chloride/diethyl ether gave the hydrochloride salt of VII. IR: $\nu_{C=O}$ 1740 cm^{-1} NMR (D_2O): δ 3.04 (t, 2 H), 3.64 (m, 2 H), 4.90 (DOH), 5.25 (s, 1 H), 6.80 (s, 1 H), and 7.14 (s, 1 H).

4,6,7-Trihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, III, was prepared in the same manner as described for VII above. The zwitterionic form of III had an IR spectrum identical to that of a sample of II, (kindly supplied by Dr. Gaignault). After exposure of the KBr pellet to HCl gas or crystallization from ethanolic hydrogen chloride, the IR spectrum displays a $\nu_{C=O}$ at 1730 cm^{-1}. NMR (DMSO-d_6 + CF_3COOH): δ 3.97 (m, 2 H), 4.74 (m, 1 H), 5.17 (s, 1 H), and 7.00 (s, 2 H).

2-Carboxymethyl-3,4-dihydro-7-hydroxy-isoquinolin-6-one, IV. Dopamine hydrobromide (0.2 g) and glyoxylic acid monohydrate (0.5 g) in *tert*-butyl alcohol (25 ml) and water (2 ml) was allowed to stand at room temperature for 20 h. The zwitterionic form of IV was then precipitated by the addition of diethyl ether. Yield: 0.2 g. The zwitterion was then transformed into IV-HCl by recrystallization from ethanolic hydrogen chloride-diethyl ether. IR: ν 1730, 1650 cm^{-1}. NMR (DMSO-d_6): δ 3.10 (t, 2 H), 4.00 (t, 2 H), 4 96 (s, 2 H), 6.95 (s, 1 H), 7.33 (s, 1 H), 9.25 (s, 1 H).

When ethanol was used as a solvent a small crop of IV-ethyl ester could be isolated from the mother liquor. NMR (DMSO-d_6): δ 1.27 (t, 3 H, $J = 7$ Hz), 3.10 (t, 2 H), 4.02 (t, 2 H), 4.27 (q, 2 H, $J = 7$ Hz), 5.04 (s, 2 H), 7.00 (s, 1 H), 7.34 (s, 1 H), 9.23 (s, 1 H). IR: ν 1740, 1640 and 1220 cm^{-1}

2-Carboxymethyl-3,4-dihydro-4,7-dihydroxyisoquinoline-6-one, V, and *2-carboxymethyl-3,4-dihydro-7-hydroxyisoquinolin-6-one-3-carboxylic acid*, VI, were prepared in the same manner as IV.

V: IR: ν 1755, 1735, 1650 cm^{-1}. NMR (DMSO-d_6): δ 4.07 ("d", 2 H), 4.90 ("t", 1 H), 4.97 (s, 2 H), 7.21 (s, 1 H), 7.44 (s, 1 H), 9.25 (s, 1 H).

VI: IR: ν 1740, 1640 and 1620 cm^{-1}. NMR (D_2O + CF_3COOD): δ 4.20 (d, 2 H), 5.53 (s, 2 H), 5.60 (m, 1 H), 6.47 (DOH), 7.57 (s, 1 H), 7.93 (s, 1 H) and 9.40 (s, 1 H).

2,4-Dicarboxymethyl-7-hydroxyisoquinolin-6-one, IX. To 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (1.3 g), dissolved in DMSO (20 ml) was added a solution of GA (2 g) in DMSO (5 ml). The orange coloured reaction mixture was allowed to stand at room temperature for 5 days, when NMR showed that all starting material had disappeared. The product was then isolated as yellow crystals by precipitation with diethyl ether and acetonitrile. NMR (DMSO-d_6 + CF_3COOD): δ 4.04 (s, 2 H), 5.45 (s, 2 H), 7.40 (s, 1 H), 7.62 (s, 1 H), 8.31 (s, 1 H), 9.40 (s, 1 H). IR: ν 1720 and 1630 cm^{-1}. MS: *m/e* 203, 189, 175, 161, 147, 140, 132.

2-(1-[Trifluoromethyl]-ethyl)-7-hydroxy-3,4-dihydroisoquinolin-6-one, XII. A solution of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrochloride (0.2 g) and trifluoroacetone (0.5 g) in DMF (10 ml) was heated in a closed vessel at 80 °C for 20 h. Diethyl ether was then added to the brown-red solution and the hygroscopic precipitate collected. Mass spectrum: *m/e 259* (M+), 230, 192, 190, 179,

178, 177, 162, 153, 148, 136, 129.5 (M^{2+}) and 124.

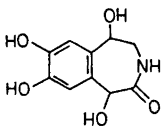
When this reaction was performed in the NMR-sample tube, the C_1-proton appeared at δ 9.3 and the $-CH_3$ protons as a doublet at δ 1.8 ($J = 8$ Hz).

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ethyl ester, XV. To dopamine-HBr (0.2 g) dissolved in water (1 ml) was added ethyl glyoxylate (0.1 g). The mixture was then allowed to stand for 20 h at room temperature. EtOH was then added and the mixture evaporated to dryness. The residue was dissolved in MeCN, filtered and to the filtrate was added Et_2O to precipitate the ester. Yield: 150 mg, m.p. 192 °C (uncorr., decomp.). IR: $\nu_{C=O}$ 1730 cm^{-1}. NMR (DMSO-d_6) δ 1.40 (t, 3 H, $J = 7$ Hz), 2.92 (m, 2 H), 3.45 (m, 2 H), 4.30 (q, 2 H, $J = 7$ Hz), 5.30 (s, 1 H), 6.67 (s, 1 H), and 6.90 (s, 1 H).

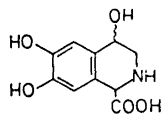
RESULTS AND DISCUSSION

A. Elucidation of the structures of the GA-catecholamine fluorophores

The reactions of noradrenaline and other similar compounds with GA in water solution have been investigated by Fourneau and coworkers.[6] They found that when equimolar amounts of noradrenaline and GA were allowed to react under "physiological" conditions, a 77 % yield of a compound claimed to be the 1,5,7,8-tetrahydroxy-tetrahydrobenzazepine-3-one-2, II, was obtained, together with a mixture of *cis*- and *trans*-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, III.

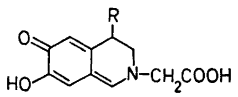


II

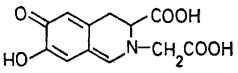


III

We found, however, that when dopamine, noradrenaline, and Dopa are allowed to react with GA in polar solvents (water, alcohols, wet DMF and DMSO), compounds with the structure IV and V and VI are formed in high yield. In contrast to the tetrahydroisoquinolines, compounds IV, V, and VI have strongly fluorescent properties, which is the basis for the use of this reaction as a histochemical tool.



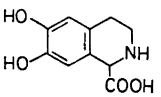
IV R = H
V R = OH



VI

On the other hand, we have not been able to demonstrate the formation of compounds like II, the formation of which also seems highly unlikely under the actual reaction conditions.

Formation of IV and V has been found to proceed *via* a rapid further reaction with GA by the initially formed tetrahydroisoquinoline-1-carboxylic acids; *i.e.* III and VII.

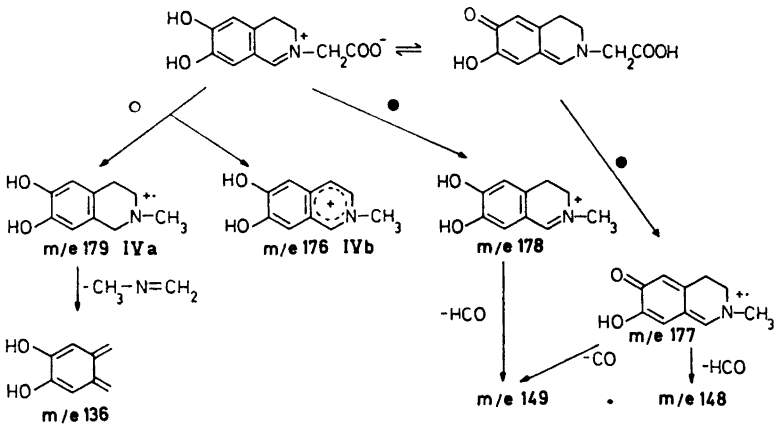


VII

The rates of fluorophore formation are rather slow in aqueous solution, while the reactions proceed very efficiently in dipolar aprotic solvents like DMF and DMSO.

Preparatively, compounds IV and V could be synthetized in high yields (~90 %) (see Experimental) after reaction of dopamine and noradrenaline, respectively, with GA in *t*-BuOH or in DMF containing 4 % water. If other alcohols were used as solvents in the reaction, esters of IV and V with these alcohols were concomitantly formed. The yields of the esters formed were usually below 25 %. The proposed structures for IV and V are in excellent agreement with their IR and NMR spectroscopic properties.

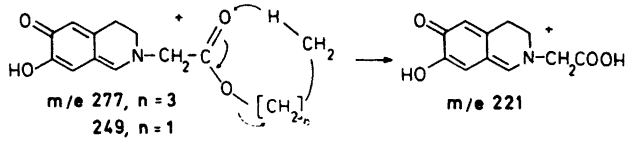
Some support for the proposed structure can also be obtained from the mass spectrum of IV, although any molecular ion cannot be detected, probably due to thermally induced decarboxylation of IV in the mass spectrometer. Generally 3,4-dihydroisoquinoline derivatives undergo decomposition and disproportionation when heated,[7] for instance in the direct inlet



Scheme 1. O, Disproportionation and decarboxylation in the direct inlet of the mass spectrometer followed by electron impact; ●, Decarboxylation in direct inlet followed by electron impact.

system of the mass spectrometer, and the spectrum will thus also contain fragmentation processes originating from the disproportionated products, *e.g.* IVa and IVb (see Scheme 1). Thus in the mass spectra of IV, the most abundant ions are found at *m/e* 179, 178, 177, 176, 149, 148, and 136, where the ions at *m/e* 176 – 9 may have been formed by loss of CO_2 from IV and its disproportionated products (see Scheme 1).

of IV and V were also recognized in the spectrum. These observations make it highly unlikely that a compound like II is formed from dopamine and noradrenaline in their reactions with GA in water solution. In our opinion, the compounds claimed to have the structure II should instead be formulated as the zwitterionic forms of the tetrahydroisoquinoline-1-carboxylic acids, III and VI, respectively, because both an authentic sample of the compound claimed



Scheme 2.

Further mass spectroscopic support for the structures IV is found in the mass spectra of the butyl and ethyl esters of IV, where a McLafferty rearrangement of the molecular ions from the esters gives rise to a common ion at *m/e* 221, while the rest of the spectra is quite similar to that obtained from IV (Scheme 2).

The spectral properties of V are quite similar to those observed for IV, the presence of an –OH group at C_4 in V being reflected in the NMR and mass spectra.

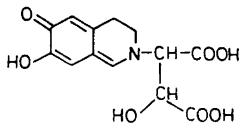
When studying the reactions of dopamine and noradrenaline with GA in D_2O in the NMR probe, formation of VI and III, respectively, were observed, and when the reaction was allowed to proceed for a longer time, formation

to have the structure II and the zwitterionic form of III reveals the C_1–H as a singlet located at δ 4.50 (DMSO-d_6) while after addition of CF_3COOH the signal for the C_1–H is shifted to δ 5.17. Such a behavior is to be expected from a structure like III, but not from a structure like II.*

Side reaction. When either dopamine or VII is allowed to react with an excess GA in neat dipolar aprotic solvents, two additional compounds are formed together with IV, as revealed by TLC. These compounds have

* *Added in proof.* At GC-MS analysis of the TMS-derivatives of III, and the compound claimed to have structure II, both compounds gave identical retention times and mass spectra with characteristic fragment ions of an isoquinoline structure.

fluorescent properties similar to those observed for IV, one of the products formed has been identified as the following structure:[8]



B. Reaction product from 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (VIII)

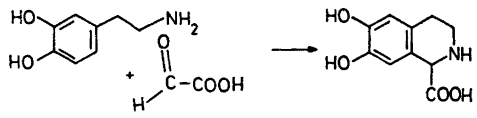
The reaction of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (VIII) with GA, the kinetics of which is also discussed below, is suggested to follow a reaction path, involving the generation of a reactive 1,2-dihydroisoquinoline intermediate,[9] which then undergoes a further reaction with GA (Scheme 3).

Compound IX is formed in almost quantitative yield (see Experimental), and any accumulation of intermediates could not be detected when the reaction was followed in the NMR sample tube.

C. Kinetics of the reaction of dopamine and some tetrahydroisoquinoline derivatives with GA

The kinetics of the reaction between GA and dopamine and VII at 30 °C has been investigated and in order to evaluate the influence of the -1-COOH- group on the rates, the reactions of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (X) and VIII with GA have also been investigated. All reactions were carried out in an excess of GA in order to obtain pseudo-first order conditions. Relevant rate constants are shown in Table 1, where also the rate constant for the reaction of 1,2,3,4-tetrahydro-β-carboline-1-COOH, (I) with GA is given for comparison.

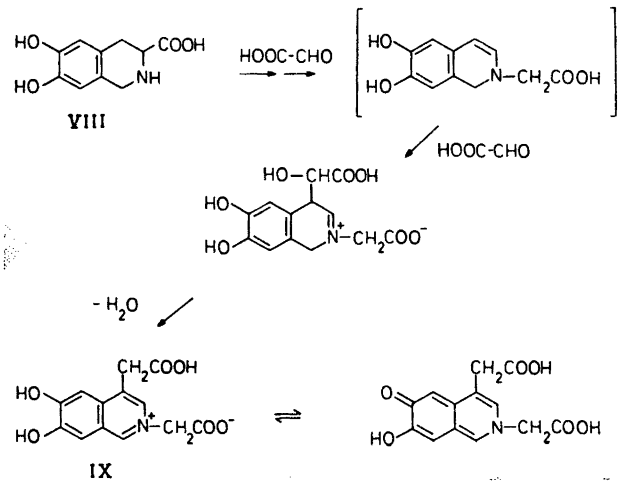
From the rate constants in Table 1 it is evident that after the introduction of a -COOH group in X, at either C_1 as in VII or at C_3 as in VIII, considerable enhancement of the reactions with GA is obtained. It can also be seen that the first step in the reaction of dopamine with GA in EtOH, *i.e.* formation of VII, (Scheme 4) is slower than its further reaction with GA.



VII

Scheme 4.

When the reaction of dopamine with GA was performed in neat DMF, only about 15 % of the calculated absorbance at 380 nm was reached. This may be due to concomitant formation of the presumably colourless lactone XI, the formation of which ought to be



IX

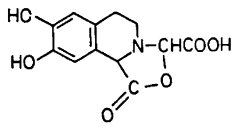
Scheme 3.

Table 1. Pseudo-first order rate constants, k_{obs} min^{-1}, at 30.0 °C for the reactions of I, VII, VIII, X, and dopamine with different carbonyl compounds.

Compound	VII				VIII	X	Dopamine		I
Solvent	EtOH	DMF	DMF + 4 % H_2O	DMF + 0.1 M HCOOH	DMF	DMF	EtOH	DMF	EtOH
Reagent									
GA	0.13[a]	38	38	–	0.15[d]	$\ll 10^{-4}$[h]	0.026[e]	0.7[f]	0.037
GA-ethyl ester	–	18	–	–	–	–	–	–	–
FA	–	0.065[b]	–	0.065[c]	–	$\ll 10^{-4}$[g]	–	–	–
$CH_3-\overset{CF_3}{\overset{\vert}{C}}=O$	–	0.02	–	–					

[a] Half the actual conc. of VII gave the same value; [b] measured at = 420 nm; [c] measured at = 278 nm; [d] measured at = 350 nm; [e] measured at = 294 nm, the isosbestic point for GA and IV; [f] rate constant for formation of VII; [g] DMF + 0.1 M HCOOH, estimated value; [h] estimated value.

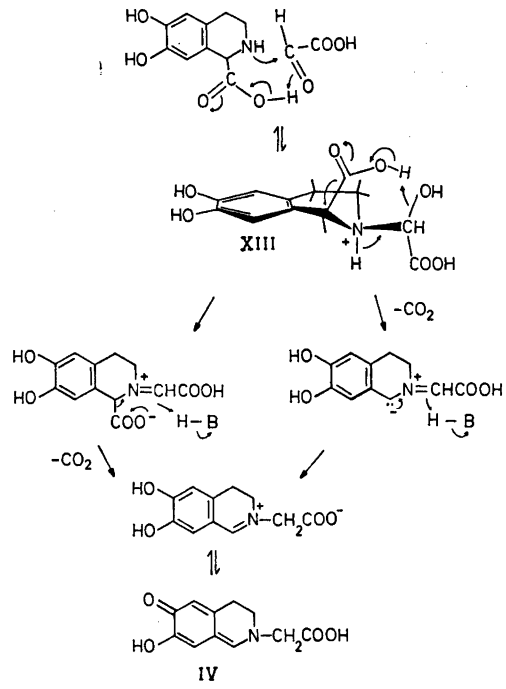
suppressed when the concentration of water in the DMF solution is increased.



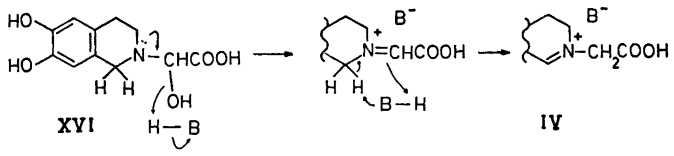
XI

The absorption *vs.* *t*-curve, however, obeyed first order kinetics and gave a rate constant of 0.7 min^{-1}. The same value was obtained in DMF containing 4 % water, where the calculated absorbance also was reached. The value of 0.7 min^{-1} for the rate constant may be taken as an approximative value for the rate constant for formation of VII from dopamine and GA. This is a fair approximation since the following step in the reaction is very fast (38 min^{-1}).

The dramatic increases in rates of reaction with GA, as observed after introduction of a -COOH group at C_1, (VII), or at C_3, (VIII), in the 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (X), is in the opposite direction to what should be expected from the increased steric hindrance from the introduced carboxyl groups and from the hence lessened nucleophilicity of the nitrogen atoms in VII and VIII compared to that in X. The high rates may therefore be due to a combination of intramolecular carboxyl group catalysis in the dehydration of the carbinolamine intermediate and facilitation of prototropy in the Schiff's base by decarboxylation of the suitably situated carboxyl group (see Scheme 5).



Scheme 5.

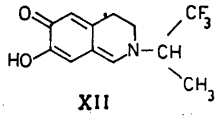


Scheme 6.

Thus, the differences in rates observed for VII and VIII in their reactions with GA are in the proper direction and may partly reflect the somewhat weaker acidity, and hence lessened catalytic efficiency of the 3-COOH group. But also the increased conformational flexibility of the 3-COOH group in VIII compared with the 1-substituted one in VII, which is obvious by inspection of molecular models, may affect the rate in the direction observed. The difference in pK_a-values for VII and VIII is supposed to be of the same order as for phenylalanine and phenylglycine, which have $pK_a = 1.80$ and 2.16, respectively.[10] The pK_a-values for the ammonium functions in VII and VIII were found to be 3.4 and 3.3, respectively.[11] Although strongly basic amines generally attack carbonyl groups without acid catalysis,[12] the juxtaposition of an amino and an acid function as in VII and VIII, may well be an effectively operating catalytic system in reactions with carbonyl compounds.[13,14]

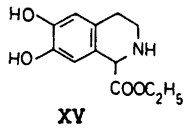
That the carboxyl group in GA is unimportant as a catalyst in this system is obvious from the small difference in rates observed for VII in its reactions with GA and GA ethyl ester. The more prominent rate difference observed for VII in its reaction with GA and FA respectively, will mainly be caused by the increased electrophilicity of the carbonyl carbon atom in GA, caused by the presence of the electron withdrawing carboxyl group. The presence of FA-polymers may, however, also be considered as a rate-affecting factor. Intermolecular acid catalysis seems to be unimportant in the reactions of VII with FA, since the rates are not significantly affected by the presence of 0.1 M formic acid.

A support for the assumption that intramolecular acid catalysis is involved in the dehydration of the carbinol-amine is found in the reactions of VII with hexa- and trifluoroacetone. Although these carbonyl compounds, owing to the strongly electron-withdrawing properties of the CF_3-groups usually form very stable carbinolamines,[15] those formed in the reaction with VII are rapidly dehydrated to yield fluorophores of the same type as those obtained from GA and FA, *i.e.* XII.



Considering the further fate of the carbinolamine intermediate (XIII), the fluorophores (IV) will be formed after decarboxylation and prototropy, according to Scheme 5. The decarboxylation reaction may take place concerted with, or following dehydration; neutralization of the positive charge on the nitrogen and gain of resonance stabilization being the driving forces for the prototropic shifts.

The involvement of decarboxylation in the further reaction of the carbinolamine (XIII) as depicted above, may be advantageous over that of the carbinolamine derived from X and GA (*e.g.* XIV) where the prototropic shifts in the Schiff's base have to solely relay on gain of resonance stabilization, probably assisted by a general base (Scheme 6). However, even if the decarboxylation reaction thus seems to play an important role in the prototropic reaction, decarboxylation alone cannot account for the dramatic rate enhancement obtained when going from X to its carboxyl substituted derivatives VII and VIII. Extremely low reactivity is also observed for the ethyl ester of VII, (XV), which in fact, does not display any visible reaction with GA when monitored in the NMR probe for two days. It thus seems like dehydration of the carbinolamine intermediate must also be an important step in the reaction leading to IV and V, but that this reaction requires an efficient catalyst,



XV

most probably supplied by the 1- or 3-COOH groups in VII and VIII.

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Oxidation of *p*-Hydroxystyrenes by Aqueous Sodium Polysulfide.*

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p-Hydroxystyrenes are formed as reactive intermediates in lignin degradation under kraft pulping conditions (heating of wood with aqueous sodium hydroxide – sodium sulfide; polysulfide formed during the cook). The reaction of *p*-hydroxystyrenes with polysulfide was investigated by heating appropriate model compounds with polysulfide liquor. They yield aromatic ketones and aldehydes, presumably by disproportionation of intermediate alkyl-hydropolysulfides. These results provide an explanation of the reaction of S^0 with lignin in kraft and polysulfide pulping of wood.

p-Hydroxyarylglycerol-*β*-aryl ether substructures are the most important site of lignin degradation in kraft pulping (heating of wood with aqueous sodium sulfide – sodium hydroxide to about 170 °C). They fragment to *p*-hydroxycinnamyl alcohols and phenols with formation of S^0 in the form of polysulfide.[1] As only small amounts of elemental sulfur are liberated on acidification of black liquors (the alkaline solution containing carbohydrates, lignin and salts which is produced in a kraft cook), the polysulfide ions must have undergone further transformations.

Because of the formation of S^0 a kraft cook is in fact a polysulfide cook with a low content of elemental sulfur. In aqueous solution, sodium oligosulfides ("sodium polysulfide") are known to disproportionate to thiosulfate and sulfide, the rate of reaction being dependent on hydroxide ion concentration.[2] Under technical cook conditions, reactions with carbohydrates and lignin compete with disproportionation.[2] Carbohydrates are oxidized by aqueous polysulfide.[2] From model experiments and IR-data it has been concluded that lignin reacts with polysulfide by oxidation of its benzylic hydroxyl groups to carbonyl groups.[3] These studies were, however, carried out which model compounds which do not sufficiently reflect the reaction modes of the *p*-hydroxyarylglycerol-*β*-aryl ether structures of lignin. We therefore decided to re-investigate the reaction of aqueous polysulfide with lignin.

In alkaline media, the ionized phenolic hydroxyl group is the functional entity of lignins that normally will most easily undergo oxidation. This is, however, not the case in polysulfide reactions as is shown by the quantitative recovery of 4-propylguaiacol from a sodium polysulfide – sodium hydroxide cook at 120 °C ("sodium polysulfide cook", although the sulfur content corresponds to a sodium disulfide cook; *cf.* Experimental).

p-Hydroxystyrenes which are formed as reactive intermediates in the reaction of lignin with aqueous sodium sulfide were considered to be another substrate that possibly could be oxidized by polysulfide. We have found that *trans*-isoeugenol (*1*) in a sodium polysulfide cook yields 60 % of 4-hydroxy-3-methoxy-propiophenone (*5*). This reaction (Fig. 1) possibly proceeds *via* the quinone methide *2*, a ketonization product of the anion of *1*. Under the same conditions *5* was also formed from 1-(4-hydroxy-3-methoxyphenyl)-1-propanol, but 1-(3,4-dimethoxyphenyl)-1-propanol was not affected. The formation of vanillin and 4-hydroxy-3-methoxy-acetophenone in polysulfide cooks of

* Part V of the series "Degradation of Lignin by Kraft Cooking"; Part IV, Ref. 1d.

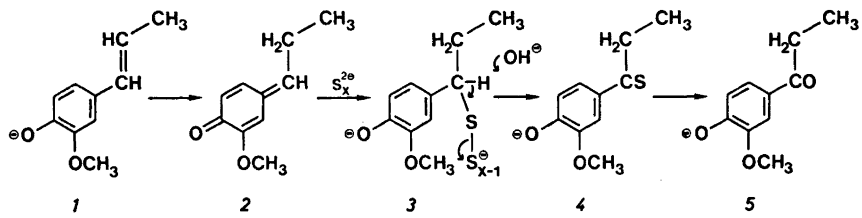


Fig. 1. Compounds *1*, *3*, *4* and *5* are depicted as anions.

vanillyl alcohol and 1-(4-hydroxy-3-methoxyphenyl-1-ethanol, respectively, has been reported.[3] 1-(4-Hydroxy-3,5-dimethoxyphenyl)-2,2-dimethyl-1-propanol, which cannot be dehydrated to a *p*-hydroxystyrene, similarly yields 1-(4-hydroxy-3,5-dimethoxyphenyl)-2,2-dimethyl-1-propanone as the main product.

The addition of polysulfide ion then should give 1-(4-hydroxy-3-methoxyphenyl)-1-propyl hydropolysulfide (*3*). The mechanism of the disproportionation of *3* to sulfide and *5* has not been ascertained. As outlined in Fig. 1, the fragmentation of the sulfur–sulfur linkage could be induced by base abstraction of a benzylic hydrogen or possibly by transformation to a sulfenic acid. The resulting thiopropiophenone *4* should then be rapidly transformed to *5*. This disproportionation mechanism would be analogous to the decomposition of a number of hydroxyperoxides,[4] peroxides,[5] and disulfides [6] by base.

trans-Coniferyl alcohol (*6*) yields vanillin (*12*) and 4-hydroxy-3-methoxy-acetophenone (*9*) as dominant mononuclear degradation products in a polysulfide cook. These two compounds, together with guaiacol, also constitute the major mononuclear degradation products from a cook of *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (guaiacylglycerol-*β*-guaiacyl ether). A reasonable explanation for their formation is given in Fig. 2.

The *retro*-aldol reactions of coniferaldehyde (*11*) giving vanillin and acetaldehyde, and of 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-1-propane (*8*) are known.[7,8] An alternative route leading to *9* (not depicted in Fig. 2) is the elimination of formaldehyde from the quinone methide *7* to give *4*-vinyl-guaiacol which is

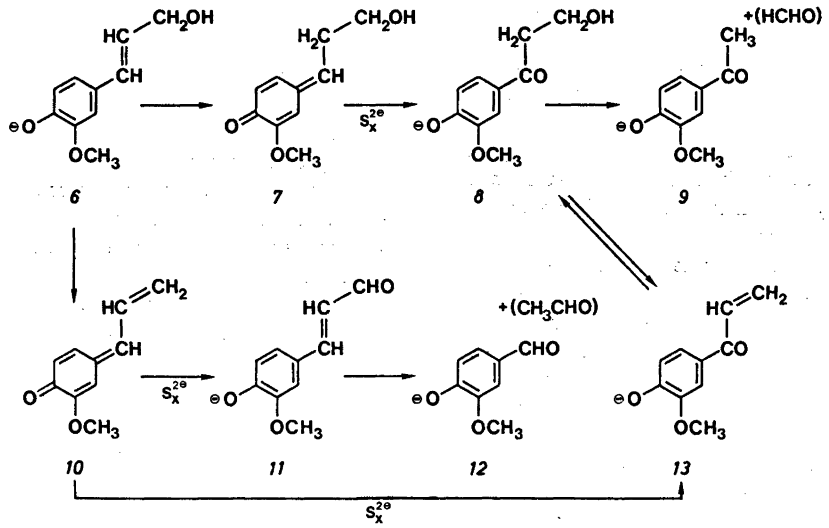


Fig. 2. Compounds *6*, *8*, *9*, *11*, *12* and *13* are depicted as anions.

oxidized by polysulfide *via* its tautomeric quinone methide. Intermediates *11* and *13* presumably react to a considerable extent with carbanions by Michael-addition, giving rise to condensation products of higher molecular weight. Similar reactions should also occur in pulping, and will partially counterbalance lignin degradation. A quantitative assessment of the contributions of the two major routes of transformation of *6* – by formation of the vinylogous quinone methide *10* and by protonation to *7* – is not possible since precise knowledge of the rate processes involved is lacking.

If *trans*-coniferyl alcohol (*6*) is heated with aqueous sodium hydropolysulfide rather than with polysulfide, the main product is 4-hydroxy-3-methoxy-acetophenone, vanillin being formed at best in trace amounts. Two other compounds, 4-hydroxy-3-methoxy-propiophenone (*5*) and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone, were encountered in minor amounts. *5* is possibly formed *via* addition of HS_x^- to 1-(4-hydroxy-3-methoxyphenyl)-2-propen-1-one (*13*), the resulting alkyl hydropolysulfide then disproportionating to *5* and S^0. This side reaction seems to be suppressed by hydroxide ion as no *5* was detected among the products of a polysulfide cook of *6*. The reaction leading to 1-(4-hydroxy-3-methoxyphenyl)-2-propanone may be initiated by nucleophilic substitution of hydropolysulfide by the anion of *6* (reaction in β-position).

The results of the hydrosulfide cook of *6* give an explanation of the results of Ashorn [8] obtained on hydrosulfide cooking of spruce wood and of guaiacylglycerol-β-guaiacyl ether. Ashorn observed the formation of *5* and *9*, as well as of small amounts of *11*, and suggested the ketol *8* as an intermediate.

Direct oxidation by aqueous polysulfide of the quinone methides formed from *p*-hydroxybenzyl alcohol substructures (primarily *p*-hydroxyarylglycerol-β-aryl ether structures) in lignin has also to be considered. This reaction mode, originally proposed by Nakano *et al.*,[3] is however ruled out by the result of a polysulfide cook of *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-phenoxy)-1-propanol. The main products are *1*, *5* and guaiacol, only small amounts of by-products being formed. Under the same conditions 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxy-phenoxy)-1-propanone gave guaiacol, but neither *1* nor *5*. These experiments demonstrate that the quinone methides formed from *p*-hydroxyarylglycerol-β-aryl ether substructures by the rate determining loss of water [9] are not oxidized by aqueous polysulfide, but are cleaved in the same way as under the conditions of a kraft cook.

The oxidation of the aldehyde groups of reducing carbohydrates by sodium polysulfide may proceed in a way analogous to the oxido-reduction depicted above. The addition of S_x^{2-} to the carbonyl groups should be followed by β-elimination of $HS_{(x-1)}^-$ from the intermediate hydroxyalkyl hydropolysulfide.

The reactions of aqueous polysulfide with the *p*-hydroxystyrenes *1* and *6* outlined in this paper provide an explanation of the reactions of S^0 with lignin in kraft and polysulfide pulping of wood.

EXPERIMENTAL

References to model and reference compounds. (4-Hydroxy-3-methoxyphenyl)-=*Gu*-; *8*;[10] *11*;[11] 1-*Gu*-2-propanone;[12] *erythro*-1-*Gu*-2-(2-methoxy-phenoxy)-1-propanol;[13] 1-*Gu*-2-(2-methoxy-phenoxy)-1-propanone;[13] *erythro*-1-*Gu*-2-(2-methoxy-phenoxy)-1,3-propanediol;[14] 1-*Gu*-1-propanol:[15] 1-(3,4-dimethoxy)-1-propanol;[15] 1-(4-hydroxy-3,5-dimethoxyphenyl)-2,2-dimethyl-1-propanol;[16] 1-(4-hydroxy-3,5-dimethoxy-phenyl)-2,2-dimethyl-1-propanone.[16]

Sodium polysulfide cooks. The model compound (0.5 mmol) in 0.2 M NaOH (3 ml) containing Na_2S (1 mol/liter) and elemental sulfur (1 mol/liter) was heated in a teflon-clad autoclave (120 °C, 6 h, N_2). The solution was then acidified to pH 5, extracted twice with ether, and the evaporation residue of the extract was dissolved in 80 % ethanol, elemental sulfur being removed by filtration. After addition of water the product was isolated by extraction with methylene chloride. After a preliminary investigation by TLC (silica gel, acetone – hexane 1:3), the product was derivatized by reaction with trimethylsilyl-trifluoroacetamide in pyridine and analyzed in a gas chromatograph – mass spectrometer (single focussing instrument with electron impact source, electron energy 70 eV, Becker-Ryhage separator at 275 °C). The components were identified by comparison of spectra with those of the trimethylsilyl derivatives of synthesized reference compounds. For GLC conditions, see below.

In the case of 1-(3,4-dimethoxyphenyl)-1-propanol, the reaction was carried out in a rotating autoclave. A preparative experiment with model *1* (0.3 g in 10 ml of cooking liquor, 120 °C, 18 h) yielded 198 mg of *5*, isolated by

preparative TLC (silica gel, acetone – hexane 1:2), m.p. 56 – 59 °C (aqueous ethanol), Lit.[17] m.p. 61 – 62 °C.

Sodium hydropolysulfide cooks. Carried out as described for the sodium polysulfide cook, except that the cooking liquor was adjusted to pH 8.5 – 9 by addition of KH_2PO_4. Precipitated sulfur was removed by filtration.

In the case of model *6*, the gas chromatographic separation was preceded by preparative thin layer chromatography (as described above). This allowed the separation of *5* from vanillin and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (not resolved). These fractions were also analyzed by GLC as acetates. *GLC.* 1.75 m stainless steel column (o.d. 6 mm) with 1.5 % OV 17 on acid-washed, HDMS-treated Chromosorb G. $T = 180$ °C, 25 ml N_2 per min. *Retention times* (acetates, min): *5*, 7.1; *9*, 5.0; 1-(4-hydroxy-3-methoxyphenyl)-2-propanone, 6.8.

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Conformational Processes in Simple Cyclic Peptides

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The elementary steps in conformational interconversion paths for cyclic tri-, tetra-, and penta-peptides are discussed. When *trans*-amide groups are present, a full exchange requires its rotation as such "through the ring" or an "outward" rotation about the CN partial double bond *via* the *cis*-amide configuration.

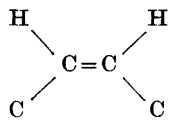
The conversion of the externally hydrogen-bonded crystal conformer of certain NH-containing cyclic pentapeptides to more stable conformers in solution, presumably internally hydrogen-bonded, can be followed at low temperatures by NMR spectroscopy.

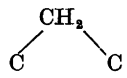
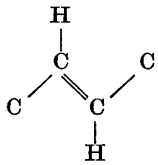
Most known cyclic peptides built up of "natural" amino acids are not amenable to studies of conformational flexibility by dynamic NMR spectroscopy, since all or most of the amino acids carry α-substituents, so that "ring inversion" will not generally lead to an identical conformer, and since the amino acids as a rule are different, so that also "pseudorotation" will lead to non-identical conformers. Cyclic peptides of glycine alone, such as the pentamer and hexamer, are the simplest examples of compounds suitable for the study of site exchange of the α-hydrogens as well as of the amino acid residues. However, the interconversion mechanism would be expected to consist of a series of several steps and lead, as in the case of cycloalkanes,[1] to both types of exchange simultaneously, since the amide bonds are here of NH-type and therefore most likely all *trans* in rings of this size. Also, the low solubility makes NMR studies difficult.

Cyclic peptides of sarcosine [2] and its combinations with glycine [3,4] are much more suitable for such studies. They are more soluble, and a cyclic tripeptide and a variety of cyclic tetrapeptides are also available. Furthermore, amide bonds of NMe-type can equally well be *cis* as *trans*, and the site exchange for the amino-acid residues in homodetic rings, and quite generally the interconversion of different conformers, must therefore involve *cis,trans*-isomerization which in open chains is found to have a barrier of around 19 kcal/mol.[5] This process can be studied separately when the site exchange for the α-hydrogens is more rapid than *cis,trans* exchange. Certain NMR-spectroscopic observations on cyclic peptides, which we have in part already reported, and some new experimental results will be interpreted in the following by analysis of the probable processes involved.

APPROACH TO THE ANALYSIS OF THE CONFORMATIONAL PROCESSES

The analysis of conformational interconversion paths, composed of a sequence of elementary processes, is simple in cycloalkanes as they consist of constitutionally identical ring elements and bonds. The situation in cycloalkenes is also relatively simple despite the more complex constitutional situation, because low-energy conformational processes leading to CH_2-hydrogen site exchange and involving barriers of the order of 5 – 15 kcal/mol, can safely be considered independent of the high-energy *cis,trans* configurational isomerization of the double bonds involving barriers of the order of 50 – 60 kcal/mol.[6]

Thus, a *cis*-olefin  is a con-

formationally rigid C_4-unit comparable to the saturated C_3-unit  and a *trans*-olefin  is a stiff conformational C_4-unit comparable to the saturated C_2-unit C–C provided it can be rotated about its long axis. On the other hand, schemes of *cis,trans*-configuration exchange can be considered separately, neglecting the detailed conformational situation in the saturated parts of the molecule during the processes.

In cyclic peptides, even though the amide groups have partial double-bond character, the energy barriers for the two types of processes are shown in the following to be much more similar, but the cycloalkene situation applies in many cases as an approximation.

CYCLIC TRIPEPTIDES

Cyclotrisarcosyl [8] is the only example of a cyclic tripeptide which can undergo a conformational process. Since in its single observable conformation all three amide groups are *cis*, only the exchange of the inner with the outer hydrogen within each of the three CH_2-groups can be observed. The corresponding AB quartet coalesces at 135 °C (100 MHz), giving a calculated free energy of activation of 20.1 kcal/mol.[7] This barrier is higher than the normal barrier for *cis,trans* isomerization of amides, so that a multi-step interconversion path through less stable conformers having one or two *trans* amide groups cannot *a priori* be excluded. This possibility seems, however, very unlikely considering that the geometrically closely similar olefinic compound, *cis,cis,cis*-cyclonona-1,4,7-triene, where the energy required to convert a *cis*- to a *trans*-double bond is expected to be of the order of 50 – 60 kcal/mol,[6] shows an activation free energy to CH_2 hydrogen site exchange [8,9] of only 14.5 kcal/mol.* A one-step synchronous ring-flattening inversion of the whole molecule is entirely out of question, although this mechanism has been favoured [8] for cyclonona-1,4,7-triene on the basis of an apparent ** very large negative entropy of activation.

It remains to consider a multi-step path with intact *cis*-amide groups. The basic elementary step in composite interconversion mechanisms for cycloalkanes smaller than cyclohexadecane is generally the local "outward" flattening at a "corner" whereby four carbon atoms are brought into coplanarity.[1] In cyclic peptides not less than five ring atoms must be in a common plane at or near the critical point of the barrier if a *cis*-amide group is to preserve its planarity. In reality there may be some temporary departure from planarity of the amide group in question, as well as of others in the molecule. It is also unclear whether in cyclotrisarcosyl it is the coplanarity of the $C_\alpha CNC_\alpha C$ system or the adjacent $NC_\alpha CNC_\alpha$ system which is reached most easily or early in a sequence of two close steps. For these reasons the passage from the initial stable conformation A (Fig. 1) to an intermediate less stable conformation B can most simply be described as the flipping of one CH_2 group from one side of the ring to the other, involving both adjacent *cis*-amide groups and hence the rough coplanarity of seven ring atoms (Fig. 1).

The intermediate conformation B has got changed dihedral angle sign in two bonds (and temporary near-eclipsing in two others) and can, by a further pseudorotation-like movement over presumably very low barriers pass to an equivalent mirror image conformation B′ whereby the sign in two further bonds is changed. Halfway between B and B′ a conformation C with a pseudo-twofold axis of symmetry is attained, which is possibly lower in energy. The final reversion to the mirror image A′ of the initial conformation goes over a barrier equivalent to the first.

This three-step cycle is analogous to, but more detailed than, the exchange mechanism proposed [9] for the "inversion" of one crown-form of cyclonona-1,4,7-triene to an identical

* The even lower reported value of 11 kcal/mol [10] is due to an error of calculation.

** "Observed" negative or extremely large activation entropies for monomolecular processes are now considered with much suspicion due to systematic errors in their determination by NMR.[11]

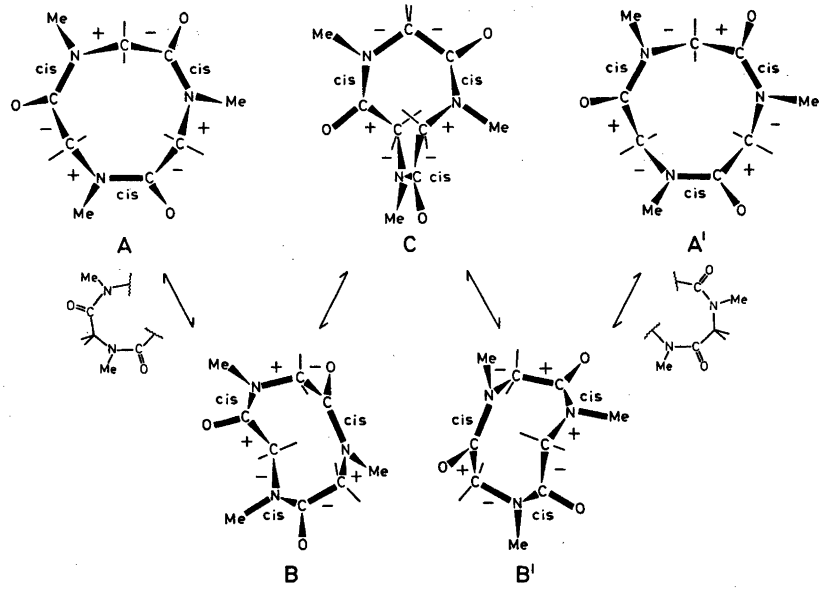


Fig. 1. Conformational interconversion scheme for c-Sar_3. Dihedral angle signs are defined with respect to the ring skeleton.

crown (note that the triolefin is not chiral). Since torsional strain in the $C_\alpha C$- and $C_\alpha N$-bonds is probably small, it is possible to get a qualitative feeling of strain on the basis of valency-angle strain in mechanical models. On this basis the first (and last) barrier is higher than any separating B and B′ (Fig. 1), hence the one which is measured by dynamic NMR spectroscopy. That the observed barrier is higher in the tripeptide than in the triolefin, can be explained by a greater transannular repulsion between the "inner" CH_2-group and the opposite

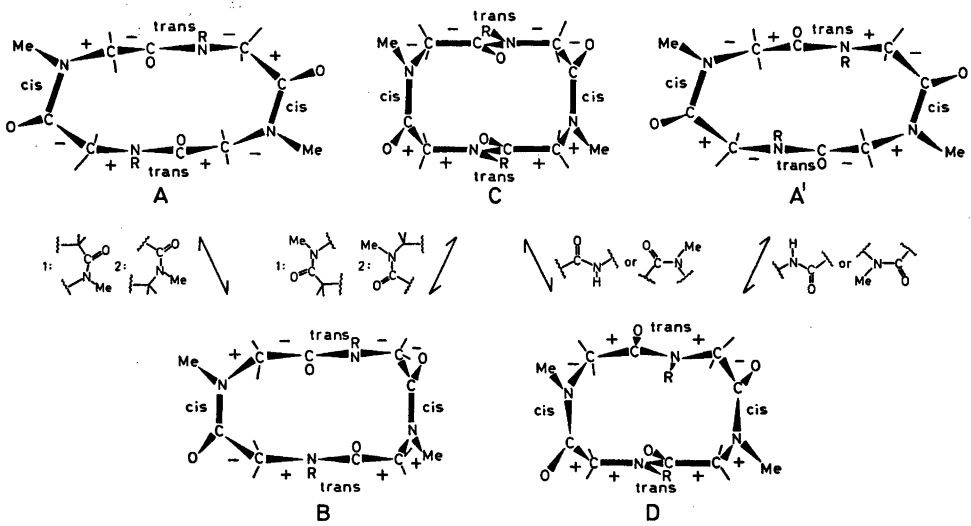


Fig. 2. Conformational interconversion scheme for c-Sar_4 (R=Me), c-$GlySar_3$ (R=Me and H), and c-GlySarGlySar (R=H).

amide group in the intermediate conformation B, and the barrier towards it, due to the presence of the carbonyl and *N*-methyl groups in the amide as compared with only hydrogen in the olefin. This is in accord with the extremely high barrier for the aromatic analogue cyclotriveratrylene; no NMR coalescence is observed [12] up to 200 °C and a conformationally chiral derivative [13] has been resolved. (See note added in proof on p. 360).

CYCLIC TETRAPEPTIDES

The uniquely preferred conformation of a large number of simple cyclic tetrapeptides has been shown both for solutions by NMR spectroscopy [2,3] and subsequently for the solid state by X-ray methods [14,15] to have a *cis,trans,cis,-trans* sequence of amide groups (A in Fig. 2). Of particular interest here are cyclotetrasarcosyl (c-Sar_4), cycloglycyltrisarcosyl (c-$GlySar_3$) and cycloglycylsarcosylglycylsarcosyl (c-GlySarGlySar). These have one, respectively both, *trans*-amide positions occupied by the *trans*-preferred NH-type amide group. The NMR-spectrum of c-Sar_4 itself [2] shows no sign of coalescence of its two AB quartets until the temperature reaches 155 °C, and by further heating the two single lines expected for the CH_2 groups if the amide groups retain their configuration do not develop. Instead, a single line is formed at the same time as also the two NMe lines coalesce to one line at ~180 °C. Thus, full exchange is obtained over a critical barrier as high as ~23 kcal/mol, and it may be concluded that α-hydrogen exchange requires either a mechanism by which also *cis-trans*-configuration is exchanged, or the passage of barriers as high as those leading to *cis,trans* exchange.*

The NMR-spectrum of c-$GlySar_3$ reveals a similar situation for this compound, with a slightly lowered coalescence temperature now observed for the CH_2-quartets only. Exchange of *cis,trans* configuration can in this case of course not be observed, since there is not a sufficient concentration of conformers with *cis* amide groups of NH-type. The NMR spectrum of c-GlySarGlySar, on the other hand, shows coalescence of the two CH_2 quartets to two single lines already at room temperature and again no change in the NMe and NH signals. The following analysis in terms of a multi-step interconversion path can rationalize these observations.

The first step in converting A to A′ (Fig. 2) may be the flattening of either the $N_tC_\alpha C_cN_cC_\alpha$ or the $C_\alpha C_cN_cC_\alpha C_t$ five-ring-atom systems at one of the *cis*-amide groups; this is essentially equivalent to flipping a CH_2 group to the other side of the ring. Whichever of the two types of CH_2 is flipping first, the other has to follow immediately to relieve strain, and therefore the two steps have been considered together in Fig. 2. Two dihedral angle signs are thereby changed to give the intermediate conformation B. The analogous two-step flipping over similar barriers of the two remaining CH_2 groups produces a second intermediate conformation C which has two further dihedral angles of changed sign. Conformation C can not be changed further by using partial-ring-flattening steps, but only be brought back to the initial situation.

In order to change the sign of the remaining four dihedral angles and obtain the inverted conformation A′, which has exchanged all outer with inner hydrogens, but is otherwise identical with the initial conformation A, it is necessary to rotate both *trans*-amide groups as such "through the ring", one after the other. Strictly, these two rotational steps might as well occur alternately with the CH_2-flipping steps according to several different schemes and probably over very similar barriers. The scheme presented in Fig. 2 has been chosen to demonstrate first those steps which involve only CH_2 flipping and can occur irrespective of whether the *trans*-amide groups are of NMe- or NH-type. The steps involving rotation of the *trans*-amide groups can occur only when the *N*-substituent is hydrogen and not the larger methyl group. It is sufficient that only one of these two steps is blocked for the whole mechanistic path to become blocked. This explains why only c-GlySarGlySar can undergo easy CH_2-exchange.

When at least one *trans*-amide group is of NMe type, it is therefore necessary to perform

* The high barrier was initially proposed [2] to be due to transannular interactions between the *trans*-amide groups, but this had to be abandoned when much lower barriers were observed for c-GlySarGlySar,[3] and when the X-ray structures [14,15] revealed normal van der Waals contacts.

a rotation about the partial double bond of the amide group through the *cis*-configuration, with both carbonyl and *N*-methyl pointing out, and further to the other *trans*-configuration (Fig. 2). This in itself requires about 19 kcal/mol,[5] and with the added accompanying ring strain, the observed value of ~ 23 kcal/mol seems reasonable. However, at the temperature necessary for this to occur rapidly, *cis,trans*-isomerization of any amide group will occur rapidly, and full *cis,trans* exchange must be observed in cyclotetrasarcosyl. Assuming that this process occurs stepwise through intermediate conformations, several interconversion paths may be followed. It can only be stated that at least four steps are required, for example the itinerary *ctct*→*ccct*→*cctt*→*cctc*→*tctc* or *ctct*→*ccct*→*cccc*→*cctc*→*tctc*. It is then assumed that increasing temporarily the number of *cis*-amide groups is more advantageous than increasing the number of *trans*-amide groups in this medium-sized ring.

Recently published data [16] on the behaviour of three proline-containing cyclic tetrapeptides also fit well with the interconversion scheme in Fig. 2. Thus, c-Gly-L-ProGly-D-Pro can take the centrosymmetric conformation A with each proline ring linking an external α-position and a *cis*-amide nitrogen and shows no NMR-spectral change with temperature, since inverted or pseudorotated conformers are not populated. On the other hand, c-Gly-L-ProGly-L-Pro does show a low-energy process (13 – 15 kcal/mol) in dynamic NMR-spectroscopy, and since conformation A would give steric problems, we propose that its lowest-energy conformation is the unsymmetrical one B (Fig. 2) which can be converted directly to an equivalent pseudorotated conformation by two successive *trans*-amide rotation steps. The NH-signal is thereby averaged between the two non-equivalent positions, but geminal site exchange in the vicinal α-CH_2 group can of course not take place. Finally, the corresponding *N*-methylated compound, c-Sar-L-Pro-Sar-L-Pro, which must also take the unsymmetrical conformation B, shows no coalescence below 70 °C. This may be explained by the much higher barrier expected for rotation of the *N*-methyl *trans*-amide groups, and we predict that coalescence should become observable in the 150 – 200 °C temperature range.

EXCHANGE PROCESSES IN HIGHER CYCLIC PEPTIDES

Cyclopentasarcosyl, which has been shown to have one unique conformation with a *cis*,-*cis,cis,trans,trans* amide sequence in the solid.[4,17] and predominantly the same in solution (see below), undergoes two separate processes in dynamic NMR spectroscopy. At about 50 °C the five CH_2 quartets coalesce to an unresolved group of presumably five lines ($\Delta G^{\ddagger} \approx 16$ kcal/mol) and by further heating this group narrows directly to a single line, at the same time as the five NMe lines coalesce to one line ($\Delta G^{\ddagger} \approx 19$ kcal/mol). The higher sarcosine homologues behave similarly; thus, cyclooctasarcosyl, which has a single conformation

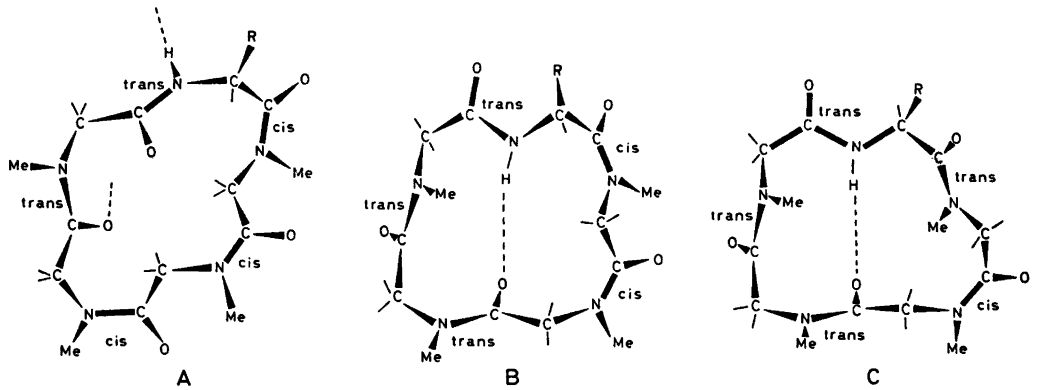


Fig. 3. Crystal conformation (A) and proposed conformations (B and C) for species formed in solution from c-AlaSar$_4$ (R = Me) and c-GlySar$_4$ (R = H).

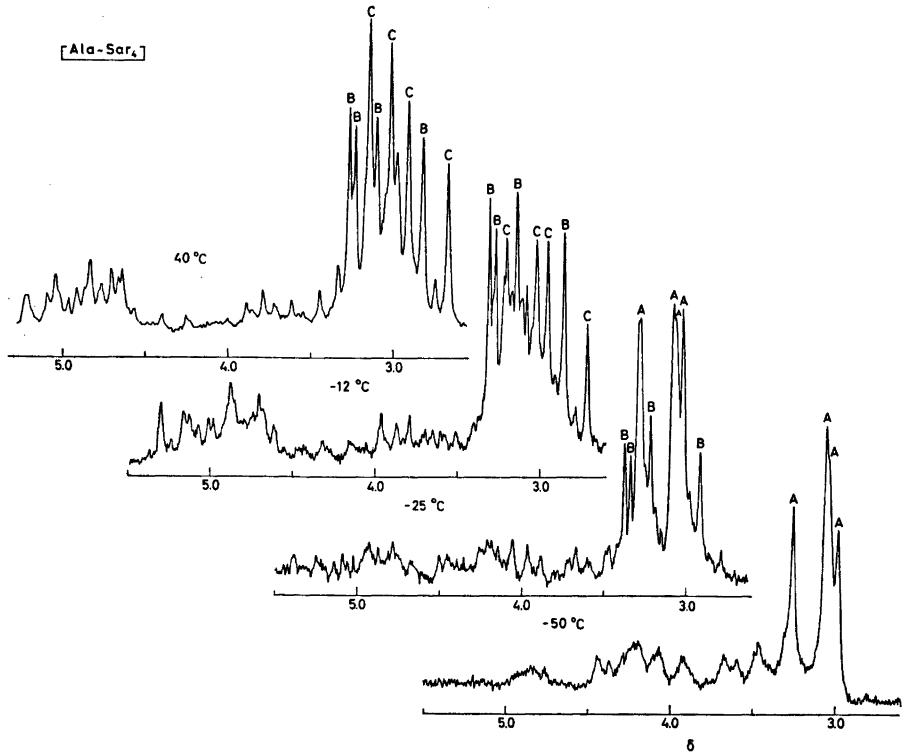


Fig. 4. ^{1}H NMR spectra at 100 MHz of c-AlaSar$_4$ (CH_2- and NMe-regions only). Crystals were dissolved in $CHFCl_2$ at -50 °C (lower spectrum) and the solution allowed to warm up in the probe. $CDCl_3$ was used as solvent at the highest temperature (40 °C).

with the *cis,cis,trans,trans,cis,cis,trans,trans* amide sequence [18] shows CH_2 quartet coalescence at 40 °C ($\Delta G^{\ddagger} \approx 15$ kcal/mol) and coalescence to single CH_2 and NMe lines at 100 °C (≈ 19 kcal/mol).

It is thus clear that the 15-membered and higher rings permit rotation of the *trans*-amide groups "through the ring" even when these are *N*-methyl substituted, the carbonyl oxygen now being the smaller substituent, thereby providing a low-barrier mechanism for geminal CH_2-hydrogen exchange. The higher barrier represents the activation energy for *cis,trans* isomerization of the amide groups leading to full exchange.

CONFORMATIONAL TRANSFORMATIONS OF SOME NH-CONTAINING CYCLIC PENTAPEPTIDES

Finally, a few examples of the conversion of one conformation to others more stable in solution will be given. The experiments were based on the following reasoning:

When, as here, the conformational interconversion barriers are so high (~ 19 kcal/mol) that temperatures of the order of 100 °C are needed to obtain life times short enough (~ 0.01 s) to observe coalescence phenomena in dynamic NMR spectroscopy, it becomes possible at temperatures below -20 °C to slow down the process sufficiently to follow by static NMR spectroscopy the interconversion between different conformers. This means that if the crystalline material is dissolved in $CDCl_3$ at -50 °C, when life-times are of the order of hours even for barriers as low as 17 kcal/mol, the NMR spectrum of the crystal conformer can be observed and identified. By heating slowly to room temperature, when life-times are of the order of seconds, the gradual appearance of other conformers may be observed. Strictly, a given *cis,trans*-sequence may

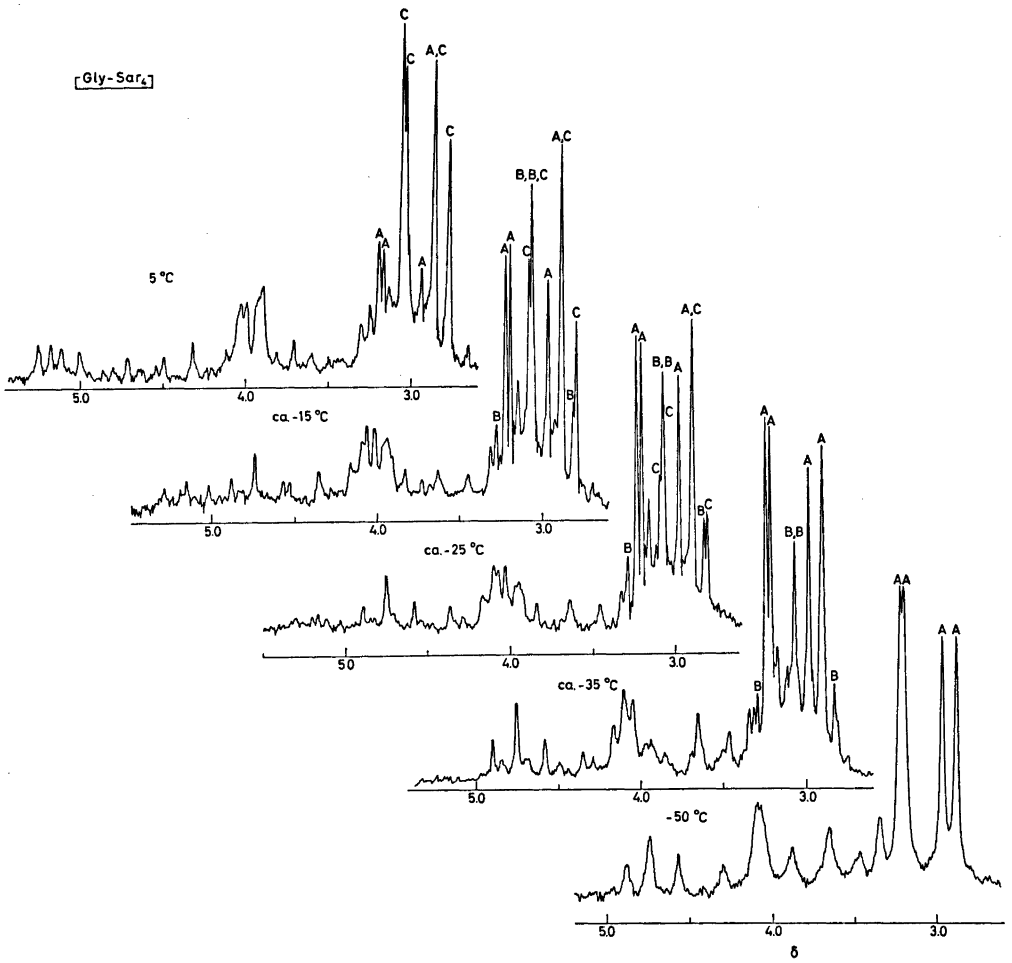


Fig. 5. ^{1}H NMR spectra at 100 MHz of c-GlySar$_4$ (CH_2- and NMe-regions only). Crystals were dissolved in $CHFCl_2$ at -50 °C (lower spectrum) and the solution allowed to warm up in the probe.

give rise to a mixture of ring conformations differing in torsional angles of the single bonds, and even when only one is observed after dissolution at low temperature, this may still be different from that found in the crystal. However, in the present type of cyclic peptides of moderate ring size a given *cis,trans*-sequence will effectively define the whole ring conformation, since the CC_α torsion angle has consistently been found close to 180 or 60° and the NC_α torsion angle roughly 90°.[14,15,17–19]

When as a first experiment crystals of c-Sar$_4$ are dissolved at -50 °C and allowed to varm up, no spectral change is observed, thus proving that this peptide is homogeneous with the same conformer in solution as in the crystal. This had already been concluded independently for c-Sar$_4$, [2,14] as well as for c-GlySar$_3$,[3,15] c-GlySarGlySar, [3,15] and c-AlaSar$_3$,[3,15] by using symmetry arguments and geminal coupling constants for the solutions, and X-ray methods for the crystals.

In the case of c-Sar$_5$ the identity of the dominant solution conformation could not be deduced from its room-temperature NMR-spectrum by symmetry arguments.[2] When now the crystals are dissolved at -50 °C, the spectrum contains just the same five CH_2 quartets

and the five strong NMe lines, and heating produces no change except for the appearance of the very weak extra NMe lines observed earlier. This shows that the crystal conformer, known to be *cis,cis,cis,trans,trans*, is identical with the one which is strongly predominant in solution.

In contrast, all other cyclic pentapeptides containing one or more glycine or alanine residues give dramatic changes on heating, whereby the crystal conformer is transformed stepwise to other conformers. Often the final mixtures contain no more of the crystal conformer, and some conformers are only of transient existence. The best example is c-Ala-Sar$_4$, since its crystal conformer (A, Fig. 3) has been determined by X-ray methods [19] and found to be exactly the same as found [17] for c-Sar$_5$ [*cis,cis,cis,trans,-trans*(NH)]. The amide group of NH-type occupies one of the *trans*-positions and makes an external hydrogen bond with the oxygen of the other *trans*-amide group of a neighbouring (identical) molecule (Fig. 3). After dissolution at -50 °C, the NMR spectrum shows four NMe lines and four CH_2 quartets, as well as the expected lines for the alanine residue;* this set of lines must belong to conformer A. At -30 °C a new set of lines due to a second conformer B appears, and its concentration grows rapidly to become equal to that of A, whereafter, on further heating, a third set appears. The corresponding conformer C dominates the final equilibrium at room temperature (~ 60 %), with the rest made up of mainly conformer B and only traces of A (Fig. 4).

We draw the conclusion that C is the most stable conformer in solution and that it must be produced through B as an intermediate. Since B accumulates temporarily, this must also be more stable than A, and the critical barrier between A and B must be lower than the critical barrier between B and C. Strictly, one cannot know whether the process goes through additional intermediate conformers, but in view of the fact that c-Sar$_5$ with no NH present does not undergo a change, the only conceivable driving force is the establishment of an internal hydrogen bond in solution to replace the external hydrogen bond of the solid. Already conformer B needs therefore a sequence of three *trans* amide groups for steric reasons to obtain the necessary internal hydrogen bond, and we propose the sequence *cis,cis,trans,trans,-trans* (NH) (Fig. 3) in analogy with the crystal conformation of c-Gly$_6$ [20] and c-Gly$_4$Ala$_2$.[21]*

The only simple way of further improving the situation is the direct change of a second *cis*-amide group to *trans*, and we prefer the sequence *trans,cis, trans,trans,trans* (NH) for C (Fig. 3). Here the sequence of the same three *trans*-amide groups resemble even more closely the situation in c-Gly$_4$Ala$_2$, and the remaining *trans,cis* sequence resembles the situation in each half of a cyclic tetrapeptide.

A very similar behaviour is observed for c-GlySar$_4$, except that B never approaches A in concentration and that the final equilibrium is more completely dominated by conformer C (80 %) with the remainder now made up of the crystal conformation A and only traces of B (Fig. 5).

Also c-Gly$_2$Sar$_3$ and c-D-Ala-L-AlaSar$_3$, which have the correct sequence of *cis*- and *trans*-preferred amide bonds, may be expected to start out from the same initial crystal conformation *cis,cis,cis,trans*(NH),*trans*(NH), and do in fact show analogous transformations upon dissolution and subsequent heating.

Added in proof. An alternative to the CH_2-flipping mechanism A⇌B⇌C⇌B′⇌A′ for c-Sar$_3$ shown in Fig. 1 is the amide-group flipping mechanism A⇌B′⇌C⇌B⇌A′. During the flipping of one amide group (A⇌B′) the other two amide groups will approach each other momentarily across the ring, and so the higher barrier for the more bulky amide group as compared to the unsubstituted olefin receives a more convincing explanation.

* Actually, the α-Me group gives rise to two doublets instead of one, but since hardly any splitting is noticeable in the NMe-lines, it is very likely that we have about equal populations of the crystal conformer and its inverted conformer with an identical ring skeleton but exchanged α-H and α-Me positions. For the present discussion these are equivalent.

* These cyclic hexapeptides have only *trans*-amide bonds of NH-type and can therefore form both external and internal hydrogen bonds in the crystals.

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The Preparation and ^1H Nuclear Magnetic Resonance Spectra of Some Acyclic Oxyarsoranes

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It has been shown that compounds of the type $R_nAs(OCH_3)_{5-n}$, $R = CH_3$ or Ph, $n = 0$, 1, 2, 3, can be prepared by allowing the corresponding halides of trivalent arsenic to add bromine and thereafter react with sodium methoxide in methanol solution. Their variable temperature ^1H NMR spectra have been examined down to -100 °C and the results interpreted in terms of pseudorotation processes among structures with trigonal bipyramidal geometry. $Ph_2As(OCH_3)_3$ was the only compound which showed NMR nonequivalence of the methoxy groups at low temperatures.

While pentasubstituted phosphorus compounds containing one or more P–O bonds have been studied rather extensively,[1–3] the research done on analogous arsenic compounds is rather limited.[1] A considerable amount of work has also been done on pentacovalent antimony compounds having Sb–O bonds.[1] On the other hand there is strong evidence that pentacovalent arsenic compounds play an important role as reactant intermediates in reactions involving nucleophilic attack on tetracoordinated arsenic. On this background we considered it valuable to attempt synthesis of pentasubstituted arsenic compounds with one or more As–O bonds (oxyarsoranes). Given the existence of oxyarsoranes, variable temperature NMR provides a possible technique for examining their ability to undergo pentatopal isomerizations (Berry pseudorotation or turnstile rotation).[1] Hopefully, the comparison with corresponding phosphorus and antimony derivatives may lead to some generalisations regarding intramolecular processes in pentasubstituted compounds of group V elements. In the present work we consider the preparation and NMR spectra of oxyarsoranes with acyclic substituents. Oxyarsoranes incorporating five- and six-membered ring systems will be the subject of a later investigation.

Some pentacoordinated arsenic compounds with *O*-alkyl- and F-substituents have been synthesized by Hass.[4–8] For example, $As(OCH_3)_5$ was obtained as one of the products from the reaction between $O=As(OCH_3)_3$ and $Pr-NH_2$.[4] However, the sequence of reactions leading to this compound can not be regarded as established and the yield is low. Alternative reaction routes are therefore desirable. The dynamic stereochemistry, in terms of pseudorotation processes, has not been considered for the above mentioned compounds.[4–8]

RESULTS AND DISCUSSION

The oxyarsoranes *1*–*6* (Table 1) were conveniently prepared according to the general reactions:

$$R_nAsX_{3-n} + Br_2 = R_nAsX_{3-n}Br_2$$

$$R_nAsX_{3-n}Br_2 + (5-n)\ Na^+OCH_3^- = R_nAs(OCH_3)_{5-n} + (5-n)\ Na^+ + (3-n)X^- + 2Br^-$$

$R = CH_3$, Ph
X = Cl, Br, I

The reactions were performed by allowing bromine to drop into a methanol solution containing the appropriate trivalent arsenic compound and sodium methoxide in the proper ratio (see experimental section for further details). The yield in the case of *1*, *2*, and *3*

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seemed to be quantitative. *1–6* are thermally stable substances, and were, with the exception of *6*, distilled at reduced pressure. As can be expected for compounds having As-O bonds, the prepared oxyarsoranes are very readily hydrolyzed. Detailed studies on the hydrolytic stability of the compounds *1–6* have not been performed, but it appears that the hydrolytic stability decreases as the number of R groups increase, regardless of whether R is methyl or phenyl. The compounds *1*, *2* and *3* (Table 1) have been stored for more than a year at ambient temperature and appear to be completely stable. They can be handled under normal atmospheric conditions without difficulty. The reaction scheme given above offers a new and simple way of preparing this type of oxyarsoranes.

^{1}H NMR data for the arsoranes *1–6* are listed in Table 1. With the exception of compound *5* there is no essential change in spectral behaviour on cooling down to −60 °C in deuteriochloroform solution. *1–4* were also recorded in carbon disulfide at −100 °C, but no qualitative change in their NMR spectra resulted.

Assuming the oxyarsoranes to have the structure of a trigonal bipyramid (TBP), the substituents can exist in two different environments, apical and equatorial. If the TBP structure is rigid, one would expect for *1*, two different signals with relative intensity 2:3. The observation of five equivalent methoxy groups is in accordance with fast intramolecular pseudorotation, *i.e.* the activation energies for the relevant intramolecular processes are low. The observed NMR equivalence of the methoxy groups could also be explained if the chemical shift difference between apically and equatorially situated substituents is small. In this case, the temperature required to observe splitting of the NMR signal would not be obtainable, even if the activation energy for the pseudorotation process is considerable, say 15 kcal mol^{-1}. The fact that a single signal results at low temperatures whether the solvent is deuteriochloroform or carbon disulfide does not support this explanation. The NMR equivalence of substituents in pentacoordinated compounds of the type AsY_5, seems to be general. Thus the fluorines in AsF_5 [9,10] and the methyl groups in $As(CH_3)_5$ [11] and $As(p\text{-}CH_3\text{-}C_6H_4)_5$ [12] give rise to only one signal in their respective NMR spectra, even at low temperatures.

It should be noted that *1* and methanol are participating in an exchange reaction which at 25 °C is fast on the NMR time scale. Thus while *1* in deuteriochloroform solution gives a resonance signal at δ 3.74 and methanol one at δ 3.47, a mixture of these two compounds does not give rise to two separate signals. Instead, one signal, having a chemical shift in between that of methanol and *1*, is observed. This result opens the possibility that traces of methanol present in the NMR tube can catalyze an intermolecular exchange of OCH_3 groups. As a consequence, NMR equivalence of substituents may result.

In the case of pentacovalent phosphorus compounds, the generalisation that the most electronegative substituents tend to occupy the apical positions in the TBP seems to be valid.[1–3] Assuming that this electronegativity rule also applies to pentacovalent arsenic compounds, a rigid TBP structure would predict the methoxy groups in *2* and *3* to show up in NMR as two signals in 1:1 ratio. The four equivalent methoxy

Table 1. ^{1}H NMR spectral data[a] for acyclic oxyarsoranes in deuteriochloroform solution at 25 °C.

Compound	Chemical shifts, δ[b]			
	O–CH_3	As–CH_3	As–Ph	
1 $As(OCH_3)_5$	3.74			
2 CH_3-$As(OCH_3)_4$	3.62	1.93		
3 Ph-$As(OCH_3)_4$	3.65		7.55,	7.85
4 $(CH_3)_2As(OCH_3)_3$	3.36	1.83		
5 $Ph_2As(OCH_3)_3$	3.24		7.58,	8.00
6 $Ph_3As(OCH_3)_2$	2.63		7.42,	8.08

[a] The spectra were recorded at 60 Mz, using a Jeol JNM-C-60H spectrometer. [b] Downfield from internal TMS.

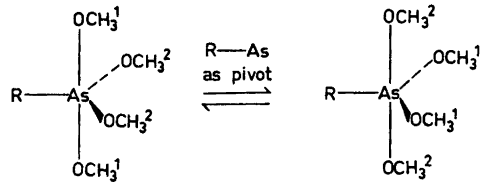


Fig. 1.

groups observed in both cases, down to −100 °C, are consistent with a low activation energy for the pseudodorotation process illustrated in Fig. 1 (R = CH_3, Ph).

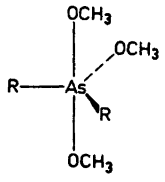
These results are in correspondence with those obtained previously for $PhAsF_4$ [9,13] and $PhP(OCH_2CH_3)_4$,[14] as the variable temperature NMR spectra of these substances showed only one signal for their respective substituents.

5 is the only oxyarsorane considered in this work which shows qualitative difference in its NMR spectrum at different temperatures. At room temperature the OCH_3 signal is very broad. Cooling causes splitting into two signals (δ 3.02, 3.98 at 40 °C) in the ratio 2:1, while heating results in a normally sharp singlet (δ 3.36 at 50 °C). The low temperature spectrum is in agreement with the structure predicted from the electronegativity rule, *i.e.* the one in which the two phenyl groups are occupying equatorial positions, I (R = Ph). This geometrical situation will have as a consequence that the equatorial methoxy group is less shielded than are the apical groups. The fact that the high intensity signal is at higher field than the low intensity signal is consistent with this deduction. The coalescence temperature for the process leading to equivalence of the methoxy groups was found to be 21 °C. From this temperature and the chemical shift difference between the two methoxy signals, 58 Hz, the free energy of activation (at the coalescence temperature) can be estimated.[15] This was found to be 14.4 kcal mol^{-1}. The variable temperature NMR spectrum of *5* is completely analogous to the one obtained for the related phosphorus compound, $Ph_2P(OCH_2CH_3)_3$.[14] The same kind of NMR behaviour is exhibited by the ^{19}F NMR spectrum of Ph_2AsF_3.

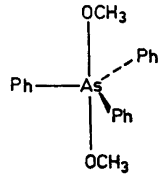
On this background one would expect compound *4* to have a NMR spectrum which on cooling changed in a manner similar to the one of *5*. This was not observed as both the methyl groups and methoxy groups each gave rise to only one signal over the temperature range studied. The result can either be explained by assuming accidental isochrony of the substituents or alternatively by postulating that the pseudorotation processes involved have activation energies which are too low to be detected by the NMR technique. There is also the possibility that traces of water can lead to exchange of methoxy groups at a rate which is rapid on the NMR time scale. It is interesting to note that $(CH_3)_2AsF_3$ has a ^{19}F NMR spectrum which is essentially invariant with temperature.[10] The single ^{19}F signal observed in this case has been attributed to an octahedral structure in which the arsenic compound acts as a Lewis acid towards the solvent molecule (acetone or acetonitrile). It is not likely that this interpretation applies to *4*, especially because of the absence of suitable donor molecules in this case.

While the low temperature spectrum of the phosphorus analogue of *4* has not been reported, the antimony analogue, $(CH_3)_2Sb(OCH_3)_3$, has been studied in detail. At −80 °C this compound showed two signals in the ratio 1:2 for the methoxy groups, an observation consistent with a structure similar to I. However, these results were, with support from molecular weight data, interpreted in terms of a dimer with octahedral structure.[16]

The NMR spectrum of compound *6* showed only one signal associated with the methoxy groups. This is in agreement with the favoured



I



II

structure in which the three phenyl groups are situated equatorially, II. There is no necessity of assuming the occurrence of fast pseudorotation processes in order to explain the observed NMR equivalence of methoxy groups, specially because the possible intramolecular processes would involve structures in which the most electronegative substituents are in equatorial positions and the least electronegative substituents in apical positions. In addition, the substantial shielding expected for the methoxy groups in II is in agreement with the high field signal found experimentally. In the case of *6*, there is, as far as NMR behaviour is concerned, complete analogy with related compounds. Thus the low temperature NMR spectra of Ph_3AsF_2,[10] $Ph_3P(OCH_2CH_3)_2$,[14] and $Ph_3Sb(OCH_3)_2$,[17] showed only one signal for the fluorine-, ethoxy-, and methoxy groups, respectively. The assumed TBP geometry has in the case of $Ph_3Sb(OCH_3)_2$ been confirmed by X-ray structure determination.[18]

In conclusion, the NMR behaviour of the oxyarsoranes considered in this study can be explained in terms of pseudorotation processes among structures having TBP geometry. There is, as far as the comparison goes, correspondence in the variable temperature NMR spectra between analogous compounds of arsenic and phosphorus. However, much work has to be done in order to determine the relative importance of the various factors governing the dynamic stereochemistry of oxyarsoranes. As a beginning, we will in future work consider oxyarsoranes containing five- and six-membered rings.

EXPERIMENTAL

Preparation of haloarsines

Diiodo(methyl)arsine was prepared by treatment of an alkaline solution of arsenic oxide with methyl iodide followed by reduction with sulfur dioxide.[19]

Dichloro(phenyl)arsine was synthesized according to Michaelis from triphenylarsine and trichloroarsine.[20]

Chlorodimethylarsine was prepared from cacodylic acid according to the procedure of Steinkopf and Mieg.[21]

Chlorodiphenylarsine was prepared from triphenylarsine and dichloro(phenyl)arsine as described by Pope and Turner.[22]

Preparation of oxyarsoranes

Pentamethoxyarsorane(1) was prepared as described previously,[23] b.p. 39 °C/2 mmHg.

Tetramethoxy(methyl)arsorane (2) was obtained by treatment of a solution of 1 mol of sodium in methanol with 0.25 mol diiodo (methyl)arsine followed by addition of 0.25 mol of bromine. The temperature of the reaction mixture was held at 0 – 5 °C during the additions. The methanol was distilled off at reduced pressure and the residue extracted with dry ether. The ether extract was freed from sodium bromide and the product distilled after evaporation of the solvent, b.p. 30 °C/0.03 mmHg.

Tetramethoxy(phenyl)arsorane(3) was obtained according to a procedure analogous to the one used for preparing *2*, b.p. 70 °C/0.01 mmHg.

Trimethoxydimethylarsorane (4). To a solution of 0.15 mol of sodium in 500 ml methanol 0.1 mol chlorodimethylarsine, and thereafter 0.1 mol of bromine was added. The reaction mixture was vigorously stirred during the additions and the temperature held at 0 °C. The reaction mixture was stirred at room temperature for 1 h after the addition was completed. Most of the methanol was distilled off and the product dissolved in ether. After evaporation of this solvent the product was distilled, b.p. 32 °C/0.2 mmHg. After brief exposure to the atmosphere the product is hydrolysed to a white solid, m.p. 195 °C, undepressed by admixture of an authentic sample of cacodylic acid.

Trimethoxydiphenylarsorane (5). This compound was prepared according to a procedure similar to the one used for *4*, b.p. 163 °C/1 mmHg. The product was readily hydrolysed when exposed to the atmosphere.

Dimethoxytriphenylarsorane (6). To a solution of sodium in methanol was added 0.05 mol triphenylarsine and thereafter 0.1 mol bromine. The temperature was held at 0 °C. Working up of the reaction mixture as described above for compound *4* resulted in a viscous material, which on exposure to air was hydrolysed to triphenylarsenic oxide. The NMR spectrum of the isolated material (Table 1) integrated in agreement with compound *6*.

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Oxidation of Carbohydrate Derivatives with Silver Carbonate on Celite. X. Identification of Three Mono-*O*-isopropylidene Derivatives of D-Galactose

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Treatment of D-galactose with anhydrous cupric sulfate in acetone and dimethylformamide gave three mono-*O*-isopropylidene derivatives, identified as 5,6-*O*-isopropylidene-D-galactofuranose (I), 3,4-*O*-isopropylidene-D-galactopyranose (II), and 4,6-*O*-isopropylidene-D-galactopyranose (III). The identification was based on oxidation of the *O*-isopropylidene-derivatives with silver carbonate on Celite; this oxidant gave in methanol *O*-isopropylidene derivatives of D-glyceraldehyde, D-lyxose, and D-threose, respectively, from I, II, and III, in addition to different esters of aldonic acids. Oxidation in benzene, or dimethylformamide and benzene, resulted in less extensive degradation of the *O*-isopropylidene-D-galactoses.

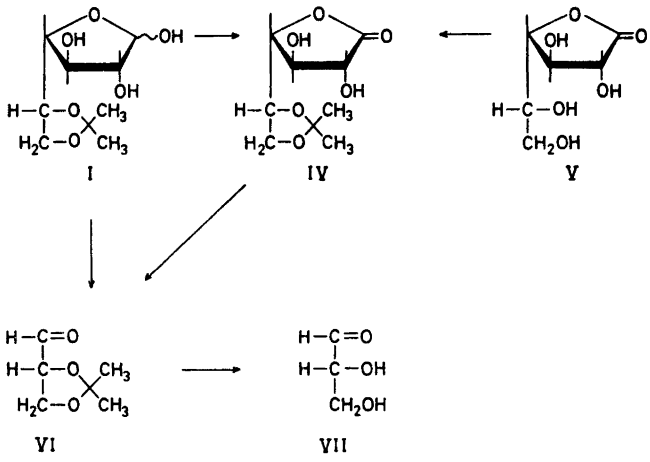
Silver carbonate on Celite has previously been shown to degrade aldoses stepwise from C-1 in methanolic solution. Compounds containing two or three carbon atoms are the final products obtained from unsubstituted aldoses,[1] whereas substituted sugars are degraded until the substituent prevents further shortening of the carbon chain.[2,3] These results suggested that the reagent might be of potential utility in location of substituents in aldoses, and in the present paper the application of the oxidant in identification of three reducing monoisopropylidene derivatives of D-galactose is described. Investigations of the applicability of the method on other types of substituted carbohydrates are in progress.

RESULTS AND DISCUSSION

Treatment of D-galactose with anhydrous cupric sulfate in acetone containing 20 % dimethylformamide at reflux temperature for 20 h, resulted in formation of three mono-*O*-isopropylidene derivatives. In addition, small amounts were also formed of two compounds which were chromatographically indistinguishable from 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose. These derivatives were previously reported to be the products when D-galactose was treated with the same reagents for 2 days in the presence of greater proportions of acetone.[4]

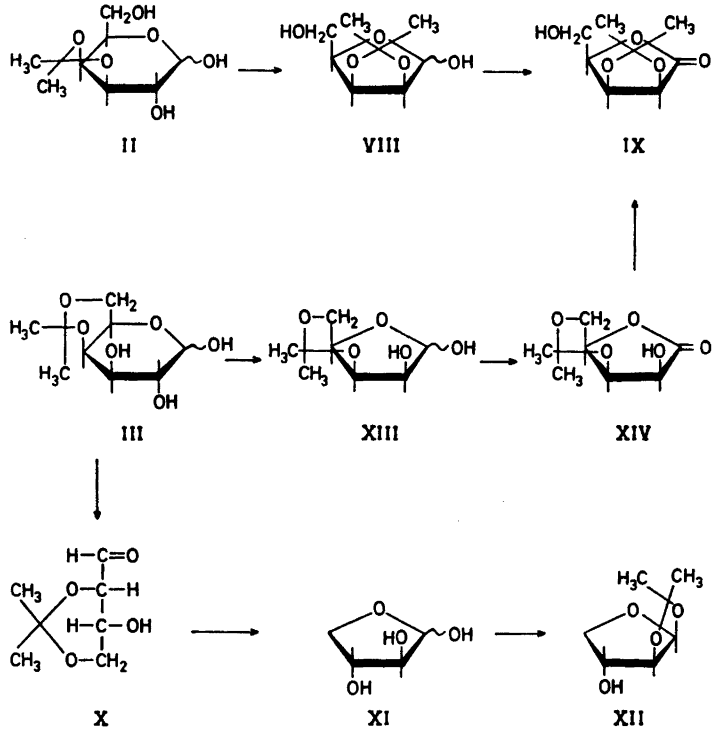
The mono-*O*-isopropylidene derivatives were separated on a silica gel column, and identified as 5,6-*O*-isopropylidene-D-galactofuranose (I), 3,4-*O*-isopropylidene-D-galactopyranose (II), and 4,6-*O*-isopropylidene-D-galactopyranose (III). The yields were 22, 15, and 13 %, respectively. The total amount of mono-*O*-isopropylidene derivatives was somewhat higher than the sum of the above yields since some fractions contained mixtures of I and II.

The identification of the products was based on oxidation with silver carbonate on Celite in methanol, benzene, or benzene and dimethylformamide. Oxidation of I in refluxing benzene gave 5,6-*O*-isopropylidene-D-galactono-1,4-lactone (IV). This compound was for comparison also prepared from D-galactono-1,4-lactone (V) with anhydrous cupric sulfate and acetone; the structure was established by infrared spectroscopy in combination with periodate oxidation at pH 7, which led to the formation of 2,3-*O*-isopropylidene-D-glyceraldehyde (VI). In methanolic solution, oxidation of I gave a product mixture



in which no aldotetrose, -pentose, or -hexose derivative could be detected; a compound chromatographically indistinguishable from 2,3-*O*-isopropylidene-D-glyceraldehyde (VI) seemed to be the main product. In addition, products which apparently were aldonic acid esters or lactones, were also formed. Acid hydrolysis of the product mixture yielded as the only detectable aldose a compound which was indistinguishable from D-glyceraldehyde (VII) by chromatography and electrophoresis.

Oxidation of II in methanol gave mainly 2,3-*O*-isopropylidene-D-lyxose (VIII), which was further oxidized in benzene with silver carbonate



on Celite to 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX).

Treatment of III with the oxidant in methanol at reflux temperature resulted in formation of a mono-*O*-isopropylidene derivative of D-threose, presumably the 2,4-*O*-isopropylidene derivative (X). Hydrolysis yielded D-threose (XI), characterized as its 1,2-*O*-isopropylidene derivative (XII). When the oxidation of III was carried out in benzene-dimethylformamide, an *O*-formyl-*O*-isopropylidene-D-lyxose was the only detectable product. Removal of the *O*-formyl group yielded a compound which apparently is 3,5,-*O*-isopropylidene-D-lyxofuranose (XIII), since oxidation of this compound with silver carbonate on Celite in benzene resulted in formation of an *O*-isopropylidene-D-lyxono-1,4-lactone, different from the 2,3-*O*-isopropylidene derivative (IX). This leaves 3,5-*O*-isopropylidene-D-lyxono-1,4-lactone (XIV) as the only realistic remaining possibility. XIV was isomerized to IX in acetone in the presence of acid.

The products obtained from I, II, and III on oxidation with silver carbonate on Celite establish their structures, as far as isomerization of the *O*-isopropylidene-D-galactoses may be excluded under the reaction conditions employed. The almost neutral or weakly basic conditions make isomerizations very unlikely, and it has not been observed in oxidations of other *O*-isopropylidene aldoses.[3] The somewhat surprising formation of a lyxose derivative (XIII) from III on oxidation in benzene and dimethylformamide, whereas oxidation in methanol yields a threose derivative, is explained by the fact that the *O*-formyl group at C-4, resulting from cleavage of III between C-1 and C-2, is stable in benzene-dimethylformamide. This prevents cyclization to a new hemiacetal, and hence also prevents further degradation or oxidation to an aldonolactone. One step degradations to lyxose derivatives have also been observed when 4,6-*O*-ethylidene- and -benzylidene derivatives of D-galactose are similarly treated with this oxidant, whereas the corresponding D-glucose derivatives give negligible amounts of *O*-alkylidene derivatives of D-arabinose.[5] Oxidation of III in boiling methanol leads to a threose derivative since the *O*-formyl group initially formed undergoes transesterifications, the hydroxyl group at C-4 in the lyxose derivative then forms a hemiacetal with the aldehyde group, and further degradation may occur. In methanol the possibility also exists that degradation occurs *via* an intermolecularly formed hemiacetal, involving a molecule of methanol, and despite earlier results have shown that formation of methyl esters is more dominating than degradation of methyl acetals of some α-hydroxy aldehydes,[1,6] this seems to be the only way by which 5,6-*O*-isopropylidene-D-galactofuranose (I) can be degraded beyond the first stage.

The usefulness of anhydrous cupric sulfate and acetone in preparation of reducing mono-*O*-isopropylidene derivatives of some aldoses has previously been observed.[7,8] In particular in the presence of dimethylformamide, the formation of *O*-isopropylidene groups involving the hemiacetal hydroxyl group was inhibited.[7] The results presented in this paper support this observation. The relatively high yield of 5,6-*O*-isopropylidene-D-galactofuranose (I) should also be noticed. Except for a shorter reaction time and a relatively greater amount of dimethylformamide, the conditions are not very different from those employed in a previously reported work concerning the preparation of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose.[4] The 5,6-*O*-isopropylidene derivative (I) is presumably the precursor in the formation of this compound. High temperature and dimethylformamide seem to have a favorable effect on the formation of furanose forms in this reaction.

EXPERIMENTAL

Thin layer chromatography (TLC) was performed on Silica Gel G in (A) benzene-ethanol 3:1 (v/v), (B) benzene-ethanol 5:1, and (C) chloroform-methanol 25:1. Paper chromatography was done with (D) butanol-pyridine-water 5:3:1 and electrophoresis with borate buffer pH 10 on Whatman No. 1 paper. As spray reagents were used diphenylamine-aniline-phosphoric acid,[9] aniline oxalate, and for esters and lactones hydroxylamine-ferric chloride.[10] Gas liquid chromatography (GLC) was performed with a Perkin-Elmer F 11 gas chromatograph, equipped with a flame ionization detector; the column was a stainless steel column, 2 m × 2.2 mm (i.d.) with 3 % OV 225 on Gas Chrom Q 80 – 100 mesh. The temperature was maintained at 150 °C for 17 min, and then increased 4 °C/min. Mass spectra were recorded with an AEI MS 902 mass spectrometer at an ionizing potential of 70 eV.

Reaction of D-*galactose with anhydrous cupric sulfate and acetone in the presence of dimethylformamide.* D-Galactose (1 g) and anhydrous cupric sulfate (3 g) were stirred in acetone (150 ml) and dimethylformamide (30 ml) at reflux temperature for 20 h. TLC (solvents A and B) indicated the presence of five products in addition to small amounts of unreacted D-galactose. The two fastest moving compounds were indistinguishable from 1,2:3,4- and 1,2:5,6-di-*O*-isopropylidene-D-galactose, the three other products I, II, and III had R_F (TLC, solvent A) 0.46, 0.40, and 0.31, respectively. The solution was filtered, benzene (150 ml) was added and the resulting solution stirred with solid sodium hydrogencarbonate (1 g) for 30 min. After filtration and evaporation of the solvents under reduced pressure, the residue was chromatographed on a silica gel G column (30 × 3 cm) in solvent A. A fraction containing the di-*O*-isopropylidene derivatives was eluted first, and then three fractions (1, 2, and 3) were obtained, containing pure I, II, and III, respectively. A fraction containing a mixture of I and II was also obtained.

Fraction 1 gave on evaporation of the solvent and treatment of the residue with ethyl acetate, crystalline 5,6-*O*-isopropylidene-D-galactofuranose (I, 267 mg, 22 %), m.p. 83 – 84 °C, $[\alpha]_D^{22}$ – 23° (*c* 2, methanol, 5 min). (Found: C 48.86; H 7.02. Calc. for $C_9H_{16}O_6$: C 49.09; H 7.27).

Fraction 2 contained 3,4-*O*-isopropylidene-D-galactopyranose (II, 187 mg, 15 %), m.p. 100 – 102 °C after crystallization from ethyl acetate, $[\alpha]_D^{22}$ +86° (*c* 0.5, methanol, 5 min). (Found: C 49.08; H 7.18. Calc. for $C_9H_{16}O_6$: C 49.09; H 7.27).

Fraction 3 on treatment with ethyl acetate gave crystalline 4,6-*O*-isopropylidene-D-galactopyranose (III, 163 mg, 13 %), m.p. 141 – 143 °C, $[\alpha]_D^{22}$ +153° (*c* 0.5, methanol, 5 min). (Found: C 48.81; H 7.40. Calc. for $C_9H_{16}O_6$: C 49.09; H 7.27). Mass spectrum: *m/e* 205 (4, $M^+ - CH_3$), 145 (2), 131 (8), 127 (1.5) 103 (3), 101 (3), 73 (14), 59 (65), 43 (100).

All the *O*-isopropylidene derivatives (I, II, and III) gave after treatment with acetone containing 1 % (volume) sulfuric acid a compound indistinguishable from 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose by TLC (solvent B) and GLC.

*Oxidation of 5,6-*O*-isopropylidene-*D*-galactofuranose (I) in benzene.* 5,6-*O*-Isopropylidene-D-galactofuranose (I, 20 mg) was stirred in benzene (25 ml) at reflux temperature for 15 min, silver carbonate on Celite (1 g) was then added, and the stirring under reflux continued for 20 min. TLC (solvent A) showed a single product, detectable with the ester reagent only, and indistinguishable from 5,6-*O*-isopropylidene-D-galactono-1,4-lactone (IV), prepared as described below. Filtration of the solution and evaporation of the solvent gave a syrupy residue (15 mg), $[\alpha]_D^{22}$ –45° (*c* 1, acetone). The infrared spectrum was identical with that of the authentic sample (strong absorption at 1780 cm^{-1} in $CHCl_3$).

*5,6-*O*-Isopropylidene-*D*-galactono-1,4-lactone (IV) from* D-*galactono-1,4-lactone (V).* D-Galactono-1,4-lactone (V, 0.5 g) was stirred with anhydrous cupric sulfate (3 g) in acetone (100 ml) for 18 h. TLC (solvent A) showed the presence of traces of starting material, and a single product with higher mobility. The solution was filtered and the solvent evaporated. Extraction of the residue with a hot mixture of benzene and ethyl acetate 3:2 afforded after removal of the solvents a chromatographically homogeneous syrup, $[\alpha]_D^{22}$ –42° (*c* 2, acetone), the infrared spectrum ($CHCl_3$) showed strong absorption at 1780 cm^{-1}, characteristic of 1,4-lactones. (Found: C 48.42; H 6.45. Calc. for $C_9H_{14}O_6$: C 49.54; H 6.42).

Treatment of the syrup with sodium periodate in water at pH 7 (adjusted by addition of sodium hydrogencarbonate), extraction of the water solution with chloroform and evaporation of the chloroform, gave a product indistinguishable (TLC, solvents B and C) from 2,3-*O*-isopropylidene-D-glyceraldehyde (VI). Hydrolysis of this compound in 30 % aqueous acetic acid at 40 °C for 2 h, yielded as the only detectable product a compound indistinguishable from D-glyceraldehyde (VII) by TLC (solvent A) and electrophoresis (M_{glc} 0.73).

*Oxidation of 5,6-*O*-isopropylidene-*D*-galactofuranose (I) in methanol.* 5,6-*O*-Isopropylidene-D-galactofuranose (I, 30 mg) in methanol (5 ml) was stirred with silver carbonate on Celite at reflux temperature for 90 min. TLC (solvent B) showed minor amounts of two compounds detectable with hydroxylamine-ferric chloride, R_F 0.54 and 0.40, and one component detectable with diphenylamine-aniline-phosphoric acid, R_F 0.48, indistinguishable from 2,3-*O*-isopropylidene-D-glyceraldehyde in this solvent and in solvent C.

Preparative chromatography of this compound (TLC, solvent B) after filtration of the solution and evaporation of the solvent gave a chromatographically pure 2,3-*O*-isopropylidene-D-glyceraldehyde (VI), $[\alpha]_D^{22}$ +60° (*c* 1, benzene) (lit.[11] –67.9° for the L-enantiomer).

Hydrolysis in 30 % aqueous acetic acid at 40 °C for 2 h, evaporation of the water and acetic acid and treatment of the residue in water with Dowex 1 (HCO_3^-) ion exchanger yielded after evaporation of the solvent a syrup. TLC (solvent A) and electrophoresis showed a single compound, detectable with diphenylamine-aniline-phosphoric acid, indistinguishable from D-glyceraldehyde (VII).

*Oxidation of 3,4-*O*-isopropylidene-*D*-galactopyranose (II).* 3,4-*O*-isopropylidene-D-galactopyranose (II, 30 mg) in methanol (5 ml) was stirred under reflux with silver carbonate on Celite (0.5 g) for 15 min. The solution was filtered and the solvent evaporated yielding a syrup; TLC (solvent B) showed the presence of a major compound, detectable with diphenylamine-aniline-phosphoric acid, indistinguishable

from 2,3-*O*-isopropylidene-D-lyxose (VIII). Minor amounts of two compounds detectable with the ester reagent, were also present. GLC also showed the presence of a major compound, retention time 10.5 min, indistinguishable from VIII.

Oxidation of the product mixture with silver carbonate on Celite (0.5 g) in benzene (10 ml) at reflux temperature for 1 h, filtration of the solution and evaporation of the solvent, gave a syrup from which crude 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX) crystallized on treatment with ethyl acetate-light petroleum (b.p. 60 – 80 °C). The yield was 15 mg, m.p. 88 – 93 °C (lit.[12] 99 – 100 °C), $[\alpha]_D^{22}$ +108° (*c* 1, acetone) (lit.[12] +106°, water). The product was indistinguishable from an authentic sample of IX by GLC (retention time 25.2 min) and TLC (solvents B and C); the infrared spectra were identical ($CHCl_3$, strong absorption at 1785 cm^{-1}).

*Oxidation of 4,6-*O*-isopropylidene-*D*-galactopyranose (III) in methanol.* 4,6-*O*-Isopropylidene-D-galactopyranose (III, 75 mg) in methanol (25 ml) was stirred at reflux temperature for 30 min with silver carbonate on Celite (2 g). TLC (solvent B) showed a single component detectable with diphenylamine-aniline-phosphoric acid, giving the same colour as 1,2-*O*-isopropylidene-β-D-threofuranose (XII); the mobility relative to that of XII was 0.75. In addition, small amounts of a slightly slower moving compound, detectable with the ester reagent, were also observed. The solution was filtered and the solvent evaporated to give a syrupy residue which was hydrolyzed in 30 % aqueous acetic acid for 2 h at 45 °C. After removal of the solvents, TLC (solvent A) showed only one compound detectable with diphenylamine-aniline-phosphoric acid, indistinguishable from D-threose (XI). Treatment of the product with acetone (10 ml) containing 1 % sulfuric acid for 2 h, neutralization with solid sodium hydrogencarbonate, filtration and evaporation of the solvent gave a syrup (35 mg) from which a few crystals of 1,2-*O*-isopropylidene-β-D-threofuranose were obtained on treatment with ethyl acetate-light petroleum, m.p. 76 – 81 °C (lit.[13] 83 – 84 °C). Preparative of TLC the syrup (solvent B) yielded chromatographically homogeneous 1,2-*O*-isopropylidene-β-D-threofuranose (XII), the yield was 14 mg, $[\alpha]_D^{22}$ −15° (*c* 1, acetone) (lit.[13] – 15.1°).

*Oxidation of 4,6-*O*-isopropylidene-*D*-galactopyranose (III) in benzene and dimethylformamide.* 4,6-*O*-Isopropylidene-D-galactopyranose (III, 95 mg) in dimethylformamide (20 ml) was poured into a stirred mixture of silver carbonate on Celite (2 g) and benzene (100 ml) at 80 °C. The stirring was continued for 25 min at reflux temperature, the solution was then filtered and the solvents evaporated. Treatment of the residue with Dowex 1 (HCO_3^-) ion exchanger in methanol and subsequent removal of the solvent yielded a chromatographically (TLC, solvent B) homogeneous syrup (62 mg), the mobility relative to that of 2,3-*O*-isopropylidene-D-lyxose (VIII) was 0.84. Hydrolysis of a part of the product (5 mg) in 3 % aqueous acetic acid at 45 °C for 2 h and subsequent removal of the solvents afforded a product indistinguishable from D-lyxose by electrophoresis (M_{glc} 0.67) and paper chromatography.

The remaining part (57 mg) of the above product was oxidized with silver carbonate on Celite (3 g) in benzene (50 ml) at reflux temperature for 75 min. The solution was filtered and the solvent removed giving a product which crystallized from ethyl acetate-light petroleum (40 mg). The compound, 3,5-*O*-isopropylidene-D-lyxono-1,4-lactone (XIV), had m.p. 137 – 138 °C, $[\alpha]_D^{22}$ +22° (c 2, acetone), the infrared spectrum showed strong absorption at 1785 cm^{-1} (in $CHCl_3$), characteristic of 1,4-lactones. The mobility (TLC, solvent C) relative to that of 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX) was 0.86. Mass spectrum: *m/e* 173 (9, $M^+ - 15$), 159 (2), 149 (1), 145 (2), 131 (7), 130 (6), 113 (4), 101 (16), 85(12), 73 (5), 59 (61), 43 (100).

The lactone XIV (30 mg) was treated with acetone (10 ml) containing 1 % sulfuric acid for 2 h. The solution was neutralized with solid sodium hydrogencarbonate and filtered. TLC showed the presence of a compound indistinguishable from 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX) (solvents B and C). In addition, substantial amounts of a compound with mobility corresponding to that of the starting material were also present.

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Reactions Between Formaldehyde and Polyhydric Alcohols. I. Five-, Six-, Seven-, and Twelve-membered Cyclic Acetals from *meso*-Erythritol and Formaldehyde

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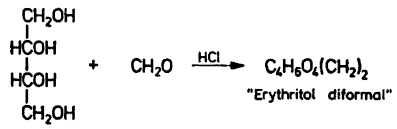
The reaction between *meso*-erythritol and formaldehyde has been studied. By using aqueous formaldehyde as reagent and hydrochloric acid as catalyst, all the possible bicyclic acetals composed of one molecule of *meso*-erythritol and two molecules of formaldehyde (II, VI, VII) were generated, as well as *E*-4-hydroxymethyl-1,3-dioxane-5-ol (V).

If the reaction takes place in benzene, with paraformaldehyde as reagent and *p*-toluenesulfonic acid as catalyst compounds II, VI, VII and one additional compound (VIII) are generated. This compound is composed of two molecules of *meso*-erythritol and four molecules of formaldehyde. Furthermore, it is shown that compound V is the precursor of compounds VI and VIII.

INTRODUCTION AND RESULTS

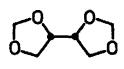
It is known that some polyvalent alcohols react with formaldehyde under acidic conditions to give cyclic acetals of varying ring size.[1] This is interesting from a mechanistic as well as a stereochemical point of view, and it was decided to study some of the reactions, using the little examined butanetetrols as substrates. These substrates appeared to present all the basic intriguing possibilities for reactions, but due to their simplicity, they also appeared to offer excellent possibilities for the understanding of the complex reaction sequences involved.

A search of the literature revealed that little attention had been paid to these substrates. In



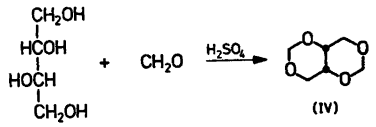
(I)

1896 it was published that the reaction between erythritol (I) and aqueous formaldehyde, with hydrochloric acid as catalyst, led to a compound called "erythritol diformal",[2] which according to Beilstein's "Handbuch der Organischen Chemie" was *meso*-4,4'-bi[1,3]dioxolane (II) (System number 3008).



(II)

More recently the formation of 1,3-dioxano-[*Z*-5,4-*d*]-1,3-dioxane (IV) from threitol (III) was reported.[3]

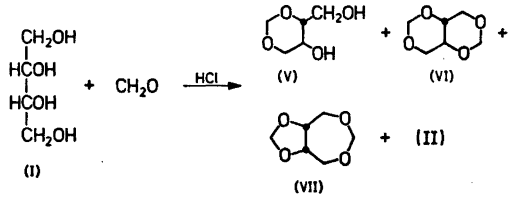


(III) (IV)

We therefore decided to examine in details the reactions between butane-1,2,3,4-tetrols and formaldehyde, and the present paper describes the reaction between erythritol and formaldehyde under various conditions.

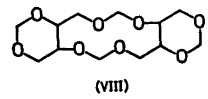
* To whom the correspondence should be addressed.

By repeating the reactions under conditions similar to those previously employed,[2] *i.e.*, 40 % aqueous formaldehyde and concentrated hydrochloric acid, we obtained a complex reaction mixture, from which three compounds were isolated in the pure state. Their structures have been determined as shown below (formulas V, VI and VII).



Furthermore, a fourth component was observed, but it could not be isolated in the pure state from the above reaction mixture. However, it was later shown to have the structure corresponding to the original suggestion for the so-called "erythritol diformal", *i.e.*, structure II, whereas compound VI has the same m.p. as that of the originally reported "diformal". Consequently we assume that "erythritol diformal" has structure VI.

The reaction between erythritol (I) and paraformaldehyde in benzene with *p*-toluenesulfonic acid as the catalyst led to a mixture of products, from which compounds II, VI and VII were isolated, as well as a new compound in low yield, which has been assigned structure VIII.

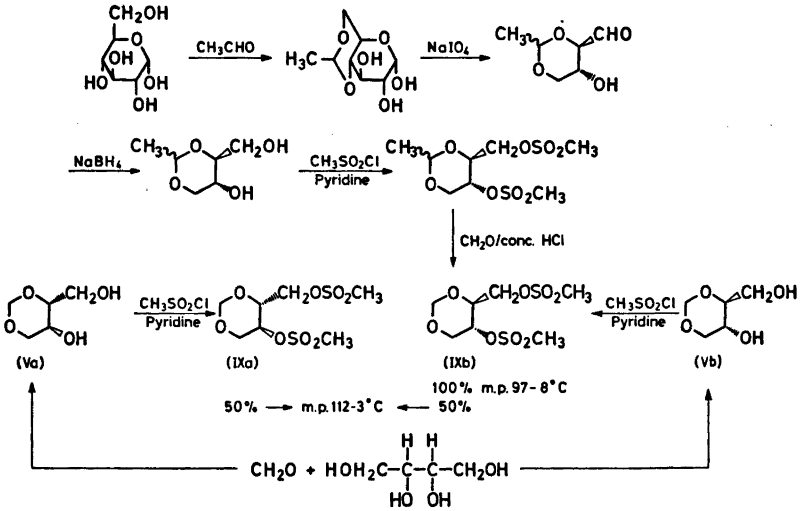

(VIII)

It should be noted that the detailed stereochemistry of VIII has not yet been determined, and that compounds VI and VIII can also be formed by the reaction of V with paraformaldehyde in benzene.

STRUCTURE DETERMINATION

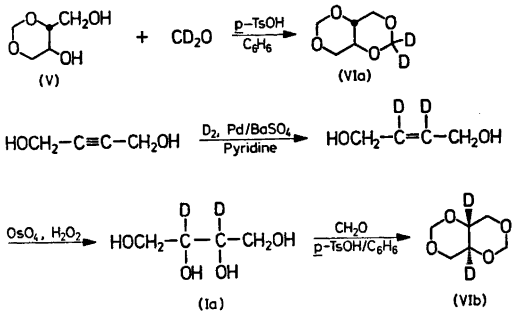
The structure of compound V is in agreement with IR, NMR, and mass spectral data, as well as with elemental analysis. Furthermore, unambiguous evidence for the assignment was found by means of chemical reactions, as shown in Scheme I.

From this scheme it is seen that α-D-glucose in a known series of reactions [4] can be converted to a compound, IXb, with the indicated absolute configuration. For comparison racemic V was treated with methanesulfonyl chloride to give a racemic mixture, which exhibited IR, NMR and mass spectra identical with those of compound IXb. This constitutes the basis for our structural assignment. It should be noted that the IR spectra are only identical when recorded in solution, and that the melting points of IXb and the product from V are different. However, this is what can be expected since compound V leads to a racemic mixture of IXa



Scheme 1.

and IXb, whereas the sample prepared from glucose consists of only one stereoisomer.



Scheme 2. (a) Preparation of: 1,3-dioxano-[*E*-5,4-*d*]-1,3-dioxane-2,2-d_2 (VIa); (b) Preparation of: 1,3-dioxano[*E*-5,4-*d*]-1,3-dioxane-4a,8a-d_2 (VIb).

The assignment of structure to compound VI is based on the following observations: (a) Compound VI can be prepared in a high yield from compound V and paraformaldehyde. (b) After reaction between perdeuterioparaformaldehyde and compound V the resulting compound VI contained only one $-CD_2$-group. This shows that compound V does not undergo ring-opening during the reaction. (c) The NMR-spectra of VI and of two different dideuterated derivatives (VIa and VIb) are in agreement with the proposed structures, as seen from Fig. 1. (d) That $J_{1,2} = -6.2$ Hz indicates that the $O-CH_2-O$ groups are part of six-membered rings (not five-membered) in chair conformations.[5-8] (e) The centro-symmetrical character of VI was indicated by ^{13}C magnetic resonance studies, which showed only three lines (proton decoupling). (f) Furthermore, the value of the dipole moment in benzene is found to be 0.97 D.

The structures of compounds II and VII are based on their NMR and mass spectra. A detailed study of their configuration and conformational equilibria is in progress and will be published later.

The gross structure of compound VIII follows

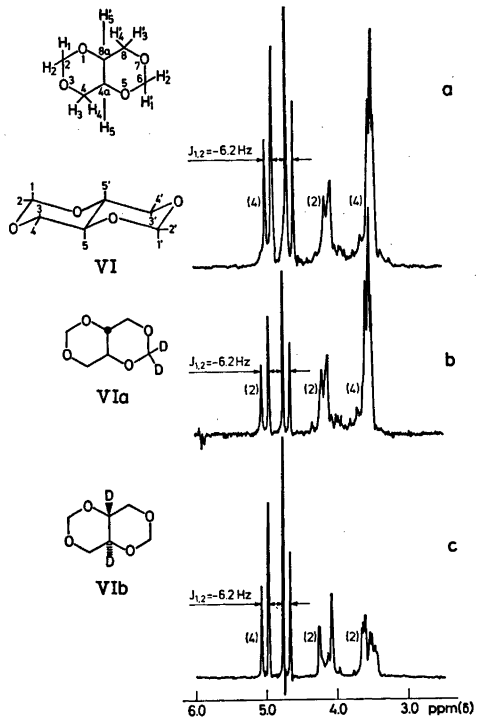


Fig. 1. The ^1H NMR spectra of (a) 1,3-dioxano-[*E*-5,4-*d*]dioxane; (b) 1,3-dioxano[*E*-5,4-*d*]-1,3-dioxane-2,2-d_2; (c) 1,3-dioxano[*E*-5,4-*d*]-1,3-dioxane-4a,8a-d_2. The figures in parentheses refer to the number of protons.

from its synthesis from compound V and paraformaldehyde, which strongly indicates the presence of two 1,3-dioxane rings. However, this independent synthesis does not permit any conclusions to be drawn about the detailed structure. *A priori*, two structural isomers, A and B, would be envisaged, each of which could exist in several diastereoisomeric forms.

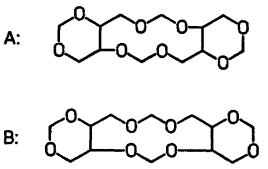
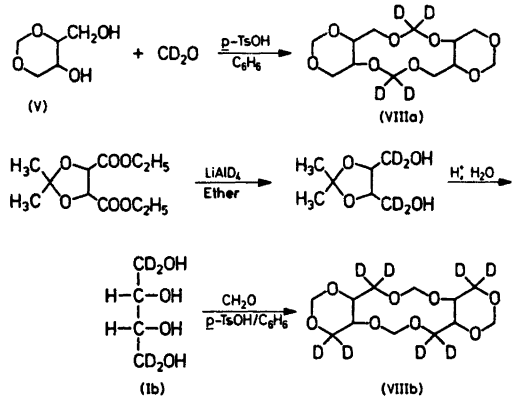


Table 1. ^{13}C chemical shifts (ppm) of VI and VIII.

VI	C-2=C-6		C-4=C-8		C-4a=C-8a	
	93.73		68.22		73.79	
VIII	C-7=C-11	C-3=C-13	C-5=C-15	C-6=C-16	C-8=C-18	C-10=C-20
	78.19	93.08	64.47	68.22	91.53	63.18

The ^{13}C and ^{1}H NMR spectra of VIII (Table 1 and Fig. 2) strongly suggest that only one pair of enantiomers is formed, and since the ^{13}C spectrum contains six lines, we assign structure A to this compound. Similarly the ^{1}H NMR spectrum of VIII and the ^{1}H NMR spectra of the two deuterated derivatives VIIIa and VIIIb are in agreement with this assignment. From these spectra it can be seen that there are only two different $O-CH_2-O$ groups in compound VIII. $J_{2,3}=J_{12,13}=-6.1$ Hz again indicates a six-membered ring in a chair form and $J_{7,8}=J_{17,18}=-7.5$ Hz points to a slightly different geometry of $O-CH_2-O$ in the twelve-membered ring. If the other pair of enantiomers B had been present, there would have been three different $O-CH_2-O$ groups. The deuterated derivatives of compound VIII were prepared as depicted below.



Scheme 3. (a) Preparation of 2,4,7,9,12,14,17,19-octaoxa-tricyclo[4.14.$0^{1,6}$.$0^{11,16}$]eicosane-8,8,18,-18-d_4; (b) Preparation of 2,4,7,9,12,14,17,19-octaoxa-tricyclo[4.14.$0^{1,6}$.$0^{11,16}$]eicosane-5,5,10,-10,15,15,20,20-d_8.

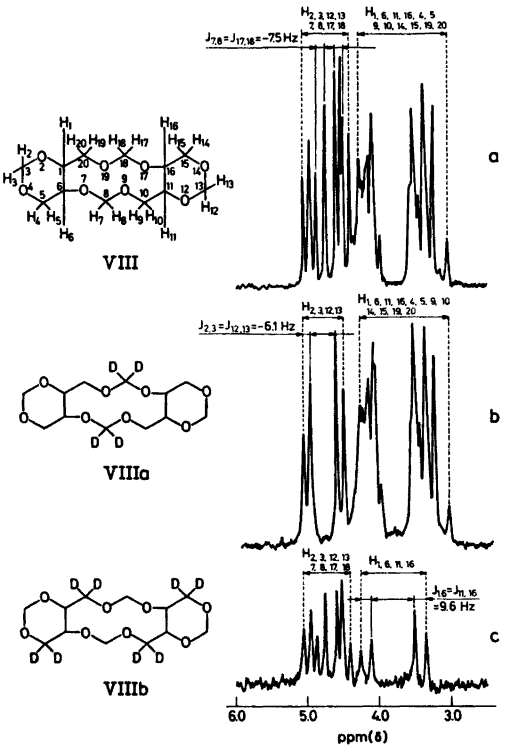
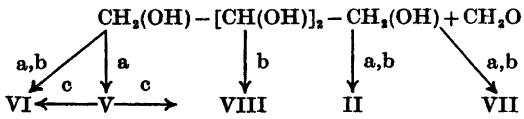


Fig. 2. The ^{1}H NMR spectra of (a) 2,4,7,9,12,-14,17,19-octaoxa-tricyclo[4.14.$0^{1,6}$.$0^{11,16}$]eicosane; (b) 2,4,7,9,12,14,17,19-octaoxa-tricyclo-[4.14.$0^{1,6}$.$0^{11,16}$]eicosane-8,8,18,18-d_4; (c) 2,4,7,9,-12,14,17,19-octaoxa-tricyclo[4.14.$0^{1,6}$.$0^{11,16}$]-eicosane-5,5,10,10,15,15,20,20-d_8. The hydrogen atoms at C_1 and C_6 and at C_{11} and C_{16} are *trans*.

EXPERIMENTAL

^{1}H NMR spectra were obtained at 60 MHz in deuteriochloroform using a Varian A-60 spectrometer at ambient temperature (40 °C). The shift reference was internal TMS. Noise-decoupled ^{13}C NMR spectra were determined with a Bruker WH-90 system (22.63 MHz) operating in the Fourier transform mode. The samples were examined at ambient temperature (40 °C) as saturated solutions in $CDCl_3$ and shieldings were measured relative to internal TMS. The IR spectra were recorded on a Perkin-Elmer 337 Grating Infrared Spectrophotometer using chloroform as solvent. The mass spectra were recorded on an AEI MS-902 mass spectrometer at 70 eV. The sample was introduced through a direct inlet system. The analytical GLC was made on a Perkin-Elmer F 11 instrument at 95 °C. Column: Silicone gum rubber (10 % on Gaschrom Q, 100/120 mesh), 3 mm × 2.0 m, flow 30 ml/min, injection temp. 130 °C. The gas chromatographic separations were made on a Perkin-Elmer "Preparative Gas Chromatograph" F 21, using a silicone rubber gum column at 95 °C (20 % on PE Chromosorb W NAV 60/80 mesh, 6 mm × 1.0 m, flow 400 ml N_2/min, injection temp. 130 °C). Retention times: Chloroform 1.1 min, VI 10.3 min and VII 12.9 min. In each cycle 2.5 µl of the mixture was injected. The dipole moment of VI was determined by measuring dielectric constants and densities of five solutions in benzene (molar fraction range 0.00 to 0.04) at 25 °C. The dielectric measurements were carried out by means of a low frequency Schering-bridge.[9]



Scheme IV.

The various reaction paths for the reaction between *meso*-erythritol (I) or DL-*E*-4-hydroxymethyl-1,3-dioxane-5-ol (V) and formaldehyde:

Method a. (I) + 35 % aq. CH_2O + conc. HCl.

Method b. (I) + paraformaldehyde + *p*-toluenesulfonic acid in benzene.

Method c. (V) + paraformaldehyde + *p*-toluenesulfonic acid in benzene.

Method a

Procedure 1. DL-E-*4-Hydroxymethyl-1,3-dioxane-5-ol (V) and 1,3-dioxano*[E-*5,4*-d]-*1,3-dioxane (VI).* A solution of *meso*-erythritol (I) (50 g, 0.41 mol) in 35 % aqueous formaldehyde (75 ml, 0.95 mol) and concentrated hydrochloric acid (50 ml, 0.60 mol) was heated under reflux for 2 h. The water was removed from the reaction mixture by distillation under reduced pressure (14 mmHg), through a 15 cm fractionating column. On standing 24 h at room temperature, white crystals of VI precipitated from the distillate. The same material was also found deposited in the column. 10 ml of absolute ethanol was added to the residue from the distillation, and after 24 h at room temperature white needles of VI separated. These crystals were collected, and the solvent was removed by distillation in the same way as mentioned above. The residue from this distillation was redistilled at 130 – 140 °C/0.04 mmHg, and 19 g of V (35 %) was obtained as a viscous liquid, which was brought to crystallize by cooling in a dry ice-acetone mixture, and after recrystallization from ether, the m.p. was 53 – 54 °C. VI was recrystallized from absolute ethanol, m.p. 100 – 101 °C. The combined yields of VI were 8.4 g (0.057 mol, 14 %).

Procedure 2. V, VI, 1,3-Dioxolano[E-*5,4*-e]-*1,3-dioxepane (VII) and* meso-*4,4'-bi*[*1,3*]-*dioxolane (II).* A solution of *meso*-erythritol (I) (100 g, 0.82 mol) in 35 % aqueous formaldehyde (150 ml, 1.9 mol) and concentrated hydrochloric acid (100 ml, 1.2 mol) was treated in the same way as described in procedure 1. The distillate was extracted continuously with ether for 24 h (ether phase 1). The residue from the distillation was extracted with 4 × 50 ml portions of ether (ether phase 2). The residue from this extraction was concentrated to dryness on a Rotavapor (100 °C/14 mmHg) and distilled at 130 – 140 °C/0.05 mmHg to give 35 g of solid, which after recrystallization from ether gave 26 g (24 %) of V. The mother liquor was saved (ether phase 3). Ether phase 1 was cooled to – 80 °C, filtered and concentrated to 10 ml. GLC on the concentrate showed the presence of three components. By means of PGLC on the concentrate 60 mg (0.05 %) of VII was isolated in the pure state. The compounds VI and II could not be separated in this way. The ether was removed from the combined ether phases 2 and 3 to give a crystalline solid. Recrystallization from absolute ethanol gave 25 g of VI (21 %).

Method b

VI, VII, II and 2,4,7,9,12,14,17,19-Octaoxatricyclo[*4.14.0.*^{1,8}*.0*^{11,16}]*eicosane (VIII).* A suspension of *meso*-erythritol (15 g, 0.12 mol), paraformaldehyde (15 g, 0.5 mol) and *p*-toluenesulfonic acid (0.2 g, 0.0012 mol) in 200 ml of benzene was stirred and gently refluxed for 20 h. In order to neutralize the acidic catalyst, Na_2CO_3 (3 g, 0.03 mol) was added to the reaction mixture, which was then stirred for 2 h. The solid material was filtered off and washed several times with acetone. The washings were added to the filtrate, and the solvents (benzene and acetone) were removed by distil-

Table 2. Melting points, molecular weights (from mass spectroscopy) and formulas of compounds II – VIII together with their composition in number of units of *meso*-erythritol(I) (or V) and CH_2O.

Compound	Formula	M	m.p. °C	Yield (%) a	b	c	Number of units *meso*-erythritol	V	CH_2O
V	$C_5H_{10}O_4$	134	53 – 54	35,[a] 24 [b]			1		1
VI	$C_6H_{10}O_4$	146	100 – 101	14,[a] 21 [b]	33		1		2
VI	$C_6H_{10}O_4$	146	100 – 101			86		1	1
VII	$C_6H_{10}O_4$	146	84 – 85		17 [c]		1		2
II	$C_6H_{10}O_4$	146	unknown		50 [c]		1		2
VIII	$C_{12}H_{20}O_8$	292	232 – 233		4.5		2		4
VIII	$C_{12}H_{20}O_8$	292	232 – 233			11		2	2

[a] Procedure 1. [b] Procedure 2. [c] Yields estimated from ^1H NMR.

lation through a short column. The residue from the distillation partly crystallized on standing. The crystals were collected by filtration and recrystallized from absolute ethanol, this gave VIII (0.8 g, 4.5 %) m.p. 232–233 °C. The ethanolic solution from the recrystallization was concentrated to dryness, and recrystallization of this from acetone gave VI (6 g, 33 %). The mother liquor from above was shown to contain mainly II and VII by ^{1}H NMR spectroscopy, corresponding to a yield of about 50 % and 17 %, respectively. However, until now, all efforts to isolate II in the pure state have been unsuccessful.

Method c

VI and VIII. A suspension of DL-*E*-4-hydroxymethyl-1,3-dioxane-5-ol (3 g, 0.022 mol), paraformaldehyde (4 g, 0.13 mol) and *p*-toluenesulfonic acid (0.1 g, 0.0006 mol) in 25 ml benzene was stirred and gently refluxed for 20 h. During the reaction formaldehyde sublimed into the reflux condensor, and at the end of the reaction the reaction mixture became clear. On cooling in an ice-water mixture a crystalline material separated. This was recrystallized twice from 96 % ethanol to give VIII (0.35 g, 11 %). The mother liquor was concentrated to dryness and recrystallized from 50 % ethanol to give VI (2.8 g, 86 %), m.p. 100–101 °C.

Racemic E-*4-hydroxymethyl-1,3-dioxane-5-ol-di*-O-*methanesulfonate (IXa+b).* DL-*E*-4-Hydroxymethyl-1,3-dioxane-5-ol (Va+b) prepared by the above described procedures 1 and 2, was converted by conventional methods (see Ref. 4) into the di-*O*-methanesulfonate. Recrystallization from ethyl acetate gave m.p. 112–113 °C.

Optically active E-*4-hydroxymethyl-1,3-dioxane-5-ol-di*-O-*methanesulfonate (IXb) (=1,3*-O-*methylene-2,4-di*-O-*methanesulfonate*-L-*erythritol).* This compound was prepared according to Barker *et al.*[4] The melting point 97–98 °C was, in spite of careful purification, constantly 20 °C below that reported.[4] The 1,3-*O*-ethylidene-L-erythritol used was prepared according to a method given by Barker and MacDonald.[10]

1,3-Dioxano[E-*5,4*-d]-*1,3-dioxane-2,2*-d_2 *(VIa)* was prepared from DL-*E*-4-hydroxymethyl-1,3-dioxane-5-ol (Va+b) and perdeuterioparaformaldehyde using method c.

2,4,7,9,12,14,17,19-Octaoxa-tricyclo-[*4.14.0*^{1,6}*.0*^{11,16}]*eicosane-8,8,18,18*-d_4 *(VIIIa)* was prepared from DL-*E*-4-hydroxymethyl-1,3-dioxane-5-ol (Va+b) and perdeuterioparaformaldehyde using method c.

2,4,7,9,12,14,17,19-Octaoxa-tricyclo-[*4.14.0*^{1,6}*.0*^{11,16}]*eicosane-5,5,10,10,15,15,20,20*-d_8 *(VIIIb)* was prepared through the following reactions: Diethyl-2,3-D-isopropylidene *meso*-tartrate was reduced with lithium aluminium deuteride analogously to the procedure of Feit [11] to give 4,5-(dihydroxyperdeuteriomethyl)-2,2-dimethyl-1,3-dioxolane. This compound was then hydrolysed with 1 M hydrochloric acid, and the reaction mixture was evaporated to dryness. Recrystallization from absolute ethanol gave *meso*-1,2,3,4-butanetetrol-1,1,4,4-d_4 (Ib). Treatment of Ib with paraformaldehyde in accordance with method b gave VIIIb.

cis-*2-Butene-1,4-diol-2,3*-d_2. This compound was prepared from 2-butyne-1,4-diol by deuteration, adopting the method given by Feit [12] for the analogous hydrogenation. The Lindlar catalyst was in this case replaced with 5 % palladium on barium sulfate.

meso-*1,2,3,4-Butanetetrol-2,3*-d_2 *(Ia)* was prepared from *cis*-2-butene-1,4-diol-2,3-d_2 by hydroxylation with hydrogen peroxide and osmium tetroxide according to Reppe.[13]

1,3-Dioxano[E-*5,4*-d]-*1,3-dioxane-4a,8a*-d_2 *(VIb)* was prepared from Ia and paraformaldehyde by means of method b.

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The Crystal and Molecular Structure of Δ^9-Tetrahydrocannabinolic Acid B

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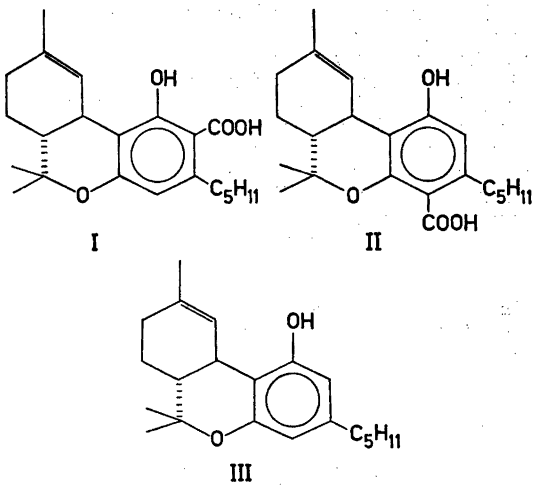
The crystal and molecular structure of the title compound $C_{22}H_{30}O_4$, has been determined by X-ray methods using 1106 reflections above background level collected by counter methods. The crystals are orthorhombic, space group $P2_12_12_1$ with cell dimensions $a=16.514(2)$ Å; $b=14.324(2)$ Å; $c=8.744(1)$ Å; there are four molecules per unit cell. The structure was refined to an R of 0.084 (weighted $R_w=0.068$).

The cyclohexene and the pyran part of the molecule occurs in the half-chair conformation. The bond distances and angles, and a slight twist of the benzene ring, indicate considerable strains in the aromatic system. Both the phenolic and carboxylic group are significantly out of the plane through the aromatic ring. The angle between this plane and a plane through the cyclohexene ring is 37.7°. The pentyl side-chain occurs in an extended *gauche* conformation, and the thermal parameters of this part of the molecule are very high.

The molecules are held together by van der Waals forces in the c-directions, and hydrogen bonds (2.688 Å) from phenolic to carboxylic groups in the $a-b$ plane. There is a short *intra*-molecular hydrogen bond (2.490 Å) from the carboxylic group to the pyran oxygen.

It is now well established that the major psycotomimetic constituent in hashish is *l*-Δ^9-tetrahydrocannabinol (Δ^9-TCH).[1] Although a great deal of information about the structure-activity relationship has been collected over the years, and empirical correlation of these data have been inferred,[2] the mode and site of action of Δ^9-THC still remains obscure.

While most cannabinoids are oily liquids or very low melting compounds, Mechoulam *et al.*[3] have isolated in some samples of hashish a crystalline isomer of Δ^9-THC acid. In order to distinguish between the two isomers, they call them, respectively, Δ^9-THC acid A (I) (the more common component of hashish) and Δ^9-TCH acid B (II). Neither of these are hallucinogenic *per se*, but are decarboxylated upon heating (smoking) to form the active Δ^9-THC (III).



To get a basis for further studies of the interrelationship between the molecular structure and pharmacological activity of cannabinoids, a structure determination would be of interest. A crystal structure investigation of Δ^9-THC acid B was therefore carried out.

EXPERIMENTAL

The product obtained from R. Mechoulam was recrystallized by slow evaporation of a chloroform solution. Colourless, prismatic crystals were formed. A crystal of dimensions $0.45\times0.30\times0.12$ mm was selected for the crystallographic work.

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Oscillation and Weissenberg diagrams indicated orthorhombic symmetry with systematic extinctions corresponding to the space group $P2_12_12_1$. Unit cell parameters were determined from diffractometer measurements of 15 general reflections. A Picker manual diffractometer was applied using Cu$K\beta$ radiation ($\lambda = 1.3922$ Å) and a take-off angle of 1°. The computer program utilized in the least-squares calculations of cell parameters, as well as programs used in all subsequent calculations, are part of a local assembly of programs for a CYBER-74 computer which is described in Ref. 4.

Three-dimensional intensity data were recorded on an automatic Picker four-circle diffractometer with graphite monochromated Mo$K\alpha$ radiation using the $\omega - 2\theta$ scanning mode with a 2θ scan speed of 1° min^{-1}. Background counting time was 40 s at each end of the scan range. The temperature was kept constant within 1° at 18 °C. The variations in the intensities of three standard reflections which were remeasured after every fifty reflections were random and less than three times their standard deviations. Accordingly no corrections were applied for these variations.

The estimated standard deviations were taken as the square root of the total count with a 2 % addition for experimental uncertainties. Of the 1854 symmetry-independent reflections measured ($2\theta_{max} = 50°$), 1109 had intensities larger than twice their standard deviations. These were regarded as "observed" reflections, and the remaining reflections were excluded from further calculations. The intensities were corrected for Lorentz and polarization effects.

The atomic scattering factors used were those of Doyle and Turner [5] for carbon and oxygen, and of Stewart *et al.*[6] for hydrogen.

CRYSTAL DATA

Δ^9-Tetrahydrocannabinolic acid B, $C_{22}H_{30}O_4$, orthorhombic. Cell dimensions: $a = 16.514(2)$ Å; $b = 14.324(2)$ Å; $c = 8.744(1)$ Å. Figures in parentheses are estimated standard deviations. $V = 2068.5$ Å^3, $M = 358.5$ amu; $D_{obs} = 1.12$ g/cm^3; $Z = 4$; $D_{calc.} = 1.151$ g/cm^3.; $F(000) = 776$. Absent reflections: ($h00$) for h odd; ($0k0$) for k odd; ($00l$) for l odd; space group $P2_12_12_1$.

STRUCTURE DETERMINATION

The phase problem was solved by a computer procedure [7] based on direct methods utilizing tangent refinement.

The structure model was refined to a conventional R of 0.19. Introduction of anisotropic thermal parameters for all nonhydrogen atoms and least-squares refinement yielded an R of 0.14. At this point the hydrogen atoms were placed in calculated positions (C–H: 1.0 Å, O–H: 0.95 Å) and included in the structure factor calculations. Three reflections [(13 4 1), (0 1 4), (3 0 7)] which were obviously mismeasured, (due to diffractometer errors), were removed from the data set (leaving 1106 F_o's).

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations ($\times 10^4$) for non-hydrogen atoms. The temperature factor is given by $\exp -(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}hl + B_{13}hl + B_{23}kl)$.

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
O1	2099(3)	1829(4)	2856(8)	51(3)	61(4)	336(14)	-17(6)	-13(12)	-45(13)
C2	1547(5)	1081(6)	2705(10)	44(4)	50(5)	193(18)	-22(8)	-29(16)	55(18)
C3	741(5)	1297(6)	3127(11)	40(4)	48(5)	285(20)	3(8)	-11(16)	-1(19)
C4	184(5)	573(7)	3041(12)	32(4)	58(7)	323(23)	-18(9)	11(16)	22(22)
C5	458(5)	-287(6)	2492(11)	51(5)	46(5)	281(20)	-12(8)	39(17)	-17(19)
C6	1228(5)	-453(6)	2005(10)	47(4)	49(5)	169(16)	1(8)	34(14)	-49(17)
C7	1832(5)	226(6)	2154(9)	44(4)	48(5)	281(16)	-4(8)	-19(15)	-33(17)
C8	2738(4)	94(6)	1746(9)	39(4)	71(6)	183(18)	9(9)	41(14)	20(18)
C9	3062(5)	-829(6)	2198(12)	45(4)	82(7)	267(21)	5(9)	28(18)	-74(21)
C10	3846(6)	-961(8)	2544(12)	54(5)	101(8)	299(22)	27(11)	57(20)	-73(24)
C11	4439(5)	-162(8)	2579(14)	48(4)	112(9)	394(27)	-21(11)	10(28)	-114(38)
C12	4111(6)	725(7)	1904(12)	57(5)	103(8)	366(27)	-58(11)	49(21)	13(26)
C13	3235(5)	862(5)	2435(10)	38(4)	74(6)	221(19)	-32(8)	21(14)	-48(21)
C14	2696(6)	1819(7)	2131(12)	53(5)	89(8)	184(21)	-38(10)	20(18)	-15(22)
O15	1429(3)	-1277(4)	1397(7)	53(3)	56(3)	263(11)	-21(5)	45(9)	-92(12)
C16	439(7)	2263(7)	3578(15)	61(6)	75(8)	355(27)	-24(12)	-1(25)	38(25)
O17	-234(4)	2455(4)	3837(11)	59(4)	68(4)	628(26)	49(7)	76(18)	22(18)
O18	1099(4)	2919(4)	3679(12)	72(4)	66(4)	794(30)	16(7)	-4(19)	-169(20)
C19	4186(6)	-1995(8)	3021(16)	81(6)	111(8)	557(39)	72(13)	75(29)	-98(32)
C20	3344(6)	2598(6)	2975(14)	88(6)	75(6)	336(26)	-59(10)	-91(23)	-22(22)
C21	2802(7)	2836(7)	492(15)	133(9)	111(8)	279(23)	-22(15)	23(25)	39(26)
C22	-709(6)	629(7)	3587(14)	66(6)	82(7)	353(25)	34(10)	-44(22)	-1(25)
C23	-1181(7)	939(8)	2315(17)	55(6)	133(10)	464(35)	-18(13)	-22(27)	-42(36)
C24	-2122(12)	989(12)	2683(25)	117(13)	198(17)	696(58)	-51(22)	-104(53)	154(51)
C25	-2506(12)	344(15)	3010(27)	113(11)	303(28)	813(58)	-87(27)	267(43)	24(72)
C26	-3453(7)	408(11)	3067(17)	63(5)	251(14)	549(39)	-66(16)	59(26)	-71(44)

Table 2. Fractional atomic coordinates (calculated) ($\times 10^3$) and isotropic thermal parameters used for hydrogen atoms. A hydrogen atom is bonded to the atom given in parentheses. (For the identities of these see Fig. 1.)

Atom	x	y	z	B
H27 (O15)	101	−171	129	6.0
H28 (O18)	157	275	351	10.0
H29 (C5)	5	−80	235	6.0
H30 (C8)	271	11	55	6.0
H31 (C9)	269	−137	219	8.0
H32 (C11)	493	−36	200	8.0
H33 (C11)	460	−1	365	8.0
H34 (C12)	410	65	69	8.0
H35 (C12)	445	127	210	8.0
H36 (C13)	322	79	360	6.0
H37 (C19)	478	−181	330	11.0
H38 (C19)	414	−233	214	11.0
H39 (C19)	389	−215	393	11.0
H40 (C20)	391	266	255	11.0
H41 (C20)	337	244	408	11.0
H42 (C20)	305	320	283	11.0
H43 (C21)	334	203	−3	11.0
H44 (C21)	254	265	35	11.0
H45 (C21)	245	154	0	11.0
H46 (C22)	−75	108	440	11.0
H47 (C22)	−89	0	385	11.0
H48 (C23)	−109	52	144	11.0
H49 (C23)	−100	159	205	11.0
H50 (C24)	−237	118	161	14.0
H51 (C24)	−221	151	336	14.0
H52 (C25)	−231	26	413	14.0
H53 (C25)	−231	−18	242	14.0
H54 (C26)	−365	−21	346	15.0
H55 (C26)	−364	47	197	15.0
H56 (C26)	−364	91	369	15.0

Full-matrix least-squares refinement of all positional and anisotropic thermal parameters for all non-hydrogen atoms resulted in a conventional R of 0.084 and a weighted R_w of 0.068.

Atomic parameters for non-hydrogen atoms are listed in Table 1, and parameters used for hydrogen atoms are given in Table 2. A listing of observed and calculated structure factors is available from the authors upon request. (May also be obtained from: Department of Chemistry, University of Oslo, Oslo 3, Norway).

Standard deviations in molecular parameters were calculated from the correlation matrix ignoring standard deviations in cell parameters.

DISCUSSION

Description of the structure. The molecular parameters and the numbering of the atoms are presented in Fig. 1. A sketch of the molecular conformation is given in Fig. 2. The absolute configuration at the chiral centers is R,R, as determined by Mechoulam.[8]

The variation of the bond angles in the benzene ring [126.6°(7) − 113.8°(7)], indicates a considerable strain in the aromatic system. This is also implied by the differences in bond lengths and the deviations from planarity (see Plane A, Table 4), which indicates a slightly twisted conformation of the benzene ring. This strain is probably due to the pyran ring. However, the short intramolecular O1 − O18 contact may also affect the conformation of the aromatic system to some extent. The phenolic oxygen is bent 0.096 Å out of the plane, and away from the C9 proton (H31). The distance between this proton and O15 is only 2.20 Å (assuming normal C − H bond length and angle). From Westheimer calculations and NMR studies on Δ^9-THC, Archer *et al.*[9] report this distance to be 2.30 Å. Their calculations also show that the phenolic group is bent out of the plane, and a slight opening of the O15 − C6 − C7 angle. The last effect is not observed here. The C6 − O15 distance, 1.337(8) Å is somewhat shorter than the normal phenolic C − O bond of 1.36 − 1.38 Å.

The carboxylic groups is also significantly out of plane A, and at the same side as the phenolic group. From spectroscopic data (carboxylic absorptions: Acid A: $\nu_{max} = 1615$ cm^{-1}; Acid B: $\nu_{max} = 1710$ cm^{-1}), Mechoulam [8] concludes that the plane of the carboxylic group is twisted with respect to the aromatic ring in Δ^9-THC Acid B. We do not observe this; the dihedral angle (C4 − C3) − (C16 − O17) is only −1.4° (2.0). This gives a short O18···O1 distance [2.490(8) Å]. Although the hydrogen atom was not located, we believe that this indicates the presence of an intramolecular hydrogen bond of the same type as in *o*-ethoxybenzoic acid.[10] There are no short contacts to other hydrogen bond acceptors from O18. We believe that in both isomers the COOH group is near coplanar with the aromatic ring, but that there are different types of intramolecular hydrogen bonds in the two compounds. In Δ^9-THC Acid B, the COOH is donor and the pyran oxygen acceptor, while in Acid A the phenolic group is probably donor and the carboxylic group acceptor, as in salicylic acid.[11] This will also explain the spectroscopic results.

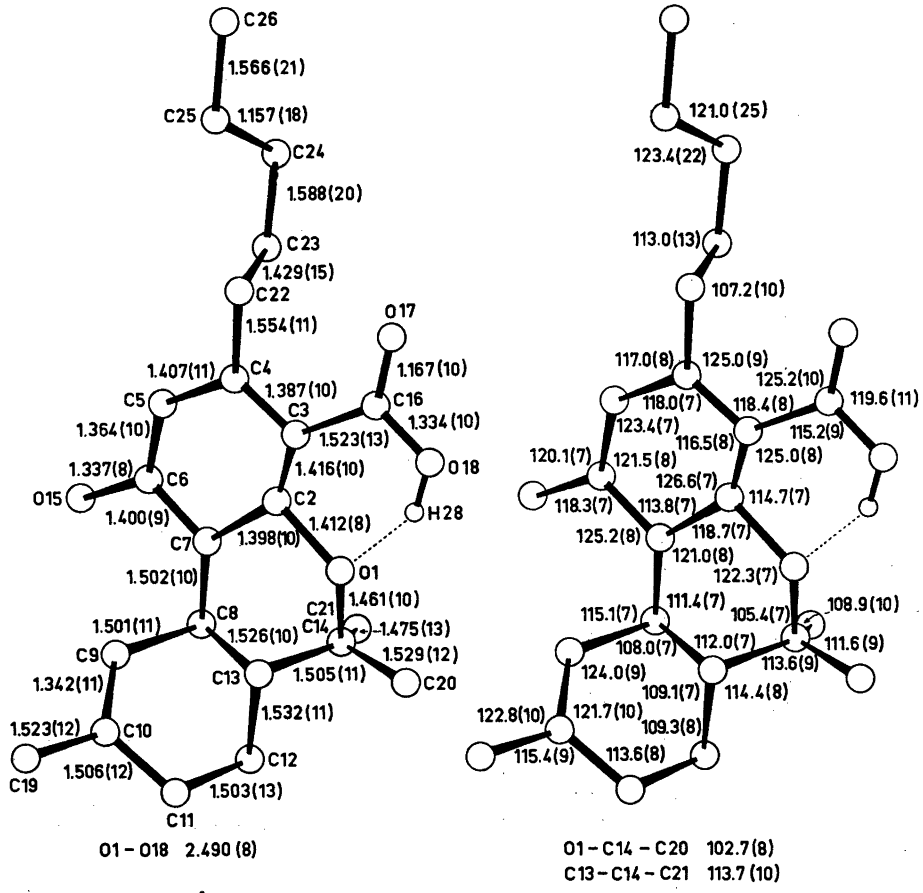


Fig. 1. Bond lengths (Å) and bond angles (°). Estimated standard deviations in the last digit listed for the corresponding parameter are given in parentheses.

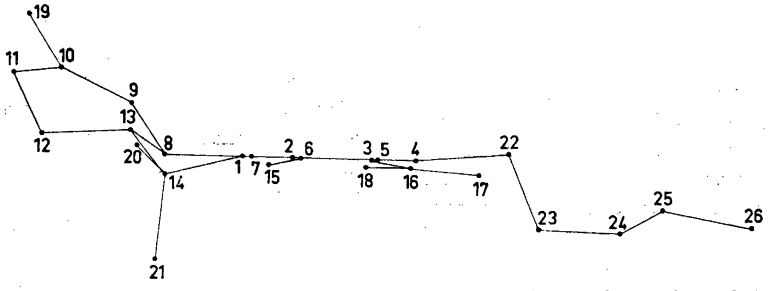


Fig. 2. A view along the plane of the benzene ring, showing the conformation of the molecule.

A least-squares plane through O1, C2, C7, C8 (Plane B, Table 4) shows a half-chair conformation of the pyran ring, with C13 0.422 Å above and C14 0.332 Å below the plane. This gives a staggered conformation around the C13 – C14 bond [the dihedral angle (C8 – C13) – (C14 – O1) is 60.8°], with the C21 methyl group in an axial and the C20 in an equatorial position. This conformation agrees well with the observations of Archer *et al.*[9] from NMR spectroscopy of Δ^9-THC in solution. The angle between plane A and plane B is only 1.0°.

The cyclohexene ring also occurs in the half-chair conformation. Archer *et al.*[9] have calculated this conformer of Δ^9-THC to be 18.8 KJ/mol more stable than the half-boat form. The bond distances and angles agree very well with the electron diffraction results for cyclohexene,[12] although the true two-fold axis through the centers of the C9–C10 and the C12–13 bonds is distorted, with C12 0.206 Å below and C13 0.591 Å above plane C (Table 4).

The conformation of the pentyl side chain (See Fig. 2 and Table 3) is extended and gauche with respect to the C23–C24 bond. The thermal vibrations in this part of the molecule are very high and anisotropic, and the accuracy in the positions low. The thermal libration cause an apparent shortening of the C22–C23 and C24–C25 bond lengths. The plane through C4–C22–C23 is perpendicular to the aromatic ring (90.1°).

Crystal packing. The low density and high thermal parameters indicate weak intermolecular forces and an open packing of the molecules in the crystal. A packing diagram of the crystal structure is given in Fig. 3. This shows that the molecules are stacked in layers perpendicular to the *c*-axis. There are only van der Waals forces between the layers, which are separated by *c*/2. As seen from the diagram, the side chain is located in a very open cave in the crystal. What apparently holds the molecules together, and is the reason for Δ^9-THC Acid B to crystallize, is the intermolecular hydrogen bond network, going from the phenolic group in one molecule to the carboxylic group in the neighbour molecule [O15···O17 2.688(8) Å].

Table 3. Selected dihedral angles (°) with estimated standard deviations. The angles are positive in a righthand screw.

Angle	(°)
C7–C2–O1–C14	14.9 (12)
C2–C7–C8–C9	140.1 (9)
C2–C7–C8–C13	16.6 (12)
C7–C8–C9–C10	−150.8 (10)
C13–C8–C9–C10	−25.6 (13)
C7–C8–C13–C12	−176.6 (8)
C7–C8–C13–C14	−48.9 (10)
C9–C8–C13–C12	56.0 (9)
C9–C10–C11–C12	−11.3 (15)
C10–C11–C12–C13	42.0 (12)
C11–C12–C13–C8	−66.4 (10)
C11–C12–C13–C14	167.2 (9)
C8–C13–C14–O1	60.8 (10)
C12–C13–C14–O1	−174.4 (7)
C2–O1–C14–C13	−44.9 (11)
C2–C3–C16–O18	−5.3 (17)
C4–C3–C16–O17	−1.4 (20)
C3–C4–C22–C23	90.1 (12)
C4–C22–C23–C24	177.5 (10)
C22–C23–C24–C25	−62.4 (30)
C23–C24–C25–C26	−168.8 (14)

Table 4. Deviations from least-squares planes (Å × 10^3). The deviations for those atoms used to define the plane are given in italicized figures. Plane equations:

A, $(-0.0127x + 0.0208y - 0.1065z)R + 2.274 = 0$
C, $(0.0132x - 0.0129y - 0.1096z)R + 0.511 = 0$
B, $(-0.0119x + 0.0201y - 0.1082z)R + 2.272 = 0$

Atom	Plane A	Plane B	Plane C
O1	*−1*	*−1*	1609
C2	*4*	*4*	
C3	*−24*	2	
C4	*22*		
C5	*2*		
C6	*−22*	−42	
C7	*18*	*−4*	693
C8	46	*2*	*1*
C9		868	*7*
C10		1460	*−20*
C11		1352	*6*
C12		326	−206
C13	463	422	591
C14	−311	−332	711
O15	−96		
C16	−174		
O17	−278		
O18	−173		
C19			*7*
C22	132		

Angle between: A and C, 37.7°; A and B, 1.0°; B and C, 36.7°.

MOLECULAR STRUCTURE AND BIOLOGICAL ACTIVITY

As mentioned in the introduction the mode and site of action of Δ^9-THC is still completely unknown. Several investigators have attempted to correlate the structure and hallucinogenic activity of compounds like Δ^9-THC, LSD, and psilocybin, and drawn attention to the structural similarities of the molecules (see, *e.g.*,

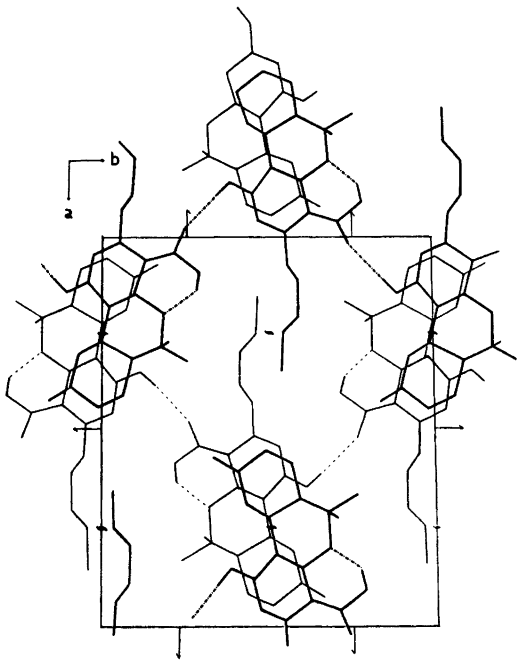


Fig. 3. Crystal packing in the unit cell, as seen along the *c*-axis.

Petrzilka [13a] or Razdan and Pars [13b]). However, the solubility properties of these drugs are quite different. While Δ^9-THC is a hydrophobic molecule, the others are easily soluble in water. This will give a different distribution and adsorption to membranes and enzyme systems in the central nervous system. Also the entire pattern of effects of Δ^9-THC is different from that of any other drugs, so another view [14] is that cannabis should be regarded as unique.

If Δ^9-THC is fixed by hydrogen bonds to a receptor there are only two possible sites for bond formation; the phenolic group and the pyran oxygen, and the distance between these is 4.76 Å. Other types of fixation might be $\pi-\pi$ interaction with the C9 – C10 double bond or the phenyl ring, or van der Waals forces.

As this work represents the first and only direct structure determination of the Δ^9-THC skeleton, and the molecule is substituted and psycotomimetic inactive, it is not possible to draw any absolute conclusions on what is the *active* conformation for Δ^9-THC. However, we want to point out that the effect of the carboxylic group on the rest of the molecule is probably small. We believe therefore that this structure may also describe the Δ^9-THC molecule well, and that the conformation of Δ^9-THC Acid B in the crystal (maybe with exception of the side chain) is that relevant to the hallucinogenic activity of Δ^9-THC.

Added in proof. Del Castillo *et al.* [*Nature* (*London*) *253* (1975) 365], draw attention to the similarities between Δ^9-THC and thujone, the active principle of *Artemisia absinthium* and the liqueur absinthe. They propose that both drugs exert their psychotomimetic effects by interacting with a common receptor in the central nervous system.

Acknowledgement. We wish to express our sincere thanks to Professor Arnold Nordal for valuable support, and to Professor Raphael Mechoulam for supplying the raw material (Δ^9-THC Acid B).

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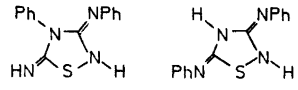
Oxidation of Selenoureas in Protic Medium. The Selenoanalogue to Hugershoff's Base

SVEND TREPPENDAHL

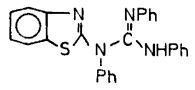
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Three substituted selenoureas have been oxidised in protic medium. The reaction products were substituted benzoselenazolylguanidines in the case of *N*-methyl-*N'*-phenyl- and *N*,*N'*-diphenylselenourea. As by-products a cyclic trimer and a cyclic dimer of the corresponding carbodiimides are formed, respectively. For phenylselenourea the only product is an open chain dimer of phenylcarbodiimide.

Oxidation of substituted thioureas in protic media results in the formation of heterocyclic bases.[1] Depending upon the substitution of the thiourea three different types of heterocycles namely Hector's (I), Dost's (II), and Hugershoff's (III) bases [1,2] are formed. In order to investigate whether the seleno analogue heterocyclic bases are formed upon oxidation of substituted selenoureas, the oxidation of phenyl-, *N*-phenyl-*N'*-methyl- and *N*,*N'*-diphenylselenourea have been carried out.



I II



III

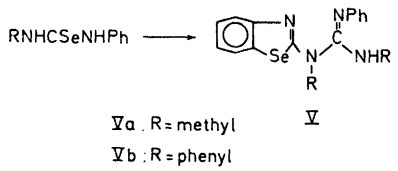
Substituted selenoureas have not previously been subjected to oxidation in protic media. Unsubstituted selenourea is analogous to thiourea, known to form formamidine diselenide.[3] In aprotic medium arylselenoureas form the well known aminobenzoselenazoles.[4]

The oxidations were carried out at room temperature with hydrogen peroxide in ethanol or water-ethanol mixtures with hydrochloric acid as a catalyst. Oxidation of phenylselenourea was expected to give a Hector type (I) heterocycle, but a non-selenium containing compound corresponding to a phenylcarbodiimide dimer was formed. No conclusive evidence for its structure has been established, but structure IV is favoured for IV' because of a very intense infrared absorption band at 2235 cm^{-1} wich is close to the cyanide region (2260 – 2240 cm^{-1})[5] but considerably higher than the carbodiimide region (2155 – 2130 cm^{-1}).[5]



IV IV'

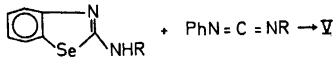
Oxidation of *N*-methyl-*N'*-phenylselenourea and *N*,*N'*-diphenylselenourea were expected to give the Dost (II) and Hugershoff (III) type compounds, respectively,[2] but in both cases Hugershoff type heterocycles were formed (Scheme 1).



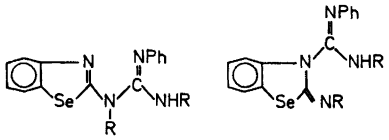
Va : R = methyl
Vb : R = phenyl

Scheme 1.

The compounds Va and Vb were characterised by elemental analysis, IR and NMR spectra. As chemical evidence for the structure V, synthesis from the aminobenzoselenazole and carbodiimide were carried out.



This synthetic pathway does not *a priori* determine the structure unambiguously, since it is know that aminobenzoselenazoles upon methylation methylate on the endocyclic nitrogen atom.[4] In order to discriminate between the two possible structures V and V′ the ^{13}C NMR spectra of Va and Vb were obtained and compared with ^{13}C NMR spectra of the model compounds 2-methylaminobenzoselenazole and 3-methyl-2-iminobenzoselenazoline.

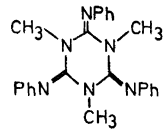


V V′

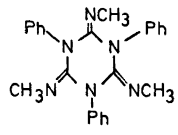
The ^{13}C NMR spectrum of 3-methyl-2-iminobenzoselenazoline shows a very characteristic high-field resonance at 110.5 ppm for one carbon in the benzoselenazoline ring system, a feature which is also observed for the benzothiazolines.[2] The analogue of this ^{13}C resonance is not seen in the spectra of 2-methylaminobenzoselenazole, Va or Vb, where the highest-field aromatic carbon signals are at 121.7, 120.2 and 119.6 ppm, respectively.

On this basis the structure V is favoured over V′ for the oxidation products of *N*-methyl-*N*′-phenyl- and *N*,*N*′-diphenylselenourea.

Besides these benzoselenazolylguanidines Va and Vb non-selenium containing heterocyclic substances are formed as by-products upon oxidation of the selenoureas. In the case of *N*-methyl-*N*′-phenylselenourea a cyclic trimer of methylphenylcarbodiimide is formed. A symmetrical 2,4,6-triiminohexahydro-1,3,5-triazine VI or VI′ is the only possibility for the structure because the ^1H NMR spectrum shows only one CH_3 signal.



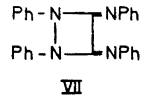
VI



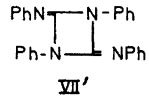
VI′

On basis of the spectroscopic evidence it is not possible to discriminate between VI and VI′.

In the case of *N*,*N*′-diphenylselenourea a cyclic dimer of diphenylcarbodiimide is formed. This has been previously isolated by Huisgen [6] without any structure elucidation. The structure must be VII or VII′, but on the basis of spectroscopic data obtained here, discrimination between the two isomers VII and VII′ was impossible.



VII



VII′

EXPERIMENTAL

IR-spectra were recorded on a Perkin-Elmer model 225 grating spectrograph, ^1H NMR spectra on a Varian A 60 or a JEOL JNM-MH-60/II instrument (chemical shifts are given in ppm relative to TMS as internal reference). ^{13}C NMR spectra were recorded on a Bruker WH 90 instrument. The mass spectra were obtained on an AEI-MS 902 mass spectrometer, operating at 70 eV. Elemental analyses were carried out by the microanalysis department of Chemical Laboratory II, University of Copenhagen, The H. C. Ørsted Institute, and melting points (uncorrected) were determined on a Büchi melting point apparatus.

Synthesis of the selenoureas

Phenyl-, *N*-methyl-*N*′-phenyl- and *N*,*N*′-diphenylselenourea were synthesised according to the procedure described by Sonoda *et al.*[7] Phenyl isocyanide was refluxed with equivalent amounts of selenium and the amine in tetrahydrofurane. The selenoureas were obtained in good yield even when undistilled phenyl isocyanide prepared in accordance with Bulka and co-workers [8] was used.

Oxidation of phenylselenourea

N-Cyano-N,N′-diphenylguanidine (IV). A solution of hydrogen peroxide (0.02 mol, 2.07 ml 30 %) in water was added dropwise to a stirred solution of phenylselenourea (0.2 mol) in ethanol

(50 ml) and four drops of conc. hydrochloric acid. After stirring for 1 h the precipitated selenium (1.51 g (0.019 mol) after drying) was removed by filtration. 200 ml of crushed ice was added to the filtrate. The precipitate was filtered off and recrystallized from ethanol. Yield 1.36 g (57 %) of shining colourless plates m.p. 103–105 °C. (Found: C 71.05; H 5.30; N 24.00. Calc. for $C_7H_6N_2$: C 71.16; H 5.12; N 23.72). ^{1}H NMR ($CDCl_3$): δ 4.55 (1 H, broad), 6.81–7.71 (5 H, m). IR (KBr): cm^{-1} 3420 (m), 3310 (m), 2235 (s), 1690 (s), 1650 (s), 1490 (s), 1365 (s). MS: 236 (M^+).

Oxidation of *N*-methyl-*N'*-phenylselenourea

N-(2-Benzoselenazolyl)-N,N'-dimethyl-N''-phenylguanidine (Va). To a stirred solution of *N*-methyl-*N'*-phenylselenourea (0.02 mol) in ethanol (30 ml) and four drops of conc. hydrochloric acid, a solution of hydrogen peroxide (0.02 mol, 2.07 ml 30 %) in water (20 ml) was added dropwise. The reaction mixture was stirred for 1 h and the precipitate was removed by filtration. 200 ml of crushed ice and 1 M sodium hydroxide were added to the filtrate until pH 10–11. The precipitate was filtered and purified by preparative thin layer technique (PLC) on silica gel plates with chloroform as eluent. Yield 2.64 g (77 %) of a light yellow amorphous compound. (Found: C 56.15; H 5.09; N 15.90. Calc. for $C_{16}H_{16}N_4Se$: C 55.98; H 4.70; N 16.32) ^{1}H NMR ($CDCl_3$): δ 2.63 (3 H, s), 3.47 (3 H, s), 3.90 (1 H, broad), 6.73–7.77(9 H, m). IR (KBr): cm^{-1} 3395 (m) 1335(s), 1585(s), 1495(s). ^{13}C NMR ($CDCl_3$): δ 164.0, 151.9, 151.6, 146.9; 135.8, 129.2, 125.8, 123.7, 122.8, 122.7, 121.1, 121.7, 37.0, 31.5.

Trimer of methylphenylcarbodiimide (VI *or* VI'). Extraction of the precipitated selenium with boiling petrolether and crystallisation resulted in 0.40 g (15 %) of colourless crystals, m.p. 120–124 °C. (Found: C 72.56; H 6.12; N 21.04. Calc. for $C_8H_8N_2$: C 72.70; H 6.10; N 21.20). ^{1}H NMR ($CDCl_3$): δ 2.92 (3 H, s), 6.67–7.33 (5 H, m). IR (KBr): cm^{-1} 1620 (s), 1580 (s), 1465 (s), 1450 (s), 1430 (s), 1395 (s). MS: 396 (M^+).

Oxidation of *N,N'*-diphenylselenourea

N-(2-Benzoselenazolyl)-N,N',N''-triphenylguanidine (Vb). A solution of hydrogen peroxide (0.01 mol, 1.04 ml 30 %) in water (10 ml), was added dropwise to a stirred suspension of *N,N'*-diphenylselenourea (0.01 mol) in an ethanol-water mixture (50 ml:20 ml) with four drops of conc. hydrochloric acid. The reaction mixture was stirred for 2 h and the precipitated selenium was removed by filtration. 200 ml of crushed ice and conc. ammonia was added to the filtrate until pH 10–11. The mixture was stirred for an hour, the precipitate was filtered off and recrystallized from ethanol. Yield 1.20 g (51 %) of colourless crystals, m.p. 156–157 °C. (Found: C 67.10; H 4.43; N 11.76. Calc. for $C_{26}H_{20}N_4Se$: C 66.87; H 4.28; N 12.00). ^{1}H NMR ($CDCl_3$): δ 6.67–7.73. IR (KBr): cm^{-1} 3400 (m), 1650 (s), 1585 (s), 1495 (s), 1480 (s), 1435 (s), 1315 (s). ^{13}C NMR ($CDCl_3$): δ 151.8, 146,3, 140.3, 129.5, 129.1, 128.8, 127.8, 126.2, 125.8, 124.9, 124.2, 123.6, 123.3, 122.5, 121.9, 121.2, 120.5, 120.2.

Dimer of diphenylcarbodiimide (VII *or* VII'). The precipitated selenium from the oxidation of *N,N'*-diphenylselenourea was extrated with boiling toluene. Evaporation of the extract to dryness yielded 0.70 g (36 %) of colourless crystals, m.p. 163–165 °C. (Found: C 80.55; H 5.33: N 14.05. Calc. for $C_{13}H_{10}N_2$: C 80.39; H 5.19; N 14.42). ^{1}H NMR ($CDCl_3$): δ 6.58–7.37. IR (KBr): cm^{-1} 1700 (s), 1680 (s), 1590 (s), 1485 (s), 1360 (s). MS: 388 (M^+).

N-(2-Benzoselenazolyl)-N,N'-dimethyl-N''-phenylguanidine. A mixture of 2-methylaminobenzoselenazole (0.005 mol) and *N*-methyl-*N'*-phenylthiourea (0.005 mol) was refluxed in 30 ml dry benzene together with 5 g lead oxide (PbO) for 1 h. The precipitated lead sulfide was removed by filtration. The filtrate was evaporated to dryness under reduced pressure. *N*-(2-Benzoselenazolyl)-*N,N'*-dimethyl-*N''*-phenylguanidine was purified by PLC and shown to have a superimposable IR spectrum upon Va arising from oxidation of *N*-methyl-*N'*-phenylselenourea.

N-(2-Benzoselenazolyl)-N,N',N''-triphenylguanidine was prepared in a similar way to *N*-(2-benzoselenazolyl)-*N,N'*-dimethyl-*N''*-phenylguanidine from phenylaminobenzoselenazole and *N,N'*-diphenylthiourea. The compound was purified by recrystallization from ethanol and shown to have a superimposable IR-spectrum upon Vb arising from oxidation of *N,N'*-diphenylselenourea.

2-Methylaminobenzoselenazole was prepared in accordance with a previously published procedure.[4] ^{13}C NMR ($CDCl_3$): δ 206.0; 170.1; 154.1; 132.8; 126.3; 124.2; 123.9; 121.3; 119.6; 32.9.

3-Methyl-2-iminobenzoselenazoline was prepared in accordance with a previously published procedure.[4] ^{13}C NMR ($CDCl_3$): δ 160.0; 142.5; 126.6; 125.0; 121.9; 121.3; 110.5; 29.9.

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Reaction of Acylated Pentoses with Acyl Bromide. Preparation of Some Bromo-deoxy Pentoses

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When tri-*O*-acetyl-D-xylopyranosyl bromide was reacted with acetyl bromide and zinc bromide bromine was introduced stereospecifically at C2 to give di-*O*-acetyl-2-bromo-2-deoxy-D-xylopyranosyl bromide (*7a*). Further treatment of the latter compound with acetyl bromide and zinc bromide gave 2,4-dibromo-2,3,4-trideoxy-D,L-*glycero*-pent-2-enopyranosyl bromide (*11*), isolated as the 1-hydroxy-compound (*10*) after hydrolysis. Analogous results were obtained when tri-*O*-benzoyl-D-xylopyranosyl bromide was treated with benzoyl bromide and zinc bromide. Other acetylated or benzoylated pentopyranoses did not give well defined products when treated with acyl bromide and zinc bromide.

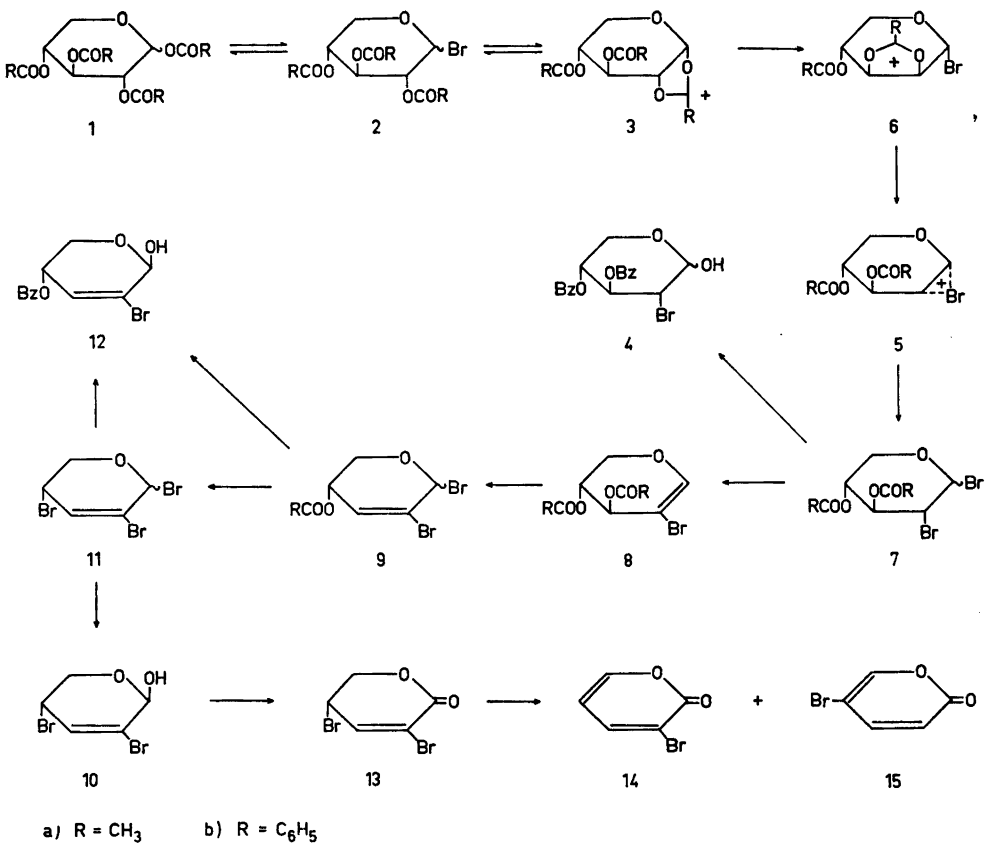
In previous papers the reaction of acylated pentopyranoses and furanoses with dibromomethyl methyl ether was studied.[1,2] It was found that brief treatment with the dibromo ether in the presence of zinc bromide gave glycosyl bromides. The latter on further reaction were converted into 2-bromo-2-deoxy-pentosyl bromides in stereospecific displacement reactions. This property of dibromomethyl methyl ether is probably connected with its ability to form an oxocarbonium ion which is the reactive species.[3,4] Since acyl halides also form oxocarbonium ions under acidic conditions it was decided to study the behaviour of acetyl and benzoyl bromide towards acylated carbohydrates.

Acetylated glycosyl bromides have previously been prepared by the reaction of free sugars or acetylated sugars with acetyl bromide.[5] Treatment of tetra-*O*-acetyl-β-D-xylopyranose (*β-1a*) with acetyl bromide and zinc bromide gave tri-*O*-acetyl-α-D-xylopyranosyl bromide (*α-2a*). In the absence of zinc bromide, or in the presence of a weaker catalyst such as boron trifluoride, the unstable β-bromide (*β-2a*) was first formed as seen from ^{1}H NMR spectra of the reaction mixtures. It subsequently underwent anomerization to the more stable α-bromide. With stronger catalysts, such as zinc bromide, mercuric chloride, or sulfuric acid, the β-bromide could not be detected, probably because of rapid anomerization.

The reaction of the tetraacetate (*1a*) with acetyl bromide and zinc bromide gives, besides the bromide (*2a*), acetic anhydride. Separate experiments showed that the bromide (*2a*) reacted with acetic anhydride and zinc bromide to give the α-tetraacetate (*α-1a*). It is therefore necessary to use a rather large excess of acetyl bromide in order to convert *1a* completely into *2a*. When (*β-1a*) was treated with acetyl chloride and zinc chloride tri-*O*-acetyl-α-D-xylopyranosyl chloride was obtained in good yield.

When the bromide *2a* was treated with acetyl bromide and zinc bromide for 3 days further reactions took place and the known 2-bromo-2-deoxy-D-xylopyranosyl bromide[1] (*7a*) was formed as seen from ^{1}H NMR spectra of the reaction mixture. The dark colour of the reaction mixture showed that extensive decomposition took place. Work up and treatment with silver benzoate gave a low yield of 3,4-di-*O*-acetyl-1-*O*-benzoyl-2-bromo-2-deoxy-β-D-xylopyranose together with the elimination product *8a* and tri-*O*-acetyl-1-*O*-benzoyl-β-D-xylopyranose.

Since benzoates are generally more stable than acetates the reaction of tri-*O*-benzoyl-α-D-xylopyranosyl bromide (*α-2b*) with benzoyl bromide and zinc bromide was also investigated. This gave a considerably better yield of the 2-bromo-



2-deoxy-bromide (*7b*), isolated as the crystalline 3,4-di-*O*-benzoyl-2-bromo-2-deoxy-D-xylose (*4*) after hydrolysis.

When tetra-*O*-acetyl-β-D-xylopyranose was treated with acetyl bromide in the presence of a larger amount of zinc bromide than that used in the experiments described above the reaction went even further to give the unstable tribromo-compound (*11*). After hydrolysis of the crude product 2,4-dibromo-2,3,4-trideoxy-β-D,L-*glycero*-pent-2-enopyranose (*10*) was isolated in 37 % yield. The same compound was obtained in 51 % yield by treatment of tri-*O*-benzoyl-α-D-xylopyranosyl bromide with benzoyl bromide and zinc bromide. A similar treatment of 3,4-di-*O*-benzoyl-2-bromo-2-deoxy-D-xylopyranosyl bromide (*7b*) also gave *10*, showing that *7* is an intermediate in the conversion of *2* into *10*.

Tri-*O*-acetyl- and tri-*O*-benzoyl-arabino-, -ribo-, and -lyxopyranosyl bromides did not give 2-bromo-2-deoxy-compounds by treatment with acetyl and benzoyl bromide, respectively. All the compounds underwent a slow decomposition, but no pure products could be isolated.

The 2-bromo-2-deoxy-xylopyranosyl bromides (*7*) are probably formed *via* the dioxolanylium ions *3* and *6* and the bromonium ion *5* as proposed for the analogous reaction with dibromomethyl methyl ether.[1,4] This mechanism requires that the acyloxy-groups at C2 and C3 are *trans*-oriented and thus explains why tri-*O*-acyl-lyxo- and -ribopyranosyl bromides do not give 2-bromo-2-deoxy compounds. It does, however, not explain the lack of reactivity of the corresponding arabinosyl bromides.

The conversion of *7* into the unsaturated tribromo-compound *11* must necessarily involve a number of intermediates. It was found that 3,4-di-*O*-benzoyl-2-bromo-1,2-dideoxy-D-*threo*-pent-1-enopyranose (*8b*), obtained from *7b* by treatment with diethylamine, could be converted into *10* in 63 % yield by treatment with

Table 1. Proton chemical shifts (δ) and coupling constants (Hz).

Compound	H1	H3	H4	H5	H5′	J_{13}	J_{34}	J_{35}	$J_{35'}$	J_{45}	$J_{45'}$	J_{55}
8b[a]	7.02	5.74	5.39	4.45	4.14	0	2.5	1.5	~0.5	2.5	1.5	12.0
10[b]	5.36	6.47	4.79	4.46	3.92	~0.5	5.5		1.1	2.6	1.1	13.3
12[b]	5.36	6.49	5.24	4.41	3.96	~0.5	5.5		1.1	2.9	1.1	13.0
13[b]		7.59	5.18	5.00	4.67		5.9		1.4	3.0	1.9	13.3

^{13}C chemical shifts (δ) and $^{1}J\{C-H\}$ values (Hz).

Compound	C1	C2	C3	C4	C5	J_{C1-H1}	J_{C3-H3}	J_{C4-H4}	J_{C5-H5}
8b[a]	147.5	94.7	68.7	68.3[c]	63.7[c]	194	155	155	149
10[a]	91.1	125.8	130.0	44.3	62.6	170	175	158	148
12[a]	92.0	130.9	126.8	67.5	61.0	171	167	151	150

[a] In deuteriochloroform; [b] in acetone-d_6; [c] may be reversed.

benzoyl bromide and zinc bromide for 24 h. The primary product of this reaction is *11* which is subsequently hydrolyzed during work-up. When *8b* was treated with benzoyl bromide and zinc bromide for only 10 min *9b* was formed in high yield, as seen from an NMR spectrum of the reaction mixture, and the corresponding 1-hydroxy-compound *12* could be isolated in 80 % yield after hydrolysis.

From these experiments it is concluded that the conversion of *7* into *11* takes place *via 8* and *9*. The presence of *8* was not observed directly, probably because it, as shown above, reacts rapidly with benzoyl bromide and zinc bromide to give *9*.

The structures of the products *10* and *12* were derived from their ^{1}H NMR and ^{13}C NMR spectra (Table 1). Only one anomer was observed of the two compounds. The small values of J_{45} and $J_{45'}$ indicated that both compounds adopt the $^{5}H_{0}$ conformation with equatorial H4. The rather large value of J_{34} (5.5 Hz) also shows that H4 is equatorial.[6] The small allylic coupling, J_{13}, indicates that H1 is equatorial [6-8] and hence that the hydroxygroup and the substituent at C4 are *trans*-oriented. The dibromo-compound *10* was obtained as a racemic form only whereas *12* was optically active. Thus the racemization takes place during the conversion of *9* to *11*. Reaction of *10* with silver benzoate yielded racemic *12*.

In order to confirm the structure of *10* it was oxidized to the lactone *13*, which gave spectral data in agreement with its structure. Reaction of *13* with triethylamine yielded a mixture of the known bromo-pyrones *14* and *15*.

The structures of *9* and *11*, together with those of similar compounds, will be discussed in detail in a forthcoming paper.

Treatment of tri-*O*-acetyl-α-D-xylopyranosyl chloride or of other pentosyl chlorides with acetyl chloride and zinc chloride did not result in formation of chloro-deoxy derivatives.

EXPERIMENTAL

Melting points are uncorrected. Preparative TLC was performed on 1 mm layers of silica gel (Merck PF_{254}). ^{1}H NMR spectra were measured on Varian A-60 and HA-100 instruments or on a Bruker HX-90E instrument using tetramethylsilane as internal reference. ^{13}C NMR spectra were obtained as previously described.[9] Optical rotations were measured on solutions in chloroform.

Tri-O-acetyl-α-D-xylopyranosyl bromide (α-2a). A mixture of tetra-*O*-acetyl-β-D-xylopyranose (*β-1a*) (995 mg), acetyl bromide (5 ml), and anhydrous zinc bromide (100 mg) was stirred for 10 min at room temperature. It was then diluted with dichloromethane (100 ml), washed with 4 N hydrochloric acid and with aqueous sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated. The residue (1.1 g) was crystallized from ether-pentane to give 833 mg (79 %) of

α-*2a*, m.p. 92 – 95 °C. One recrystallization gave a product with m.p. 96 – 97 °C, $[\alpha]_D^{20}$ +206° (*c* 2.3) (reported [19] m.p. 102 °C, $[\alpha]_D$ +212°). A ^{1}H NMR spectrum further confirmed the structure.

Tri-O-*acetyl*-α-D-*xylopyranosyl chloride* was prepared in the same way by treating *β-1a* (1.01 g) with acetyl chloride (5 ml) and zinc chloride (80 mg) for 10 min. Work up and crystallization from ether-pentane gave 616 mg (67 %) of product, m.p. 93 – 96 °C. Two additional recrystallizations gave a product with m.p. 99 – 100 °C, $[\alpha]_D^{20}$ +167.8° (*c* 3) (reported [11] m.p. 105 °C, $[\alpha]_D$ +171.2°).

Reaction of tri-O-*acetyl*-α-D-*xylopyranosyl bromide with acetyl bromide.* To a solution of α-*2a* (983 mg) in dichloromethane (3 ml) was added acetyl bromide (1 ml) and zinc bromide (965 mg) and the mixture was stirred for 3 days at +5 °C. Dichloromethane (50 ml) was then added and the solution was washed with 4 N hydrochloric acid and aqueous sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated. The residue (1.0 g) was dissolved in acetonitrile (50 ml) and stirred with silver benzoate (2.0 g) for 16 h at room temperature. The mixture was filtered through carbon and evaporated, the residue was dissolved in dichloromethane, washed with aqueous sodium hydrogencarbonate and dried. Evaporation gave a syrup (900 mg) which was separated into 5 fractions by preparative TLC using ether-pentane (2:1) as eluent.

The fastest moving fraction (100 mg) was a mixture of products as seen from a ^{1}H NMR spectrum and was not investigated further. The second fraction (70 mg) consisted mainly of 3,4-di-*O*-acetyl-2-bromo-1,2-dideoxy-D-*threo*-pent-1-enopyranose (*8a*).[1] It was only characterized through its NMR spectrum. The third fraction gave 208 mg (18 %) of 3,4-di-*O*-acetyl-1-*O*-benzoyl-2-bromo-2-deoxy-*β*-D-xylopyranose, recrystallized from ether-pentane, m.p. 119 – 121 °C, $[\alpha]_D^{20}$ −24.7° (*c* 2.6). A mixed m.p. and an NMR spectrum proved its identity with the product described previously.[1] The fourth fraction gave 217 mg (20 %) of tri-*O*-acetyl-1-*O*-benzoyl-*β*-D-xylopyranose, recrystallized from ether – pentane, m.p. 144 – 145 °C, $[\alpha]_D^{20}$ −68.1° (*c* 2.0) (reported [12] m.p. 147 – 148 °C, $[\alpha]_D$ −68.2°). The last fraction (48 mg) consisted of hexa-*O*-acetyl-aldehyde-D-xylose as seen from an NMR spectrum.[13]

3,4-Di-O-*benzoyl-2-bromo-2-deoxy*-D-*xylopyranose (4).* A solution of tri-*O*-benzoyl-α-D-xylopyranosyl bromide [1] (5.03 g) in dichloromethane (5 ml) was stirred with benzoyl bromide (10 ml) and zinc bromide (2 g) for 5 days at +5 °C. The mixture was then diluted with dichloromethane (100 ml) and stirred for 3 h with 100 ml of 4 N hydrochloric acid. The organic phase was washed with hydrochloric acid and aqueous sodium hydrogencarbonate, dried and evaporated. The semicrystalline residue (10 g) was shown by ^{1}H NMR and TLC to be a mixture of *7b* and benzoic anhydride. It was dissolved in acetone (100 ml) and water (10 ml) and stirred with silver carbonate (5 g) over night. The mixture was then filtered through carbon and evaporated, dissolved in dichloromethane, washed with water, dried and evaporated. The residue (7.4 g) was separated into 2 fractions by chromatography on a column of silica gel using chloroform as eluent.

The first fraction consisted of benzoic anhydride. The next fraction gave 2.0 g (50 %) of *4*, m.p. 143 – 146 °C, $[\alpha]_D^{20}$ −51.5° (10 min) → −29.7° (4 days) (*c* 3.2). An NMR spectrum was identical with that of the product described previously.[1]

2,4-Dibromo-2,3,4-trideoxy-β-D,L-glycero-*pent-2-enopyranose (10). A. From tetra*-O-*acetyl-β*-D-*xylopyranose.* To a solution of *β-1a* (5.0 g) in dichloromethane (15 ml) was added acetyl bromide (5 ml) and zinc bromide (2.3 g) and the mixture was refluxed for 3 h (the same result was obtained when the mixture was stirred for 3 days at room temperature). The solution was then poured into a mixture of ice (50 g), conc. hydrochloric acid (50 ml), and ether (100 ml) and stirred for 30 min. The ether phase was washed with 4 N hydrochloric acid and aqueous sodium hydrogencarbonate, dried, filtered through carbon, and evaporated. The residue (2.56 g) was crystallized from tetrachloromethane to give 967 mg (24 %) of *10* as colourless needless, m.p. 187 – 190 °C, $[\alpha]_D^{20}$ 0.0° (*c* 5, acetone). Recrystallization gave the pure product, m.p. 187 – 188 °C. (Found: C 23.19; H 2.06; Br 62.12. Calc. for $C_5H_5Br_2O_2$: C 23.37; H 1.96; Br 62.21). Preparative TLC of the material in the mother liquor gave an additional 517 mg (13 %) of *10*.

B. From tri-O-*benzoyl*-α-D-*xylopyranosyl bromide.* A solution of α-*2b* (5.01 g) in dichloromethane (5 ml) was stirred for 5 days at room temperature with benzoyl bromide (10 ml) and zinc bromide (2.0 g). Work up as described above gave 5.8 g of a product which was separated into two fractions by chromatography on a column of silica gel using chloroform as eluent. The first fraction consisted of benzoic anhydride. The second fraction was a mixture of *10* and benzoic acid. It was dissolved in dichloromethane, washed with aqueous sodium hydrogencarbonate and dried. Evaporation of the solvent gave 1.25 g (51 %) of *10* which was recrystallized from tetrachloromethane, m.p. 186 – 189 °C. An NMR spectrum showed that it was identical with the product described above.

C. From di-O-*benzoyl-2-bromo-2-deoxy-β*-D-*xylopyranosyl bromide.* A solution of *β-7b* [1] (501 mg) in dichloromethane (0.5 ml) was stirred with benzoyl bromide (1 ml) and zinc bromide (330 mg) for 4 days at room temperature. Work up and chromatography as described above gave 146 mg (55 %) of *10*, m.p. 185 – 187 °C.

D. From 3,4-di-O-*benzoyl-2-bromo-1,2-dideoxy*-D-threo-*pent-1-enopyranose.* The same product

was obtained in 63 % yield by treatment of *8b* with benzoyl bromide and zinc bromide for 24 h as described above, m.p. 185 – 188 °C, $[\alpha]_D^{22}$ 0.0° (*c* 4, acetone).

*4-O-Benzoyl-2-bromo-2,3-dideoxy-β-*D-glycero-*pent-2-enopyranose (12).* A solution of *8b* (501 mg) in dichloromethane (0.5 ml) was stirred at room temperature for 10 min with benzoyl bromide (1 ml) and zinc bromide (245 mg). Work up as described above and purification by preparative TLC (chloroform) gave 295 mg (80 %) of *12*, m.p. 134 – 140 °C, $[\alpha]_D^{20}$ +99° (*c* 3.0). One recrystallization from ether-pentane gave a product with m.p. 135 – 137 °C, $[\alpha]_D^{20}$ +102° (*c* 1.2.) (Found: C 48.28; H 3.79; Br 26.94. Calc. for $C_{12}H_{10}BrO_4$: C 48.34; H 3.78; Br 26.81).

Racemic 12. A solution of *10* (499 mg) in acetonitrile (10 ml) was stirred for 1 h at room temperature with silver benzoate (1.03 g). The mixture was then filtered through carbon, evaporated, dissolved in dichloromethane, washed with aqueous sodium hydrogencarbonate, dried and evaporated. The residue (438 mg) was separated into two fractions by preparative TLC (ether-pentane 1:1).

The fast moving fraction gave 310 mg (54 %) of racemic *12* which was recrystallized from ether-pentane, m.p. 146 – 148 °C. A ^{1}H NMR spectrum and an IR spectrum in chloroform solution were identical with those of the D-form described above. IR spectra of the two forms in the solid phase (KBr) were different.

The second fraction gave 55 mg (14 %) of 1-*O*-benzoyl-3,4-dideoxy-pent-3-enopyranosulose, m.p. 71 – 72 °C (recorded [14] m.p. 74 – 75 °C). NMR and IR spectra were identical with those of the product previously described.

*3,4-Di-*O*-benzoyl-2-bromo-1,2-dideoxy-*D-threo-*pent-1-enopyranose (*8b*).* A solution of *7b* (1.017 g) in dichloromethane (2 ml) was reacted with diethylamine (2 ml) for 15 min at 0 °C. The solution was then diluted with dichloromethane (50 ml), washed with 4 N hydrochloric acid and aqueous sodium hydrogencarbonate, dried and evaporated. The residue was crystallized from ether-pentane to give 634 mg (78 %) of *8b*, m.p. 136 – 137 °C. One recrystallization gave m.p. 138 – 139 °C, $[\alpha]_D^{20}$ −296° (*c* 1.3). (Found: C 56.49; H 4.04; Br 19.50. Calc. for $C_{19}H_{15}BrO_5$: C 56.59; H 3.75; Br 19.82).

Racemic 3,5-dibromo-5,6-dihydro-2-pyrone (13). A solution of *10* (505 mg) in dichloromethane (20 ml) was stirred at room temperature for 3 h with water (50 ml) saturated with bromine. The organic phase was then washed with 5 % aqueous sodium thiosulfate and water, dried and evaporated. The product (469 mg) was purified by preparative TLC (ether-pentane 3:1) to give 330 mg (66 %) of *13*, m.p. 61 – 64 °C. Recrystallization from ether-pentane gave the pure product, m.p. 64 – 65 °C. (Found: C 23.57; H 1.65; Br 62.32. Calc. for $C_5H_4Br_2O_2$: C 23.47; H 1.58; Br 62.45). An IR spectrum (KBr) showed a carbonyl band at 1730 cm^{-1}; a UV spectrum gave λ_{max} 250 nm, ε 7700 (acetonitrile).

3- and 5-Bromo-2-pyrone, (14 and 15), respectively. A solution of *13* (101 mg) in dichloromethane (10 ml) was cooled to 0 °C and triethylamine (0.5 ml) was added. After 16 h at 0 °C the solution was evaporated and the residue was separated into two fractions by preparative TLC (ether-pentane 2:1).

The fast moving fraction gave 17 mg (25 %) of *15*, m.p. 58 – 60 °C (reported [15] m.p. 60 – 61 °C). The next fraction gave 44.5 mg (64 %) of *14*, m.p. 62 – 63 °C after recrystallization from ether – pentane (reported [15] m.p. 63.5 – 64 °C). UV, IR, and NMR spectra of both products were in agreement with those published.[15,16]

Microanalyses were made by Dr. A. Bernhardt's Microanalytical Laboratory.

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Short Communications

Reactions of Aromatic Anion Radicals and Dianions. Part III.[1] Electrolyte and Solvent Effects on the Kinetics of Electron Transfers to Cyclooctatetraenes

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The first electron transfer to cyclooctatetraene (COT) to generate the anion radical has been reported to be unusually slow while reduction of the anion radical proceeds at a rate comparable to that observed for benzenoid hydrocarbons.[2] This effect was attributed to the large activation energy associated with distortion of COT to a transition state resembling planar COT.[2] Electron transfer kinetics to COT were investigated more thoroughly using A. C. polarography by Huebert and Smith[3] and a semiquantitative study by Anderson *et al.* indicated that the first charge transfer to a related system, 2-methoxyazocines, is even slower than that to COT.[4] More recent studies have led to the curious conclusions that COT anion radical is electrochemically irreducible[5] in acetonitrile and THF and that benzocyclooctatetraene (BCOT) and *sym*-dibenzocyclooctatetraene (DBCOT) undergo reversible one electron transfers in THF to the anion radicals which are not further reducible.[6] By using "super dry" solvents[7] and taking advantage of the effect of small supporting electrolyte cations[1] on the electrochemical reversibility of cathode processes we have been able to conclusively demonstrate that the conclusions of the latter studies[5,6] are not valid. Furthermore, the kinetics of the COT reductions were found to be markedly dependent upon the nature and concentration of the supporting electrolyte cation. Under the most favorable conditions the first electron transfer to COT approaches reversibility which suggests that the flattening energy does not contribute as much to the electron transfer kinetics as previously reported.[2,3]

The cyclic voltammogram of BCOT in carefully purified THF[8] passed over neutral alumina immediately before use is illustrated in Fig. 1a (supporting electrolyte; 0.2 M Bu_4NBF_4). The shape of the voltammogram is indicative of two closely spaced one electron transfers.* When

* The same conclusion can be arrived at by examing the voltammogram of BCOT shown in Fig. 2 of Ref. 6.

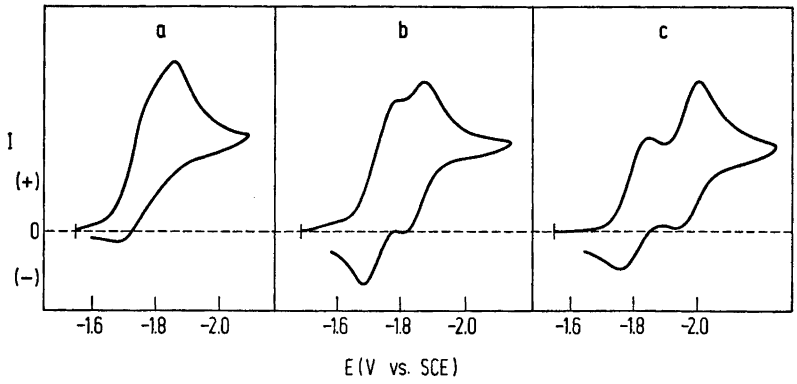


Fig. 1. Cyclic voltammograms of BCOT on mercury at 65 mV/s. (a) In THF containing Bu_4NBF_4 (0.2 M). (b) As in (a) with neutral alumina added. (c) In DMF containing Bu_4NBF_4 (0.2 M) saturated with Me_4NI.

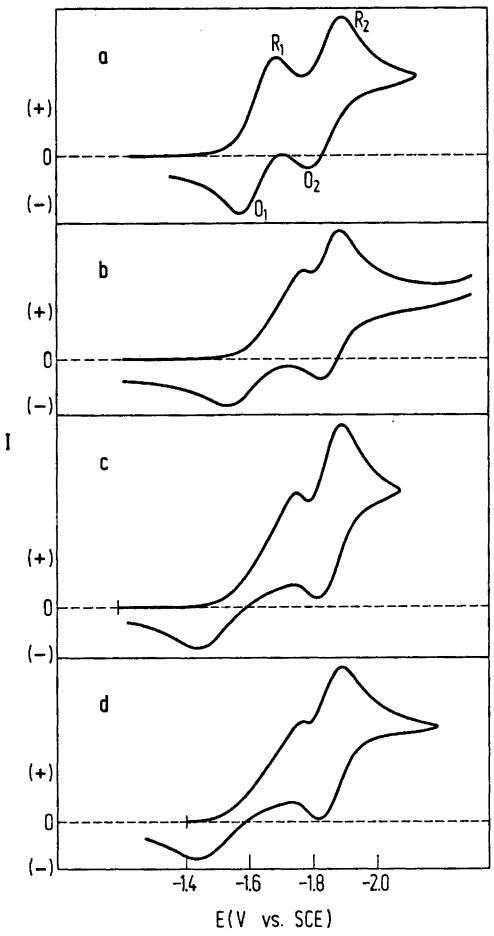


Fig. 2. Cyclic voltammograms of COT on platinum in DMF at 131 mV/s. supporting eleetrolyte (a) Me_4NI (13.5 °C), (b) Et_4NClO_4 (27.5 °C), (c) Pr_4NI (47 °C), and (d) Bu_4NBF_4 (45 °C).

all residual water is removed by adding neutral alumina to the cell and stirring immediately before recording the voltammogram, the two electron transfers are distinctly separated (Fig. 1b). The latter effect is readily understood by considering the effect of a follow up chemical reaction of the BCOT dianion. Thus the protonation of $BCOT^{2-}$ by residual water shifts the peak for the second electron transfer so that it merges with the first peak. The two peaks are completely resolved during voltammetry in DMF when the small cation, Me_4N^+, is present in the voltammetric solution (Fig. 1c). To further substantiate that the two consecutive reversible charge transfers involve one electron, an equimolar amount of azobenzene, which is reduced by two consecutive, well spaced one electron transfers, was added to the solution. The height of the first peak for azobenzene was nearly identical to the for BCOT while the peaks for the second charge transfers from both compounds overlapped and appeared as a single peak. Thus, it is apparent that BCOT is reduced by two consecutive one electron transfers to first the anion radical and then the dianion, the difference in potentials for the two processes being dependent upon the voltammetric conditions.

The latter results suggested that the reduction of COT should be re-examined. Fig. 2a–d show the cyclic voltammograms of COT in DMF at a platinum electrode containing Bu_4N^+ (d), Pr_4N^+ (c), Et_4N^+ (b), and Me_4N^+ (a) ions.* The effect is dramatic, k_s (heterogeneous rate constant for electron transfer) as judged by the separation of oxidation and reduction peaks for the first electron transfer,[9] increased uniformly as the size of the cation decreased. In fact, the rate of charge transfer in the presence of Me_4N^+ was calculated to be of the order of 10^3 times greater than when Bu_4N^+ was the supporting electrolyte cation. Electron transfer is also fast when the supporting electrolyte cation was Bu_4N^+ in the presence of low concentrations of Me_4N^+.

Apparent heterogeneous rate constants (k_{app}) for the first electron transfer to COT in DMF on platinum were determined by cyclic voltammetry[9] and data at various concentrations of Bu_4NBF_4 are summarized in Table 1. According to diffuse double layer theory[10] k_{app} is related to the electrolyte concentration (C) by eqn. (1). Using the value determined ($\alpha = 0.4$) for the transfer coefficient,[3] relative k_{app} values were

* Electron transfer to COT is faster on mercury than on platinum and on either electrode the rate increases with increasing temperature.

Table 1. Supporting electrolyte concentration effect of COT electron transfer rate in DMF.

$[Bu_4NBF_4]$	k_{app}^{rel}(Exp) [a]	k_{app}^{rel}(Calc) [b]
0.01	0.4	0.5
0.05	1.0	1.0
0.10	1.4	1.3
0.15	1.6	1.6
0.30	2.2	2.0
0.75	3.1	3.0

[a] Calculated from cyclic voltammograms according to Ref. 9. Voltage sweep rate = 131 mV/s
[b] Calculated according to eqn. (1), $\alpha = 0.4$.

$$k_{app} = k_s(e^{\alpha nF/RT})\ C^{\alpha} = k_s(\text{constant})C^{\alpha} \quad (1)$$

calculated and found to agree remarkably well with the observed values. These data show that when Bu_4NBF_4 is the supporting electrolyte, a significant portion of the apparent activation energy for the first electron transfer is due to the double layer effect and not to a conformational change.

It is evident that studies which involved the use of large tetraalkylammonium ions, Pr_4N^+ [2] and Bu_4N^+ [3] as supporting electrolyte cations during kinetic studies on the electron transfer to COT, were not under optimum conditions and the conformational effect may not be as important as previously [2,3] reported. Our results do show that the electrolyte and double layer effects are greater than any conformational effects but more detailed kinetic studies under optimum conditions are necessary in order to completely clarify the role of the conformational barrier to electron transfer to COT. Further studies on these and related systems are under way.

In conclusion we draw attention to the cyclic voltammogram of COT in acetonitrile containing Me_4N^+ (Fig. 3). The height of the initial reduction peak (R) was nearly identical to that of an equimolar solution of azobenzene in acetonitrile. The reduction obviously involves two consecutive one electron transfers as it does in DMF. It is not clear what the previous report, indicating that the anion radical is "irreducible" in acetonitrile, was founded on.

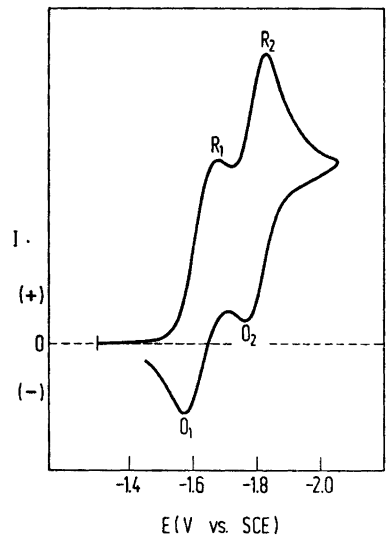


Fig. 3. Cyclic voltammogram of COT on mercury in acetonitrile (13 °C) containing Bu_4NBF_4 (0.2 M) saturated with Me_4NI. Voltage sweep rate 131 mV/s.

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Some Binding Properties of Human Serum Albumin as Studied by the Spin Labels 12-Doxyl Stearic Acid and its Methyl Ester

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Serum albumin has the ability to bind a large variety of substances in complexes which involve non-covalent bonds. By this means long chain fatty acids, bilirubin, and many drugs, otherwise of a very low solubility in the water phase, are dissolved and transported in the blood.

The study of this subject is of importance from several points of view. Thus the binding ability of serum albumin is a unique property of this protein. Further, the competition for binding sites between different substances can produce toxic concentrations of substances such as bilirubin and certain drugs when released from the serum albumin. Numerous studies have been devoted to these problems, mainly using ultrafiltration and equilibrium dialysis.[1]

The binding of a nitroxide spin label [2] to a large slowly rotating molecule such as serum albumin is expected to produce a considerable deviation from the sharp three-line ESR spectrum characteristic for the unbound spin label, and a complete immobilization relative to the serum albumin molecule is expected to produce a powder spectrum. The spin label method has so far been used for studying the motional and conformational properties of serum albumin after the attachment of the spin label through a covalent bond.[3] Spin labeling of biological membranes has been performed by the use of serum albumin as a carrier of spin labeled fatty acids of low solubility in the water phase.[4] In connection with the latter studies it was observed that spin labeled fatty acids gave rise to a powder spectrum when attached to serum albumin.

In this note it is demonstrated that the anisotropic properties of stable nitroxide radicals complexed with serum albumin can be very conveniently utilized for the study of the binding and competition of various low-weight molecular substances to serum albumin in addition to ultrafiltration and equilibrium dialysis.

In the present study some preliminary results are described which were obtained by the use of the stable nitroxide spin label 12-doxyl stearic acid (the *N*-oxyl-4′,4′-dimethyloxazolidine derivative of 12-oxostearic acid) and the corresponding methyl ester. The experiments were performed with a 0.5, 2, or 5 % solution of human serum albumin (HSA) in 0.2 M phosphate buffer pH 7.4, a pH at which the carboxylic group of 12-doxyl stearic acid is completely dissociated. The concentration of the spin label was between 0.1×10^{-6} and 2.0×10^{-6} mol/ml sample solution. Some experiments were also performed with the Cohn fractions IV and II, roughly corresponding to α- and β-globulins, respectively, and with human γ-globulin.

A. HSA. The experimental results are described for 12-doxyl stearic acid-HSA as follows, but were essentially identical with those obtained with its methyl ester.

(1) 12-Doxyl steric acid – HSA gave rise to a powder spectrum with no trace of a sharp three-line spectrum. However, when the relative concentration of the spin label was increased so that the molar ratio of the spin label to HSA exceeded a value of about 2, a superposition of the sharp three-line spectrum just appeared (Fig. 1 A), which increased upon a further increase of the relative concentration of the spin label.

(2) Treatment of the system 12-doxyl stearic acid – HSA exhibiting a pure powder spectrum with active charcoal as described by Chen [5] for removing bound fatty acids from serum albumin, reduced the amount of bound spin label to about 1 – 2 % of the original value.

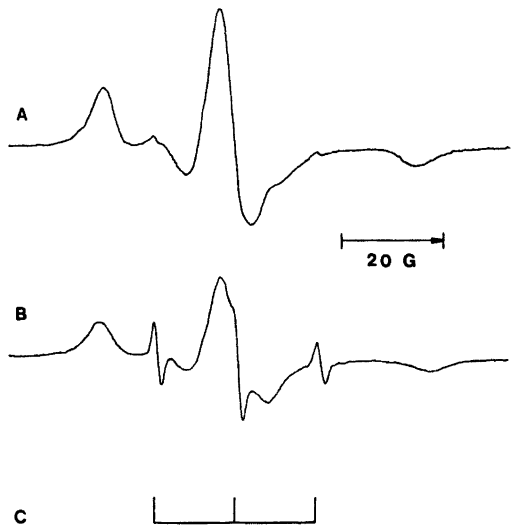


Fig. 1. ESR spectrum of the spin label 12-doxyl stearic acid in a 2 % solution of human serum albumin (HSA), 0.2 M phosphate buffer pH 7.4. *A*. Molar ratio spin label/HSA ~1.7. *B*. After heating of *A* at 80 °C for 10 min. *C*. Stick diagram of the sharp three-line spectrum.

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(3) Heating a solution of 12-doxyl stearic acid – HSA, which originally exhibited only the powder spectrum, at 80 °C for 5 to 10 min, brought about the appearance of the sharp three-line spectrum accompanied by a decrease of the powder spectrum (Fig. 1 B). The same result was obtained when the solution of HSA was heated prior to the addition of the spin label.

(4) After the digestion of HSA by pepsin at pH 3 for 3 h, the ESR spectrum exhibited a superposition of powder spectrum and sharp three-line spectrum when 12-doxyl stearic acid was added to the reaction mixture so that the molar ratio of the spin label to HSA was 2.

(5) Competition experiments were performed with substances such as sodium benzoate, sodium salicylate, and sodium decanoate. When these substances were added in excess to the system of 12-doxyl stearic acid – HSA originally exhibiting only a powder spectrum, the sharp three-line spectrum appeared. An increase of the sharp three-line spectrum was always accompanied by a decrease of the powder spectrum. A similar result was obtained after the addition of ethanol to a final concentration of about 10 %.

B. α- and β-Globulins. 12-Doxyl stearic acid and its methyl ester respectively, gave rise to overlaps of sharp three-line spectra and powder spectra for all concentrations investigated, and it was not possible to remove the sharp three-line spectra by decreasing the concentration of the spin label relative to the α- or β-globulin, or by ultrafiltration of the solution, a separation which could be accomplished for the system 12-doxyl stearic acid – HSA.

C. γ-Globulin. Only a very small amount of powder spectrum could be detected in the system 12-doxyl stearic acid (or its methyl ester) – γ-globulin.

The powder spectra indicated a complete immobilization of 12-doxyl stearic acid relative to the HSA molecule. This would mean that the spin label is attached not only by means of the dissociated carboxylic group at the end of the molecule, but that it is also attached in the whole of its length to the HSA molecule, or at least by means of the oxazolidine ring carrying the free radical function. The almost identical result obtained with the methyl ester of 12-doxyl stearic acid seems to reduce further the importance of the dissociated carboxylic group for the attachment of 12-doxyl stearic acid to HSA.

The finding that about 2 molecules of 12-doxyl stearic acid could be firmly bound to each HSA molecule is in agreement with the number of strong binding sites for fatty acids established by equilibrium dialysis.[6] The decrease of the powder spectrum followed by an increase of the sharp three-line spectrum upon heating, or digestion by pepsin, reflects the loss of binding sites when the protein was denaturated or partially split, respectively.

The competition experiments showed that 12-doxyl stearic acid could be displaced from HSA by an excess of substances such as decanoate and salicylic acid. Experiments are in progress for utilizing the competition between a spin label and a variety of low-weight molecular substances as a quantitative method for the evaluation of their relative binding ability to HSA.

The binding of 12-doxyl stearic acid and its methyl ester to α- and β-globulins seems to be much weaker than to HSA. It is possible that the binding sites are localized to the lipid part of these globulins.

The very low binding of 12-doxyl stearic acid to γ-globulin seems to be in agreement with the known binding properties of this globulin when no hapten – antibody relationship is present.

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Reactions of Lone Pair Electron Donors with Unsaturated Electrophiles. III. Addition of Tetrahydrofuran to Dimethyl Maleate[1]

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In a previous paper,[2] it was shown that tetrahydrofuran (THF) and oxetane add thermally and photochemically to dimethyl acetylenedicarboxylate in a free radical chain reaction. Here, the radical chain was presumed to be initiated by electron and proton transfer from the ether to the electrophile. Furthermore, it is known that THF adds to maleic anhydride and diethyl maleate to give α-substituted tetrahydrofurans (*1*) initiated by dibenzoyl peroxide [3] or by direct [3-5] or sensitized irradiation.[5]

In the present study it was found that THF adds thermally to dimethyl maleate in the presence of oxygen to give the adducts *1* and *2* even though initiators such as dibenzoyl peroxide are absent. Typically, oxygen was bubbled for a few minutes through a solution of dimethyl maleate in a 3 – 10 fold excess of THF.[6] This mixture was then kept at 120 °C in a sealed pyrex tube for 12 h. The adducts (*1* and *2*) were isolated by evaporation of excess THF under reduced pressure followed by vacuum-distillation (*1*: b.p. 0.05 mmHg 90 – 100 °C, *2*: b.p. 0.05 mmHg 160 – 180 °C). The yields of the adducts *1* and *2* are 75 % and 25 %, respectively. The structures of *1* and *2* were determined by their spectral properties (IR, NMR, mass spectra). *Analyses: 1*, Found: C 55.27; H 7.31. Calc. for $C_{10}H_{16}O^5$: C 55.54; H 7.46. *2*, Found: C 52.96; H 6.55. Calc. for $C_{16}H^{24}O_9$: C 53.33; H 6.71. NMR ($CDCl_3$) *2*: δ 1.85 (m, 4 H), 2.5 – 3.1 (m, 6 H), 3.70 (two s. 12 H), 4.0 (m, 2 H). Alkaline hydrolysis of *1* gave its corresponding acid (as compared with an authentic sample [5]).[7]

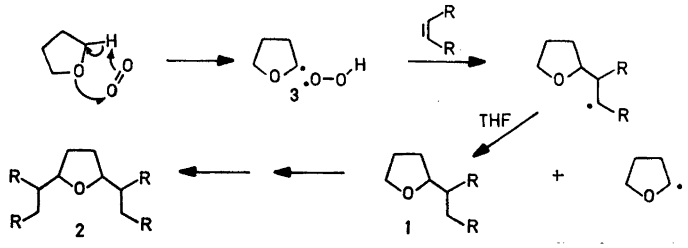
This reaction does not take place in the absence of oxygen. Furthermore, small amounts of a free radical scavenger such as *p*-dinitrobenzene [8] completely inhibit the reaction; in both cases the maleate is virtually completely recovered. When the reaction is initiated with dibenzoyl peroxide at lower temperatures, only a moderate yield of *1* is formed and no formation of the adduct *2* is detected.

The effect of *p*-dinitrobenzene suggests that *1* and *2* are formed in a free radical chain reaction, and decomposition of THF peroxide formed by reaction of the dissolved oxygen with THF may be responsible for the radical initiation. Alternatively, and in consonance with what has been suggested for a related system,[2] THF radicals may be generated directly by a possibly concerted electron and proton transfer from the ether oxygen and α-position, respectively, to the electrophilic oxygen as indicated in Scheme 1. Diffusion of the radical pair *3* would then generate a free radical chain reaction. Abstraction of the hydroperoxy radical hydrogen by a chain-carrying radical would account for termination of the chain and the apparently catalytic action of oxygen.

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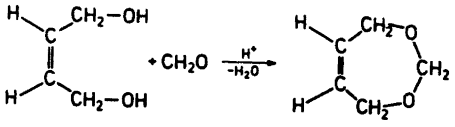
Scheme 1.

Reactions between Formaldehyde and Polyhydric Alcohols. II.* A Fourteen-membered Cyclic Acetal from (Z)-2-Butene-1,4-diol and Paraformaldehyde

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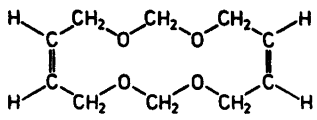
It was previously described that the reaction between (*Z*)-2-butene-1,4-diol and paraformaldehyde led to 2*H*-4,7-dihydro-1,3-dioxepin (I) and a viscous residue.[2]



I

We have reinvestigated this reaction and isolated a novel fourteen-membered cyclic acetal (II) from the residue.

Elemental analysis and mass spectrometry of the new compound corresponded to the molecular composition $C_{10}H_{16}O_4$. The signals of the proton magnetic resonance spectrum of compound II have almost the same chemical shifts as those found in the spectrum of compound I.[3] However, compound II shows larger splittings of the signals from the vinylic and allylic protons, thus allowing the identification of these protons as an $AA'M_2M_2'$ system. From these findings compound II was assigned the structure (5*Z*,12*Z*)-1,3,8,10-tetraoxacyclotetradeca-5,12-diene.



II

The conformation of saturated cyclic compounds containing the 1,3-dioxa-grouping has been studied extensively,[4] and the conformation of 1,3,8,10-tetraoxacyclotetradecane (the saturated analogue of II) was recently established by X-ray crystallography.[5] To our knowledge, no conformational analysis has been carried out on unsaturated compounds containing the 1,3-dioxa-grouping, and we are now investigating the conformation of II.

Experimental. (5Z,12Z)-1,3,8,10-Tetraoxacyclotetradeca-5,12-diene (II). (*Z*)-2-Butene-1,4-diol (176 g, 2 mol), paraformaldehyde (60 g, 2 mol of CH_2O), benzene (25 ml) and *p*-toluenesulfonic acid (0.1 g) was heated on an oil bath kept at 86 °C for 24 h. The water formed by the reaction was removed by means of a Dean-Stark-separator. The reaction mixture was cooled to room temperature and stirred for 24 h with solid sodium hydroxide (5 g). The sodium hydroxide was removed and the reaction mixture dissolved in dichloromethane and filtered through silica gel. Distillation through a 20 cm column packed with Fenske rings gave 115 g (57 %) of pure 2*H*-4,7-dihydro-1,3-dioxepin (b.p. 127 – 129 °C) leaving a viscous residue (41 g). The distillation was continued at diminished pressure (0.3 Torr) with the residue heated to 170 – 180 °C on an oil bath. During 24 h a product solidified in the condenser. Recrystallization from abs. ethanol gave 1.0 g of analytically pure II, m.p. 114.5 – 115.0 °C. (Found: C 60.03; H 8.06. Calc. for $C_{10}H_{16}O_4$: C 59.98; H 8.05). Mass spectra were recorded on an AEI MS902 instrument at 70 eV. Compound I: *m/e* 100 (M^+ 5 %), 70 (56), 69 (19), 43 (19), 42 (100), 41 (56). Compound II: *m/e* 200 (M^+ 0.3 %), 100 (12), 70 (40), 69 (100), 68 (24), 53 (32), 42 (25), 41 (21), 40 (25). NMR spectra were recorded on a Bruker HX 90 E instrument. Compound II ($CDCl_3$, *ca.* 30 °C): δ 5.87 [=C–H (A/A')], 4.13 [=C–CH_2 (M_2/M_2')], 4.72 (s, O–CH_2–O). $^3J_{AA'} = 11.0$ Hz, $^5J_{MM'} = 0$ Hz, $^3J_{AM} = \pm 7.5$ Hz, $^4J_{AM'} = \mp 1.1$ Hz.

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* Part I, see Ref. 1.

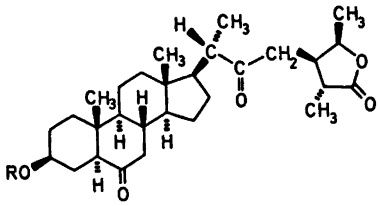
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Studies on Orchidaceae Glycosides. 1. The Constitution and Relative Configuration of Dendrosteroside, a Steroid Glycoside from *Dendrobium ochreatum* Lindl.

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Three closely related crystalline glycosides have been isolated from *Dendrobium ochreatum* Lindl.[1] One of the glycosides ($C_{41}H_{64}O_{15}$), named dendrosteroside (I), crystallised from water as colourless needles, m.p. 239–245 °C (decomp.), $[\alpha]_{578}^{23}$ −32.5° (*c* 1.27, methanol). Sugar and methylation analysis [2] showed that the glycoside (I) contains a disaccharide moiety composed of two D-glucose residues, connected by a (1→6)-linkage. On enzymatic hydrolysis with emulsin, dendrosteroside (I) gave first a monoglycoside (II), m.p. 235–240 °C (decomp.), $[\alpha]_{578}^{23}$ −12.5° (*c* 1.39, methanol) and finally the aglycone III, named dendrosterone, m.p. 240–245 °C (decomp.), $[\alpha]_{578}^{23}$ +19.6° (*c* 0.74, methanol). This



I : R= ß-gentiobiosyl
II : R= ß-D-glucopyranosyl
III: R= H

evidence, together with the application of Hudson's rules of isorotation,[3] indicates that the sugar moiety in I is gentiobiose, β-linked to the aglycone (III).

The structure of dendrosterone (III) $C_{29}H_{44}O_5$, was determined by an X-ray direct phase determination procedure. The compound crystallises from methanol in the monoclinic space group $P2_1$ with $a = 17.551(2)$, $b = 7.280(1)$ and $c = 10.654(3)$ Å, $\beta = 104.64(1)°$, $Z = 2$. X-Ray intensity data were collected with the computer-controlled diffractometer Philips PW 1100. The phase determination was carried out by the direct phase method. A number of *E*-maps gave probable partial structures located in different parts of the unit cell. Phases calculated from one of these partial structures, the A, B and C ring-system, were used as a basic set for further phase determination. A new *E*-map was calculated which additionally displayed the five-membered ring and the lactone ring. The remainder of the structure was revealed by a combination of difference Fourier syntheses and least-squares refinements. The coordinates were refined to an *R* value of 0.058 with anisotropic thermal parameters assigned to all atoms other than hydrogen and fixed coordinates and isotropic thermal parameters assigned to the hydrogen atoms. Fig. 1 shows a perspective view of dendrosterone (III).

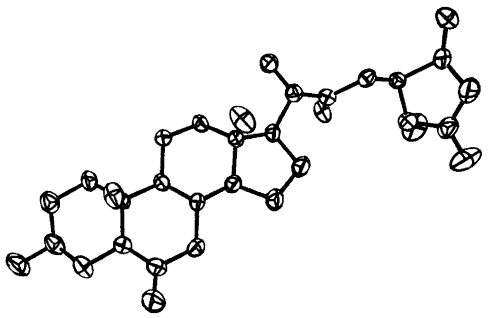


Fig. 1. A perspective view of dendrosterone (III).

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The Chemistry of Dibenzo-[*d,f*][1,3]diazepines. 4.*Sterically Determined Water Loss from Dibenzo[*d,f*][1,3]diazepines upon Electron Impact

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The electron-impact-induced loss of H_2O from the molecular ion of 6-methyl-5,7-dihydroxy-6,7-dihydro-5-*H*-dibenzo[*d,f*][1,3]diazepine (*A*) was shown [2] – by deuterium labeling – preferentially to involve the loss of the hydrogen atoms from the two OH-groups. Inspection of Dreiding models of *A* further indicated, that in one of the conformations (I, Fig. 1) the two OH-groups could approach each other closely.

In this conformation a hydrogen rearrangement through a six-membered ring transition

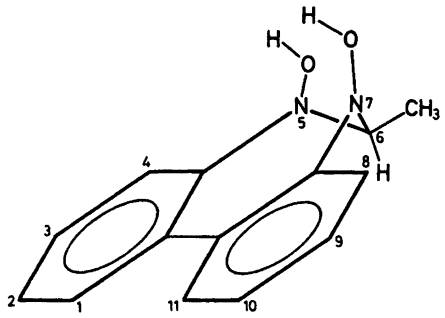


Fig. 1. I

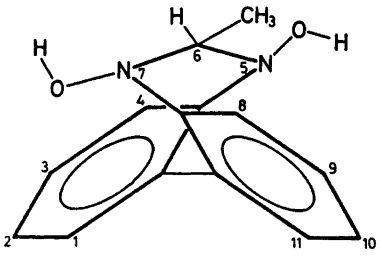


Fig. 2. II

* See Ref. 1. ** Present address as a.

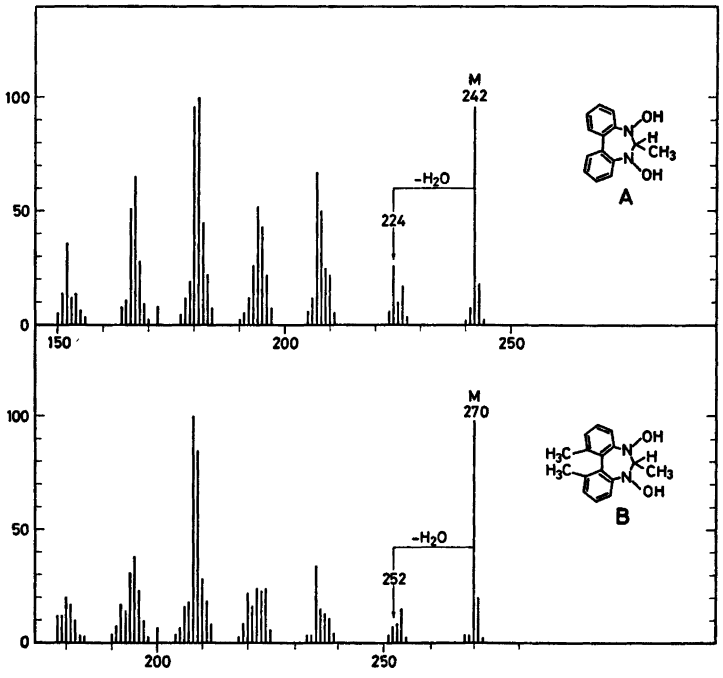


Fig. 3. The mass spectra of *A* and *B*.

state may lead to the ejection of a molecule of water.

In stereochemical studies it was found that substituted biphenyls bridged by three atoms may be resolved.[3] Furthermore, the optical stability was reported to be increased considerably when the biphenyl system was substituted in the 1- and 11-positions. Correspondingly it could be expected that conformation I is impossible when substituents – large enough to prevent the planar arrangement of the biphenyl – are introduced in the 1- and 11-positions. In those cases the two OH-groups are separated considerably (*e.g.* confirmation II, Fig. 2).

If this effect is preserved in the molecular ion formed upon electron impact a less abundant $[M-H_2O]^{+\cdot}$ ion should be expected in the mass spectrum of a 1,11-substituted compound than found in that of *A*. However, the structure of a molecular ion may differ to a great extent from that of the "ground state" due to the large amount of excess energy ($\sim$5eV [4]).

In order to investigate the influence of these factors the mass spectrum of 1,6,11-trimethyl-5,7-dihydroxy-6,7-dihydro-5*H*-dibenzo[*d*,*f*][1,3]diazepine [1] (*B*) was recorded.

A comparison with the mass spectrum of *A* [2] (Fig. 3) shows a significant decrease in the abundance of the $[M-H_2O]^{+\cdot}$ ion (from 25 % to 7 %) and also of the daughter ions of this fragment.

This leads to the conclusion that the considerations regarding the conformation of the "ground state" of these compounds appear to be valid also for their molecular ions. Thus a relation between the stereochemistry of these compounds and ease of water elimination upon electron impact is highly probable.

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The Effect of Nitrous Acid on the Nitrodeiodination of 2,4-Diiodomesitylene and of 2,4-Diiodo-3,5-dimethylanisole

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It has been found that the rate of nitrodeiodination of 2,4-diiodo-3,5-dimethylanisole increases with the concentration of nitrous acid in the reaction medium. This fact indicates a two-step mechanism for the nitrodeiodination reaction. The first step is a nitrosodeiodination, followed by an oxidation of the nitroso group to a nitro group. In the case of 2,4-diiodomesitylene, however, the nitrodeiodination is somewhat retarded by nitrous acid. This gives evidence for a direct nitrodeiodination.

It has been demonstrated that two different mechanisms can operate when an electrophilic aromatic nitrodeiodination takes place. One of them is composed of an exchange of the iodine atom for a nitroso group, followed by an oxidation of the nitroso group to a nitro group. Butler and Sanderson [1] have demonstrated this mechanism in the case of 4-iodoanisole. The other mechanism is a direct exchange of the iodine atom for a nitro group in complete analogy with the well-known m echanism of the nitrodeprotonation reaction. The latter mechanism has been shown to operate in the case of 2-iodo-1,3,5-trineopentylbenzene.[2]

The reasons why one, and not the other of the two mechanisms operates in a particular case are not clear. It has been suggested [2] that the two mechanisms have different *ortho:para* ratios, leading to the dominance of one or the other depending on the substrate. It could also be an effect caused by the specific properties of a methoxy substituent in the aromatic iodo compound.

The present paper deals with questions concerning which properties of the aromatic compound are responsible for the realization of a particular mechanism.

In order to test the idea of different *ortho:para* ratios for the two mechanisms, the two compounds 2,4-diiodomesitylene (I_2M) and 2,4-diiodo-3,5-dimethylanisole (I_2Me_2A) were investigated. These compounds are very similar; if one of the methyl groups of I_2M is replaced by a methoxy group, I_2Me_2A is obtained. The iodine atoms of both compounds have two *ortho* substituents and, moreover, the iodine atoms at position 4 in both compounds have identical surroundings.

RESULTS

Qualitative results. The substrates were treated with 90 % nitric acid in nitromethane solution at 0 °C. From I_2M three different products were obtained: 2-iodo-4-nitromesitylene from nitrodeiodination, 2,4-diiodo-6-nitromesitylene from nitrodeprotonation, and 2,4,6-triiodomesitylene from iodination of unreacted starting material by the iodine liberated during the nitrodeiodination. Four products from I_2Me_2A could be detected: 2-iodo-3,5-dimethyl-4-nitroanisole and 4-iodo-3,5-dimethyl-2-nitroanisole from nitrodeiodination, 2,4,6-triiodo-3,5-dimethylanisole (I_3Me_2A) from iodination, and traces of 2,4-diiodo-3,5-dimethyl-6-nitroanisole ($I_2Me_2NO_2A$) from nitrodeprotonation.

The relative amounts of the different products were constant throughout the course of the reactions, which means that the competing reactions all followed the same kinetics.

The substrates were also treated with nitric

acid containing nitrous acid. This was done by dissolving sodium nitrite in the nitric acid before the latter was added to the nitromethane solution of the aromatic substrate. In the case of I_2M the same products were obtained in the same proportions whether nitrous acid was present or not. In the case of I_2Me_2A, however, there was a difference. I_3Me_2A appeared only in the absence of nitrous acid. I_3Me_2A is probably formed as a result of a reaction in which I_2Me_2A acts as a nucleophilic species which accepts the positive iodine expelled in the nitrodeiodination reaction. When nitrous acid is present, other species are possibly more prone to react with positive iodine, and thus no I_3Me_2A is formed. Another reason may be that the nitrous acid present catalyses a nitrodeiodination of the I_3Me_2A that is formed, and thus no detectable amount of this compound accumulates. The latter explanation cannot be the only one, however, because the observed amount of $I_2Me_2NO_2A$ did not correspond to the lost amount of I_3Me_2A. The fact that no similar dependence of the composition of the product mixture on the presence of nitrous acid could be detected with I_2M may be explained if nitrous acid has no catalyzing effect on nitrodeiodination of I_3M.

The conditions were not identical for the two substrates. As I_2Me_2A is much more reactive towards electrophiles than I_2M and reasonable reaction rates were desired in order to make possible determinations of the rate constants, it was necessary to use a reaction solution with a lower concentration of nitric acid in the case of I_2Me_2A than in the case of I_2M. This means also that the water concentrations had to be different, if the relation between the concentrations of nitric acid and water was to be the same for both substrates.

Quantitative results. The reaction rates were determined by measuring photometrically the increasing amount of liberated molecular iodine. All the iodine expelled in the nitrodeiodination reactions does not appear as molecular iodine, but the amount of free iodine is proportional to the molar amount of aromatic reaction products. This was shown by comparing the rate of appearance of iodine with the rate of consumption of substrate. The material balance with respect to aromatic compounds showed no deficit.

Table 1. Nitration of 2,4-diiodomesitylene (I_2M) and of 2,4-diiodo-3,5-dimethylanisole (I_2Me_2A) with an excess of nitric acid containing varying amounts of sodium nitrite. For conditions, see Experimental section. The rate constants k are the pseudo first-order rate constants of the total reaction of the substrate.

Substrate	mol $NaNO_2$/ mol HNO_3	mol $NaNO_2$/ mol of substrate	$k \times 10^3$ (s^{-1})
I_2M	0	0	2.0
	0.003	130	1.8
	0.007	260	1.4
	0.011	440	1.1
	0.016	650	1.0
I_2Me_2A	0	0	0.3
	0.003	8	1.2
	0.007	16	2.1
	0.011	27	2.9
	0.016	40	3.6

The dependence of the rate of reaction on addition of nitrous acid was determined for both substrates. It was found that the reaction rate for I_2Me_2A increased with increasing amounts of nitrous acid, while the rate for I_2M decreased. The results are summarized in Table 1.

In a separate experiment, it was found that sodium nitrate causes a decrease of the rate of reaction for I_2M of the same order of magnitude as that caused by the addition of sodium nitrite. It is thus impossible to state whether the decrease of the rate of reaction for I_2M, with increasing concentrations of nitrous acid, is analogous to the decrease found for the rate of nitrodeprotonation under the same circumstances,[3] or if it is merely an effect of the nitrate ions which are formed when sodium nitrite is added.

DISCUSSION

The results found indicate a difference in mechanism between the nitrodeiodination reactions of the two compounds. In the case of I_2Me_2A, a nitrosodeiodination is apparently the first step, followed by an oxidation, giving altogether a nitrodeiodination. In the case of I_2M, a direct nitrodeiodination is operating. Thus two different mechanisms seem to operate for two substrates which are sterically very similar but which differ widely in electronic

properties. The idea that different *ortho:para* ratios are responsible for the operation of different reaction mechanisms can be ruled out since the two substrates used in the present investigation have similar patterns of substitution around the reacting iodine atoms.

One possible explanation of the difference in mechanism is that the methoxy group has some special properties which favour the nitrodeiodination reaction. In such a case, the oxygen atom may play a key role, but its action is not limited to the neighbouring positions since both iodines were affected similarly (*cf.* Experimental section).

Another explanation is based on the fact that the two substrates differ widely in their reactivity towards electrophiles. In such a case, one of the two competing reactions, nitrodeiodination and nitrosodeiodination, may dominate for the most reactive species and the other for the less reactive one. The Hammett equation [4] is assumed to be applicable to the two different reactions (ni = nitro; no = nitroso):

$$\log (k^{ni}/k_0^{ni}) = \varrho^{ni}\sigma \quad (1)$$

$$\log (k^{no}/k_0^{no}) = \varrho^{no}\sigma \quad (2)$$

The symbols have the following meanings: k^{ni} is the rate constant for the nitrodeiodination reaction of a substrate of interest, k_0^{ni} is the corresponding constant for a standard substrate, ϱ^{ni} is the reaction parameter of the nitrodeiodination reaction and σ is the substituent parameter of the substrates at hand. The symbols of eqn. (2) have the corresponding meanings.

If eqn. (2) is subtracted from eqn. (1), the following expression is obtained:

$$\log (k^{ni}/k^{no}) = \log (k_0^{ni}/k_0^{no}) + (\varrho^{ni} - \varrho^{no})\sigma \quad (3)$$

As $\log (k_0^{ni}/k_0^{no})$ is a constant, it can be seen from eqn. (3) that $\log (k^{ni}/k^{no})$ can be either positive or negative, *i.e.* either nitrodeiodination or nitrosodeiodination is dominating, depending on the value of σ, provided that the difference between the values of ϱ^{ni} and ϱ^{no} is sufficiently large.

A necessary condition for a change in σ to result in a change of sign, from positive to negative, of $\log (k^{ni}/k^{no})$ can be expressed in the following way:

$$(\varrho^{ni} - \varrho^{no})\sigma_1 > -\log (k_0^{ni}/k_0^{no}) > (\varrho^{ni} - \varrho^{no})\sigma_2 \quad (4)$$

where σ_1 is the substituent parameter for the compound which mainly undergoes nitrodeiodination (in the present case I_2M) and σ_2 has the corresponding meaning for the other compound (I_2Me_2A).

In the present case, the standard compound corresponding to k_0^{ni} and k_0^{no} is 2,4-diiodo-1,3-dimethylbenzene. This means that σ_1 is the substituent parameter for a methyl group and σ_2 that for a methoxy group. Both groups have an activating effect on electrophilic aromatic substitutions and thus σ_1 and σ_2 are negative, and since the methoxy group has the strongest activating effect, σ_2 has the largest absolute value. From eqn. (4) it can thus be concluded that the expression $(\varrho^{ni} - \varrho^{no})$ is positive, *i.e.* $\varrho^{ni} > \varrho^{no}$. As ϱ^{ni} and ϱ^{no} both refer to electrophilic aromatic substitutions, their values are negative, and thus ϱ^{no} has a larger absolute value than ϱ^{ni}.

The difference in reactivity parameters for the two reactions may explain the difference in behaviour of the two compounds studied. If this assumption is correct, the methoxy group, as such, would not be crucial for the dominant operation of the nitrosodeiodination. The effect of the methoxy group in such a case is merely a general activation of the substrate towards electrophiles. It might thus be possible to find substrates with a methoxy group which are not reactive enough to undergo nitrosodeiodination, and substrates without methoxy groups which undergo this reaction because they are sufficiently activated towards electrophiles for some other reason.

EXPERIMENTAL

The NMR analyses were performed on a Varian A 60 NMR spectrometer. About 10 % (by weight) solutions in carbon tetrachloride were used. The probe temperature was 35 °C. The chemical shifts are reported in ppm downfield from tetramethylsilane as internal standard. The peaks were all singlets, indicated by (s). No other peaks than those reported could be detected, which indicates that the compounds prepared were free from isomers.

Gas chromatographic (GLC) analyses were made on a Perkin-Elmer 900 instrument fitted with columns packed with SE-30 silicon gum rubber. Photometric measurements were made on a Beckman DB UV spectrophotometer using a thermostatted cuvette holder.

The mass spectra (MS) were determined (at the Department of Medical Chemistry, University of Göteborg) on an LKB 9000 instrument fitted with a gas chromatograph. The electron energy used was 70 eV. The intensities of the peaks are reported in parentheses as percentage of the base peak. Only peaks with an intensity of more than 10 % of the base peak are reported, together with the isotope peaks corresponding to the base peaks.

The melting points were determined on a Kofler micro hot stage. The purity of the substances prepared was checked by gas chromatography and was found to be at least 99.5 % as estimated from the peak heights.

The fuming nitric acid used was obtained according to Bennett *et al.*[5] The concentration was adjusted to 90 % by mixing with a proper amount of concentrated nitric acid. The acid did not contain nitrous acid within an experimental accuracy of 1 mol %.[2]

2,4-Diiodomesitylene was obtained from mesitylene *via* reaction with iodine monochloride in acetic acid with zinc(II) chloride as catalyst. The reaction has been described by Keefer and Andrews.[6] The physical properties were identical with those reported previously.[7]

3,5-Dimethylanisole was prepared from 3,5-dimethylphenol according to a method described by Brändström and Junggren.[8] Tetrabutylammonium hydrogen sulfate (34 g, 0.1 mol) and 8 g (0.2 mol) of sodium hydroxide were dissolved in 100 ml of water, and 150 ml of dichloromethane was added. 3,5-Dimethylphenol (12.2 g, 0.1 mol) and 14.2 g (0.1 mol) of iodomethane were added. The mixture was stirred vigorously at room temperature for 15 min. The two layers were separated and 400 ml of diethyl ether was added to the organic layer. Tetrabutylammonium iodide precipitated and the crystals were filtered off. Small amounts of unreacted starting material were present in the solution and were removed by washing with an aqueous sodium hydroxide solution, water, aqueous acetic acid and water, in that order. The solution was dried with magnesium sulfate and the solvent was evaporated yielding 7.5 g (55 %) of 3,5-dimethylanisole. NMR: δ 6.37 (s, 3 H, aromatic), 3.67 (s, 3 H, methoxy), 2.24 (s, 6 H, methyl).

2,4-Diiodo-3,5-dimethylanisole was obtained from 3,5-dimethylanisole according to the method described by Keefer and Andrews.[6] NMR: δ 6.49 (s, 1 H, aromatic), 3.82 (s, 3 H, methoxy), 2.86 (s, 3 H, methyl), 2.46 (s, 3 H, methyl). MS: 39(15), 51(13), 63(13), 65(12), 91(35), 104(13), 119(17), 127(10), 246(37), 388(100), 389(9). M.p. 124 – 125 °C, reported [9] 125 °C.

Nitration of 2,4-diiodomesitylene (I_2M). A solution of 42 mg of I_2M in 50 ml of nitromethane was prepared. In a cuvette 1.25 ml of the solution (containing 2.8×10^{-3} mmol of substrate) was placed and cooled to 0 °C. Then 0.50 ml (11 mmol) of 90 % nitric acid (cooled to 0 °C) was added. The concentration of I_2M was 1.6×10^{-3} M, that of nitric acid 6.3 M, and that of water 2.4 M in the reaction solution. (The volume of the reaction solution was assumed to be equal to the sum of the volumes of the nitromethane solution and the nitric acid.)

The absorption of iodine (at 477 nm) was measured as a function of time. Approximately half of the expelled iodine could be detected in the form of molecular iodine. The appearance of the iodine was found to follow a first-order rate equation, and the rate constant (estimated accuracy ± 10 %) was calculated using the Guggenheim method.[10] The experiment was repeated with differing amounts of sodium nitrite added to the nitric acid. The results are presented in Table 1.

In separate experiments under conditions similar to the above-mentioned (both with and without sodium nitrite added to the nitric acid) it was proved that the appearance of iodine and the consumption of I_2M follow pseudo first-order kinetics and have the same rate constant. Since no aromatic material was lost (see below), the latter must apply also to the formation of total aromatic product. The iodine content was measured photometrically as described above, and the consumption of I_2M was monitored by taking aliquots, quenching with a small amount of water, and analyzing by means of GLC. The gas chromatograms also show that the relative ratios between the products were constant during the course of reaction.

The dependence of the rate constant on the addition of sodium nitrate was checked in one experiment with conditions similar to those used in the experiments concerning addition of sodium nitrite. A decrease in the rate of reaction by one third was found when 16 mmol of sodium nitrate was added per mol of nitric acid. As can be seen from Table 1, a decrease by one half was found in the case of sodium nitrite.

The products obtained were identified by means of mass spectrometry. For this purpose I_2M was nitrated as described above (no nitrite was added), and after completion of the reaction, a small amount of water was added to the solution. The new solution was extracted with cyclohexane, and the organic phase was washed with water and dried with magnesium sulfate. After evaporation of the solvent, the product was analyzed by means of combined gas chromatography and mass spectrometry. The products were identified as 2-iodo-4-nitromesitylene (INO_2M), 2,4-diiodo-6-nitromesitylene (I_2NO_2M) and 2,4,6-triiodomesitylene (I_3M) in the approximate relative ratio 85:11:4, as determined from the peak heights of the gas chromatogram. (When the reaction was carried out with nitrous acid present, the same product ratio was obtained.) No other products could be found by means of GLC or NMR studies of the crude product mixture.

The material balance was checked by weighing the crude products after completed reaction.

The molar yield of aromatic products was quantitative in the presence, as well as in the absence, of nitrous acid.

MS for INO_2M: 30(16), 39(33), 50(16), 51(37), 52(14), 63(23), 65(26), 75(12), 77(37), 78(19), 89(12), 91(77), 92(14), 103(30), 104(19), 115(46), 116(21), 117(79), 118(79), 119(81), 120(12), 127(35), 137(40), 274(100), 291(95), 292(9).

MS for I_2NO_2M: 89(10), 91(16), 115(21), 116(14), 117(32), 118(26), 127(12), 207(10), 244(25), 245(21), 273(14), 400(77), 417(100), 418(9).

MS for I_3M: 51(12), 105(27), 107(33), 117(25), 244(25), 371(23), 498(100), 499(11).

Nitration of 2,4-diiodo-3,5-dimethylanisole (I_2Me_2A). This substrate was nitrated as described for I_2M with the exception that 2.00 ml of a nitromethane solution containing 7.2×10^{-3} mmol of I_2Me_2A was placed in the cuvette. The 90 % nitric acid added amounted to 0.080 ml (1.7 mmol). The concentration of I_2Me_2A was 3.5×10^{-3} M, that of nitric acid 0.82 M, and that of water 0.32 M in the reaction solution. The temperature was kept at 0 °C and the rate constants were determined in the same manner as for I_2M. The results are summarized in Table 1. As in the case of I_2M, it was checked (both with and without nitrite added) that the same rate constant applied to the formation of free iodine and to the formation of total aromatic product. The relative ratios of the different products were also constant during the course of reaction in the presence, as well as in the absence, of nitrous acid, as seen from the gas chromatograms.

The products obtained were identified in the following way. I_2Me_2A was nitrated under the conditions just described, and the reaction mixture was then worked up as described for the nitration of I_2M. The mass spectra were determined by means of combined GLC and MS, and the different compounds could be identified as 2-iodo-3,5-dimethyl-4-nitroanisole, 4-iodo-3,5-dimethyl-2-nitroanisole, and 2,4,6-triiodo-3,5-dimethylanisole (I_3Me_2A) which were the main products. Traces (less than 1 %) of 2,4-diiodo-3,5-dimethyl-6-nitroanisole ($I_2Me_2NO_2A$) could also be detected. When sodium nitrite had been added to the nitric acid used, no I_3Me_2A could be detected in the product mixture.

In order to make possible an identification of the two isomers of iodo-3,5-dimethyl-nitroanisole, the I_3Me_2A was removed from the mixture of reaction products by means of column chromatography (silica gel, cyclohexane as eluent). A good separation of the two isomers was not achieved, however, and the NMR spectrum of the mixture of them was determined. From this spectrum it was possible to deduce the NMR spectrum of each compound, determine the relative abundance of the two compounds, compare this figure with the corresponding one obtained from GLC and then assign the proper mass spectrum to each isomer.

The relative amounts of 2-iodo-3,5-dimethyl-4-nitroanisole, 4-iodo-3,5-dimethyl-2-nitroanisole and I_3Me_2A were found to be 72:24:4 as determined from the NMR spectrum of the product mixture before any separation of the products had been done. The nitric acid used contained no nitrite in this case. When nitrite was present, the relative amounts of the two isomers of iodo-3,5-dimethyl-nitroanisole were the same as in the case just mentioned.

The crude product was weighed and the yield of aromatic products was found to be quantitative after completed reaction in the presence, as well as in the absence, of nitrous acid.

Spectral characteristics of 2-iodo-3,5-dimethyl-4-nitroanisole. NMR: δ 6.46 (s, 1 H, aromatic), 3.89 (s, 3 H, methoxy), 2.41 (s, 3 H, methyl), 2.26 (s, 3 H, methyl). MS: 39(13), 51(10), 65(18), 77(12), 91(19), 119(10), 135(16), 246(16), 290(100), 307(75), 308(9).

Spectral characteristics of 4-iodo-3,5-dimethyl-2-nitroanisole. NMR: δ 6.74 (s, 1 H, aromatic), 3.85 (s, 3 H, methoxy), 2.49 (s, 3 H, methyl), 2.37 (s, 3 H, methyl). MS: 39(20), 51(16), 63(16), 65(28), 77(19), 78(13), 91(37), 104(23), 107(20), 119(17), 135(14), 246(22), 290(55), 307(100), 308(11).

Spectral characteristics of I_3Me_2A. NMR: δ 3.81 (s, 3 H, methoxy), 2.94 (s, 6 H, methyl). MS: 63(10), 89(12), 90(11), 245(16), 372(34), 499(15), 514(100), 515(11).

MS of $I_2Me_2NO_2A$: 39(13), 41(22), 43(41), 51(11), 53(35), 57(38), 63(17), 67(13), 69(22), 71(15), 77(17), 81(11), 83(15), 89(22), 90(17), 97(15), 103(13), 217(11), 372(51), 416(39), 433(100), 434(11).

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The Crystal Structure of 1,4-Ethyleno-2,8-dichloro-2,4,6,8-tetramethyloctahydronaphthal-5-ene-3,7-dione

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The crystal structure of the title compound, $C_{16}H_{18}Cl_2O_2$, has been determined in three dimensions by X-ray diffraction, from 2093 independent intensities. The crystals belong to the triclinic space group $P\bar{1}$ with two molecules per unit cell of dimensions $a = 8.848$ Å, $b = 9.215$ Å, $c = 10.115$ Å, $\alpha = 98.03°$, $\beta = 92.86°$, and $\gamma = 113.80°$. The structure was solved by a combination of heavy-atom and direct methods and refined by full-matrix least-squares to a final R value of 0.052.

The Diels-Alder product 1,4-ethyleno-2,8-dichloro-2,4,6,8-tetramethyloctahydronaphthal-5-ene-3,7-dione is a dimer of 6-chloro-2,6-dimethylcyclohexa-2,4-dien-1-one, formed upon oxidation of 2,6-dimethylphenol by chlorine dioxide. Two molecules with the same absolute configuration about the tertiary carbon atom have dimerised. The structure is rather strained with $C(sp^3) - C(sp^3)$ bond lengths of 1.589 and 1.560 Å for the bonds C(4) – C(4a) and C(4a) – C(8a), respectively.

Oxidation of 2,6-xylenol in carbon tetrachloride solution by chlorine dioxide gives in a low yield the Diels-Alder product 1,4-ethyleno-2,8-dichloro-2,4,6,8-tetramethyloctahydronaphthal-5-ene-3,7-dione which is a dimer of 6-chloro-2,6-dimethylcyclohexa-2,4-dien-1-one.

Table 1. Crystal data.

Lattice constants	$a = 8.848(2)$ Å
	$b = 9.215(3)$ Å
	$c = 10.115(4)$ Å
	$\alpha = 98.03(1)°$
	$\beta = 92.86(1)°$
	$\gamma = 113.80(1)°$
Cell volume	$V = 742.08$ Å^3
Density (calculated)	$d = 1.40$ g cm^{-3}
Molecules per unit cell	$Z = 2$
Space group	$P\bar{1}$

The steric orientation of the chloro group in the cyclohexenone moiety was established [1] from the high value (1700 cm^{-1}) of the stretching frequency of the conjugated carbonyl group.

The purpose of this investigation, in a program of crystal structure studies of oxidation products of phenols, was to establish the steric configuration at carbon atom C(2) and to confirm the structure of the dimer.

EXPERIMENTAL

Two crystals of the approximate volumes 0.0044 and 0.0014 mm^3 were mounted arbitrarily on the computer-controlled Philips diffractometer PW 1100 equipped with a graphite monochromator to reflect Cu$K\alpha$ radiation. Preceding the measurement of the reflexion intensities, the least-squares unit cell parameters, and crystal-orientation parameters were determined, through measurement of the angular positions of 25 accurately centered reflexions by the "Peak-Hunting Program". Table 1 contains the refined cell parameters.

The intensities of 2562 independent reflexions out to $\theta = 65°$ were measured by the moving-crystal, moving-counter technique ($\theta - 2\theta$ scan). Backgrounds were estimated by stationary counting at $\pm 0.75°$ 2θ from the peak maxima. The scan speed was 1.2° min^{-1}. Three monitor reflexions were measured every 90 min, which showed that the crystals were not stable to X-rays. Two crystals were used, each with a decrease in intensity of about 10 to 15 % during the period of data collection. Lorentz and polarization factors were applied but not correction for absorption. Only the 2093 reflexions for which $\sigma(I)/I \leq 0.25$ were used in refinement of the structure. $\sigma(I)$ is the estimated standard

Table 2. Observed and calculated structure amplitudes. The columns use the running index l, $10|F_o|$, and $10|F_c|$, respectively.

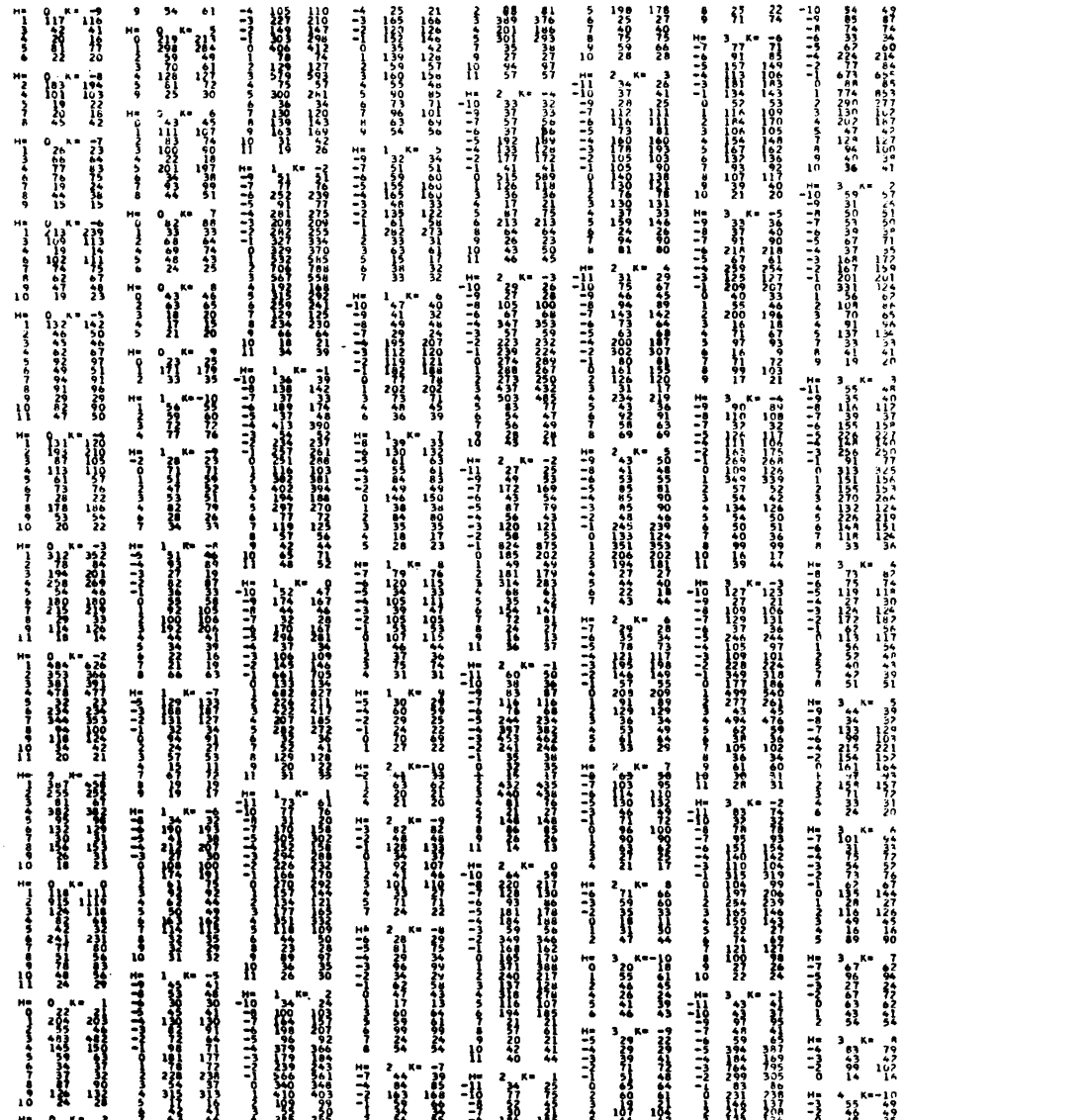


Table 2. Continued.

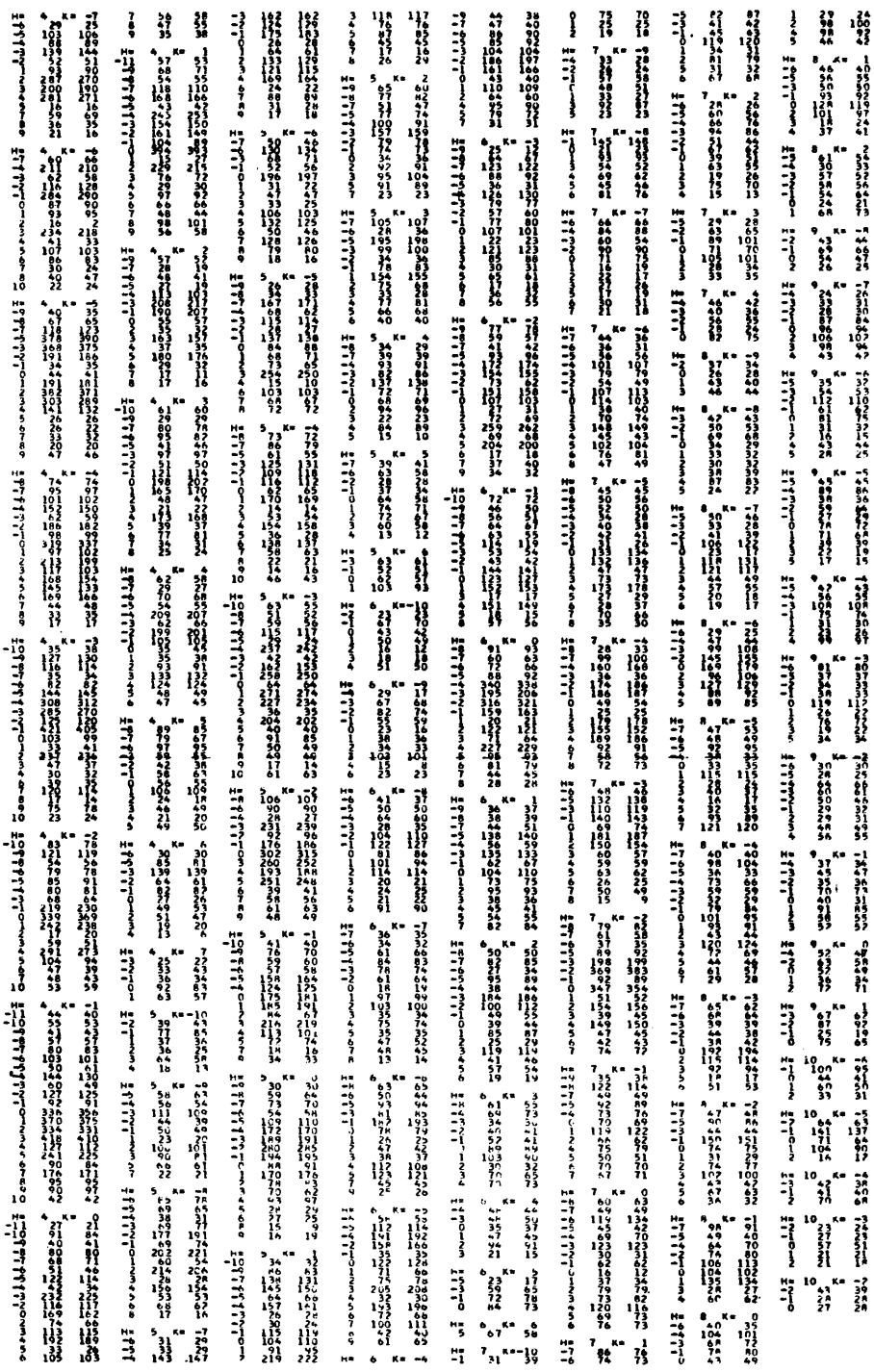


Table 3. Positional and anisotropic thermal parameters of the non-hydrogen atoms. The β-values refer to the temperature factor expression exp $[-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+hk\beta_{12}+hl\beta_{13}+kl\beta_{23})]$. Estimated standard deviations are given in parentheses. Values are $\times 10^4$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	3375(3)	2821(3)	3005(3)	93(4)	101(4)	81(3)	92(6)	−2(5)	21(5)
C(2)	3265(4)	1096(4)	2602(3)	132(5)	125(4)	85(3)	149(7)	19(6)	24(6)
C(3)	1431(4)	−41(3)	2254(3)	148(5)	102(4)	79(3)	112(7)	3(6)	30(5)
C(4)	262(3)	769(3)	2583(3)	106(4)	101(4)	88(3)	67(7)	23(5)	47(5)
C(4a)	612(3)	2065(3)	1621(3)	98(4)	100(4)	60(3)	80(6)	3(5)	16(5)
C(5)	−553(3)	2848(4)	1878(3)	85(4)	129(4)	95(3)	90(7)	22(5)	57(6)
C(6)	−132(4)	4318(4)	2563(3)	133(5)	138(4)	78(3)	139(7)	62(6)	64(6)
C(7)	1642(4)	5379(3)	2947(3)	165(5)	101(4)	79(3)	135(7)	6(6)	36(5)
C(8)	2760(3)	5001(3)	1987(3)	86(4)	99(4)	99(3)	45(6)	3(5)	70(5)
C(8a)	2498(3)	3223(3)	1833(3)	90(4)	110(4)	65(3)	94(6)	32(5)	33(5)
C(9)	2425(4)	2781(4)	4198(3)	147(5)	121(4)	65(3)	144(7)	−7(6)	11(5)
C(10)	865(4)	1731(4)	3990(3)	150(5)	147(5)	69(3)	150(8)	57(6)	65(6)
C(11)	4108(5)	596(5)	3699(4)	178(6)	150(5)	134(4)	197(10)	−43(8)	44(8)
Cl(12)	4268(1)	956(1)	1103(1)	206(2)	213(2)	129(1)	271(3)	124(2)	31(2)
O(13)	961(3)	−1438(3)	1759(3)	208(5)	112(4)	157(3)	132(6)	−35(6)	−15(5)
C(14)	−1535(4)	−454(4)	2403(4)	122(5)	136(5)	148(5)	43(8)	21(7)	90(8)
C(15)	−1381(5)	4958(5)	2985(4)	206(7)	196(6)	130(4)	280(11)	127(9)	82(9)
O(16)	2134(4)	6503(3)	3870(3)	266(5)	137(4)	118(3)	162(7)	−34(6)	−37(5)
C(17)	2429(5)	5412(5)	610(4)	165(6)	186(6)	117(4)	173(10)	77(8)	164(8)
Cl(18)	4896(1)	6312(1)	2594(1)	107(2)	132(2)	233(2)	5(2)	−62(3)	122(2)

Table 4. Positional and isotropic thermal parameters of the hydrogen atoms, with estimated standard deviations in parentheses.

	$x\times 10^3$	$y\times 10^3$	$z\times 10^3$	$B\times 10^2$
H(C1)	445(4)	350(4)	319(3)	283
H(C4a)	35(4)	142(4)	77(3)	242
H(C5)	−158(4)	220(4)	160(3)	287
H(C8a)	295(4)	299(3)	110(3)	230
H(C9)	295(4)	352(4)	505(3)	281
H(C10)	13(4)	166(4)	464(3)	311
H1(C11)	526(5)	133(4)	385(4)	378
H2(C11)	354(4)	54(4)	449(4)	378
H3(C11)	405(4)	−41(4)	347(4)	378
H1(C14)	−193(5)	−84(5)	157(4)	441
H2(C14)	−164(4)	−134(5)	287(4)	441
H3(C14)	−226(5)	8(5)	274(4)	441
H1(C15)	−254(5)	420(5)	252(4)	432
H2(C15)	−134(5)	508(5)	383(4)	432
H3(C15)	−115(5)	596(5)	257(4)	432
H1(C17)	244(4)	631(5)	65(4)	362
H2(C17)	328(5)	541(4)	9(4)	362
H3(C17)	129(5)	458(4)	16(3)	362

deviation, based on counter statistics, of the net intensity I.

Structure determination and refinement

The structure was solved by a combination of heavy-atom and direct methods. The positional parameters of the two chlorine atoms were found from a 3-D Patterson synthesis. The structure factors were converted to normalized $|E|$'s using an approximate absolute scale and average temperature factor determined by Wilson's [2] method. The atomic positions of the chlorine atoms were used to calculate structure factors. The phases of 10 reflexions with $|F_c| \geq 0.2|F_o|$, where $|F_c|$ and $|F_o|$ are calculated and observed structure factor magnitudes, respectively, were used as starting phases together with two reflexions as variables. These

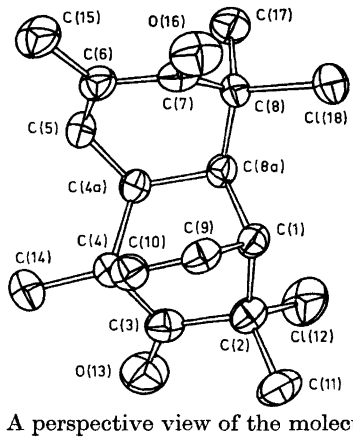


Fig. 1. A perspective view of the molecule.

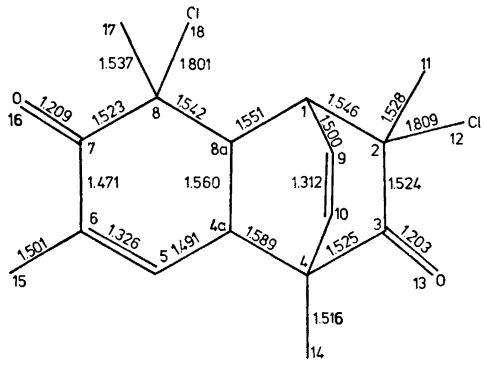


Fig. 2. Bond distances in the molecule.

Table 5. Interatomic angles (°) with estimated standard deviations in parentheses.

C(2) – C(1) – C(8a)	108.9(2)
C(2) – C(1) – C(9)	105.9(2)
C(8a) – C(1) – C(9)	108.8(2)
C(1) – C(2) – C(3)	107.6(2)
C(1) – C(2) – C(11)	112.6(3)
C(1) – C(2) – Cl(12)	110.2(2)
C(3) – C(2) – C(11)	111.7(3)
C(3) – C(2) – Cl(12)	107.2(2)
C(11) – C(2) – Cl(12)	107.4(2)
C(2) – C(3) – C(4)	113.4(2)
C(2) – C(3) – O(13)	123.0(3)
C(4) – C(3) – O(13)	123.6(3)
C(3) – C(4) – C(4a)	105.4(2)
C(3) – C(4) – C(10)	106.8(2)
C(3) – C(4) – C(14)	111.3(3)
C(4a) – C(4) – C(10)	105.5(2)
C(4a) – C(4) – C(14)	113.3(3)
C(10) – C(4) – C(14)	113.9(3)
C(4) – C(4a) – C(5)	107.1(2)
C(4) – C(4a) – C(8a)	109.6(2)
C(5) – C(4a) – C(8a)	115.7(2)
C(4a) – C(5) – C(6)	125.2(3)
C(5) – C(6) – C(7)	118.9(3)
C(5) – C(6) – C(15)	123.2(3)
C(7) – C(6) – C(15)	117.9(3)
C(6) – C(7) – C(8)	113.0(2)
C(6) – C(7) – O(16)	122.8(3)
C(8) – C(7) – O(16)	124.0(3)
C(7) – C(8) – C(8a)	112.0(2)
C(7) – C(8) – C(17)	109.3(3)
C(7) – C(8) – Cl(18)	109.0(2)
C(8a) – C(8) – C(17)	110.3(3)
C(8a) – C(8) – Cl(18)	110.2(2)
C(17) – C(8) – Cl(18)	105.8(2)
C(1) – C(8a) – C(4a)	109.2(2)
C(1) – C(8a) – C(8)	114.8(2)
C(4a) – C(8a) – C(8)	111.4(2)
C(1) – C(9) – C(10)	114.9(3)
C(4) – C(10) – C(9)	116.0(3)

phases were extended and refined by use of "variance-weighted" $\sum_2$-relationships.[3] Signs were determined for 178 reflexions ($|E| > 1.8$) without any contradictions in the solution with the highest "reliability index". An E-map based on these phases revealed the complete structure, except for hydrogens. The positional and isotropic thermal parameters of these atoms were refined by full-matrix least-squares computations to an R value of 0.162. Upon assignment of anisotropic thermal parameters to all atoms the value of R fell after three cycles of refinement to 0.075.

The hydrogen atoms were then located from a difference Fourier synthesis. Refinement was continued including all atoms. For the heavy atoms anisotropic temperature factors were used and for the hydrogen atoms isotropic temperature factors equal to those of the final isotropic values of their parent atoms, were applied. The R value converged to 0.052.

The full-matrix least-squares refinements were performed by a modified version of program LALS.[4] Hughes' weighting scheme [5] was applied with $|F_{o,min}| = 1.75$. The atomic scattering factors for oxygen and carbon were taken from Freeman,[6] that for chlorine from *International Tables for X-Ray Crystallography* [7] and that for hydrogen from Stewart, Davidson and Simpson.[8] The observed and calculated structure amplitudes are given in Table 2. The positional and thermal parameters of the non-hydrogen atoms with estimated standard deviations are listed in Table 3 and those for the hydrogen atoms in Table 4.

RESULTS AND DISCUSSION

A perspective view of the molecule together with the numbering of atoms is given in Fig. 1. The stereochemistry of the structure is consonant with considerations given by Adler and Holmberg [9] and agrees with that found through X-ray investigations of related structures by Karlsson, Pilotti and Wiehager.[10,11] Intramolecular bond distances and bond angles involving non-hydrogen atoms are given in Fig. 2 and Table 5, respectively.* No corrections for

* The estimated standard deviations, based solely on least-squares parameters, are about 0.004 Å for for the bonds.

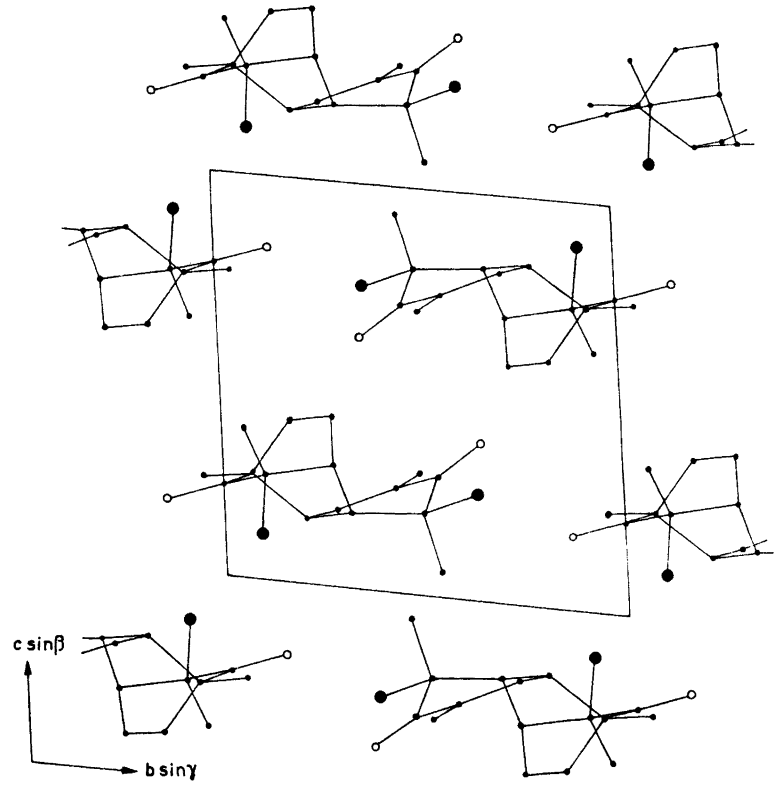


Fig. 3. The unit cell in projection along the *a* axis. •, carbon; ○, oxygen; ●, chlorine.

Table 6. Intermolecular distances (Å) less than 3.8 Å.

Code for symmetry related atoms

Superscript	Coordinates	Superscript	Coordinates
None	$x;\ y;\ z$	iv	$1-x;\ -y;\ -z$
i	$x;\ -1+y;\ z$	v	$1-x;\ -y;\ 1-z$
ii	$-1+x;\ -1+y;\ z$	vi	$-x;\ 1-y;\ -z$
iii	$-x;\ -y;\ -z$	vii	$-x;\ 1-y;\ 1-z$
		viii	$1-x;\ 1-y;\ 1-z$

C(11) – O(16^{i})	3.492(4)	Cl(12) – Cl(12^{iv})	3.285(2)
O(13) – C(7^{i})	3.570(3)	C(11) – C(11^{v})	3.523(7)
O(13) – C(15^{i})	3.565(4)	C(15) – C(17^{vi})	3.641(5)
O(13) – O(16^{i})	3.425(3)	C(9) – C(15^{vii})	3.670(5)
O(13) – C(17^{i})	3.703(4)	C(15) – O(16^{vii})	3.612(5)
C(14) – Cl(18^{ii})	3.393(3)	C(9) – Cl(18^{viii})	3.703(3)
C(4a) – O(13^{iii})	3.503(3)	C(11) – O(16^{viii})	3.797(4)
C(5) – O(13^{iii})	3.673(4)		

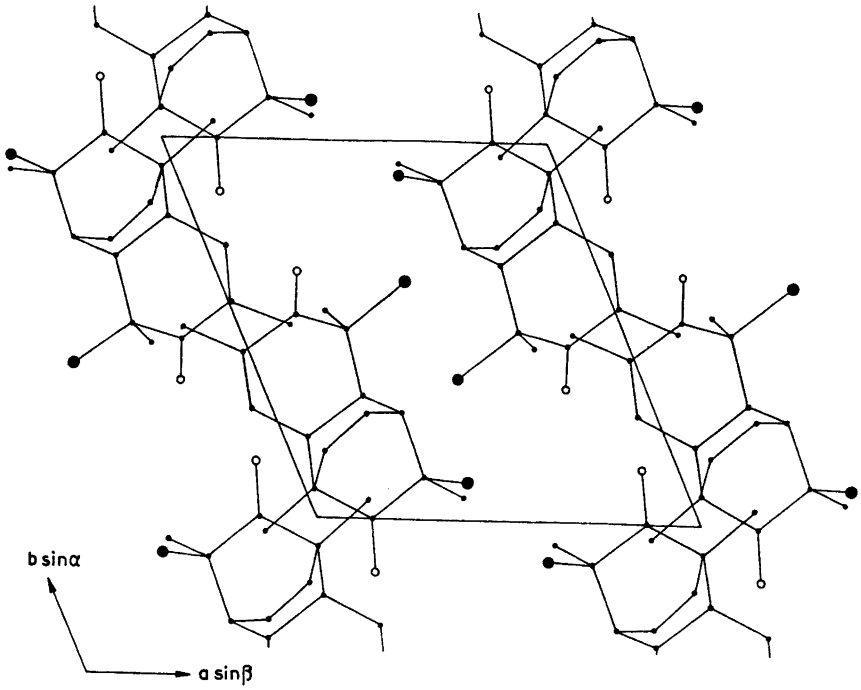


Fig. 4. The unit cell in projection along the *c* axis. •, carbon; ○, oxygen; ●, chlorine.

Table 7. Least-squares planes and deviations. The planes are described in terms of normalized equations in the orthogonal coordinate system (m,n,p) having $p \parallel c$, n in the bc plane and m in the abc octant.

Plane A	$-0.0307m-0.3701n+0.9285p=0.4227$
Plane B1	$-0.1205m+0.3597n+0.9252p=1.7490$
Plane B2	$0.1245m-0.6029n+0.7880p=2.2637$
Plane B3	$-0.2549m+0.9538n+0.1590p=1.6508$

Plane A		Plane B1	
Atom	Deviation (Å)	Atom	Deviation (Å)
C(4a)[a]	0.198	C(1)[a]	0.742
C(5)	0.001	C(2)	0.034
C(6)	0.001	C(3)	−0.035
C(7)	0.001	C(4)[a]	0.758
C(8)[a]	−0.650	C(4a)	0.034
C(8a)	0.001	C(8a)	−0.033
Plane B2		**Plane B3**	
Atom	Deviation (Å)	Atom	Deviation (Å)
C(1)[a]	−0.740	C(1)[a]	−0.667
C(2)	0.031	C(4)[a]	−0.729
C(3)	−0.031	C(4a)	0.021
C(4)[a]	−0.632	C(8a)	−0.021
C(9)	−0.036	C(9)	0.025
C(10)	0.036	C(10)	−0.025

Angles between the normals to the planes (°).	A ∧ B1	60
	A ∧ B2	77
	A ∧ B3	78

[a] These atoms were omitted from the calculations of the least-squares planes.

thermal motion have been made. The bond lengths and angles are comparable with values observed in similar structures.[10,11] Bonds C(4) – C(4a) (1.589 Å) and C(4a) – C(8a) (1.560 Å) are significantly longer than the average value, 1.532 Å, of the other C(sp^3) – C(sp^3) bonds in the structure, probably in consequence of internal strain in the molecule. Some of the C(sp^2) – C(sp^3) bond lengths significantly exceed the standard value 1.501 ± 0.004 Å;[12] those of C(2) – C(3), C(3) – C(4), and C(7) – C(8) are 1.524, 1.525, and 1.523 Å, respectively, perhaps because each involves a highly substituted carbon atom. The effect of conjugation is apparent in the C(6) – C(7) bond, 1.471 Å, which is adjacent to double bonds. The two C – Cl bond lengths of 1.801 and 1.809 Å agree with the "normal" value of 1.81 Å given by Gade and Glusker.[13]

The arrangement of the molecules as viewed along the *a* and *c* axis is shown in Figs. 3 and 4, respectively. The molecules are held together in three-dimensional space by van der Waals forces. There are, however, some close intermolecular contacts, a C···Cl distance of 3.393 Å and a Cl···Cl distance of 3.285 Å (Table 6).

Parameters of least-squares planes through selected atoms of the molecule are given in Table 7. The four atoms C(5), C(6), C(7), and C(8a) in the cyclohexene ring are coplanar within ± 0.001 Å. Atoms C(4a) and C(8) deviate by 0.198 and – 0.650 Å from the plane, respectively. Atom C(8) is significantly out of the plane as it is in the dimers of 2,4- and 2,6-dimethyl-*o*-quinol.[10,11] The three rings containing the ethyleno moiety are all boat-shaped. This rather strained three-ring system is connected to the cyclohexene ring in accordance with the endo rule; the angle between the normals to the planes containing atoms C(5), C(6), C(7), and C(8a) and atoms C(4a), C(8a), C(9), and C(10), respectively, is 77.7°.

All hydrogen atoms lie in chemically reasonable positions with apparent bond distances ranging from 0.80 to 1.05 Å, mean value 0.93 Å, in good agreement with values found by Stewart, Davidson and Simpson.[8]

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Purification and Properties of Cellobiose: Quinone Oxidoreductase from *Sporotrichum pulverulentum*

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Cellobiose: quinone oxidoreductase was purified by ammonium sulfate precipitation, SP-Sephadex C-50 chromatography, and hydroxylapatite column chromatography. The purified enzyme is homogeneous by ultracentrifugal and SDS-gel electrophoretic analyses. The enzyme is a flavoprotein with FAD as the prosthetic group and produces cellobiono-δ-lactone as the product of cellobiose oxidation. Cellopentaose is also oxidized but no oxidation of cellulose could be detected. The enzyme oxidizes lactose and 4-β-glucosylmannose but not 4-β-mannosylglucose which implicates the C-2-hydroxyl of the non-reducing end of the disaccharide as important for substrate specificity.

White-rot fungi degrade both polysaccharides and lignin in wood; however, the enzymic mechanism of lignin degradation is still very poorly understood. In two previous papers,[1,2] we reported the discovery and some properties of an extracellular enzyme from the white-rot fungi, *Polyporus versicolor* and *Sporotrichum pulverulentum*, which catalyzes the oxidation of cellobiose with simultaneous reduction of a quinone. The enzyme was given the systematic name cellobiose:quinone oxidoreductase and the trivial name cellobiose dehydrogenase. As the enzyme utilizes a carbohydrate and an oxidized phenol of cellulose and lignin origin, respectively, it was suggested [1,2] that the enzyme may play an important role both in lignin and cellulose degradation by fungi.

The culture solution from *S. pulverulentum* contains, in addition to cellobiose dehydrogenase, a lactonase and large amounts of cellulose degrading enzymes. Therefore to better understand the function and substrate specificity of the enzyme, a pure preparation is needed and this paper reports purification and some properties of cellobiose:quinone oxidoreductase from *S. pulverulentum*.

EXPERIMENTAL

Organism and cultivation. The fungus *Sporotrichum pulverulentum*, Novobranova was formerly called *Chrysosporium lignorum*. Recent investigations have revealed that it is identical with *S. pulverulentum* and the latter name should be used. The origin of the organism and the method of cultivation was described previously.[2]

Enzyme assay. Cellobiose:quinone oxidoreductase was measured spectrophotometrically.[2] One enzyme unit was defined as the amount of enzyme that reduced 1 μmol/min of 3-methoxy-5-*tert*-butyl-benzoquinone-(1,2) at pH 4.5 and 25 °C.

Concentration and ammonium sulfate precipitation. The culture filtrate was concentrated about 30 times by ultrafiltration (membrane 4M-10 Amicon, Cambridge, Mass., U.S.A.). The concentrated solution was centrifuged at 14 600 *g*; then solid ammonium sulfate was added to the supernatant fluid to 60 % saturation. The resulting precipitate was collected by centrifugation at 14 600 *g* for 30 min.

Isoelectric focusing. Isoelectric focusing was carried out as described in the LKB manual (LKB, Bromma, Sweden). The density gradient was formed with 1,2-ethanediol (0 – 75 % w/v) [3] and the carrier ampholyte (pH 3 – 10) was used at a concentration of 1 % in a total volume of 110 ml. After 20 h at 10 °C, focusing was terminated and the column emptied in 2.4 ml fractions. The pH of each fraction was measured at 4 °C.

Desalting and buffer exchange. Gel filtration using a column of Sephadex G-25 (15 × 260 mm) was generally used for desalting and change of buffer system.

Ion exchange chromatography. SP-Sephadex C-50 was used in the first chromatography step. The column (20 × 340 mm) was eluted (36 ml/h) with sodium acetate buffer (0.02 M, pH 4.3) and a linear gradient of NaCl. The total volume of salt eluent was 400 ml with a final concentration of 0.5 M NaCl.

Hydroxylapatite column chromatography. The second chromatography procedure used a column (15 × 110 mm) of Bio-Gel HT (Bio-Rad Lab., Richmond, U.S.A.) eluted (5 ml/h) stepwise initially with phosphate buffer (0.02 M, pH 7.4) and finally with 0.3 M NaCl.

Concanavalin A chromatography. Chromatography with a Concanavalin A Sepharose column (Pharmacia Fine Chemicals, Uppsala, Sweden) (10 × 20 mm) in sodium acetate buffer (0.1 M, pH 5.0) was also employed. Glycoproteins retained by the column were eluted (12 ml/h) with a solution of 10 % α-methyl D-mannoside in starting buffer.

Gel electrophoresis. Polyacrylamide-sodium-dodecylsulfate (SDS) gel electrophoresis [4] were run for 2.5 h at pH 7.1 with a current of 0.2 mA/mm² (6 mA/gel). Proteins were stained with Coomassie Brilliant Blue.[5]

Ultracentrifugation. Determination of the molecular weight was carried out in an ultracentrifuge Spinco Model E by the sedimentation-equilibrium method in an Yphantis doublesector cell. The enzyme was dissolved in phosphate buffer (0.02 M, pH 7.4) and determination carried out at 30 000 rpm for 24 h at 20 °C.

Absorption spectrophotometry. The ultraviolet and visible spectra were determined in phosphate buffer (pH 7.4) in a Cary 118 recording spectrophotometer with a 1 cm light path.

Thin-layer chromatography of flavins. Thin-layer chromatography was performed with 5 % Na_2HPO_4 in water and *t*-butyl alcohol:water 6:4 (v/v) as developing solvents.[6] Flavins were detected by their fluorescence.

Determination of lactones. Lactones were measured colorimetrically [7] as described earlier.[2]

Chemicals. Flavin-adenine dinucleotide (FAD), flavin-adenine mononucleotide (FMN) and α-methyl D-mannoside were purchased from Sigma Chemical Co. Mannodextrins and cellopentaose were gifts (see Acknowledgements).

RESULTS

Purification

Isoelectric focusing. Isoelectric focusing studies on the enzymes from the culture filtrate resolved three peaks of cellobiose dehydrogenase activity with isoelectric points of 4.0, 5.7, and 6.4, respectively. This result as well as data from other fractionation experiments suggests that at least three isoenzymes of cellobiose dehydrogenase are produced by the fungus *S. pulverulentum.*

Purification procedure. A summary of the purification steps is presented in Table 1. The dialysed precipitate from ammonium sulfate fractionation of culture solution (29 l) was first chromatographed on a SP-Sephadex C-50 column at pH 4.3. The bulk of the protein as well as 68 % of the cellobiose dehydrogenase activity were not retarded by the column (Fig. 1 and Table 1). The enzyme that chromatographed on the column was eluted with a salt gradient and was further purified on a hydroxylapatite column. This cellobiose dehydrogenase fraction was readily adsorbed on the hydroxylapatite column while a great deal of contaminating proteins passed unretarded as shown in Fig. 2. A yellow zone became visible on the top of the column and on elution cellobiose dehydrogenase was

Table 1. Purification procedure.

Purification step		Volume ml	Total activity units (U)	Total A_{280}	Specific activity U/A_{280}	Yield %	Fold purification
Culture filtrate		29000	521	6558	0.08	100	1
Ultrafiltrate		930	438	1987	0.220	84	3
$(NH_4)_2SO_4$ precipitation Step 1		17	379	802	0.47	73	6
SP-Sephadex C-50 chromatography	eluate	21	99	83	1.19	19	15
acetate pH 4.3	void	360	256	656	0.39	49	5
Step 2 Hydroxylapatite chromatography		9.8	31.4	2.45	12.8	6	160

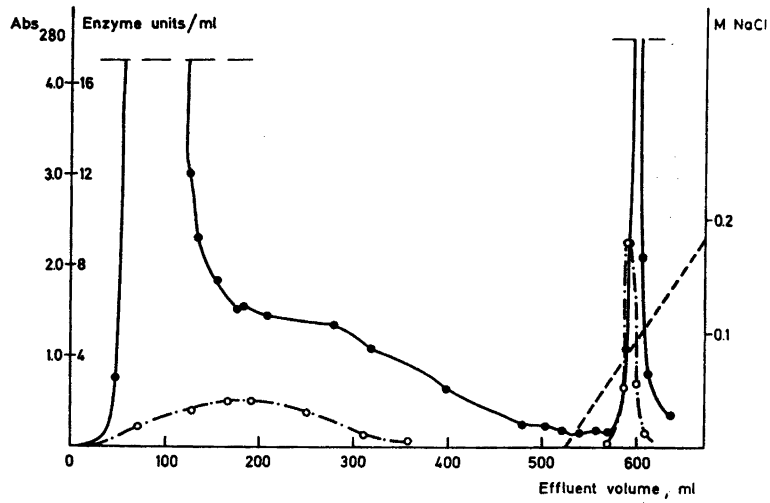


Fig. 1. Chromatography of cellobiose dehydrogenase on SP-Sephadex C-50, pH 4.3. Fractions 9 ml/tube for the starting buffer and 3.5 ml/tube in the gradient. ○, Activity of cellobiose dehy-hydrogenase; ●, absorbance at 280 nm; - - -, concentration of NaCl.

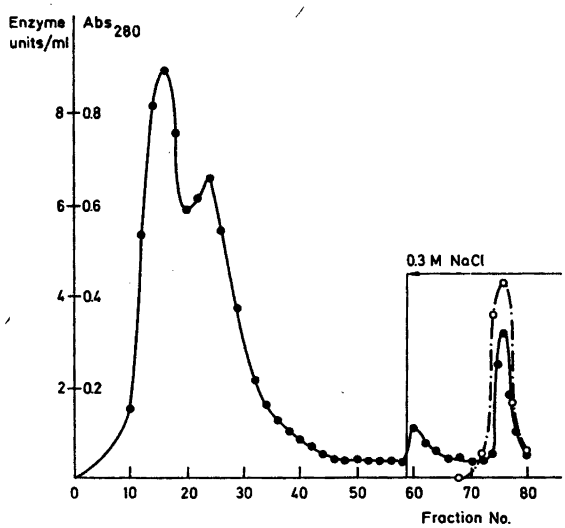


Fig. 2. Adsorption chromatography on hydroxylapatite. Fractions 3.25 ml/tube. ○, Activity of cellobiose dehydrogenase; ●, absorbance at 280 nm.

found in the yellow fractions. It is likely that the purified isoenzyme thus obtained is the most basic of the isoenzymes but no isoelectric focusing was undertaken to show this. This enzyme preparation was inactivated by freezing although crude material could be lyophilized.

Properties of the enzyme

Purity and molecular weight. The enzyme from the hydroxylapatite column showed one single band when run under denaturating conditions on polyacrylamide-SDS gel electrophoresis (Fig.



Fig. 3. SDS-polyacrylamide gel electrophoresis at pH 7.1 of the purified cellobiose dehydrogenase. The enzyme had an absorbance of 0.2 at 280 nm and 0.1 ml was applied.

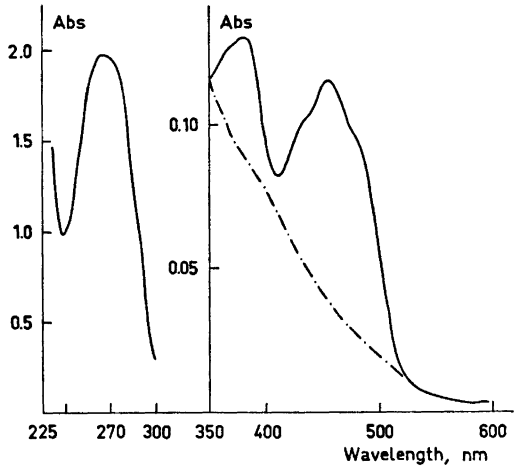


Fig. 4. Ultraviolet and visible spectrum of purified cellobiose dehydrogenase. ———, The spectrum of the oxidized enzyme; —.—, the visible spectrum after addition of the substrate cellobiose.

3). Ultracentrifugation also demonstrated that the enzyme was homogeneous with a molecular weight of 58 000 ± 2 000. An assumed partial specific volume of 0.72 was used in the calculations.

Spectrum of cellobiose dehydrogenase. The visible spectrum of the enzyme (Fig. 4) contains maxima at 375 – 380 and 457 nm and shoulders at about 435 and 475 nm. This type of spectrum is typical for a flavoprotein. The enzyme is visibly yellow and on addition of its substrate cellobiose, the yellow color disappears as well as the absorption maxima in the visible region (Fig. 4). The spectrum after addition of cellobiose is the normal for a reduced flavoprotein, although the absorption at 450 nm is somewhat high.

Identification of the prosthetic group. The prosthetic group was released from the protein by heating the enzyme in a boiling water-bath in darkness for 20 min. The precipitate was removed by centrifugation and the supernatant was analysed by thin-layer chromatography with flavin-adenine dinucleotide (FAD) and flavin-adenine mononucleotide (FMN) as reference substances. The released flavin from cellobiose dehydrogenase had the same R_F as authentic FAD in the two solvent systems used.

Binding to Concanavalin A. The enzymes retarded on the SP-Sephadex C-50 column were applied to a Concanavalin A-Sepharose column. As can be seen (Fig. 5) the cellobiose dehydrogenase enzyme bound to the Concanavalin A-Sepharose column was readily eluted by

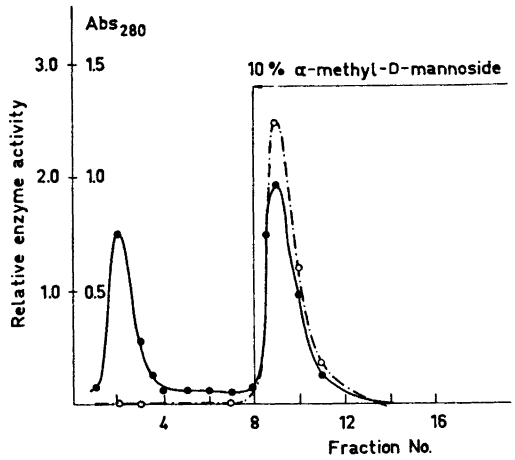


Fig. 5. Concanavalin A Sepharose chromatography of partially purified enzyme. Fractions 1.4 ml/tube. ○, Activity of cellobiose dehydrogenase; ●, absorbance at 280 nm.

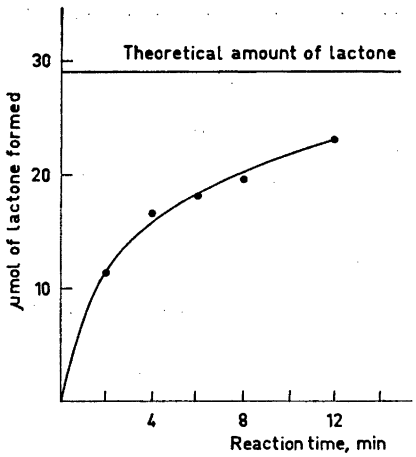


Fig. 6. Formation of cellobiono-δ-lactone. The reaction mixture contained 500 μmol of acetate buffer, pH 5.0, 29 μmol 3 methoxy-5-*tert*-butyl benzoquinone-(1,2), 58 μmol cellobiose, and 6.8 units of cellobiose dehydrogenase in a total volume of 10 ml. The reaction was started by addition of cellobiose.

a 10 % solution of α-methyl D-mannoside. The enzyme yield was about 70 % but no extensive purification occurred as the contaminating proteins were also bound to the column. However, binding to Concanavalin A strongly suggests [8] that the cellobiose dehydrogenase is a glycoprotein which contains terminal α-D-glucosyl and/or α-D-mannosyl or sterically similar residues.

Formation of cellobiono-δ-lactone from oxidation of cellobiose. As described in a previous paper [2] cellobionic acid was identified as a reaction product of the crude cellobiose dehydrogenase preparation. It is, however, more likely that cellobiono-δ-lactone is the first reaction product which then is destroyed by lactonase in the crude preparation. With purified cellobiose dehydrogenase, an accumulation of a lactone in the reaction mixture was demonstrated when the enzyme was incubated with cellobiose and quinone (Fig. 6). When all the quinone was reduced about 80 % of the stoichiometrical amount of lactone was found. The lactone is spontaneously hydrolyzed at pH 5.0 but the short reaction time gave little spontaneous hydrolysis.

Substrate specificity. As described in the previous paper [2] and Table 2 this paper, a number of mono- and disaccharides as well as cellopentaose and cellulose were investigated as possible enzyme substrates. Only cellobiose, cellopentaose, 4-β-glucosylmannose, and lactose were oxidized (Table 2).

DISCUSSION

The function of the different isoenzymes of cellobiose dehydrogenase is unknown; in fact it must still be established that these are true isoenzymes. Purified cellobiose dehydrogenase shows the same substrate specificity as the crude culture filtrate with regard to oxidation of carbohydrates. The same fungus, S. *pulverulentum*, also produces five endo-1,4-β-glucanases which have small but significant structural differences.[9] In particular, they differ in molecular weight (± 4 500) and in isoelectric point (± 1.1 pH unit). All but one are glycoproteins with varying amounts of carbohydrates of dif-

Table 2. Oxidation of different substrates. The reaction mixture contained purified cellobiose dehydrogenase, 300 mmol acetate buffer (pH 4.5), 1 μmol 3-methoxy-5-*tert*-butyl-benzoquinone-(1,2), 2 μmol of sugar in a total reaction volume of 3 ml. The cellulose sample had a degree of polymerization of 150 and about 8 μmol of reducing endgroups.

Substrate	Relative rate of oxidation
Cellobiose	100
Cellopentaose	100
Cellulose	0
Lactose (4-*O*-β-D-galactopyranosyl-D-glucose)	44
4-β-Mannosylglucose (4-*O*-β-D-mannopyranosyl-D-glucose)	0
4-β-Glucosylmannose (4-*O*-β-D-glucopyranosyl-D-mannose)	36
Mannobiose (4-*O*-β-D-mannopyranosyl-D-mannose)	0

ferent composition. There are also differences in their degradative and synthetic activities.[10]

The studied cellobiose dehydrogenase from *S. pulverulentum* has many properties in common with glucose dehydrogenase from *Aspergillus oryzae*.[11] For instance, both enzymes, as do many other sugar dehydrogenases, contain FAD. Glucose dehydrogenase, like cellobiose dehydrogenase, is a glycoprotein which uses certain redox dyes and quinones but not oxygen as a hydrogen acceptor. Glucose dehydrogenase is a soluble intracellular enzyme which is induced by certain quinones;[12] however, small amounts of the enzyme are also excreted extracellularly. The cellobiose dehydrogenase is extracellular and is not induced by quinones but by cellulosic substrates and it has a pronounced specificity for oxidation of degradation products from cellulose while most sugar dehydrogenases can oxidize a number of similar mono- and disaccharides.

From the ability or inability of cellobiose dehydrogenase to oxidize the disaccharides tested (Table 2) some understanding of the enzyme-substrate interaction can be gained. 4-β-Mannosylglucose differs from cellobiose only in the C-2 hydroxyl in the non-reducing end of the substrate yet it cannot be oxidized by cellobiose dehydrogenase. 4-β-Glucosylmannose, on the other hand, which has the C-2 hydroxyl of the reducing sugar unit inverted, is oxidized. However, oxidation of lactose demonstrates that inversion of the hydroxyl at C-4 of the non-reducing end does not prevent oxidation. This may be expected as this hydroxyl is involved in the β-glucosidic bond to the next glucose unit in cellodextrins. Xylobiose which differs from cellobiose by lacking hydroxymethyl groups at both C-5 positions is not oxidized.[2] These results indicate that the C-2 of the non-reducing end of the disaccharide substrates must have the glucose conformation but that inversion may occur at C-4 position. This may explain why monosaccharides are not oxidized. Furthermore, the enzyme needs a hydroxymethyl group at one or both C-5 atoms in the disaccharide. A β-linkage between the two sugar units seems also to be a requirement for activity since maltose is not oxidized.[2] From the substrate specificity and the mode of induction it is obvious that degradation products from cellulose are the proper substrates for the enzyme.

Acknowledgements. We want to thank Drs. G. O. Aspinall, York University, Ottawa, Canada and T. E. Timell, State University of New York, Syracuse, U.S.A. for the mannosylglucose, Dr. W. Brown, Uppsala University, Sweden for supplying the cellopentaose, Dr. H. Lundström of this department for the glucosylmannose and Dr. E. T. Reese, Natick Laboratories, Natick, Mass., U.S.A. for the mannobiose.

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Volatile Constituents Obtained by the Extraction with Alcohol-Water Mixture and by Steam Distillation of Coriander Fruit

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The composition of the volatile oil of Coriander fruit *(Coriandrum sativum* L.) isolated by distillation with steam and by extraction and distillation with an alcohol-water mixture was investigated by means of gas chromatography, column chromatography, infra red spectrophotometry and mass spectrometry. 45 compounds were identified including 19 hydrocarbons, 10 alcohols, 7 aldehydes and, in the alcoholic distillate only, 6 ethyl esters of long chain fatty acids.

Coriander is an annual herb originating in the Mediterranean countries, and is nowadays mostly grown in Italy, Morocco, India and Eastern Europe. Two types of coriander are distinguished according to the size of the fruit, *Coriandrum sativum* L. var. *vulgare* Alef. (fruit diameter 3-5 mm) and *Coriandrum vulgare* L. var. *microcarpum* DC. (fruit diameter 1.5 – 3 mm). Steam distillation of the fruit yields 0.15 – 1.7 % of volatile oil,[1] which is used in food industry, perfumery and pharmacy. The alcoholic extracts and distillates of dried fruits are used extensively for flavouring liquors.

The examination of the chemical composition of Coriander oil started at the end of the last century and in 1893 Barbier [2] identified its main component as *d*-linalool. In 1909 Walbaum and Müller [3] published quite a detailed study of the oil. They found the amount of linalool to be 60 – 70 % and identified 16 additional compounds.

Since 1960 gas chromatographic studies of the composition of coriander oil have been made by Ikeda *et al.*,[4] Schratz and Qadry,[5] Akimov and Voronin,[6] Paukov *et al.*,[7] Karlsen *et al.*[8] and Rasmussen *et al.*[9] The identification of most of the 31 compounds reported in these studies are based on the gas chromatographic retention times. In all, the following compounds have been found in coriander oils of various origin: α-pinene, β-pinene, camphene, sabinene, 3-carene, myrcene, α-terpinene, γ-terpinene, α-phellandrene, β-phellandrene, limonene, α-thujene, *cis*-ocimene, *trans*-ocimene, terpinolene, *p*-cymene, linalool, borneol, geraniol, nerol, citronellol, α-terpineol, terpinenol-4, linalyl acetate, geranyl acetate, bornyl acetate, camphor, decanal, 2-tridecenal, thymol, anethol, acetic acid, and decanoic acid.

It is obvious that the method of separation may have some effect on the composition of an essential oil. In the present study the chemical composition of both steam distilled oil and an alcoholic coriander distillate was investigated.

EXPERIMENTAL

Apparatus. A Hewlett-Packard 7620 A with FI detector was used for the analytical gas chromatography. The packed columns used were 5 % LAC-2R-446 on 70/80 mesh Chromosorb G AW DMCS, 15 % Apiezon L on 60/80 mesh Chromosorb W AW and 5 % SE-30 on 60/80 mesh Chromosorb G all in 6 mm O.D. × 3 m stainless steel tubes. A 0.28 mm I.D. × 25 m SP 1000 glass capillary column was also used in the same gas chromatograph fitted with a glass inlet splitter of S.G.E. The detector temperature was maintained at 290 °C and the injector temperature at 240 °C. Helium flow was 80 ml/min with packed columns and 0.7 ml/min with the capillary column. Peak areas were measured with an Infotronics CRS-11 HSB/42 integrator.

For the preparative gas chromatography the same packed columns and the same instrument were used with TC detector at 240 °C. The fractions eluting from the gas chromatograph

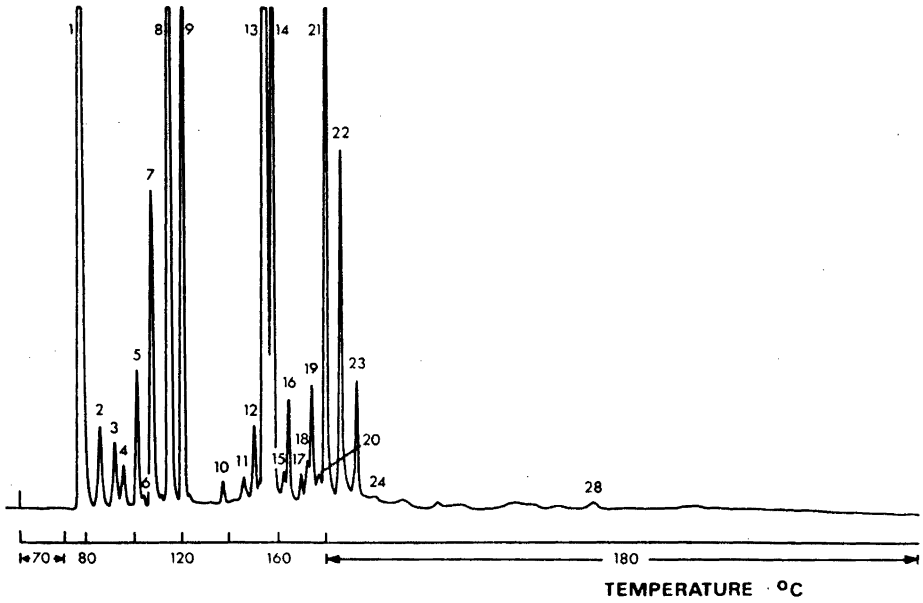


Fig. 1. Gas chromatogram of steam distilled coriander oil on 6 mm × 3 m LAC-2R-446 column.

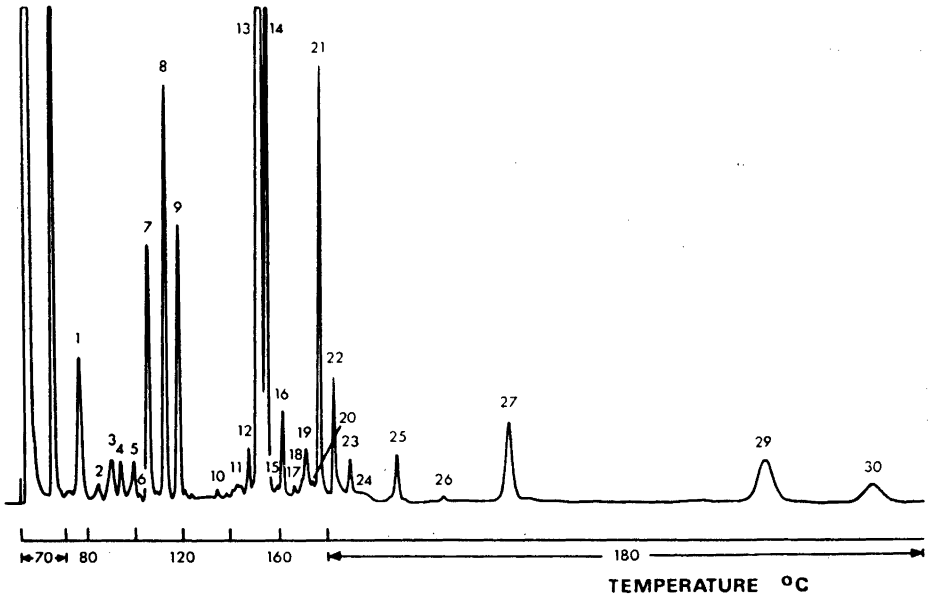


Fig. 2. Gas chromatogram of the pentane extract of the alcoholic coriander distillate on 6 mm × 3 m LAC-2R-446 column.

Table 1. Compounds identified in the steam distilled oil and the alcoholic distillate of coriander fruit.

Peak No.	Compound	Percentage		Evidence for identification
		Steam distilled oil	Extract of the alcoholic distillate	
1	α-Pinene	6.5	2.1	GLC, IR,[a] MS[a]
	α-Thujene	+	+	GLC
2	Camphene	0.39	0.11	GLC, MS[a]
3	β-Pinene	0.27	0.30	GLC, MS[a]
4	Sabinene	0.15	0.32	GLC, MS[a]
5	Myrcene	0.65	0.32	GLC, MS[a]
	α-Phellandrene	+	+	GLC
	3-Carene	+	+	GLC
6	α-Terpinene	0.05	0.02	GLC, MS[a]
7	Limonene	1.7	3.1	GLC, MS,[a] IR[a]
	β-Phellandrene	0.05	0.02	GLC, MS[a]
8	γ-Terpinene	10.1	4.5	GLC, MS,[a] IR[a]
	cis-Ocimene	+	+	GLC
	trans-Ocimene	+	+	GLC
9	*p*-Cymene	3.7	2.8	GLC, MS,[a] IR[a]
	Terpinolene	0.1	0.02	GLC, MS[a]
10	Nonanal	0.07	0.04	MS [12]
11	Linalool oxide	0.08	0.05	MS [13]
12	Decanal	0.31	0.38	GLC, MS [12]
13	Linalool	65	66	GLC, MS,[a] IR[a]
14	Camphor	5.0	6.7	GLC, MS,[a] IR[a]
15	Caryophyllene	0.06	0.01	GLC, MS[a]
	Undecanal			MS [12]
16	Terpinenol-4	0.31	0.67	GLC, MS[a], IR[a]
17	*trans*-2-Decenal	0.07	0.03	MS,[11] IR
18	Borneol	0.13	0.05	GLC, MS,[14] IR[15]
19	α-Terpineol	0.46	0.70	GLC, MS,[a] IR[a]
	Decanol			GLC, MS.[16] IR[a]
	Dodecanal			MS [12]
	Ethyl undecanoate			MS
	Heptadecane	+		MS [17]
20	Citronellol	0.02	0.03	GLC, MS, IR[a]
21	Geranyl acetate	2.6	3.8	GLC, MS, IR[a]
	Nerol	+	+	GLC, MS [14]
	trans-Citral	+	+	GLC, MS [18]
22	Geraniol	1.7	1.3	GLC, MS,[14] IR[a]
	Octadecane	+	+	MS [17]
23	*trans*-2-Dodecenal	0.44	0.25	MS, IR
24	Dodecanol	+	+	GLC, MS
25	Ethyl myristate		0.59	GLC, MS, IR[a]
26	Ethyl pentadecanoate		0.02	MS
27	Ethyl palmitate		2.3	GLC, MS,[17] IR[a]
28	Myristicin	0.05		MS,[19] IR[19]
29	Ethyl stearate		2.1	GLC, MS, IR[a]
30	Ethyl linoleate		0.64	GLC, MS, IR[a]

[a] = spectrum of an authentic sample.

were collected in 1 mm I.D. × 20 cm glass tubes cooled with dry ice.

The IR spectra were measured between KBr plates using a Perkin-Elmer 521 spectrophotometer fitted with a beam condenser.

The mass spectra were run on a Perkin-Elmer GLC-MS model 270 using a 15 m FFAP SCOT column or on a Varian CH7 GLC-MS using a 2 mm I.D. × 2 m SE-30 glass column in the gas chromatograph. A Spectro-System 100 MS computer was coupled to the Varian mass spectrometer.

Materials and procedure. The alcoholic distillate studied in this work was produced in Alko's flavour distillery from Moroccan coriander (fruit diameter 3 – 5 mm). The distillate is prepared by percolating the spice with 43 % alcohol-water mixture for two weeks and by distilling the percolate under reduced pressure. In the distillate the essential oil of coriander is present as a dilute alcoholic solution and for analyses it was concentrated by extracting with pentane as described previously.[10]

Another sample of coriander oil was obtained by three hours' steam distillation of 500 g of pulverized coriander fruit (from the same batch of herb as used for the alcoholic distillate.) The distillate was extracted with pentane, the extract dried over Na_2SO_4 and the pentane evaporated to leave about 2 g of oil.

The percentage composition of the samples was determined with LAC-2R-446 column from peak areas without using response factors. The composition of some unresolved monoterpene hydrocarbon peaks was determined with the capillary column.

The initial preparative fractionation of the steam distilled oil and the pentane extract of the alcoholic distillate was performed with an LAC-2R-446 column. The fractions obtained were further chromatographed on the SE-30 column to collect pure compounds. The GLC-MS analyses were made from the initial fractions using the SE-30 column.

The hydrocarbons were also analysed after separation from the oxygenated compounds in a silica gel column as described previously.[10] GLC-MS analysis was made directly from this fraction using an FFAP SCOT column.

RESULTS AND DISCUSSION

The gas chromatograms of steam distilled coriander oil and the pentane extract of the alcoholic distillate obtained with the LAC-2R-446 column are shown in Figs. 1 and 2. For the most part the numbered peaks correspond to the preparative fractions. The compounds identified, with their approximate percentage contribution and identification methods, are presented in Table 1.

Peaks 1 – 9 in Figs. 1 and 2 represent monoterpene hydrocarbons. These constitute about 24 % of the steam distilled oil and about 14 % of the aroma compounds of the alcoholic distillate. The same 16 compounds were found in both samples: α-pinene, β-pinene, camphene, sabinene, myrcene, limonene, β-phellandrene, α-terpinene, γ-terpinene, *p*-cymene, terpinolene, α-thujene, 3-carene, α-phellandrene, *cis*-ocimene, and *trans*-ocimene. The last five compounds were present in trace amount and were identified by gas chromatographic retention times only.

Some higher boiling compounds were isolated from the hydrocarbon fraction of the steam distilled oil. The largest of these (about 0.03 % of the oil) was identified as caryophyllene. Three other unidentified sesquiterpene hydrocarbons were detected by GLC-MS analysis. Sesquiterpenes have not previously been reported as constituents of coriander oil. Also found in this fraction were non-terpenic compounds heptadecane and octadecane, and two other saturated hydrocarbons, which according to IR and MS analyses would be bicycloalkanes of molecular weight 180 and 208.

The most important group of compounds in the aroma of coriander are the oxygen containing monoterpenes, which are distributed from peak 13 to 22 in the gas chromatograms. Linalool was found to form about 65 % of both samples. The other monoterpene alcohols, geraniol, α-terpineol, borneol, terpinenol-4, citronellol, and nerol, constituted together about 3 %, camphor 5 – 7 %, and geranyl acetate 3 – 4 %. *trans*-Citral and linalool oxide were also identified as minor components. Linalyl acetate and bornyl acetate, which are reported in many earlier papers,[3,6–8] could not be found in the samples studied.

Several non-terpenic oxygenated compounds were found as minor constituents of coriander oil. Four saturated aldehydes, nonanal, decanal, undecanal, and dodecanal, were identified. In addition, two unsaturated aldehydes, *trans*-2-decenal and *trans*-2-dodecenal, represented by peaks 17 and 23, were found. The IR spectrum of compound 17 exhibited a strong absorption band at 1690 cm^{-1} typical for α,β-unsaturated aldehyde and absorptions at 1630 and 970 cm^{-1} characteristic for a *trans*-disubstituted double bond. The mass spectrum matched well with the spectrum published for *trans*-2-decenal.[11]

The IR spectrum of compound 23 also had absorption bands (at 3020, 2810, 2720, 1688, 1632, 970 cm^{-1}) implying the presence of an aldehyde carbonyl group conjugated with a *trans*-disubstituted double bond. The mass spectrum showed the molecular peak at *m/e* 182 and the fragmentation pattern closely resembled that of *trans*-2-decenal. Accordingly, compound 23 was identified as *trans*-2-dodecenal. Two further aliphatic compounds, decanol and dodecanol, were found in trace amounts. Of these compounds decanal has been reported earlier,[3,5,7] while Schratz and Qadry [5] have shown the presence of *trans*-2-tridecenal in a sample of bulgarian oil.

Besides *p*-cymene, myristicin was the only aromatic compound identified. Neither thymol, found by Schratz and Qadry [5] in Indian coriander oil, nor anethol reported by Rasmussen *et al.*[9] were detected.

The ethyl esters of undecanoic, myristic, pentadecanoic, palmitic, stearic, and linoleic acids were found in the alcoholic distillate of coriander fruit. Together they constituted about 6 % of the aroma fraction. These compounds were absent from the steam distilled oil and are obviously formed during the preparation of the alcoholic distillate. Other qualitative differences between the two samples investigated proved to be small.

Acknowledgements. The authors are indebted to Dr. J. Kärkkäinen, Department of Medical Chemistry, University of Helsinki, and Mr. P. Karvonen, Department of Forest Products, Helsinki University of Technology, for running the mass spectra.

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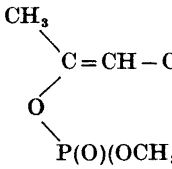
Studies on Organophosphorus Compounds. X.* Phosphorylation of Enolates. Stereochemical Investigations of the Reaction between Salts of β-Dicarbonyl Compounds and *O,O*-Diethyl Phosphorochloridothioate**

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Sodium salts of β-dicarbonyl compounds are found to yield mainly *Z*-isomers and tetrabutylammonium (TBA) salts mainly *E*-isomers of vinyl phosphorothioates when reacted with *O,O*-diethyl phosphorochloridothioate. The structure of the products is ascribed to the geometry of the anion of the salts: the W-shape yields *E*-isomers and the U-shape *Z*-isomers. At elevated temperature decomposition and *Z/E* isomerization are observed. *O,O*-Diethyl *O*-(2-acetyl-1-phenylvinyl) phosporothioate (*6*) undergoes a thermal rearrangement to *O,O*-diethyl *O*-(2-benzoyl-1-methylvinyl) phosphorothioate (*5*) and the reverse rearrangement is also observed.

Enol phosphates and related compounds are known as powerful insecticides [4-6] and the physiological effect is dependent on the *Z/E* ratio.[5] It is possible to separate the isomers by chromatography,[6] and for Phosdrin the *E*-isomer is found to be the most active one.[5,6] In recent years much effort has been made to design specific reactions, and this has been achieved through variations of solvent, concentration and counter ion of the enolates.

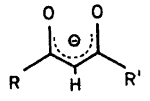


Phosdrin

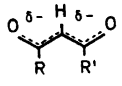
O,O-Diethyl phosphorochloridothioate

Reactions of enolate ions of β-dicarbonyl compounds and similar species with phosphorylating agents such as *O,O*-dialkyl phosphorochloridothioate or *O,O*-dialkyl phosphorochloridate occur solely on oxygen to give stable vinyl phosphorothioates or phosphates [1,4] while normal enolates in few cases give keto phosphonates as co-products.[8] Even metal derivatives of malonic ester are postulated to give diethyl 2-ethoxycarbonyl-1-ethoxyvinyl phosphate by *O*-phosphorylation when reacted with diethyl phosphorochloridate.[16,22] Also the reaction of α-halo ketones with trialkyl phosphites (Perkow reaction) in most cases leads to the *O*-phosphorylated product.[8]

It is found that the ratio of geometrical isomers procuded changes remarkably as the polarity of the solvent is varied.[1] This is accounted for by the shape of the enolate ion. When the enolate ion is U-shaped the phosphorylated product is the *Z*-isomer and when W-shaped,



U-shape



W-shape

* Part IX, Pedersen, E. B. and Lawesson, S.-O. *Acta Chem. Scand. B 28* (1974) 1045.

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the product is the *E*-isomer. In aprotic, polar solvents (DMSO, HMPA) the nucleophilic species is the non-solvated dissociated anion in W-shape.[13] Formation of products of *Z*-configuration in nonpolar, aprotic solvents is due to chelation of the anion in U-shape with the counterion, while formation of *E*-isomers in polar, aprotic solvents is due to reaction of the free, unchelated anions in W-shape.[1,2] The same configuration is achieved if the size of the cation is enlarged to tetrabutylammonium (TBA^+).[2,13] Tetraalkylammonium salts are postulated to exist largely as free ions even in relatively nonpolar solvents. This is concluded from the hypothesis that *E*-isomers exclusively are formed from free anions [1] which should also explain why more dilute solutions give higher percentage of *E*-configuration.[1] However, in relatively nonpolar solvents the presence of the free anions must be questioned and the *E*-isomer must be ascribed to the geometry of an ion pair. The smaller cations give much higher percentages of *Z*-isomers [1,9] due to fixation of the anion in U-shape.

It is postulated that the protonic or non-protonic nature of the solvent should have only little effect on the geometry of the products.[1] That is rejected by Kurts *et al.*,[15] arguing that in polar aprotic solvents W-shape conformations are more stable while in protic solvents enolate ions of β-dicarbonyl compounds adopt the alternative U-shaped conformation. This should be ascribed to hydrogen bond stabilization by the protic solvent of the anionic ground state and it means that the *O*-phosphorylated product in a protic solvent should be the *Z*-isomer.

If the vinyl phosphates are prepared according to the Perkow reaction the products are mixtures of the *Z*- and *E*-isomers.[9]

Previous investigations have supplied us with some helpful spectroscopic tools for the determination of the structure of vinyl phosphates and vinyl phosphorothioates. All compounds assigned to *E*-configuration have the vinyl proton resonance appearing downfield from those assigned to *Z*-configuration [1–3,5,6] and if there is a methyl group attached to the double bond at carbon 1, *e.g.* in the products from alkyl acetoacetate, the methyl proton resonance also occurs at lower field in the *E*- than in the *Z*-isomers.[1,5,6] In some previous investigations [3,6] the coupling constant in the *E*-isomer between the vinylic proton and P is found to be greater than the corresponding constant in the *Z*-isomer and in the products from acetoacetates the coupling constant between the protons in the methyl group and P is found to be greater in the *Z*-isomer than in the *E*-isomer.[3] From the above it is obvious that it should be possible to synthesize *Z*-isomers by reacting the sodium salts of β-dicarbonyl compounds in a suitable alcohol with a suitable electrophilic reagent and the corresponding *E*-isomer by reacting the TBA-salts in methylene chloride. This paper concerns reactions between salts of β-dicarbonyl compounds and *O,O*-diethyl phosphorochloridothioate, $(C_2H_5O)_2P(S)Cl$.

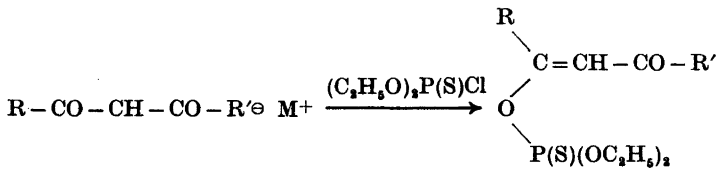
FORMATION OF SALTS OF β-DICARBONYL COMPOUNDS

The TBA-salts of the β-dicarbonyl compounds used in the present investigation (Table 1) were synthesized according to the method described by Brändström and Junggren.[17] However, a more clean reaction was obtained by using methylene chloride instead of chloroform as solvent. The ammonium salts were isolated and, except for III and IV, purified before use. The sodium salts (Table 1) were not isolated before the subsequent reactions and they were generated by adding the β-dicarbonyl compound to a freshly prepared alcoholic solution of sodium alcoholate.

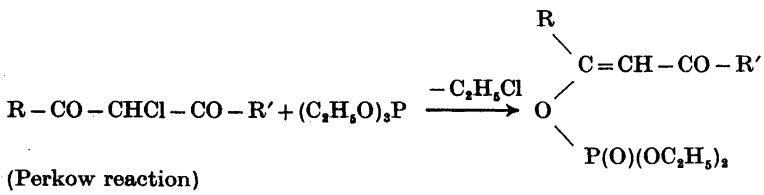
PHOSPHORYLATION, STRUCTURE ELUCIDATION AND REARRANGEMENTS

The phosphorylation reactions were performed by adding $(C_2H_5O)_2P(S)Cl$ to solutions of the salts in the respective solvents. Some of the mixtures were heated to reflux temperature (see experimental). The reactions of the sodium salts in alcoholic solutions yielded the vinyl phosphorothioates in lower yields. Other products isolated were the parent β-dicarbonyl compound and trialkyl phosphorothiates. After workup the product distibution (Table 1) in the reaction mixture was determined by NMR spectroscopy. In Table 2 are shown the NMR chemical shifts and coupling constants of the vinylic and other protons of the vinylic part of the phosphorothioates. Also the products obtained by the Perkow reaction of triethyl phosphite with

Table 1. The product distribution in the vinyl phosphorothioates from the reaction between TBA- and sodium salts of β-dicarbonyl compounds and *O,O*-diethyl phosphorochloridothioate and in the vinyl phosphates from the reaction between triethyl phosphite and 2-chloro-1-phenyl-1,3-butanedione.



Salt No.	R	R′	M+	*Z* (%)	*E* (%)	Prod. No.
I	CH_3	CH_3	TBA^+	30	70	*1*
II	CH_3	OCH_3	TBA^+	~0	100	*2*
III	CH_3	OC_2H_5	TBA^+	~0	100	*3*
IV	C_6H_5	OC_2H_5	TBA^+	25	75	*4*
V	CH_3	C_6H_5	TBA^+	0	100	*5*
	C_6H_5	CH_3	TBA^+	0	0	*6*
VI	CH_3	CH_3	Na^+(EtOH)	90	10	*1*
VII	CH_3	OCH_3	Na^+(EtOH)	90	10	*2* + *3*
			(MeOH)	75	25	*2*
VIII	CH_3	OC_2H_5	Na^+(EtOH)	100	0	*3*
IX	C_6H_5	OC_2H_5	Na^+(EtOH)	100	0	*4*
	CH_3	C_6H_5	Na^+(EtOH)	72	21	*5*
X	C_6H_5	CH_3	Na^+(EtOH)	7	0	*6*



(Perkow reaction)

	CH_3	C_6H_5	Ether	50	10	*7*
	C_6H_5	CH_3		10	30	*8*

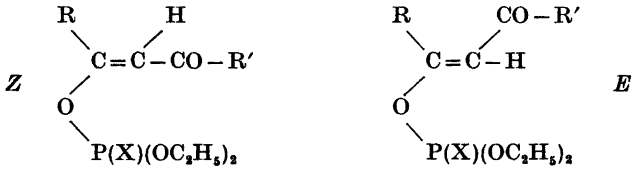
chlorobenzoylacetone (2-chloro-1-phenyl-1,3-butanedione) are included in Tables 1 and 2.

One of the products from the reaction between the TBA-salts and $(C_2H_5O)_2P(S)Cl$ is TBA^+Cl^-. For determination of the yields it is also of interest to isolate this product, but as the chloride is hygroscopic it is converted to the TBA^+I^- by treating the reaction mixture with a water solution of an iodide. For TBA^+Cl^- and TBA^+I^- log E_{TBAX} from the equation

$$\frac{[TBA^+X^-]_{org}}{[TBA^+]_{aq}\,[X^-]_{aq}} = E_{TBAX}$$

is 0.5 and 3.34, respectively, when the organic phase is CH_2Cl_2.[14] According to that, tetra-

Table 2. NMR chemical shifts and coupling constants of the protons from the vinylic part of the vinyl phosphorothioates and phosphates generated by the reactions depicted in Table 1 ($CDCl_3$).



	R	R′	X	R	R′	H_E	H_Z
1E	CH_3	CH_3	S	t, 2.35, (J = ~0.5 Hz)	s, 2.20	m, 6.19	–
1Z	CH_3	CH_3	S	dd, 2.18, (J_1 = ~2.0 Hz, J_2 = ~1.0 Hz)	s, 2.31	–	m, 5.56
2E	CH_3	OCH_3	S	t, 2.38, (J = ~0.5 Hz)	s, 3.71	m, 5.78	–
2Z	CH_3	OCH_3	S	dd, 2.14, (J_1 = ~2.0 Hz, J_2 = ~1.0 Hz)	s, 3.68	–	m, 5.40
3E	CH_3	OC_2H_5	S	t, 2.38, (J = ~0.5 Hz)	CH_2: q, 4.19 (J = 7.1 Hz) CH_3: t, 1.28 J = 7.1 Hz)	m, 5.77	–
3Z	CH_3	OC_2H_5	S	dd, 2.14, (J_1 = ~2.0 Hz, J_2 = ~1.0 Hz)	CH_2: q, 4.17 (J = 7.2 Hz) CH_3: t, 1.26 (J = 7.2 Hz)	–	m, 5.39
4E	C_6H_5	OC_2H_5	S	m, 7.3 – 7.8	CH_2: q, 4.09 (J = 7.0 Hz) CH_3: t, 1.37 (J = 7.0 Hz)	d, 6.06, (J = ~2.2 Hz)	–
4Z	C_6H_5	OC_2H_5	S	m, 7.3 – 7.8	CH_2: q, 4.06 (J = 7.0 Hz) CH_3: t, 1.32 (J = 7.0 Hz)	–	d, 6.00 (J = ~2.2 Hz)
5E	CH_3	C_6H_5	S	t, 2.44 (J = ~0.7 Hz)	2H: m, 7.8 – 8.1 3H: m, 7.4 – 7.7.	m, 6.97	–
5Z	CH_3	C_6H_5	S	dd, 2.23, (J_1 = ~2.0 Hz, J_2 = ~1.0 Hz)	2H: m, 7.8 – 8.1 3H: m, 7.3 – 7.6	–	m, ~6.2
6Z	C_6H_5	CH_3	S	2H: m, 7.8 – 8.1 3H: m, 7.3 – 7.6	s, 2.56	–	m, ~6.2
7E	CH_3	C_6H_5	O	d, 2.46, (J = ~0.8 Hz)	2H: m, 7.8 – 8.1 3H: m, 7.3 – 7.7	m, 7.02	–
7Z	CH_3	C_6H_5	O	dd, 2.29, (J_1 = ~1.5 Hz, J_2 = ~1.0 Hz)	5H: m, 7.3 – 8.0	–	m, ~6.18
8E	C_6H_5	CH_3	O	5H: m, 7.3 – 8.0	s, 2.06	d, 6.40 (J = ~1.9 Hz)	–
8Z	C_6H_5	CH_3	O	5H: m, 7.3 – 8.0	s, 2.44	–	d, 6.18 (J = ~1.0 Hz)

butylammonium ion is found as TBA^+I^- in the organic phase when the mixture is treated with iodide. Evaporation of the organic phase followed by treatment with ether yields TBA^+I^- as clean, nonhygroscopic, and white crystals suitable for regeneration of $TBA^+HSO_4^-$. The vinyl phosphorothioate is in the ether phase. That iodide is postulated to be a "catalyst poison" by some authors [11,12] is due to the equilibrium constant above. For chloride and bromide (log $E_{TBABr} = 1.23$ [14]) some TBA^+X^- is found in the water phase and TBA^+ can then serve as catalyst.

The TBA-salt of acetylacetone (I) was refluxed in methylene chloride with $(C_2H_5O)_2P(S)Cl$ yielding 75 % of *1Z* and *1E* (3:7). Distillation enhanced the content of the *E*-isomer to 75 % and the expected conversion [19,26] to products of the type C_2H_5-S-P was also observed. The isomers were separated by column chromatography. Addition of $(C_2H_5O)_2P(S)Cl$ to a solution of the sodium salt of acetylacetone (VI) yielded after a few minutes reflux 90 % of *1Z* and *1E* (9:1).

The reaction of the TBA-salt of methyl acetoacetate (II) with $(C_2H_5O)_2P(S)Cl$ was exothermic and the yields of *2Z* and *2E* were 93 % (apparent ratio 0:100). However, by column chromatography a fraction of 2.13 g containing *2E* and a fraction of 0.08 g containing 50 % *2E* and 50 % *2Z* was isolated from 2.40 g product. This corresponds to less than 2 % of the *Z*-isomer in the crude product. Addition of $(C_2H_5O)_2P(S)Cl$ to the sodium salt generated from methyl acetoacetate in ethanol yielded after reflux for a few minutes 82 % vinyl phosphorothioate (90 % *Z*- and 10 % *E*-isomer). A neat *Z*-fraction containing 43.5 % methyl ester (*2Z*) and 56.5 % ethyl ester (*3Z*) was isolated by column chromatography. Transesterification of the carboxylic ester group has occured. Refluxing diethyl 2-methoxycarbonyl-1-methylvinyl phosphorothioate (*2Z* and *E*) in ethanol did not give diethyl 2-ethoxycarbonyl-1-methylvinyl phosphorothioate (*3*) as shown by gas chromatography. Even ethanolysis of the vinyl phosphorothioate [18] was not observed to any remarkable extent as alkyl acetoacetate or triethyl phosphorothioate were only detected in an amount less than 1 % in the refluxed mixture.

When methanol was used as solvent for the generation and subsequent phosphorylation of the sodium salt of methyl acetoacetate (VII) the product isolated was *2* (75 % *2Z* and 25 % *2E*). If the sodium salt of methyl acetoacetate (VII) was generated in benzene from sodium hydride the yield of the vinyl phosphorothioate after addition of $(C_2H_5O)_2P(S)Cl$ was only 9 % even after reflux for several hours and the added $(C_2H_5O)_2P(S)Cl$ was recovered in 75 % yield. The poor yield must be ascribed to the low solubility of the salt in benzene.[1]

When the TBA-salt of ethyl acetoacetate (III) was reacted with $(C_2H_5O)_2P(S)Cl$ the reaction was exothermic and yielded 84 % of *3E* and *3Z* (apparent ratio 100:0). However, a fraction of 1.23 g *3E* and a fraction of 0.06 g *3Z* was isolated from 1.7 g of product by column chromatography. Addition of $(C_2H_5O)_2P(S)Cl$ to the sodium salt of ethyl acetoacetate (VIII) in hot ethanol yielded *3Z*.

When the TBA-salt of ethyl benzoylacetate (IV) was reacted with $(C_2H_5O)_2P(S)Cl$ at room temperature the product was a mixture of *4Z* (25 %) and *4E* (75 %). The sodium salt of ethyl benzoylacetate (IX) when refluxed with $(C_2H_5O)_2P(S)Cl$ in ethanol gave *4Z* as the only product.

Phosphorylation of salts of benzoylacetone (V,X) can yield two products due to the non-symmetry of the salts. In Table 1, V and X are depicted in two ways to explain the two products *5* and *6*, each with the two possible isomers *Z* and *E*. However, *6E* is not isolated. When the TBA-salt of benzoylacetone (V) was reacted with $(C_2H_5O)_2P(S)Cl$ at room temperature a slight elevation of the temperature was observed. The only product observed was diethyl 2-benzoyl-1-methylvinyl phosphorothioate (*5E*). Distillation of *5E* gave to a great extent new products as shown in Fig. 1. The NMR spectra of the distillation products also showed the expected conversion [19,26] to products of the type C_2H_5-S-P. In Fig. 1 the β-diketone and the monothio-β-diketone are depicted in a similar way rather than necessarily in the most likely form. (About the true structure see Refs. 20 and 23). Simple heating of *5E* in bromobenzene (155 °C) yielded *5E* and *5Z* as well as the rearranged product *6Z* (Fig. 1). The product *6* together with *5E* and *5Z* is only formed from the sodium salt X and only one of the isomers *6E* and *6Z* is found (Table 1). This can be

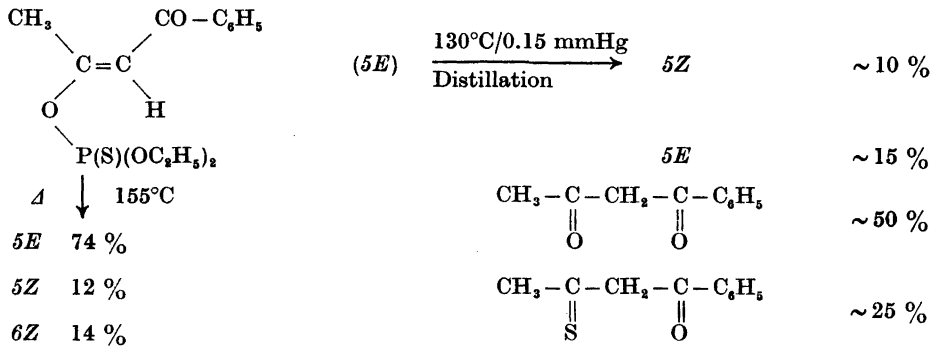


Fig. 1. Rearrangements and decomposition of diethyl 2-benzoyl-1-methylvinyl phosphorothioate (*5E*).

accounted for if *6* is generated only from the U-shaped anion. The product must then be expected to be *6Z* and the NMR data of *6* do also correspond better to *8Z* than to *8E*. Heating a benzene solution of *5Z*, *5E*, and *6Z* shows that *5Z* is transformed to *5E* while *6Z* seems to have reached an equilibrium concentration or to be stable towards rearrangements at that temperature. Heating a chlorobenzene solution of the same compounds gave almost the same product distribution as when neat *5E* was heated in chlorobenzene (Fig. 1). It is thought to be an equilibrium mixture.

Reaction of 2-chloro-1-phenyl-1,3-butanedione with triethyl phosphite (Perkow reaction) yielded the products *7* and *8* (Table 1). By column chromatography *7E* was isolated neat. Refluxing a mixture of *7Z*, *8E*, and *8Z* in chlorobenzene yielded a mixture of benzoylacetone, *7Z*, *7E*, *8Z*, and *8E*, where the *E*-isomers were the most abundant of the vinyl phosphates.

So far only the vinyl phosphorothioates have been looked on as products, but also the parent β-dicarbonyl compounds were isolated together with trialkyl phosphorothioate from the reactions between salts of β-dicarbonyl compounds and $(C_2H_5O)_2P(S)Cl$. In Table 3 is shown the distribution between those products. The trialkyl phosphorothioate is the *O,O,O*-triethyl

Table 3. The yields from the reactions between TBA- and sodium salts of β-dicarbonyl compounds and *O,O*-diethyl phosphorothioate.

Salt	Solvent	Vinyl phosphorothioate %	β-Dicarbonyl compound %	*O,O,O*-Trialkyl phosphorothioate %
I	CH_2Cl_2	75		
II	CH_2Cl_2	93		
III	CH_2Cl_2	84	16	
IV	CH_2Cl_2	95		
V	CH_2Cl_2	85	15	
VI	EtOH	90		
VII	EtOH	82 [a]		18
VII	MeOH	32	37	58
VIII	EtOH	70		30
IX	EtOH	32	64	64
X	EtOH	51	36	48

[a] 2-methoxycarbonyl-1-methylvinyl phosphorothioate (*2*) 27 % 2-ethoxycarbonyl-1-methylvinyl phosphorothioate (*3*) 55 %.

ester except when the solvent was methanol. In this case it was *O,O*-diethyl *O*-methyl phosphorothioate.

DISCUSSION

Polar aprotic solvents and bulky cations should give *E*-isomers when salts of β-dicarbonyl compounds are reacted with diethyl phosphorochloridothioate. In this investigation TBA-salts of β-dicarbonyl compounds produced mainly the *E*-isomers (Table 1), although methylene chloride is not a very polar solvent. If it is only the enolate anion in W-shape that gives the *E*-isomers the anion in the TBA-salts must adopt this W-shape when dissolved in methylene chloride although the ion pair is expected to be maintained. In the introduction it was also mentioned that *Z*-isomers were achieved through phosphorylation of enolate anions in U-shape. This U-shape should be found when the cation was small and nonpolar solvents were used. However, also protic solvents should generate an U-shaped anion. From these predictions the products shown in Table 1 were expected when sodium salts of β-dicarbonyl compounds

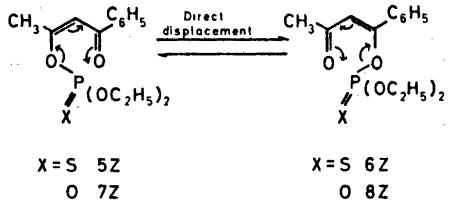


Fig. 2. A mechanism for the rearrangements of diethyl 2-benzoyl-1-methylvinyl phosphorothioate (*5*) to diethyl 2-acetyl-1-phenylvinyl phosphorothioate (*6*) (or vice versa) and of diethyl 2-benzoyl-1-methylvinyl phosphate (*7*) to diethyl 2-acetyl-1-phenylvinyl phosphate (*8*) (or *vice versa*).

were reacted with diethyl phosphorochloridothioate.

In an earlier work [4,18] the structure of the products from the reaction between X and $(C_2H_5O)_2P(S)Cl$, the reaction between X and $(C_2H_5O)_2P(O)Cl$ and the reaction between 2-chloro-1-phenyl-1,3-butanedione and triethyl phosphite are postulated to be *6* and *8*, respectively. This assumption is made from isolating methyl glyoxal and benzoic acid when the products were ozonized. However, the products were purified by distillation before the ozonization and, as shown in the present investigation, this will increase the amounts of the compounds *6* and *8* in the respective mixtures, *i.e.* there is no evidence for the products to have been entirely *6* and *8*.

The rearrangement from *5* to *6* or from *7* to *8* is expected to go *via* the *Z*-isomers as depicted in Fig. 2 (Direct displacement [21]). Two other mechanisms (elimination-addition and addition-elimination) suggested for nucleophilic substitution on phosphorus in phosphates [21] are not considered as likely. The first is excluded because the rearrangements are performed under neutral conditions. The latter involves a charged intermediate probably with a trigonal bipyramidal structure.

The formation of the monothio-β-diketone when diethyl 2-benzoyl-1-methylvinyl phosphorothioate (*5E*) was distilled (Fig. 1) can be expected to go through the intermediate shown in Fig. 3. This is parallel to the finding of products of the type C_2H_5-S-P which are also found after distillation of 1.

The cleavage yielding a β-dicarbonyl compound was also observed when *7* and *8* were heated in chlorobenzene.

As shown in Table 3 phosphorylation of the sodium salts in alcoholic solutions yielded some

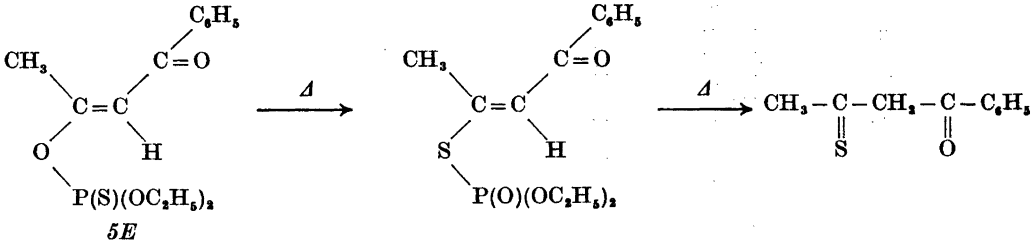


Fig. 3. A reaction sequence for the formation of the monothio-β-diketone from diethyl 2-benzoyl-1-methylvinyl phosphorothioate (*5E*).

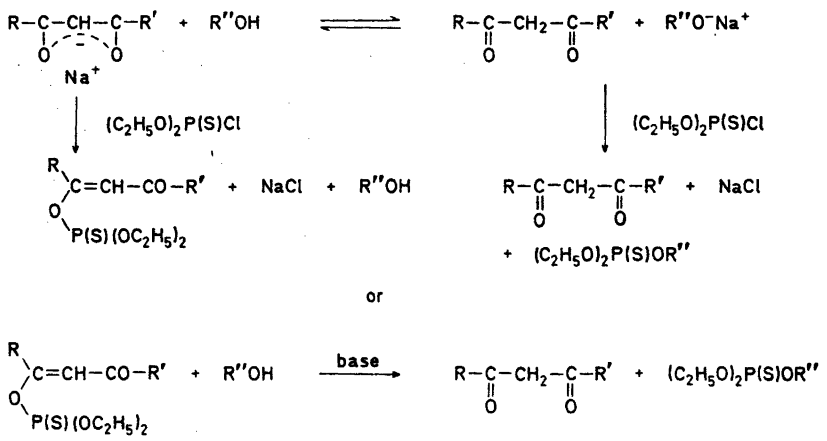


Fig. 4. Reaction sequences for the formation of the products shown in Table 3 from the reaction between $(C_2H_5O)_2P(S)Cl$ and sodium salts of β-dicarbonyl compounds.

trialkyl phosphorothioate and the parent β-dicarbonyl compound. The products can be accounted for by the equilibrium existing between the sodium salt of the β-dicarbonyl compound and sodium alcoholate as shown in Fig. 4. The amounts of β-dicarbonyl compound and *O,O,O*-trialkyl phosphorothioate can also be ascribed to base-catalyzed alcoholysis [18] (Fig. 4), and for the reaction between IX and $(C_2H_5O)_2P(S)Cl$ where the mixture was refluxed for several hours in ethanol, this might account for the poor yields of vinyl phosphorothioate. However, refluxing of *2* in ethanol as mentioned previously only yielded small amounts of the ethanolysis product. The isolation of β-dicarbonyl compound from III and V (Table 3) may be accounted for by hydrolysis of the respective vinyl phosphorothioates (*3* and *5*) during the work-up procedure.

The present investigation shows methods for preparing vinyl phosphorothioates, enriched either in the *Z*- or the *E*-isomer. For the vinyl phosphate phosdrin it is found that the *E*-isomer was 10 to 100 times more toxic to insects and mammals than the *Z*-isomer [5,6] and our procedures should thus prove to be the methods of choice for selective synthesis. Preliminary experiments have been made on the reaction between $(Me_2N)_2P(O)Cl$ and salts of β-dicarbonyl compounds [24] and the results were the same as in the present work.

EXPERIMENTAL

The IR spectra were recorded on a Perkin-Elmer model Infracord Spectrophotometer and the NMR spectra at 60 MHz on a Varian A-60 spectrometer using TMS as internal standard. The chemical shifts are expressed in δ-values. The UV spectra were recorded on a Perkin-Elmer 402 Ultraviolet-Visible Spectrophotometer. Microanalyses were carried out by Micro Analytical Laboratory, Novo A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark, and by Micro Analytical Laboratory, Løvens Kemiske Fabrik, DK-2750 Ballerup, Denmark. Column chromatography was made on silica gel (Kiselgel 60; 0.063 – 0.200 mm; 70 – 230 mesh ASTM; (Merck)). B.p.'s and m.p.'s are uncorrected.

TBA-salt of 2,4-pentanedione (acetylacetone), (I). Compound I was synthesized [17] in 95 % yield. However, methylene chloride was used instead of chloroform. M.p. 151 – 154 °C (acetone).[17]

TBA-salt of methyl acetoacetate, (II). Compound II was synthesized as I in 98 % yield, as yellow crystals, m.p. 87 – 90 °C (AcOEt). (Found: C 69.43; H 12.16; N 3.91. $C_{31}H_{43}NO_3$ requires: C 70.54; H 12.12; N 3.92). IR: ν_{max}($CHCl_3$) 3000, 1655, 1515, 1380, 1150, 1125, 1057, 1000 and 885 cm^{-1}. UV-Visible (EtOH) λ_{max} (log ε): 245 nm (3.031). NMR ($CDCl_3$): δ 0.7 – 1.2 (12 H, m), 1.2 – 2.0 (16 H, m), 2.24 (3 H, broad s), 3.1 – 3.7 (8 H, m), 3.53 (3 H, s), 7.55 (1 H, broad s).

TBA-salt of ethyl acetoacetate, (III). Compound III was synthesized as I in 100 % yield. The compound did crystallize in a deep-freezer and in vacuum, but as it was too soluble in all attempted solvents it could not be recrystallized before use. IR: ν_{max}($CHCl_3$) 2960, 1630, 1505, 1375, 1160, 1065, 1012, 914 and 882 cm^{-1}.

UV (EtOH) λ_{max} (log ε): 210 nm (2.929) and 283 nm (3.441). NMR ($CDCl_3$): δ 0.7 – 1.2 (12 H, m), 1.2 – 1.9 (16 H, m), 1.25 (3 H, t, J 7.1 Hz), 2.34 (3 H, s), 3.0 – 3.5 (8 H, m), 4.10 (2 H, q, J 7.1 Hz), 7.55 (1 H, s).

TBA-salt of ethyl 3-oxo-3-phenylpropionate (ethyl benzoylacetate), (IV). Compound IV was synthesized as I in almost 100 % yield. As the compound was too soluble in all attempted solvents to be recrystallized it was not further purified before use. NMR ($CDCl_3$): δ 0.8 – 1.6 (31 H, m), 2.9 – 3.3 (8 H, m), 4.02 (2 H, q, J 7.0 Hz), 7.1 – 7.5 (3 H, m), 7.46 (1 H, s), 7.7 – 8.0 (2 H, m).

TBA-salt of 1-phenyl-1,3-butanedione (benzoylacetone), (V). Compound V was synthesized as I in almost 100 % yield. Red-orange crystals, m.p. 100 – 101 °C. (AcOEt). (Found: C 77.26; H 11.31; N 3.48. $C_{26}H_{45}NO_2$ requires: C 77.36; H 11.24; N 3.47). IR: ν_{max}($CHCl_3$) 2950, 1605, 1565, 1495, 1460, 1415, 1370, 1275, 987 and 867 cm^{-1}. ν_{max}(KBr) 2950, 1580, 1555, 1495, 1430, 1380 (sh), 1340, 1295, 1270, 1190, 1172, 1143, 1057, 1021, 987, 880, 865, 798, 758 and 707 cm^{-1}. UV (EtOH) λ_{max} (log ε): 208 nm (3.916), 246 nm (3.803) and 313 nm (4.189). NMR ($CDCl_3$): δ 0.7 – 1.1 (12 H, m), 1.1 – 1.6 (16 H, m), 2.46 (3 H, s), 2.8 – 3.2 (8 H, m), 7.1 – 7.4 (3 H, m), 7.49 (1 H, s), 7.6 – 8.0 (2 H, m).

Phosphorylation of the TBA-salts I-V. Equivalent amounts of TBA-salt and diethyl phosphorochloridothioate were mixed in methylene chloride (25 ml to about 0.01 mol of salt). The product mixture from I was refluxed for about 7 h. The other reactions were observed to be more or less exothermic and therefore only stirred for a couple of hours at room temperature.

I→1. The reaction mixture of 0.0146 mol of I and $(C_2H_5O)_2P(S)Cl$ was treated with a solution of 0.02 mol NaI in water. The methylene chloride phase was separated, dried (Na_2SO_4) and the solvent evaporated. TBA^+I^- (0.0134 mol, 92 %) was then precipitated by adding ether. Evaporation of the ether phase yielded 2.77 g of *1* (75 %) with 30 % *1Z* and 70 % *1E* as confirmed by NMR spectroscopy (Table 2). Distillation enhanced the content of *1E* to 75 % and also small amounts of a product containing the structure element $-P-S-C_2H_5$ were observed in NMR ($CDCl_3$): δ 2.88 (2 H, broad q, J 7.3 Hz). (Found: C 42.69; H 6.86; P 12.26. $C_9H_{17}O_4PS$ requires: C 42.85; H 6.79; P 12.28). The isomers were isolated by column chromatography and the NMR spectra shown in Table 2 were recorded. The groups C_2H_5-O-P- showed the known NMR-patterns.[10] IR(*1E*): ν_{max}(CCl_4) 2985, 1705, 1625, 1410, 1380, 1350, 1210, 1135, 1023, 948 and 862 cm^{-1}. ν_{max}(CS_2) 2980, 1695, 1615, 1375, 1350, 1210, 1135, 1021, 948, 862 and 818 cm^{-1}.

II→2. The reaction mixture was treated as above yielding 93 % of almost neat *2E* and 99 % of TBA^+I^-. A fraction of 2.40 g of the product was separated by silica gel column chromatography (CH_2Cl_2) to yield 2.13 g of neat *2E* and 0.08 g of a mixture of *2E* and *2Z* (1:1). Data of *2E*: (Found: C 39.65; H 6.30; P 11.63. $C_9H_{17}O_5PS$ requires: C 40.29; H 6.39; P 11.55). $n_D^{20.5} = 1.4760$. For NMR data, see Table 2. UV(EtOH) λ_{max} (log ε): 227 nm (3.984). IR: ν_{max}(CCl_4) 3010, 1740, 1665, 1435, 1385, 1335, 1230, 1127, 1025, 965, 944, 891, 853 and 822 cm^{-1}. ν_{max}(CS_2) 3020, 1740, 1665, 1385, 1335, 1230, 1127, 1025, 968, 946, 893, 855 and 822 cm^{-1}.

III→3. The reaction mixture was treated as above and yielded 84 % of almost neat *3E* and 16 % of ethyl acetoacetate, which is likely generated by hydrolysis of *3E*. TBA^+I^- was isolated in 92 % yield. However, by silica gel column chromatography (CH_2Cl_2) 0.06 g of *3Z* and 1.23 g of *3E* was isolated from 1.70 g of *3*. (Found C 42.52; H 6.77; P 11.08. $C_{10}H_{19}O_5PS$ requires: C 42.55; H 6.78; P 10.97). For NMR data, see Table 2. UV(EtOH) λ_{max} (log ε): 225 nm (3.993). IR: ν_{max}(CCl_4) 2970, 1720, 1650, 1430, 1375, 1355, 1325, 1223, 1157, 1120, 1090, 1020, 968, 897, 855 and 822 cm^{-1}. ν_{max}(CS_2) 2975, 1725, 1655, 1380, 1360, 1330, 1225, 1160, 1123, 1093, 1025, 970, 896, 857, 822 and 730 cm^{-1}.

IV→4. The reaction mixture was treated as above and yielded 95 % of *4E* and *4Z* (3:1) and traces of ethyl benzoylacetate which is likely generated by hydrolysis of *4* during work-up. TBA^+I^- was isolated in 88 % yield. Ethyl benzoylacetate was separated from *4* by silica gel column chromatography (CH_2Cl_2), but separation of *4E* and *4Z* did not succeed. The NMR spectra of *4E* and *4Z* are shown in Table 2. They are found to correspond to the spectra of the *Z* and *E* isomers of diethyl 2-ethoxycarbonyl-1-phenylvinyl phosphate.[7] IR(*4E* and *4Z*; 3:1): ν_{max}(CCl_4) 2965, 1725, 1640, 1435, 1385, 1360, 1330, 1265, 1220, 1155, 1087, 1030, 968, 882, 822 and 692 cm^{-1}. ν_{max}(CS_2) 2970, 1725, 1640, 1375, 1355, 1260, 1215, 1155, 1087, 1034(sh), 1018, 968, 882, 822 and 691 cm^{-1}.

V→5E *(and 5Z + 6Z).* The reaction mixture was treated as above and yielded quantitatively a mixture of 85 % *5E* and 15 % benzoylacetone. The yield of TBA^+I^- was 98 %. *5E* was isolated by silica gel column chromatography (CH_2Cl_2). (Found: C 53.53; H 6.05; P 9.85. $C_{14}H_{19}O_4PS$ requires: C 53.50; H 6.09; P 9.85). For NMR-data, see Table 2. IR: ν_{max}(CCl_4) 2970, 1675, 1615, 1440, 1385, 1340, 1235, 1150, 1022, 965, 908 and 697 cm^{-1}. ν_{max}(CS_2) 2970, 1670, 1615, 1380, 1335, 1240, 1150, 1090, 1020, 965, 905, 818 and 698 cm^{-1}. The products obtained when neat *5E* was heated in bromobenzene (155 °C) for 2 h. (*5E*, *5Z*, and *6Z*, Fig. 1) were determined by NMR spectroscopy. Distillation of *5E* onto a cold finger (130 °C/0.15 mmHg) yielded the products shown in Fig. 1 and traces of a product containing the structure element C_2H_5-S-P. They were determined by NMR spectroscopy, also the monothio-β-diketone [20] for which the

molecular weight after separation by silica gel column chromatography (benzene) was determined by MS to be 178. (The mass spectrum was recorded on a CEC 21-104 mass spectrometer operating at 70 eV using the direct inlet). The fragments $C_6H_5-CO^+$ ($m/e=105$) and CH_3-CS^+ ($m/e=59$) were observed in the mass spectrum.

Phosphorylation of the sodium salts VI–X. Equivalent amounts of diethyl phosphorochloridothioate were added to refluxing alcoholic solutions of the sodium salts VI–X. The mixture from IX was refluxed for about 3 h, while the other mixtures were allowed to cool to room temperature after adding $(C_2H_5O)_2P(S)Cl$.

VI→1. Sodium chloride was filtered from the ethanolic reaction mixture of 0.024 mol VI and $(C_2H_5O)_2P(S)Cl$ and the solvent evaporated. The last traces of sodium chloride were precipitated by ether. Evaporation yielded 5.40 g (0.021 mol, 90 %) of *1E* and *1Z* (1:9) as confirmed by NMR spectroscopy (Table 2). IR [*1E* and *1Z* (1:9)]: $\nu_{max}(CCl_4)$ 2985, 1695, 1615, 1430, 1380, 1350, 1155, 1135, 1090, 1023, 963 and 865(sh) cm^{-1}. $\nu_{max}(CS_2)$ 2970, 1695, 1615, 1375, 1345, 1150, 1135, 1090, 1020, 960, 865(sh) and 818 cm^{-1}.

VII→2 *(and 3)*. By the procedure as above from 0.028 mol VII in ethanol was isolated 7.73 g of a mixture of 2-methoxycarbonyl-1-methylvinyl phosphorothioate (2) (27 % yield), 2-ethoxycarbonyl-1-methylvinyl phosphorothioate (3) (55 % yield), and *O,O,O*-triethyl phosphorothioate (18 % yield). The *E*-isomers formed 10 % of the product and the *Z*-isomers 90 %. The *Z*-isomers were isolated neat by silica gel column chromatography (CH_2Cl_2) and the NMR spectrum revealed a distribution between the just mentioned compounds *2Z* and *3Z* which was 43.5 %: 56.5 %. (Found: C 41.55; H 6.60. 43.5 % $C_9H_{17}O_5PS$ and 56.5 % $C_{10}H_{19}O_5$ requires: C 41.57; H 6.61). IR: $\nu_{max}(CCl_4)$ 2965, 1725, 1665, 1430, 1375, 1315, 1205, 1143, 1092, 1046, 1020, 972, 912 and 823 cm^{-1}. $\nu_{max}(CS_2)$ 2965, 1725, 1665, 1375, 1320, 1270, 1205, 1143, 1093, 1046, 1020, 970, 912 and 823 cm^{-1}. Refluxing the mixture of the methyl and ethyl ester in EtOH for 5 3/4 h did not change the ratio of the amounts of the two components as confirmed by GLC (Hewlett-Packard 5711 A Gas Chromatograph, 15 % PEG 4000, 130 °C for 4 min and then 130–200 °C (8 °C/min), N_2-flow: 30 ml/min). However, about 0.5 % of ethyl acetoacetate showed up to be present in the heated solution. Refluxing neat *2E* in EtOH did not give 2-ethoxycarbonyl-1-methylvinyl phosphorothioate (*3*) as confirmed by GLC.

From 0.080 mol of VII prepared in abs. methanol was isolated 18.81 g of a mixture of 0.026 mol 2-methoxycarbonyl-1-methylvinyl phosphorothioate (*2Z*+*2E*) (32 % yield), 0.031 mol methyl acetoacetate (37 % yield), and 0.046 mol *O*-methyl-*O,O*-diethyl phosphorothioate (58 % yield). Most of the two latter compounds were removed by vacuum at room temperature leaving a fraction containing 85 % of *2Z*+*2E* (3:1) and 15 % of *O*-methyl-*O,O*-diethyl phosphorothioate.

The reaction of VII (prepared from sodium hydride and methyl acetoacetate) in benzene with $(C_2H_5O)_2P(S)Cl$ yielded 9 % of *2*, a poor yield corresponding to previous statements.[1]

VIII→3*Z*. By a procedure as above from 0.046 mol VIII in ethanol was isolated 11.82 g of a mixture of 0.032 mol of 2-ethoxycarbonyl-1-methylvinyl phosphorothioate (*3Z*) (70 % yield) and 0.014 mole of triethyl phosphorothioate (30 % yield). For NMR data of *3Z*, see Table 2.

IX→4Z. The refluxed reaction mixture of 0.054 mol of IX and 0.054 mol of $(C_2H_5O)_2P(S)Cl$ in ethanol yielded by the procedure described above 19.32 g of a mixture containing *4Z* (32 % yield), ethyl benzoylacetate (64 % yield) and triethyl phosphorothioate (64 % yield). Distillation of 8 g of this mixture yielded three fractions: 1.59 g (37 °C/0.05 mmHg; triethyl phosphorothioate (confirmed by NMR spectroscopy[10])), 2.54 g (82–83 °C/0.05 mmHg; 39 % triethyl phosphorothioate and 61 % ethyl benzoylacetate) and 2.10 g (115–138 °C/0.08 mmHg; 36 % ethyl benzoylacetate and 64 % *4Z*). By silica gel column chromatography (CH_2Cl_2) 4.15 g of the above mentioned mixture was separated yielding 1.27 g of *4Z*. (Found: C 52.22; H 6.12; P 8.82. $C_{15}H_{21}O_5PS$ requires: C 52.32; H 6.15; P 8.99). IR: $\nu_{max}(CCl_4)$ 2975, 1730, 1645, 1440, 1385, 1355, 1320, 1265, 1153, 1093, 1021, 968, 913, 827 and 692 cm^{-1}. $\nu_{max}(CS_2)$ 2985, 1730, 1645, 1385, 1355, 1320, 1265, 1152, 1092, 1020, 967, 912, 825, 767 and 689 cm^{-1}.

X→5 *and* 6. By a procedure as above, starting from 0.057 mol X in ethanol was isolated 17.93 g of a mixture of *5E*, *5Z*, and *6Z* (distribution shown in Table 1) (51 % yield), 1-phenyl-1,3-butanedione (36 % yield) and triethyl phosphorothioate (48 % yield). By silica gel column chromatography (CH_2Cl_2) it was possible to get fractions enriched in either of the components of the reaction mixture. A rapid treatment of the mixture with 5 M NaOH + CH_2Cl_2 removed 1-phenyl-1,3-butanedione and triethyl phosphorothioate. Heating a benzene solution of a mixture of *5Z*, *5E*, and *6Z* (77, 6, and 17 %) (80 °C) for 5 1/4 h yielded a mixture of the components with the distribution 47, 37, and 16 %. Heating a chlorobenzene solution of a mixture of *5Z*, *5E*, and *6Z* (10, 71, and 19 %) (132 °C) for 1 h yielded a mixture of the components with the distribution 8, 76, and 16 %.

Preparation of 7 *and* 8. A mixture of *7* and *8* was synthesized [4] from 1-phenyl-2-chloro-1,3-butanedione [25] and triethyl phosphite in 77 % yield without distillation of the product mixture recovering 23 % of 1-phenyl-2-chloro-1,3-butanedione and 15 % of triethyl phosphite. Based on the NMR spectrum *7Z*, *7E*, *8Z*, and *8E* showed 50 %, 10 %, 10 %, and 30 %

appearance. Treatment of the reaction mixture with 5 M NaOH + CH_2Cl_2 removed the β-dicarbonyl compound and triethyl phosphite yielding *7Z*, *7E*, *8Z*, and *8E* with the distribution 11, 45, 33, and 11 %. By silica gel column chromatography (ether) it was possible to isolate neat *7E* from the just mentioned mixture. Refluxing *7* and *8* in chlorobenzene yields 1-phenyl-1,3-butanedione (benzoylacetone): Refluxing a mixture of *7Z*, *8Z*, and *8E* (13, 65, and 22 %) in chlorobenzene (132 °C) for 1 1/4 h yielded benzoylacetone, *7Z*, *7E*, *8Z*, and *8E* (50, 15, 19, 7, and 9 %).

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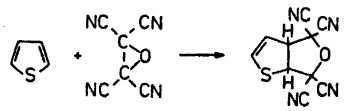
The Addition of Tetracyanoethylene Oxide to Thiophene and Related Compounds

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The cycloaddition of tetracyanoethylene oxide to thiophene and some substituted thiophenes and to furan, selenophene, benzo[*b*]thiophene, and benzo[*b*]furan has been investigated. The NMR and mass spectra of the adducts have been studied. Through competitive experiments the following order of reactivity was obtained: furan > 2-methylthiophene ~ benzo[*b*]furan > benzo[*b*]thiophene > selenophene > thiophene > 2-chlorothiophene. The mechanism of this cycloaddition is discussed.

Ten years ago Linn at Dupont discovered that tetracyanoethylene oxide (TCNEO) could be added to olefins, acetylenes and aromatics such as benzene or thiophene.[1,2] With ethylene, 2,2,5,5-tetracyanotetrahydrofuran was obtained while thiophene gave 1,1,3,3-tetracyano-1,3,3a,6a-tetrahydrothieno[2,3-*c*]furan (I) in 50 % yield when reacted with TCNEO at 130 °C



I

in 1,2-dichloroethane. Linn and Benson [2] found, by studying more than 30 different olefins, that the addition showed electrophilic properties, electron-donating substituents increasing the rate and electron-attracting groups decreasing the rate of addition.

They also found that the addition was stereospecific. From kinetic studies it was suggested [3] that the addition of TCNEO to olefins takes place in two steps, the first of which is a thermal promotion of the expoide to an activated species (TCNEO*). Depending upon the reactivity of the olefin different rate-expressions were found.

It has been suggested that TCNEO* is formed from TCNEO by ionic cleavage of the C–C bond to give a resonance hybrid of zwitterionic structures [3,4] or by homolytic cleavage to give a spin-paired diradical.[5]

Huisgen,[6–8] however, has presented strong evidence for the zwitterionic (1,3-dipolar) nature of TCNEO* and considers its addition to olefins as a 1,3-dipolar cycloaddition. This view has been supported by measurements of secondary isotope effects.[9–12]

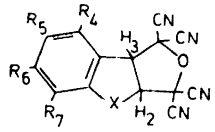
It seems therefore quite obvious from these results that the second step in the addition of TCNEO to double bonds is a 1,3-dipolar concerted addition. However, very few investigations of the addition of TCNEO to different heterocycles have been carried out. Only thiophene,[2,13] 2-chlorothiophene,[2] and furan [2,13] have previously been used as substrates.

In order to obtain more information on the scope and limitations of the addition of TCNEO to heterocycles, we have studied a number of monosubstituted thiophenes, furan, selenophene, and benzofused systems. We have also studied the spectroscopic properties of the adducts and made attempts to aromatize them.

In the normal preparative procedures hitherto used for the addition of TCNEO to olefins or aromatic compounds, either 1,2-dichloroethane [2]

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Table 1. Chemical shifts and coupling constants for the TCNEO adducts of benzo[*b*]furan and benzo[*b*]-thiophene.



Compound No.	Substituent X	R_2	R_3	M.p. °C	Solvent	Shift H_2	H_3	Aromatic hydrogens 3 H	1 H	Coupling constants H_2H_3	H_3-R_4
X	O	H	H	238.5–240	$(CD_3)_2CO$	6.55	5.73	7.25	7.80	7.6	
X	O	H	H		$(CD_3)_2SO$	6.42	5.54	7.20	7.80	7.6	
XI	O	D	H	241–243	$(CD_3)_2SO$	–	5.56	7.20	7.88	–	
XII	S	H	H	198–199	$(CD_3)_2CO$	6.06	5.75	7.44	7.70	8.2	0.8
XIII	S	D	H	196–197	$(CD_3)_2CO$	–	5.81	7.48	7.73	–	

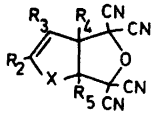
or benzene [13] is used as solvent and the reactions are carried out at high temperatures in closed vessels. Alternatively, refluxing 1,2-dibromoethane has been used as reaction medium.[2] We have tried all three methods and found that the addition is very sensitive to reaction conditions. Local overheating (by heating mantles) must be avoided, as this catalyzes decompositions and polymerization. We used an oil-bath for heating. We also found that closed glass tubes gave better results than autoclaves of stainless steel. The purity of the reagents used also appears to be of great importance. Thus benzo[*b*]furan gave only a 5 % yield of adduct in refluxing 1,2-dibromoethane, while in a closed glass vessel in 1,2-dichloroethane at 150 °C the yield was increased to 29 %. In another experiment, when highly purified reagents were used, benzo[*b*]thiophene gave only 25 % yield of adduct in refluxing 1,2-dibromoethane, but 50 % in 1,2-dichloroethane in a closed glass vessel at 150 °C.

α-Deuteriated benzo[*b*]furan, and also benzo[*b*]thiophene, prepared by metalation of the parent compounds with butyllithium [14,15] followed by reaction with deuterium oxide, were used to simplify structure determination of the adducts by NMR spectroscopy. It is not certain *a priori* that addition occurs over the 2,3-bond in these heterocycles. Although the addition of TCNEO to olefins has electrophilic characteristics, it is known that benzo fusion diminishes the reactivity of furan and thiophene in electrophilic substitution.[16] Furthermore, it is known that photochemical addition of the carbene from ethyldiazo acetate occurs over the 4,5-bond of benzo[*b*]thiophene.[17] Thermally generated carbenes, however, add to the 2,3-bond of both benzo[*b*]furan and benzo[*b*]thiophene.[18] The NMR spectra (*cf.* Table 1) of the adducts and their α-deuteriated isomers proved that addition of TCNEO also occurs over the 2,3-bond.

The adducts of furan, thiophene and 2-chlorothiophene were prepared according to the literature.[2,13] The selenophene adduct was obtained in 15 % yield by reaction in anhydrous benzene at 145 °C (glass ampoule). The low yield might be due to the quality of the selenophene used. The adducts of 3-bromothiophene and 2- and 3-iodothiophene were obtained in 50 % yield in refluxing 1,2-dibromoethane. The best yield of the adduct of 2-methylthiophene (36 %) was obtained in anhydrous benzene at 150 °C, and the adduct from 3-methylthiophene was obtained in 39 % yield in 1,2-dichloroethane at 145 °C. The 2- and 3-methoxythiophene did not give normal adducts with TCNEO. Instead, 2-methoxy-5-thenoylcyanide and 3-methoxy-2-thenoylcyanide were obtained.[19] Strongly deactivated thiophene, such as 2-cyano- and 2-nitrothiophene, gave no adducts in refluxing 1,2-dibromoethane or 1,1,2-trichloropropane or in 1,2-dichloroethane in glass ampoules.

Brown and Cookson [13] found that durene gave an adduct with TCNEO, albeit in low yield. We therefore tried to add TCNEO to 2,5-di-

Table 2. Chemical shifts and coupling constants for the TCNEO adducts of monocyclic heterocycles. Solvent: acetone, except for III–V, for which hexadeuterioacetone was used.



Compound No.	Substituent X	R_2	R_3	Shift, δ-values R_2	R_3	H_4	H_5	Coupling constants R_2R_3	R_2R_4	R_3H_4	H_4H_5
I	S	H	H	6.85	5.93	5.31	5.67	6.0	2.2	2.4	9.2
II	O	H	H	6.82	5.48	5.17	6.23	2.6	2.0	2.6	8.5
III	Se	H	H	7.30	6.32	5.22	5.68	6.2	2.4	2.4	8.8
IV	S	Cl	H	–	6.08	5.45	5.94	–	–	2.3	9.4
V	S	H	Br	7.20	–	5.50	6.14	–	1.2	–	10.6
VI	S	I	H	–	6.32	5.32	5.79	–	–	2.6	9.2
VII	S	H	I	7.16	–	5.35	6.02	–	1.2	–	10.4
VIII	S	CH_3	H	2.11	5.60	5.19	5.55	1.5	2.0	2.0	8.6
IX	S	H	CH_3	6.47	2.6	5.10	5.90	1.3	1.3	0.8	9.4

methylthiophene, but without success. The reaction of TCNEO and 2,5-dibromothiophene, on the other hand, proceeded in an unexpected manner, yielding 2,5-bis(dicyanomethylene)-2,5-dihydrothiophene.[20]

The adducts which were prepared are listed in Tables 1 and 2, together with their NMR parameters. We have made many different attempts to rearomatize the thiophene adducts, but they have hitherto been unsuccessful (*cf.* Ref. 21).

NMR spectra. A systematic upfield shift of the 3-hydrogen resonance is observed on going from the selenophene adduct to the furan adduct, which is in accordance with the order of the +M-effect of these chalcogen atoms; O > S > Se. On the other hand, for the 5-hydrogen bonded to sp^3-hybridized carbon, the shift of the furan adduct is at lowest field due to the larger –I-effect of ether oxygen. The shifts of the 4-hydrogens are, as expected, only affected to a small extent. The effects of the substituents (Cl, Br, I, and CH_3) are of the magnitude and direction expected from earlier studies of substituted olefins (*cf.* Ref. 22). The homoallylic coupling (J_{CH_3-4}) in VIII is of the same magnitude (2.0) as observed earlier in 5-methyl-4-thiolen-2-one [23] and the magnitude of J_{45} (~9 Hz) is in accordance with the *cis*-orientation (*cf.* the Karplus equation [24]). In the NMR spectrum of the benzofused systems, additional splittings (≤ 0.8 Hz) are observed in the 3-hydrogen band, which are most probably due to long-range coupling of the benzylic type to the 4-hydrogen in the benzene ring.[25] The resonances of the benzenic hydrogens are strongly coupled, but a one-hydrogen resonance at lower field can be recognized.

Mass spectra. We have also briefly studied the mass spectra of the adducts. The fragmentation pattern shows some very characteristic trends. All adducts split carbonyl cyanide from the molecular ion, whereupon usually dicyanocarbene and hydrogen cyanide are eliminated. In the furan series, the fragmentation differs somewhat: after the elimination of carbonyl cyanide mainly carbon oxide is split off,* giving the ion *E* (Fig. 1).

The elimination of carbon oxide is quite normal in furans and has been suggested to give cyclopropenyl ions.[26] The structures indicated in Fig. 1 should be considered as one of the alternatives for a given composition. The figures under the structures are the *m/e* values and relative intensities (in % of the base peak). For the selenophene adduct the *m/e* of the ions with the ^{80}Se isotope are given. For comparison the quite similar fragmentation of the benzene adduct is indicated. The ion *D* is the base peak

* It could also be hydrogen cyanide, but this seems highly unlikely.

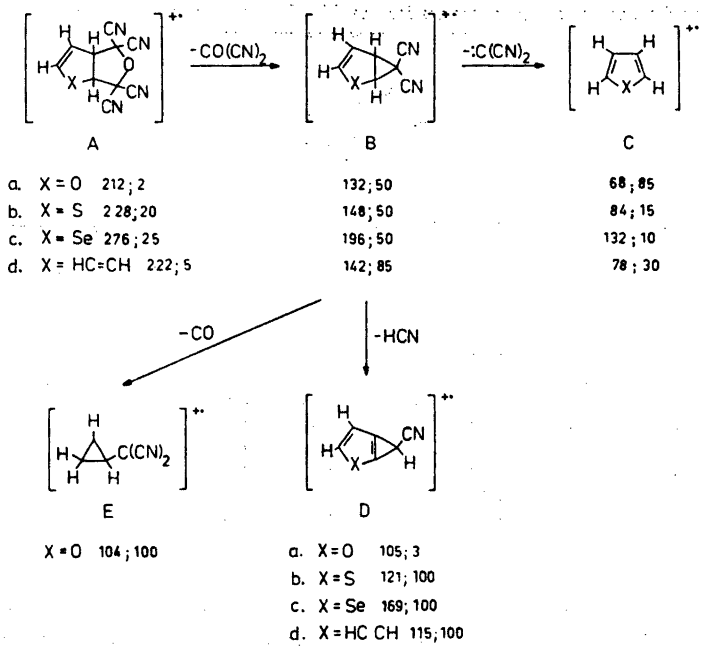


Fig. 1. Mass spectral fragmentation pattern for TCNEO adducts of furan, thiophene, selenophene, and benzene.

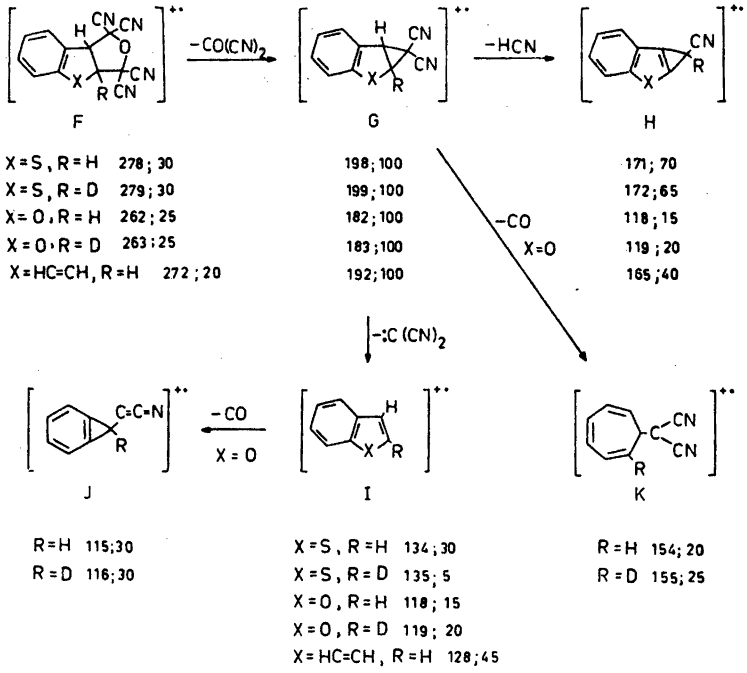


Fig. 2. Mass spectral fragmentation pattern for TCNEO adducts of benzo[*b*]thiophene, benzo-[*b*]furan, and naphtalene.

in all four adducts except for that of furan.

The 2- and 3-halosubstituted thiophene adducts (IV – VII) also primarily split off carbonyl cyanide. This ion then loses the halogen to give the most intense ion $C_6H_4N_2S^+$ or splits dicyanocarbene to give the halogenated thiophene or radical ion.

The two methyl-substituted adducts (VIII and IX) fragment somewhat differently. The molecular ion loses carbonyl cyanide as usual, but then the loss of methyl becomes more important than that of dicyanocarbene and hydrogen cyanide. In the 3-methyl case, the ion $C_6H_4N_2S^+$ is the base peak.

The fragmentation of the benzo[*b*]thiophene adduct is quite analogous to that of the thiophene adduct. However, due to the availability of the α-deuteriated derivative an interesting observation was made. In the elimination of hydrogen cyanide from the ion *G* to *H*, it is the β-hydrogen which is selectively removed (*cf.* Fig. 2). We also found that the naphthalene adduct fragmented quite similarly.

The main fragmentation pattern of the benzo[*b*]furan adducts is quite different from that of the furan adduct (*cf.* Fig. 2). The base peak is the ion formed by loss of carbonyl cyanide, and the loss of C=O is not so dominant. As in the benzo[*b*]thiophene adduct, the transformation of *G* to *H* most probably occurs by selective loss of hydrogen from the 3-position, although we cannot differentiate between the loss of DCN and C=O with our mass spectral equipment.

Kinetic measurements. In the addition of TCNEO to thiophene or other aromatic systems, aromaticity is lost and it seems therefore likely that addition of the activated TCNEO* to these systems is much slower than to styrenes or stilbenes. If this deactivation is great enough, it could occur that this addition becomes rate determining as observed for 2-nitrostyrene by Linn.[3] We therefore studied the kinetics of the reaction of thiophene with TCNEO using the technique of Linn[3] in his investigation of stilbene. The reaction was carried out at 150 °C in 1,2-dichloroethane in glass ampoules. Residual TCNEO was determined by iodometric titration. That the TCNEO was consumed at the same rate as the adduct was formed was checked in a few parallel experiments by VPC analysis of the adduct using an internal standard, as described below.

Table 3. Second-order rate constants for the addition of tetracyanoethylene oxide (TCNEO) to thiophene at 150.2 °C in 1,2-dichloroethane.

$[TCNEO]_0$ mol/l	[thiophene] mol/l	k_2 $l \times mol^{-1}s^{-1} \times 10^{-3}$
0.0210	0.0126	3.22
0.0204	0.0126	3.33
0.0408	0.0143	3.32
0.0201	0.0504	2.49

Table 4. Relative rates of some heterocycles in TCNEO cycloaddition (A) and in electrophilic substitution [acetylation (B) and trifluoroacetylation (C)].

Compound	A	B	C
2-Methyl thiophene	9.66	35.2	380
Furan	10.4	11.9	140
Selenophene	3.59	1.9	6.5
Thiophene	1	1	1
2-Chlorothiophene	0.64	0.14	0.58
Benzo[*b*]furan	9.82	0.036	0.11
Benzo[*b*]thiophene	6.82	0.04	0.12

We found that if the initial concentration of TCNEO was larger than that of thiophene, second-order rate constants could be obtained (Table 3), as was observed for *p*-nitrostyrene. As in that case, if the concentration of the substrate becomes larger, the K_2 [olefin] term in the rate equation cannot be neglected and the second-order rate constant falls off. In the latter case, a first-order rate constant (in TCNEO) can be obtained ($k = 1.09 \times 10^{-4}$ s^{-1}). It is thus obvious that also in the reaction between thiophene and TCNEO the addition of the TCNEO* to thiophene is so slow, and of a rate comparable to the reversible formation of TCNEO*, that a complex kinetic expression is obtained (*cf.* Ref. 3).

We have therefore carried out competitive experiments (*cf.* Ref. 27), using thiophene, 2-chloro- and 2-methylthiophene, furan, selenophene, benzo[*b*]furan, and benzo[*b*]thiophene in

order to obtain information about the relative rates of these substrates with the activated intermediate TCNEO*. The competitive experiments were carried out at 150 °C in 1,2-dichloroethane. The reactions were stopped by rapid cooling in ice. The amounts of adduct formed were analyzed by VPC using an internal standard (*cf.* experimental part). Details of the competitive experiments are given in Ref. 21. From these values the average values of relative rates, which are given in Table 4, were obtained, which are also corrected for the statistical factor. (The unsubstituted parent compound has two sides which can be attacked.) For comparison, the relative rates obtained in $SnCl_4$-catalyzed acetylation [28,29] and trifluoroacetylation [30,31] are given, both being examples of electrophilic aromatic substitution. With the exception of the reversal of the reactivity of furan and 2-methylthiophene, the effects of the structural changes in the monocyclic system in the addition of TCNEO, and especially $SnCl_4$-catalyzed acetylation, are quite similar. For the trifluoroacetylation the sensitivity to structural changes is much greater. It is interesting to note that the order of reactivity in the cycloaddition of TCNEO to monosubstituted thiophenes is opposite to that found by Huisgen *et al.*[32] in the cycloaddition of diphenylnitrileimine to *p*-substituted styrenes, for which the following relative rates were found: *p*-chlorostyrene, 2.8; styrene, 1.62 and *p*-methylstyrene, 1.29. In the methylacrylate series a rate-enhancing effect was also found upon chlorosubstitution: methyl α-chloroacrylate, 57; methyl acrylate, 48; and methyl methacrylate, 17.[32] The rates are given relative to ethyl crotonate in Huisgen's investigations. It is of course questionable whether comparison of substituent effects in such widely different systems is meaningful. It is perhaps more relevant to compare the reaction between the thiophenes and TCNEO with the ozonization of the corresponding benzenes. The ozonization is considered to be a 1,3-cycloaddition,[33] and Wibaut [34] found that toluene reacted *ca.* 8 times faster than benzene, which in turn reacted 5 times faster than chlorobenzene. Thus, this reaction is quite similar to the TCNEO addition.

The effect of benzofusion on the rate of TCNEO addition is quite confusing. It is evident that in the furan series the rate is decreased while in the thiophene series the rate is increased. It thus differs from electrophilic substitution, as Marino has found that benzofusion both in the thiophene series and in the furan series leads to extensive deactivation, especially of the reactivity of the 2-position.[16] The reason for this difference is not obvious.

In summarizing it is thus clear that the cycloaddition of tetracyanoethylene oxide to aromatic heterocycles, as well as to styrene, is not activated by neither electron-donating nor electron-attracting groups, in contrast to most cycloadditions to olefins but shows many similarities to electrophilic aromatic substitution. Huisgen [4] has pointed out that 1,3-dipolar additions not necessarily are completely concerted. It is therefore possible that the carbonyl ylide formed from TCNEO initially attacks as an electrophilic reagent, but that the ring-closure is then so fast that the Wheland intermediate does not lose a proton.

EXPERIMENTAL

1,1,3,3-Tetracyano-1,3,3a,6a-tetrahydrothieno-[2,3-c]furan was prepared from thiophene and tetracyanoethylene oxide [35] (TCNEO) according to Linn and Benson.[2] For NMR data see Table 2. M.p. 159 – 160 °C; literature value 159 – 160 °C.[2] Mass spectrum (*m/e*, %) 228,22; 149,7; 148,51; 147,10; 123,6; 122,14; 121,100; 84,17: 58,8; 54,8; 45.17.

1,1,3,3-Tetracyano-1,3,3a,6a-tetrahydrofuro-[2,3-c]furan was prepared according to Brown and Cookson [13] from furan and TCNEO. For NMR data see Table 2. M.p. 161 – 162 °C; literature value [13] m.p. 161 – 162 °C. Mass spectrum (*m/e*, %) 132,50; 104,10; 103,100; 102,6; 78,7; 77,84; 76,17; 75,6; 68,37; 64,7; 55,5; 54,15; 53,9; 52.8; 51.9; 50.9; 40.8.

5-Chloro-1,1,3,3-tetracyano-1,3,3a,6a-tetrahydrothieno[2,3-c]furan was prepared from 2-chlorothiophene [36] and TCNEO according to Ref. 2. For NMR data see Table 2. M.p. 178 – 179 °C; literature value [2] m.p. 178 – 179 °C. Mass spectrum (*m/e*, %) 264,2; 262,5; 184,6; 182,18; 149,5; 148,10; 147,100; 120,7; 118,9; 54,8.

1,1,3,3-Tetracyano-1,3,3a,7a-tetrahydroisobenzofuran was prepared from benzene and TCNEO according to Ref. 2. M.p. 165 – 166 °C; literature value [2] m.p. 167 – 168 °C. Mass spectrum (*m/e*, %) 222,5; 143,11; 142,87; 141,11; 116,15; 115,100; 114,12; 105,8; 88,8; 78,31; 77,11; 54,15; 51,10; 50,5.

1,1,3,3-Tetracyano-1,3,3a,9a-tetrahydronaphtho[1,2-c]furan was prepared from naphthalene and TCNEO according to Ref. 2. M.p. 172 – 174 °C; literature value [2] m.p. 172.5 – 173 °C. Mass spectrum (*m/e*, %) 272,19; 193,16; 192,100;

191,18; 166,10; 165,42; 164,17; 142,10; 141,10; 138,5; 129,5; 128,47; 127,9; 100,10; 99,10; 63,5; 54,10.

1,1,3,3,-Tetracyano-1,3,3a,6a-tetrahydroselenolo[2,3-c]furan. A solution of 13.1 g (0.10 mol) of selenophene,[37] and 5.0 g (0.035 mol) of TCNEO in 100 ml of anhydrous benzene was heated at 145 °C in a glass ampoule for 17 h. After cooling, a black polymer was filtered off and the filtrate evaporated to a volume of 20 ml. 1.7 g of the adduct was obtained, which was recrystallized from benzene (charcoal) giving 1.4 g (15 %) of the title compound, m.p. 166 – 167 °C, which did not change upon sublimation. For NMR data see Table 2. Mass spectrum (*m/e*, %) 278,4; 276,22; 274,11; 273,4; 272,4; 198,9; 197,5; 196,50; 195,10; 194,23; 193,12; 192,10; 171,17; 170,11; 169,100; 168,7; 167,50; 166,17; 165,17; 142,5; 132,10; 130,6; 117,5; 116,13; 115,12; 106,8; 105,6; 93,14; 91,7; 90,11; 89,6; 88,11; 80,9; 76,5; 64,9; 63,7; 54,29; 51,10; 50,7. [Found: C 44.46; H 2.15; N 19.79; O 5.55; Se 28.00. Calc. for $C_{10}H_4N_4OSe$ (275.1): C 43.66; H 1.46; N 20.37; O 5.81; Se 28.70].

6-Bromo-1,1,3,3-tetracyano-1,3,3a,6a-tetrahydrothieno[2,3-c]furan. A solution of 16.3 g (0.10 mol)of 3-bromothiophene[38] and 5.0 g (0.035 mol) of TCNEO in 60 ml of 1,2-dibromoethane was refluxed for 16 h. The hot solution was filtered and evaporated to a volume of 40 ml. Upon cooling 6.3 g of adduct was obtained, which upon two recrystallizations from 1,2-dichloroethane (once with charcoal) gave 5.9 g (55 %) of the title compound as colourless crystals, m.p. 187.5 – 188.5 °C. For NMR data see Table 2. Mass spectrum (*m/e*, %) 308,6; 306,5; 228,3; 226,3; 164,13; 162,13; 149,6; 148,10; 147,100; 120.7; 83.11; 82,7; 81.6; 76,6; 75,6; 69,16; 64,14; 58,56; 57,11; 54,33; 51,6; 50,6; 45,47. [Found: C 38.7; H 1.10; Br 26.3; N 18.3; S 10.5. Calc. for $C_{10}H_3BrN_4OS$ (307.1): C 39.1; H 0.99; Br 26.0; N 18.2; S 10.4].

5-Iodo-1,1,3,3-tetracyano-1,3,3a,6a-tetrahydrothieno[2,3-c]furan. A solution of 21.0 g (0.10 mol) of 2-iodothiophene[39] and 5.0 g (0.035 mol) of TCNEO in 50 ml of 1,2-dibromoethane was refluxed for 16 h. The mixture was filtered hot. Upon cooling, the adduct separated and was recrystallized from 1,2-dichloroethane, yielding 7.2 g (58 %) of the title compound, m.p. 192 – 193 °C. For NMR data see Table 2. Mass spectrum (*m/e*, %) 354,11; 275,5; 274,48; 210,8; 149,7; 148,11; 147,100;127,5; 120,8; 69,6; 57,6; 54,12; 45,9. [Found: C 33.8; H 0.83; I 37.6; N 15.7. Calc. for $C_{10}H_3IN_4OS$ (354.1): C 33.9; H 0.85; I 35.8; N 15.8].

6-Iodo-1,1,3,3,-tetracyano-1,3,3a,6a-tetrahydrothieno[2,3-c]furan. From 7.7 g (0.037 mol) of 3-iodothiophene,[40] 5.0 g (0.035 mol) of TCNEO and 50 ml of 1,2-dibromoethane, 4.8 g (39 %) of the title compound, m.p. 180 – 181 °C was obtained in the same way as described above. For NMR data see Table 2. Mass spectrum (*m/e*, %) 355,7; 354,55; 274,39; 247,5; 210,17; 149,6; 148,12; 147,100; 127,5; 120,7; 83,5; 54,9; 45,14. [Found: C 34.1; H 0.73; I 35.3; N 15.9. Calc. for $C_{10}H_3IN_4OS$ (354.1): C 33.9; H 0.85; I 35.8; N 15.8].

5-Methyl-1,1,3,3-tetracyano-1,3,3a,6a-tetrahydrothieno[2,3-c]furan. A mixture of 9.8 g (0.10 mol) of 2-methylthiophene[41] and 5.0 g (0.035 mol) of TCNEO in 100 ml of anhydrous benzene was heated at 150 °C for 10 h in a glass ampoule. After evaporation and chromatography on silica gel using chloroform:benzene (9:1) as eluent, 2.9 g (36 %) of the title compound, m.p. 180 – 181 °C after recrystallization from 1,2-dichloroethane, was obtained. For NMR data see Table 2. Mass spectrum (*m/e*, %) 242,28; 164,6; 163,12; 162,100; 161,24; 149,5; 148,10; 147,92; 136,11; 135,64; 129,7; 98,19; 97,30; 69,7; 64,5; 59,12; 58,6; 54,8; 53,5; 51,5; 45,18. [Found: C 53.7; H 2.23; N 23.0; S 13.2. Calc. for $C_{11}H_6N_4OS$ (242.3): C 54.5; H 2.50; N 23.1; S 13.2].

6-Methyl-1,1,3,3-tetracyano-1,3,3a,6a-tetrahydrothieno[2,3-c]furan. A mixture of 9.8 g (0.10 mol) of 3-methylthiophene and 5.0 g (0.035 mol) of TCNEO in 150 ml of 1,2-dichloroethane was heated at 145 °C for 22 h in a glass ampoule. After cooling, the solution was evaporated to a volume of 40 ml, whereupon the adduct crystallized on further cooling. Recrystallization from 1,2-dichloroethane gave 3.3 g (39 %) of the title compound, m.p. 180 – 181 °C. For NMR data see Table 2. Mass spectrum (*m/e*, %) 243,8; 242,50; 163,9; 162,71; 161,24; 149,5; 148,10; 147,100; 136,12; 135,65; 134,5; 133,10; 129,5; 98,33; 97,32; 91,5; 71,6; 69,7; 64,5; 63,5; 54,7; 45,26. [Found: C 54.2; H 2.40; N 22.8; S 13.2. Calc. for $C_{11}H_6N_4OS$ (242.3): C 54.5; H 2.50; N 23.1; S 13.2].

1,1,3,3-Tetracyano-1,3,3a,8b-tetrahydrobenzo-[b]*furo[2,3-c]furan.* A mixture of 11.8 g (0.01 mol) of benzo[*b*]furan,[42,43] and 5.0 g (0.035 mol) of TCNEO in 100 ml of 1,2-dichloroethane was heated in a glass ampoule at 150 °C for 10 h. After cooling, the ampoule was opened, the contents heated and a black polymer mass filtered off. The filtrate was evaporated to 40 ml and the adduct filtered off and recrystallized from 1,2-dichloroethane, yielding 21.9 g (29 %) of the title compound, m.p. 238.5 – 240 °C. For NMR data see Table 1. Mass spectrum (*m/e*, %) 262,23; 183,14; 182,100; 156,11; 155,27; 154,19; 153,7; 128,5; 127,29; 118,14; 103,5; 100,6; 90,7; 89,8; 72,5; 71,6; 70,6; 65,11; 64,6; 63,9; 54,8; 51,9; 50,7. [Found: C 63.84; H 2.53; N 20.63; O 12.88. Calc. for $C_{11}H_6N_4O_2$ (262.2): C 64.13; H 2.31; N 21.37; O 12.20].

[2-D]Benzo[b]*furan.* To a solution of 18.9 g (0.16 mol) of benzo[*b*]furan in 50 ml of anhydrous ether, 135 ml of 1.42 N butyllithium in hexane was added dropwise with cooling. The mixture was stored for 40 min at room temperature and 10 ml of deuterium oxide was added carefully with vigorous stirring. After 5 min, the reaction mixture was poured onto ice-water. The organic phase was separated, washed with water, dried and fractionated to give 13.3 g (70 %) of the title compound, b.p. 65 – 67 °C/22 mmHg.

NMR (CCl_4): $\delta_3 = 6.62$ ppm.

3a-Deuterio-1,1,3,3-tetracyano-1,3,3a,8b-tetrahydrobenzo[b]*furo*[2,3-c]*furan*. A mixture 4.3 g (0.036 mol) of [2-D]benzo[*b*]furan and 1.1 g (0.0075 mol) of TCNEO in 50 ml of 1,2-dichloroethane was heated in a glass ampoule at 150 °C for 2 h. In this case no polymerization occurred, and after cooling 1.9 g (95 %) of colourless crystals of the title compound was isolated, with a m.p. of 241 – 243 °C, which did not change upon recrystallization from 1,2-dichloroethane. For NMR data see Table 2. Mass spectrum (*m/e*, %) 264,5; 263,24; 184,16; 183,100; 182,7; 157,11; 156,25; 155,29; 154,5; 129,6; 128,25; 127,10; 119,17; 102,5; 100,5; 99,5; 91,8; 90,7; 77,5; 76,7; 75,6; 64,10; 63,9; 54,9; 52,6; 51,10; 50,8.

1,1,3,3-Tetracyano-1,3,3a,8b-tetrahydrobenzo[b]*thieno*[2,3-c]*furan*. A mixture of 13.4 g (0.10 mol) of benzo[*b*]thiophene and 5.0 g (0.035 mol) of TCNEO in 50 ml of 1,2-dibromoethane was refluxed for 27 h. The mixture was filtered hot, and after evaporation to a volume of 20 ml, the adduct crystallized. Recrystallization from 1,2-dichloroethane gave 2.3 g (25 %) of the title compound, m.p. 198 – 199 °C. For NMR data see Table 1. Mass spectrum (*m/e*, %) 279,6; 278,32; 200,6; 199,13; 198,100; 197,11; 172,15; 171,71; 170,10; 134,29; 127,8; 100,22; 99,6; 90,6; 89,8; 85,7; 69,10; 63,7; 51,6; 50,6; 45,10; [Found: C 60.64; H 2.40; N 19.67; S 11.02. Calc. for $C_{14}H_6N_4OS$ (278.3): C 60.42; H 2.17; N 20.13; S 11.52].

3a-Deuterio-1,1,3,3-tetracyano-1,3,3a,8b-tetrahydrobenzo[b]*thieno*[2,3-c]*furan*. A mixture of 4.8 g (0.036 mol) of [2-D]benzo[*b*]thiophene [44] and 1.1 g (0.0076 mol) of TCNEO in 50 ml of 1,2-dichloroethane was heated in a glass ampoule at 150 °C for 2 h. After cooling and evaporation to 20 ml, the adduct was filtered off and recrystallized from 1,2-dichloroethane which yielded 1.1 g (51 %) of the title compound, m.p. 196 – 197 °C. For NMR data see Table 1. Mass spectrum (*m/e*, %) 280,5; 279,28; 201,12; 200,20; 199,100; 198,23; 197,5; 174,5; 173,21; 172,65; 135,5; 128,8; 99,6; 91,5; 90,6; 80,5; 69,10; 64,5; 63,7; 54,37; 51,7; 50,5; 46,6; 45,8.

Kinetic experiments

The TCNEO used was recrystallized three times from 1,2-dichloroethane; m.p. 177 – 178 °C. Thiophene was dried over magnesium sulfate and distilled through an efficient column; the fraction with b.p. 84.0 – 84.1 °C was taken. 1,2-Dichloroethane was dried over calcium chloride and distilled. A fore-run of 25 % was discarded and a middle fraction, b.p. 84.0 – 84.2 °C, was used. The concentration of TCNEO was determined according to Linn *et al.*[45] The TCNEO-containing solution was added to an acidic aqueous solution of potassium iodide. Cyanogen iodide is formed, which with excess iodide gives iodine. The iodine concentration was determined by titration with thiosulfate. The method was checked by carrying out the titration in the presence of thiophene, and the thiophene adduct and these compounds were found not to interfere. The kinetic experiments were carried out in 10 ml glass ampoules. Stock solutions of TCNEO and of thiophene in 1,2-dichloroethane were prepared, and exact volumes of reactants were transferred to 50 ml volumetric flasks and made up to volume with 1,2-dichloroethane. In each glass ampoule, 5 ml of solution was placed and the ampoules were cooled to – 70 °C and sealed. After the ampoules had reached room temperature, they were placed in an oil-filled thermostat at 150.2 °C. The ampoules were shaken during the experiments and it never took more than 30 s before the oil-bath had regained its temperature after inserting the ampoules. Each rate constant was calculated from 3 to 8 ampoules with the same initial concentrations of TCNEO and thiophene. The ampoules were taken from the oil-bath at appropriate intervals (600 – 10 000 s), and the reaction was quenched by immersion in a bath of dry ice and ethanol. After the ampoules had reached room temperature, they were broken and the titration carried out exactly as described in Ref. 3. The initial concentrations and the second-order rate constants are collected in Table 3.

Competitive experiments

Thiophene, furan, selenophene, 2-chlorothiophene, 2-methylthiophene, benzo[*b*]furan, and benzo[*b*]thiophene were distilled before use through an efficient column, and a middle fraction with a small boiling range was used. TCNEO and 1,2-dichloroethane were purified as described above. All competitive experiments were carried out in 100 ml glass ampoules in an oil-filled thermostat at 150.0 ± 0.2 °C. The ampoules were shaken during the experiments and the oil-bath retook the pre-set temperature within 60 s. The concentrations of the adducts formed were determined by VPC analyses using an internal standard. All analyses were carried out with a 3 % OV 101 column (3 m) and the internal standard used was tetrabromothiophene in the experiments with thiophene-2-chlorothiophene and furan-thiophene, hexachlorobenzene for furan-2-methylthiophene, selenophene-thiophene and thiophene-benzo[*b*]thiophene and finally pentachlorobenzene for the competitive experiments between benzo[*b*]furan and benzo[*b*]thiophene. All the adducts were carefully purified by repeated crystallizations, solutions of known concentrations of adducts and standards were mixed, and strictly linear calibration curves were obtained. Stock solution of the different heterocycles and of TCNEO in 1,2-dichloroethane were prepared and suitable aliquots were transferred to 50 ml volumetric

flasks and made up to volume with the solvent. The solutions were transferred to glass ampoules, which were cooled to −70 °C and sealed. After the ampoules had reached room temperature they were immersed in the oil-bath and shaken for 1 – 6 h. The reactions were stopped by immersion in ice-water. After the contents had been thermostated at 25 °C, the same volume of internal standard was added as in the calibrations and the end concentrations of the adducts could be obtained by VPC analysis (digital integrator). The initial concentrations used and relative rate constants which were obtained together with more experimental details are given in Ref. 21.

VPC analyses were carried out with a Perkin-Elmer 900 gas chromatograph equipped with a flame-ionization detector and connected to a Varian integrator model 480.

NMR spectra were recorded with a Varian A-60 spectrometer and mass spectra with an LKB 9000 mass spectrometer. The compounds were injected through a direct inlet or *via* a gas chromatograph. The energy of the ion beam was 70 eV and the electron current was set to 60 μA.

IR spectra were recorded on a Perkin-Elmer 257 Grating Infrared Spectrophotometer and UV spectra on a Unicam SP 800 B UV spectrophotometer. The elemental analyses were carried out at the Analytical Department of the Chemical Institute, University of Lund, by Miss Ilse Beetz, Mikroanalytisches Laboratorium, Kronach, and by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mülheim.

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Studies on Electrolytic Substitution Reactions. IX.* Anodic Cyanation of Aromatic Ethers and Amines in Emulsions with the Aid of Phase Transfer Agents

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The anodic cyanation of di- and trimethoxybenzenes and *N*,*N*-dimethylbenzylamine has been studied in an emulsified system of methylene chloride, tetrabutylammonium sulfate, substrate, and aqueous sodium cyanide. The emulsification technique proved useful from the points of view of easy work-up and low applied voltage as compared to the corresponding homogeneous systems. The use of methylene chloride as the organic solvent component results in a less selective process than in acetonitrile/tetraalkylammonium salt, as evidenced by the fact that both methoxyl and hydrogen substitution take place, that dicyanation products are formed, and that *N*,*N*-dimethylbenzylamine is substituted in both the methyl and methylene group in the former solvent.

The anodic cyanation of organic compounds takes place by substitution of a ring hydrogen [1–6,8,9,12,13] or methoxyl group [8] in an aromatic compound, by substitution of a hydrogen α to the nitrogen atom of a tertiary amine,[8] or by addition across a system of double bonds in a few cases.[7,11] Mechanistically, the reaction is of the direct type, most likely proceeding *via* an ECE mechanism.[14,15]

In practice, the reaction is performed either in methanol/sodium cyanide [1–7,9,11–13] or acetonitrile/tetraethylammonium cyanide [6,8,10] at a platinum anode, generally with low yields of cyanation products except for a few cases of rather special substrates, such as methoxybenzenes,[8] diphenylacetylene,[9] 9,10-dialkylanthracenes,[10] and uracil.[11] The most often encountered side-reaction is anodic methoxylation when the reaction is performed in methanolic medium.

We have recently shown [16] that the use of an emulsified aqueous-organic system, consisting of aqueous sodium cyanide/methylene chloride-substrate-phase transfer reagent (such as tetrabutylammonium ion, added in the form of the sulfate), results in a clean, high-yield cyanation process. The reaction was exemplified by the monocyanation of naphthalene and anisole in maximally 69 and 57 % isolated yields, respectively, on the 0.05 mol scale. The phase transfer reagent is added in essentially catalytic amounts and serves for the transport of cyanide ion from the aqueous to the organic phase.[17] As an additional bonus, the aqueous phase acts as a high-conductivity medium for the transport of charge, as shown by the 5 – 15 fold decrease in cell voltage as compared to an entirely non-aqueous system of the same composition as the organic phase of the two-phase system.

Since this technique appears to hold promise for synthetic applications of organic electrochemistry,[16] we have now extended these studies to the cyanation of a number of other substrates, mainly di- and trimethoxybenzenes, as outlined in the following report.

RESULTS AND DISCUSSION

Electrolysis procedure. Generally, electrolyses were performed in a jacketed vessel between a platinum foil anode (in a few experiments, it was established that graphite electrodes do give

* Part VIII, see *Acta Chem. Scand. B 29* (1975) 168.

Table 1. Anodic cyanation of dimethoxybenzenes. Electrolyte: Aqueous 1 M NaCN (100 ml), methylene chloride (100 ml), Bu_4NSO_4H (10 mmol), substrate (10 mmol).

Dimethoxybenzene	Products	Yield,[a] % Emulsion system[b]	Yield,[a] % CH_3CN/Et_4NCN[c]
1,2-	2-Methoxybenzonitrile	26	94
	3,4-Dimethoxybenzonitrile	11	
	Other products[d]	< 5	
1,3-	2,4-Dimethoxybenzonitrile	58[e]	12
	4,6-Dimethoxyisophthalonitrile	2[e]	
1,4-	4-Methoxybenzonitrile	40	90 – 95
	2,5-Dimethoxybenzonitrile	3	
	Other products[f]	< 5	

[a] Analyzed by GLC and based on unrecovered starting material. [b] This work; 3 F/mol of substrate passed. [c] Ref. 8. [d] According to mass spectral analysis, these products are probably 2,3-dimethoxybenzonitrile, an isomer of dimethoxydicyanocyclohexadiene, and an isomer of dicyanoanisole. [e] In an experiment in which 6 F/mol of substrate were passed, 33 and 12 % of mono- and dicyanation product, respectively, were obtained. [f] According to mass spectral analysis, these products are probably two isomers of dimethoxydicyanocyclohexadiene.

similar results, albeit with much lower current efficiency) and a stainless steel cathode. This cathode material is advantageous because it does not support the cathodic reduction of methylene chloride to chloride ion, a troublesome side-reaction in an undivided cell [18–20] due to subsequent chlorine formation at the anode. Unless otherwise noted, the aqueous phase was 1 M in sodium cyanide and emulsified with an equal volume of the organic phase, a solution of the substrate in methylene chloride. The phase transfer agent, tetrabutylammonium hydrogen sulfate, was first neutralized and then added in an amount so as to make the whole system 0.05 M. Emulsification was achieved by a special high-speed stirrer, constructed in our Laboratory after the principles used for the commercially available "Ultra-Turrax" stirrer.[21] Constant current conditions were used throughout.

Dimethoxybenzenes. Table 1 shows products and yields from the emulsion electrolysis of the three dimethoxybenzenes, as well as those obtained in the homogeneous system, acetonitrile/tetraethylammonium cyanide.[8] Generally, the results indicate a more selective cyanation process in the latter electrolyte system, as evidenced by the almost quantitative yield of products derived by methoxyl substitution from 1,2- and 1,4-dimethoxybenzene. In the emulsion system, both substitution of methoxyl and hydrogen takes place, and besides, dicyanation occurs to some extent.

The lower selectivity of the emulsion system is most likely a reflection of the properties of methylene chloride/tetrabutylammonium cyanide as a supporting electrolyte and not of the emulsion system in itself. We investigated this problem somewhat more in detail by electrolyzing *N,N*-dimethylbenzylamine in methylene chloride/tetrabutylammonium cyanide (0.1 M). This reaction was deemed very well suited for this purpose, since Andreades and Zahnow [8] had found that cyanation in acetonitrile/tetraethylammonium cyanide takes place exclusively in the methyl group:

$$PhCH_2N(CH_3)_2 \xrightarrow[Et_4NCN]{CH_3CN} PhCH_2N(CH_3)CH_2CN$$

This result is the more surprising considering the fact that anodic methoxylation takes place both in the methylene and methyl groups of this substrates.[22–25] We checked these results in acetonitrile, using tetrabutylammonium instead of tetraethylammonium cyanide, and obtained exclusive methyl substitution product, exactly as found by Andreades and Zahnow.[8] However, in methylene chloride/tetrabutylammonium cyanide the selectivity for methyl group substi-

Table 2. Anodic cyanation of 1,3-dimethoxybenzene, using different ratios between the two phases. Aqueous phase, 1 M NaCN; organic phase, methylene chloride; total volume, 200 ml; amount of substrate, 10 mmol.

Volume ratio aqueous/organic phase	Tetrabutyl-ammonium ion, mmol	Charge passed, F/mol	Yield[a] of 2,4-dimethoxy-benzonitrile, %	Current yield
1.0	10	3	58	38
1.0	10	2	69	60
1.7	10	3	60	28
3.0	10	3	45	16
7.0	10	3	36	14
1.0	20	3	49	33
1.0	20	2	63	52
1.7	20	2	51	33
3.0	20	2	42	24

[a] Analyzed by GLC and based on unrecovered starting material.

tution disappeared a 50:50 mixture of methylene and methyl substitution product being obtained. This shows that there must be an intrinsic difference between acetonitrile and methylene chloride as solvents for anodic cyanation, and that the additional complication of an emulsion system in the latter case probably has no influence of its own.

Current yields for the cyanations reported in Table 1 generally fall in the region between 20 and 40 % in both systems.[8] In order to study the influence upon product yields of changing the volume ratios between the two phases, 1,3-dimethoxybenzene was chosen as the substrate for a series of such experiments. Table 2 shows that an increase of the aqueous phase at the expense of the organic one leads to decreasing current yields but leaves the material yields largely unchanged. For some reason, a higher concentration of phase transfer agent decreases both material and current yield.

A preparative experiment under the same conditions (2 F/mol of substrate, 10 mmol of phase transfer agent) as those given in Table 2, but with 50 mmol of substrate, led to the isolation of 2,4-dimethoxybenzonitrile in 61 % yield.

Trimethoxybenzenes. Table 3 shows products and yields from the emulsion electrolysis of the

Table 3. Anodic cyanation of trimethoxybenzenes. Electrolyte: Aqueous 1 M NaCN (100 ml), methylene chloride (100 ml), Bu_4NSO_4H (10 mmol), substrate (10 mmol).

Trimethoxy-benzene	Products	Yield, %	
		Emulsion system[b]	CH_3CN/ Et_4NCN[c]
1,2,3-	2,6-Dimethoxybenzonitrile	28[a]	86[a]
	2,6-Dimethoxyterephthalonitrile	1.4[d]	
1,3,4-	2,4-Dimethoxybenzonitrile	38[a]	
	3,4-Dimethoxybenzonitrile	1[a]	
1,3,5-	2,4,6-Trimethoxybenzonitrile	56[d,e,f]	

[b] Analyzed by GLC and based on unrecovered starting material. [c] This work; 3 F/mol of substrate passed. [d] Ref. 8. [e] Isolated yield, based on unrecovered starting material. [f] With 20 mmol of phase transfer agent added, this yield drops to 40 %. [g] Dicyanation takes place to same extent (see text).

three trimethoxybenzenes. Again, we can establish the acetonitrile system as being the more selective one (only replacement of methoxyl group, no dicyanation).

Dicyanation. The cyanation reaction has previously been reported to be selective towards monocyanation, as would be expected in view of the deactivating effect of electron-withdrawing substituents upon anodic substitution.[14,15] However, prolonged electrolysis has been shown to give dicyanation products from anisole [13] and now a few other cases of dicyanation have been demonstrated (Tables 1 and 3). In order to find out the extent of this process, we electrolysed 2,4-dimethoxybenzonitrile by itself in the emulsion system until 3 F/mol of substrate had passed and found a 28 % material and 8 % current yield of 4,6-dimethoxyisophthalonitrile; in a similar experiment, 2,4,6-trimethoxybenzonitrile gave the corresponding dinitrile in 53 % material and 17 % current yield.

EXPERIMENTS

Materials. Most chemicals used in this investigation are commercially available and have been used without further purification. The following substances have been synthesized by diazotation of the appropriate dimethoxyaniline followed by treatment with copper(I) cyanide:[26] 3,4-dimethoxybenzonitrile, yield 39 %, m.p. 65 – 67 °C (lit. 67 – 68 °C),[27] 3,5-dimethoxybenzonitrile, yield 15 %, m.p. 85 – 86 °C (lit. 87 – 88 °C),[28] and 2,5-dimethoxybenzonitrile, yield 25%, m.p. 81 – 82 °C (lit. 80 °C).[29] 2,6-Dimethoxybenzonitrile was synthesized from 1,3-dinitrobenzene [30] in 17 % yield, m.p. 119 – 120°C (lit 117 – 118 °C).[30]

Electrolysis equipment. Electrolyses were carried out in an open jacketed vessel, 300 ml in volume. The cell was equipped with a platinum foil anode (55 cm^2) and a stainless steel cathode. A high-speed stirring device [16] was placed in the middle of the cell between the electrodes. Electrolyses were run at constant current maintained by a Radiak power supply.

General electrolysis procedure. The electrolyte consisted of 10 mmol of substrate, 100 ml of dichloromethane, 100 ml of 1 M sodium cyanide and 10 or 20 mmol of tetrabutylammonium hydrogen sulfate dissolved in 15 ml of water and neutralized with sodium hydroxide. Electrolyses were run at a constant current of 0.75 A, resulting in a current density of 0.014 A/cm^2. Charge equivalent to 2, 3, or 6 F/mol was passed. The very efficient stirring kept the electrolyte well emulsified during the run. The potential drop across the cell varied between 12 and 20 V. When starting up the electrolysis the stirrer had to be turned on for a few seconds and then off and left for half a minute and then turned on again in order to get the voltage over the cell to rise from the initial value of 4 – 5 V. This is probably due to a wetting phenomenon.

General workup procedure. After the stirrer had been switched off the two phases separated rapidly and the organic layer was evaporated to dryness. The residue was taken up in 15 ml of water and extracted with 3 x 20 ml of ether. The combined ether layers were dried over magnesium sulfate and reduced in volume to about 15 ml. Finally, products were analyzed by GLC using a 2 m x 3 mm 5 % neopentylglycol succinate column.

1,2-Dimethoxybenzene. Experiments were carried out according to the general procedure. Besides 2-methoxybenzonitrile and 3,4-dimethoxybenzonitrile three other products were found in less than 5 % total yield. MS analysis indicated that these products were 2,3-dimethoxybenzonitrile, one isomer of the addition product, dicyanodimethoxycyclohexadiene, and one isomer of dicyanoanisole. Yields are given in Table 1.

1,3-Dimethoxybenzene. Electrolyses were carried out according to the general procedure. During workup a solid, insoluble in ether, precipitated. After recrystallization from ethanol, the substance was shown to be 4,6-dimethoxyisophthalonitrile (see cyanation of 2,4-dimethoxybenzonitrile). The main product, 2,4-dimethoxybenzonitrile, was isolated and recrystallized from ligroin (b.p. 80 – 110 °C), m.p. 90 – 91 °C (lit.[29] 89 °C). NMR and mass spectra were in accordance with the proposed structure. The crude product was also analysed according to the general workup procedure. Yields, determined by GLC, are given in Tables 1 and 2.

1,4-Dimethoxybenzene. Experiments were carried out according to the general procedure. GLC/MS analysis showed, besides 4-methoxybenzonitrile and 2,5-dimethoxybenzonitrile, two isomers of dicyanodimethoxycyclohexadiene. Yields are given in Table 1.

1,2,3-Trimethoxybenzene. Experiments followed the general procedure. The yield of the main product, 2,6-dimethoxyterephthalonitrile, precipitated. The substance was recrystallized from ethanol and then sublimed *in vacuo*. 2,6-Dimethoxyterephthalonitrile sublimed below the melting point. NMR: δ 4.0 (s, 6 H), 6.85 (s, 2 H). IR: Strong band at 2235 cm^{-1}. MS: *m/e* (%), 189 (12), 188 (100); 187 (12); 145 (42); 131 (13); 128 (63). Elemental analysis: Calc.: C 63.8; H 4.26; N 14.9. Found: C 63.7; H 4.39; N 15.0.

1,2,4-Trimethoxybenzene. Experiments were carried out according to the general procedure. Yields are given in Table 3.

1,3,5-Trimethoxybenzene. Electrolyses were carried out according to the general procedure. The product, 2,4,6-trimethoxybenzonitrile, was extracted into ether. The solvent was evaporated and the residue was recrystallized from

ligroin (b.p. 80 – 110 °C), m.p. 143 – 144 °C (lit.[31] 143 – 144 °C). NMR, IR, and mass spectra were in accordance with this structure. During workup a solid precipitated that was recrystallized from ethanol. It was shown to be 2,4,6-trimethoxyisophthalonitrile (see cyanation of 2,4,6-trimethoxybenzonitrile). Yields are given in Table 3.

2,4-Dimethoxybenzonitrile. Electrolyses were run according to the general procedure. During workup a solid precipitated between the ethereal and aqueous layers and was collected by filtration. The product, 4,6-dimethoxyisophthalonitrile, was recrystallized from ethanol and sublimed *in vacuo*, m.p. 51 – 52 °C. NMR: δ 4.1 (s, 6 H), 7.0 (s, 1 H), 8.0 (s, 1 H). IR: Strong band at 2235 cm^{-1}. MS: *m/e* (%), 189 (13); 188 (100); 187 (15); 159 (34); 158 (14); 145 (24). Elemental analysis: Calc.: C 63.8; H 4.26; N 14.9. Found: C 63.9; H 4.30; N 14.5.

2,4,6-Trimethoxybenzonitrile. Electrolyses were carried out according to the general procedure. The product precipitated as described for 4,6-dimethoxyisophthalonitrile and was recrystallized from ethanol. Prior to combustion analysis the substance was sublimed *in vacuo*. The yield of 2,4,6-trimethoxyisophthalonitrile, m.p. 250 – 1 °C, was 53 % (material) and 17 % (current). NMR: δ 4.05 (s, 6 H), 4.2 (s, 3 H), 6.7 (s, 1 H). IR: Strong band at 2235 cm^{-1}. MS *m/e* (%) 219 (13); 218 (100); 217 (8); 175 (15). Elemental analysis: Calc.: C 60.6; H 4.59; N 13.1. Found: C 60.7; H 4.64; N 13.1.

N,N-*Dimethylbenzylamine.* This compound was anodically cyanated in three different ways.

1. According to the general procedure, 10 mmol of substrate was electrolyzed with 10 mmol of tetrabutylammonium salt present. Charge equivalent of 3 F/mol was passed. Workup according to the general procedure and analysis by NMR and GLC/MS gave a 46:54 ratio of *N,N*-dimethylphenylglycinonitrile and *N*-benzyl-*N*-methylglycinonitrile.

When 50 mmol of substrate and 20 mmol of supporting electrolyte were electrolyzed as above, a 46 % yield of products was isolated in a current efficiency of 31 %. The isomer ratio was the same as above.

2. Electrolyses were run in a divided cell with 10 mmol of substrate and 20 mmol of tetrabutylammonium cyanide in 100 ml of methylene chloride as the anolyte. The catholyte consisted of 0.2 M tetrabutylammonium tetrafluoroborate in methylene chloride. A ceramic cup was used as a cell divider. Two platinum foil electrodes were used, the anode area being 60 cm^2. A charge equivalent to 2 F/mol was passed at 0.3 A. Tetrabutylammonium fluoroborate (5 mmol) was added during electrolysis in order to increase the conductivity of the anolyte. Workup according to the general procedure followed by GLC analysis showed a 54:46 ratio of *N,N*-dimethylphenylglycinonitrile and *N*-benzyl-*N*-methylglycinonitrile.

3. Substrate (10 mmol) and tetrabutylammonium cyanide (20 mmol) in acetonitrile were electrolyzed at a platinum foil anode and a stainless steel cathode. Charge equivalent to 2 F/mol was passed at 0,75 A Conventional workup and GLC analysis showed that substitution had taken place exclusively in methyl position.

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Heteroaromatic Boron Compounds. XI. On the Nitration of 3,2-Borazaropyridines under Non-acidic Conditions

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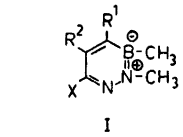
Derivatives of 3,2-borazaropyridines, *i.e.* 6,7-dimethyl-7,6-borazarothieno[3,2-*c*]pyridine (IIa), 3,4-dimethyl-4,3-borazoisoquinoline (IVa) 2,3-dimethyl-4-ethyl-3,2-borazaropyridine (Ia) and 2,3-dimethyl-5-ethyl-3,2-borazaropyridine (Ib), were conveniently nitrated in high yield, when *N*-nitropicolinium tetrafluoroborate in acetonitrile was used as nitrating agent. Substitution occurred exclusively in the position α to the pyridinic nitrogen, except in Ib where 82 % of the 6-nitro and 18 % of the 4-nitro derivatives were formed. No oxidation of the $B-CH_3$ groups occurred. The nitro derivatives could be reduced to the corresponding amino derivatives, using hydrazine and palladium on charcoal.

It has previously been demonstrated that 3,2-borazaropyridines such as Ia and Ib [1,2] and their thiophene-fused derivatives (IIa) and (III),[3,4] from which they are prepared, are stable aromatic systems. They can be halogenated and nitrated.[1,2,4–6] In our previous investigations, fuming nitric acid in acetic anhydride-acetic acid was used as nitrating agent.[2,4] With this reagent IIa and III were nitrated in the boron-nitrogen containing ring (the 4-position of IIa and the 7-position of III, respectively), while Ia was nitrated in the 6-position and Ib gave a mixture of the 6- (predominantly) and the 4-nitro derivative.[2] The great disadvantage of this reagent was that it caused partial oxidation of the $B-CH_3$ groups to yield the $B-OH$ derivatives, which made purification and characterization more difficult. It was therefore of interest to find a milder and less oxidative nitrating agent. Dewar and von Rosenberg [7] attempted the nitration of 4-methyl-4,3-borazaroisoquinoline with nitronium tetrafluoroborate in tetramethylene sulfone, but could only recover starting material. However, Cupas and Pearson [8] reported in 1968 that *N*-nitro-pyridinium tetrafluoroborates could be used as nitrating agents for benzene and toluene. These authors recommended 2-picoline as pyridine base. It is possible that the failure in the nitration with nitronium tetrafluoroborate is due to complexation with the pyridinic nitrogen of the borazaropyridine and that this can be avoided by using the 2-picoline complex. The base strength of 2-picoline is much greater than that of borazaropyridines.[2] Our hope that borazaropyridines could be nitrated with *N*-nitro-2-picolinium tetrafluoroborate was also strengthened by the fact that the former compounds are smoothly brominated in pyridine-carbon tetrachloride.[6]

Our expectations were fulfilled, as 3,4-dimethyl-4,3-borazaroisoquinoline (IVa) [9] gave 3,4-dimethyl-1-nitro-4,3-borazaroisoquinoline (IVb) in 82 % yield as the only isomer. The structure was established by elemental analyses and oxidative degradation to benzoic acid. Dewar and von Rosenberg [7] have shown that under strongly acidic conditions (fuming nitric acid in conc. sulfuric acid), 4-methyl-4,3-borazaroisoquinoline is substituted in the benzene ring, yielding 8-nitro-4-methyl-4,3-borazaroisoquinoline. 6,7-Dimethyl-7,6-borazarothieno-[3,2-*c*]pyridine (IIa) with *N*-nitro-2-picolinium-tetrafluoroborate gave exclusively 6,7-dimethyl-4-nitro-7,6-borazarothieno[3,2-*c*]pyridine (IIb).

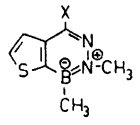
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** Taken in part from the Ph. D. thesis of A. Maltesson, University of Lund 1974.



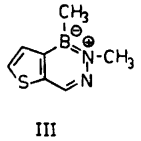
I

a. $R^1 = C_2H_5$; $R^2 = H$; X = H
b. $R^1 = H$; $R^2 = C_2H_5$; X = H
c. $R^1 = C_2H_5$; $R^2 = H$; $X = NO_2$
d. $R^1 = H$; $R^2 = C_2H_5$; $X = NO_2$
e. $R^1 = NO_2$; $R^2 = C_2H_5$; X = H
f. $R^1 = H$; $R^2 = C_2H_5$; $X = NH_2$

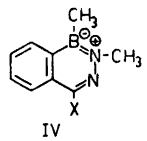


II

a. X = H
b. $X = NO_2$
c. $X = NH_2$



III



IV

a. X = H
b. $X = NO_2$

The structure followed directly from the NMR spectrum, as it still shows two thiophenic doublets, with the characteristic coupling constant of 5.0 Hz.[1a] Also 2,3-dimethyl-4-ethyl-3,2-borazaropyridine (Ia) gave only one nitro derivative, which by NMR was shown to be 2,3-dimethyl-4-ethyl-6-nitro-3,2-borazaropyridine (Ic). Compared to the parent compound,[2] the 5-hydrogen resonance is shifted 0.97 ppm towards lower field, which is of the expected order for nitro-caused *ortho* shifts in aromatic systems.[10] An assignment of the structure to the 5-nitro isomer would lead to an unreasonable *ortho*-nitro-caused shift of 0.12 ppm.

The nitration of Ib led, according to combined GLC-mass spectrometry, to two mononitro derivatives. It was not possible to separate these isomers by fractional distillation. NMR spectrometry showed that the mixture consisted of 80 % of 2,3-dimethyl-5-ethyl-6-nitro-3,2-borazaropyridine (Id) and 20 % of 2,3-dimethyl-5-ethyl-4-nitro-3,2-borazaropyridine (Ie). The structure assignments could easily be carried out with the aid of chemical shift considerations, as mentioned above. In addition, the 6-nitro isomer could also be identified by the presence of the characteristic long-range coupling $J_{CH_3\text{-}H\text{-}4}$.[2]

It is thus evident that for nitration, as well as for bromination [6] under non-acidic conditions, the order of reactivity of the three carbon positions of 3,2-borazaropyridine is $6 > 4 \gg 5$. It also appears that nitration is somewhat more selective than bromination. A more detailed discussion of the possible mechanisms of these substitution reactions will be given in a following, paper where also some other examples of substitution reactions will be presented.

No nitro derivatives were obtained when the isoelectronic 2,3,4-trimethyl- and 2,3,5-trimethylpyridine were reacted with *N*-nitro-2-picolinium tetrafluoroborate under the same conditions. However, as starting material was not recovered it is probable that ring-opening to glutacone aldehyde derivatives occurred, similar to that described by Olah and coworkers [11] for the reaction of *N*-nitropyridinium fluoroborate with excess pyridine.

The nitro derivatives described above could be transformed to amino derivatives, which constitute starting materials of great utility. We found that hydrazine in the presence of palladium on charcoal,[12,13] which has also been used by Dewar and Kubba [14] for the reduction of various nitro-9,10-borazaraphenantrenes, was an excellent reagent for this purpose. Thus IIb gave 4-amino-6,7-dimethyl-7,6-borazarothieno-[3,2-c]pyridine (IIc) in high yield. It is also interesting to note that the mixture of Id and Ie gave pure 6-amino-2,3-dimethyl-5-ethyl-2,3-borazaropyridine (If) in 52 % yield. This could indicate that 4-amino-2,3-dimethyl-5-ethyl-3,2-borazaropyridine is an unstable compound, which decomposed after formation. For comparative purposes 6-nitro-2,3,5-trimethylpyridine [2] was also reduced to the 6-amino derivative.

EXPERIMENTAL

6,7-Dimethyl-4-nitro-7,6-borazarothieno[3,2-c]-pyridine (IIb). 1.32 g (0.010 mol) of nitronium tetrafluoroborate (weighed under dry nitrogen atmosphere in a glove box) was suspended in 20 ml of dry acetonitrile under nitrogen. To this mixture, 0.93 g (0.010 mol) of dry 2-picoline, distilled over barium oxide and stored over molecular sieves, was addded dropwise. The pale yellow solution, maintained at about 0 °C, was pressed into a dropping funnel by dry nitrogen. The solution of *N*-nitro-picolinium tetrafluoroborate was added dropwise to a stirred solution of 1.04 g (0.010 mol) of 6,7-dimethyl-7,6-borazarothieno[3,2-c]pyridine [2] in 20 ml of dry acetonitrile, at 0 °C. When the addition was complete,

the cooling bath was removed and the mixture was allowed to reach room temperature. The mixture was then stirred for an additional 4 h. The solvent was removed under reduced pressure and to the remaining red-brown crystalline cake water was added. The water solution was extracted four times with ether. The combined ether fractions were dried and the ether removed under reduced pressure. The resulting crystalline cake was recrystallized from 60 % ethanol-water. These crystals contained about 10 % of starting material according to GLC. A second recrystallization gave analytically pure material as white needles. Yield: 1.42 g (68 %) of the title compound. M.p. 129.0–131.0 °C. NMR [$(CD_3)_2CO$]: δ 8.23 and 7.93 (thioph., $J_{2,3}$ 5.0 Hz), 3.87 (NCH_3), 1.05 (BCH_3). [Found: C 40.12; H 3.90; B 5.11; N 20.14; S 15.46. Calc. for $C_7H_8BN_3O_2S$ (209.0): C 40.22; H 3.86; B 5.17; N 20.10; S 15.33.]

3,4-Dimethyl-1-nitro-4,3-borazaroisoquinoline (IVb). 1.58 g (0.010 mol) of 3,4-dimethyl-4,3-borazaroisoquinoline [9] was treated with *N*-nitropicolinium tetrafluoroborate as described above. The mixture was worked up according to the procedure above. The crude product (2.33 g) was recrystallized from 50 % ethanol-water, leaving 1.66 g (82 %) of the title compound; m.p. 152–153 °C. NMR [$(CD_3)_2CO$]: δ 8.08 (m, arom.), 3.80 (NCH_3), 1.07 (BCH_3). [Found: C 53.56; H 5.08; B 5.13; N 20.21. Calc. for $C_9H_{10}BN_3O_2$ (203.0): C 53.25; H 4.97; B 5.32; N 20.70.]

Degradation of the nitro product. 3,4-Dimethyl-1-nitro-4,3-borazaroisoquinoline (IVb) was oxidized with $KMnO_4$ in alkaline medium as described for 1-bromo-4-methyl-4,3-borazaroisoquinoline.[15] This oxidation gave after work-up 35 % of benzoic acid identical with authentic material.

2,3-Dimethyl-4-ethyl-6-nitro-3,2-borazaropyridine (Ic). 1.36 g (0.010 mol) of 2,3-dimethyl-4-ethyl-3,2-borazaropyridine was treated with *N*-nitropicolinium tetrafluoroborate as described above. The mixture was worked up according to the procedure above. The crude product was fractionated, yielding 1.05 g (58 %) of the title compound; b.p. 79–82 °C/2 mmHg. NMR ($CDCl_3$): δ 1.20 (CH_3CH_2), 2.70 (CH_3CH_2), 3.85 (NCH_3), 7.88 (H-5), 0.86 (BCH_3). [Found: C 46.54; H 6.79; B 5.80; N 23.09. Calc. for $C_7H_{12}BN_3O_2$ (181.0): C 46.45; H 6.68; B 5.97; N 23.21.]

2,3-Dimethyl-5-ethyl-6-nitro-3,2-borazaropyridine (Id) and 2,3-dimethyl-5-ethyl-4-nitro-3,2-borazaropyridine (Ie). 1.36 g (0.010 mol) of 2,3-dimethyl-5-ethyl-3,2-borazaropyridine [2] was treated with *N*-nitropicolinium tetrafluoroborate as described above. The mixture was worked up according to the procedure above. The crude product contained two isomers according to combined GLC-mass spectroscopy in the proportion 18:82. NMR showed that 20 % consisted of 2,3-dimethyl-5-ethyl-4-nitro-3,2-borazaropyridine (Ie) and 80 % of 2,3-dimethyl-5-ethyl-6-nitro-3,2-borazaropyridine (Id). Fractional distillation gave 1.16 g (64 %) of a mixture of Id and Ie; b.p. 74–77 °C/1.8 mmHg. NMR ($CDCl_3$) of Id: δ 1.21 (CH_3CH_2), 2.63 (CH_3CH_2), 3.73 (NCH_3), 7.18 (H-4), 0.78 (BCH_3), J_{CH_2-H-4} 1.0 Hz. NMR ($CDCl_3$) of Ie: δ 1.21 (CH_3CH_2), 2.63 (CH_3CH_2), 3.80 (NCH_3), 7.92 (H-6), 0.72 (BCH_3). [Found: C 46.50; H 6.73; B 5.86; N 23.16. Calc. for $C_7H_{12}BN_3O_2$ (181.0): C 46.45; H 6.68; B 5.97; N 23.21.]

4-Amino-6,7-dimethyl-7,6-borazarothieno[3,2-c]pyridine (IIc). 3.35 g (0.016 mol) of 6,7-dimethyl-4-nitro-7,6-borazarothieno[3,2-*c*]pyridine (IIb) was dissolved in 100 ml abs. ethanol and 0.5 g of palladium (10 %) on charcoal was added. The mixture was refluxed and stirred and 10 ml of hydrazine hydrate (99–100 %) carefully added. During this addition, the reaction became rather exothermic and the yellow colour of the solution disappeared. After the addition of hydrazine hydrate the reaction mixture was refluxed for 30 min. After cooling, the catalyst was filtered off and washed with abs. ethanol. The solvent was evaporated and the residue was extracted with ether. The combined ether fractions were dried over magnesium sulfate and the ether was evaporated. The residue, a white crystalline cake, was recrystallized from 30 % ethanol-water. Yield after recrystallization: 2.4 g (84 %) of the title compound; m.p. 121.0–122.0 °C. NMR ($CDCl_3$): δ 7.68 and 7.47 (thioph., $J_{2,3}$ 4.8 Hz), 3.58 (NCH_3), 0.85 (BCH_3). [Found: C 47.00; H 5.67; N 23.38; B 5.99. Calc. for $C_7H_{10}N_3BS$ (179.0): C 46.96; H 5.63; N 23.47; B 6.04.]

6-Amino-2,3-dimethyl-5-ethyl-3,2-borazaropyridine (If). 1.81 g (0.010 mol) of 2,3-dimethyl-5-ethyl-6-nitro-3,2-borazaropyridine (Id) (containing 18 % of the 4-isomer) was dissolved in 100 ml of abs. ethanol, and 0.25 g of palladium (10 %) on charcoal was added. The mixture was treated as mentioned above and 6 ml of hydrazine hydrate was added. The mixture was worked up according to the last procedure. The crude product was distilled at reduced pressure; b.p. 69–72 °C/1 mmHg. The product crystallized in the receiver. GLC (BDS 10 %, 90–200 °C 10°/min) showed only one peak in addition to solvent. The product was recrystallized from 10 % ethanol-water, yielding 0.79 g (52 %) of the title compound, m.p. 70.0–71.5 °C. NMR ($CDCl_3$): δ 1.22 (CH_3CH_2), 2.41 (CH_3CH_2), 3.50 (NCH_3), 6.78 (H-4), 0.62 (BCH_3), 3.67 (NH_2), J_{CH_2-H-4} = 1.0 Hz. [Found: C 55.54; H 9.42; N 27.76; B 7.18. Calc. for $C_7H_{14}N_3B$ (151.0): C 55.67; H 9.34; N 27.82; B 7.26.]

6-Amino-2,3,5-trimethylpyridine. 1.00 g (0.006 mol) of 6-nitro-2,3,5-trimethylpyridine was dissolved in 150 ml of ethanol. To this solution 0.2 g of palladium (10 %) on charcoal and 10 ml of hydrazine hydrate were added as described above. The mixture was worked up as described above. The crystalline product was recrystallized from ligroin (80–120 °C), yielding 0.81 g (99 %) of the title compound, m.p. 105.0–

105.5 °C. NMR ($CDCl_3$): δ 6.98 (H-4), 4.38 (NH_2), 2.05, 2.10 and 2.30 [CH_3(2,3,5)]. [Found: C 70.4; H 9.0; N 20.7. Calc. for $C_8H_{12}N_2$ (136.2): C 70.55; H 8.88; N 20.57.]

GLC analyses were carried out with a Varian model 1400 gas chromatograph. NMR spectra were recorded with a Varian A 60 NMR spectrometer and mass spectra were obtained with a LKB A-9000 mass spectrometer. Elemental analyses were carried out by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mülheim/Ruhr.

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Heteroaromatic Boron Compounds. XII. On the Halogenation of 3,2-Borazaropyridines under Non-acidic Conditions

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Derivatives of 3,2-borazaropyridines can conveniently be brominated by bromine in pyridine-carbon tetrachloride and iodinated by iodine chloride in pyridine-acetonitrile. The benzo- and thieno-fused ring systems react at the remaining C–H group of the boron-nitrogen containing ring. While derivatives of 5-ethyl-3,2-borazaropyridine give a mixture of the 4- and 6-isomer upon attempted monobromination, iodination occurs selectively in the 4-position. The 5-ethyl derivative easily gives the 4,6-dibromo and 4,6-diiodo derivatives, when excess reagents are used. 4-Ethyl-3,2-borazaropyridines are only monobrominated or iodinated in the 6-position.

The isoelectronic pyridines are not halogenated with the above mentioned reagents. Possible mechanism for the facile halogenation of 3,2-borazaropyridines are discussed.

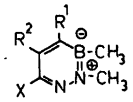
In a previous short communication we mentioned that the new aromatic boron-containing heterocycle 3,2-borazaropyridine could be brominated by molecular bromine in carbon tetrachloride-pyridine as solvent.[1] From 2,3-dimethyl-4-ethyl-3,2-borazaropyridine (Ia), the 6-bromo derivative (Ib) was obtained, while 2,3-dimethyl-5-ethyl-3,2-borazaropyridine (Ic) gave a mixture of approximately the same amounts of the 4- (Id) and 6- (Ie) bromo derivatives, in addition to the 4,6-dibromo derivative (If). It has previously also been found that bromine in acetic acid brominates benzo-[2] and thieno-fused 3,2-borazaropyridines[3] in the boron-nitrogen containing ring.

We have now undertaken a more detailed study of the bromination and iodination of 3,2-borazaropyridines and their benzo- and thieno-fused derivatives under non-acidic conditions.

We found that both B–OH derivatives, such as 4-hydroxy-5-methyl-4,5-borazarothieno[2,3-*c*]pyridine (IIa) and 7-hydroxy-6-methyl-7,6-borazarothieno[4,3-*c*]pyridine (IIIa), and B–CH_3 derivatives, such as 4,5-dimethyl-4,5-

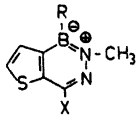
* To whom correspondence should be addressed.

** Taken in part from the Ph. D. thesis of A. Maltesson, University of Lund 1974.



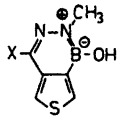
I

a. $R^1 = C_2H_5$; $R^2 = H$; X = H
b. $R^1 = C_2H_5$; $R^2 = H$; X = Br
c. $R^1 = H$; $R^2 = C_2H_5$; X = H
d. $R^1 = Br$; $R^2 = C_2H_5$; X = H
e. $R^1 = H$; $R^2 = C_2H_5$; X = Br
f. $R^1 = Br$; $R^2 = C_2H_5$; X = Br
g. $R^1 = C_2H_5$; $R^2 = H$; X = I
h. $R^1 = I$; $R^2 = C_2H_5$; X = H
i. $R^1 = I$; $R^2 = C_2H_5$; X = I



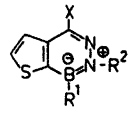
II

a. R = OH; X = H
b. R = CH_3; X = H
c. R = OH; X = Br
d. R = CH_3; X = Br



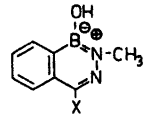
III

a. X = H
b. X = Br



IV

a. $R^1 = CH_3$; $R^2 = CH_3$; X = H
b. $R^1 = CH_3$; $R^2 = CH_3$; X = Br
c. $R^1 = OH$; $R^2 = H$; X = H
d. $R^1 = OH$; $R^2 = H$; X = Br
f. $R^1 = CH_3$; $R^2 = CH_3$; X = I



V

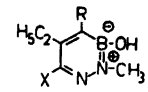
a. X = H
b. X = Br

borazarothieno[2,3-*c*]pyridine (IIb) and 6,7-dimethyl-7,6-borazarothieno[3,2-*c*]pyridine (IVa), react with one equivalent of bromine in pyridine-carbon tetrachloride to give monobromo derivatives IIc, IIIb, IId, and IVb, respectively, in 70–80 % yield. In all cases substitution occurred at the only C–H position of the boron-nitrogen containing ring. The structures followed directly from the NMR spectra.

However, N–H derivatives such as 7-hydroxy-7,6-borazarothieno[3,2-*c*]pyridine (IVc) gave a low yield of the 4-bromo derivative (IVd), when one equivalent of bromine was used, and much starting material was recovered. This could be circumvented by using two equivalents of bromine, but the yield was still only 52 %. It is possible that *N*-bromination complicated the reaction. Also 4-hydroxy-3-methyl-4,3-borazaroisoquinoline (Va) [4] was brominated to the 1-bromo derivative (Vb) in high yield with one equivalent of bromine under these conditions. The structure was determined by comparison of its NMR spectrum with that of 1-bromo-4-methyl-4,3-borazaroisoquinoline [2,5] and by oxidative degradation to benzoic acid. In general the bromination method described above gave higher yield than bromination in acetic acid.

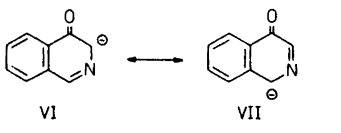
We made several attempts to obtain dibromination by using excess bromine and longer reaction time, but it was not possible to introduce bromine in the fused thiophene or benzene part with this reagent. This is also in accordance with the observation by Roos [6] that 5,7-dimethyl-4-hydroxy-4,5-borazarothieno-[2,3-*c*]pyridine, in which the reactive 7-position is blocked by a methyl group, is not brominated by the bromine-pyridine adduct in carbon tetrachloride.

In order to obtain some information on the mechanism of this bromination, we reacted 3,4-dimethylisoquinoline [7] which bromine in pyridine-carbon tetrachloride, but only starting material was recovered. It is possible that this is due to the fact that the base strength of 3,4-dimethylisoquinoline* is greater than that of pyridine and that the pyridine bromine adduct is only transformed to the isoquinoline adduct. We therefore used 2,4,6-trimethylpyridine,[8] which should be a stronger base than 3,4-dimethylisoquinoline, as solvent. However, also in this case no bromination occurred. On the other hand, it has been shown that 4-hydroxyisoquinoline in aqueous sodium hydroxide is smoothly monobrominated to 3-bromo-4-hydroxyisoquinoline by bromine in aqueous sodium hydroxide, and dibrominated to the 1,3-dibromo derivative with excess bromine.[10] However, IIa did not react under these conditions and was recovered unchanged. It is probable that the activation for electrophilic substitution in the phenolate ion, as expressed in resonance structures VI and VII, is not effectively transmitted in the 3,2-borazaropyridine system (VIII and IX), as indicated by structure IX, which involves several charged atoms.**

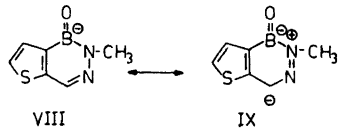


X

a. R = H ; X = H
b. R = Br ; X = H
c. R = H ; X = Br
d. R = Br ; X = Br



VI VII



VIII IX

As mentioned above bromination of Ic led to a mixture of 4- and 6-bromosubstituted derivatives, which could not be separated by fractional distillation. Similarly bromination of the B–OH derivative 5-ethyl-3-hydroxy-2-methyl-3,2-borazaropyridine (Xa) gave with one equivalent of bromine a mixture of 4-bromo- (Xb), 6-bromo- (Xc), and 4,6-dibromo-5-ethyl-3-hydroxy-2-methyl-3,2-borazaropyridine (Xd) in

* The base-strength of this compound is estimated from that of isoquinoline and the known effects of methyl groups (*cf.* Ref. 8).

** It should be pointed out that the similarity of the UV spectra of the B–OH derivatives of 3,2-borazaropyridines in acid and alkaline media has been taken as evidence that these acids are proton acids and not Lewis acids.[4,11]

the proportions 1:1:2. In this case, it was even more difficult to separate the isomers, as they could not be distilled and the B – OH compounds partially formed anhydrides during the work-up.

With excess bromine, dibromination of both Ic and Xa, in the 4- and 6-position could be achieved to give If and Xd, respectively, in high yields. However, the purification of the latter compound caused some difficulty. Attempts to substitute Ia in the 5-position by using excess bromine and more forced conditions failed. Neither did the isoelectronic 2,3,4-[12] and 2,3,5-trimethylpyridines[13] react under these conditions.

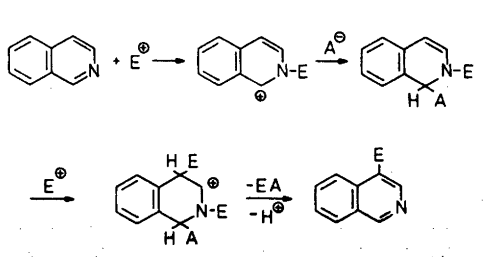
We then investigated the possibility of iodination of 3,2-borazaropyridines. Our hope was also to obtain crystalline derivatives suitable for X-ray analysis of these new heterocyclic systems. Attempts to react IVa with iodine in carbon tetrachloride-pyridine failed, perhaps due to the poor solubility of iodine in this solvent system. We found, however, that smooth iodination of IVa occurred, using iodine chloride in acetonitrile-pyridine as solvent, yielding 6,7-dimethyl-4-iodo-7,6-borazarothieno[3,2-*c*]pyridine (IVf) in 92 % yield. In analogy with the bromination reaction, it is assumed that the iodine chloride-pyridine complex is the iodinating agent. We found that the monocyclic derivative Ia was also smoothly iodinated with this reagent, yielding 2,3-dimethyl-4-ethyl-6-iodo-3,2-borazaropyridine (Ig). The use of excess iodine chloride and forced conditions did not give disubstitution. According to GLC and NMR Ic is selectively iodinated in the 4-position, giving 2,3-dimethyl-5-ethyl-4-iodo-3,2-borazaropyridine (Ih) in 60 % yield. The structure follows from its NMR spectrum [δ 7.57 (H-6)]. The use of excess iodine chloride in the reaction with Ic gave the crystalline (m.p. 37 – 38 °C) 4,6-diiodo-2,3-dimethyl-5-ethyl-3,2-borazaropyridine (Ii) in 74 % yield. An X-ray analysis of this compound is in progress.

From the investigations on the substitution reactions of 3,2-borazaropyridines hitherto carried out, the following picture is emerging. In strongly acidic solutions in which the 3,2-borazaropyridines are highly protonated, the monocyclic derivatives do not react,[14] while the benzo- or thieno-fused systems undergo substitution in the benzene[5] or thiophene ring.[15] The isoelectronic monocyclic pyridines, in contrast to the 3,2-borazaropyridines, undergo electrophilic substitution smoothly under these conditions.[14] It thus seems clear that the 3,2-borazaropyridinium ion is more deactivated towards electrophilic substitution than the pyridinium ion. Under slightly acidic or non-acidic conditions, on the other hand, as shown above, and in previous papers, both the monocyclic and fused 3,2-borazaropyridines react in the boron-nitrogen containing ring,[1,3,16] while the corresponding isoelectronic pyridines do not react.

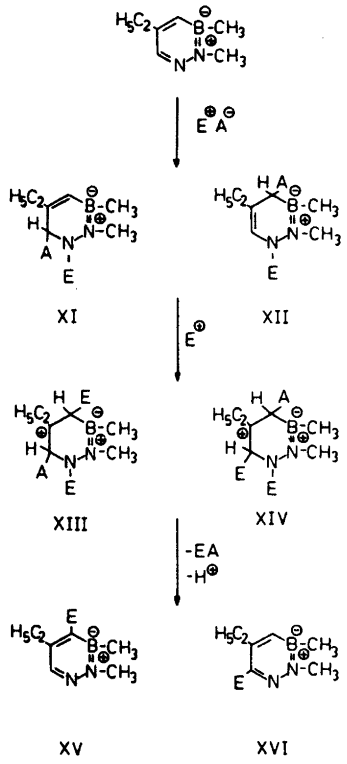
It appears probable that substitution of the pyridines in strongly acidic solution follows the normal mechanism for electrophilic substitution *via* the Wheland intermediate. The mechanism of the substitution under non-acidic conditions is more uncertain. A direct electrophilic substitution on the free bases seems somewhat unlikely, as it is then difficult to understand why protonation should reverse the order of reactivity of the monocyclic 3,2-borazaropyridines compared to the isoelectronic pyridines. Also the dependence of the isomer distribution on the nature of the reagent, substitution occurring predominantly in the 6-position upon nitration and in the 4-position on iodination, with intermediate results for bromination, is hardly expected for "normal" electrophilic substitution through a Wheland intermediate.

However, HMO calculations on the parent 3,2-borazaropyridine system using the ω-technique gave the highest π-electron densities and super-delocalizabilities for the 4- and 6-positions. This indicates that these positions should be the most reactive in electrophilic substitution.

In isoquinoline different substitution patterns are obtained under acidic and non-acidic conditions. Thus, bromination of isoquinoline in the presence of aluminium chloride gives a mixture



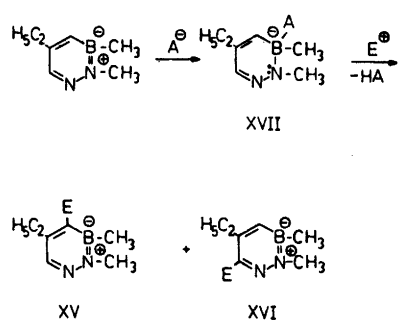
Scheme 1.



Scheme 2.

of 5-bromo- and 5,8-dibromoisoquinoline,[17] while bromination with bromine in pyridine-carbon tetrachloride yields 4-bromoisoquinoline.[18] For the latter reaction Brown and Harcourt [19] have suggested an addition-substitution-elimination mechanism shown in Scheme 1.

A somewhat analogous mechanism can be perceived for the substitution of 3,2-borazaropyridines (Scheme 2). 1,2- and 1,4-Addition to the formal conjugated azomethine bond give XI and XII, respectively, which are attacked by the electrophile in positions 4- and 6- to give intermediates such as XIII and XIV, which lose EA and H^+ to give the products. The reason that the isoelectronic pyridines do not react in the same way may depend upon the fact that the primary addition is slow and/or that the more basic pyridines attack the α-position of the *N*-pyridine complex, giving ring-opened products. Except for *N*-nitropyridinium tetrafluoroborate, which with pyridine gives a derivative of glutaconaldehyde,[20] similar ring-



Scheme 3.

opened products have also been observed from the adduct of cyanogen bromide and pyridine [21,22] and from the adducts of pyridine with sulfur trioxide, chlorosulfonic acid and ethyl chlorosulfonate.[23,24]

Another possible mechanism which utilizes the characteristic properties of boron is indicated in Scheme 3. The nucleophile attacks at boron, transforming the 3,2-borazaropyridine in principle into a cyclic α,β-unsaturated hydrazone (XVII). It is known that certain hydrazones are nitrated with amylnitrite [25] and brominated with bromine in acetic acid at the azomethine bond.[26] Therefore, substitution at the 6-position to give XVI and at the vinylogous position to give XV could also be a possibility. We have in vain tried to detect intermediate addition products by the NMR technique. However, this does not exclude the above mechanism, since intermediate addition products can be present in low "steady-state" concentrations.

It appears to us that the problem of proving the mechanism(s) which are valid under non-acidic conditions is a difficult task, demanding extensive theoretical and experimental studies. Knowledge of the reaction mechanism is obviously of great importance for a deeper understanding of the isomer distributions obtained in nitration and halogenation of 3,2-borazaropyridines.

EXPERIMENTAL

7-Bromo-4-hydroxy-5-methyl-4,5-borazarothieno[2,3-c]pyridine (IIc). To a mixture of 9 ml of anhydrous pyridine (dried and distilled from potassium hydroxide) and 15 ml of carbon

tetrachloride (distilled from phosphorus pentoxide), 1.66 g (0.10 mol) of 4-hydroxy-5-methyl-4,5-borazarothieno[2,3-*c*]pyridine [11] was added, and 1.70 g (0.011 mol) of bromine in 20 ml of anhydrous carbon tetrachloride was added dropwise with stirring during 15 – 20 min. A yellow precipitate formed, and the mixture was refluxed for 1.5 h. The precipitate was filtered off and washed with carbon tetrachloride. The combined carbon tetrachloride phases were extracted with water, dried over magnesium sulfate and the solvent evaporated. The residual sirupy oil (2.3 g) which upon scratching crystallized, was recrystallized from 35 ml (30 %) of aqueous acetic acid, yielding 1.8 g (73 %) of the title compound, m.p. 93 – 95 °C. After solidification it melted at 204.5 °C probably due to anhydride formation during the melting process. NMR (CD_3COCD_3): δ 7.85 (H-2 and H-3), 3.63 (NCH_3). [Found: C 29.44; H 3.08; Br 33.01; N 10.68; S 12.72. Calc for C_6H_6BBrOS (244.9): C 29.43; H 2.47; Br 32.63; N 11.48; S 13.08.]

7-Bromo-4,5-dimethyl-4,5-borazarothieno[2,3-c]pyridine (IId). From 1.64 g (0.10 mol) of 4,5-dimethyl-4,5-borazarothieno[2,3-*c*]pyridine [11] and 1.70 g (0.011 mol) of bromine, 2.3 g of crude product was obtained in the same way as described above. Recrystallization from 75 % aqueous ethanol gave 1.9 g (78 %) of the title compound, m.p. 75.0 – 76.0 °C. NMR ($CDCl_3$): δ 7.63 (H-2 and H-3), 3.80 (NCH_3), 0.93 (BCH_3). [Found: C 34.42; H 3.26; Br 32.96; N 11.45; S 13.22. Calc. for C_7H_8BBrS (242.9): C 34.61; H 3.32; Br 32.89; N 11.53; S 13.20.]

4-Bromo-6,7-dimethyl-7,6-borazarothieno[3,2-c]pyridine (IVb). In the same way as described above, 2.3 g of crude product was obtained from 1.64 g (0.010 mol) of 6,7-dimethyl-7,6-borazarothieno[3,2-*c*]pyridine,[14] 1.70 g (0.011 mol) of bromine and the appropriate amounts of pyridine and carbon tetrachloride. Recrystallization from 75 % aqueous ethanol yielded 2.0 g (82 %) of the title compound, m.p. 79.0 – 80.5 °C. NMR ($CDCl_3$): δ 7.47 and 7.73 (H-2 and H-3 or H-3 and H-2, J_{23} 5.0 Hz), 3.75 (NCH_3), 0.90 (BCH_3). [Found: C 34.64; H 3.29; B 4.39; Br 32.92; N 11.50; S 13.25. Calc. for $C_7H_8BBrN_2S$ (242.9): C 34.61; H 3.22; B 4.45; Br 32.89; N 11.53; S 13.20.]

4-Bromo-7-hydroxy-7,6-borazarothieno[3,2-c]pyridine (IVd). In the same way as described above, 1.52 g (0.010 mol) of 7-hydroxy-7,6-borazarothieno[3,2-*c*]pyridine [27] and 3.2 g (0.020 mol) of bromine in pyridine-carbon tetrachloride gave after recrystallization from 30 % aqueous acetic acid 1.2 g (52 %) of the title compound, m.p. 95.0 – 98.0 °C. When only 1.7 g (0.011 mol) of bromine was used, 1.1 g of a 1:1 mixture of the title compound and 7-hydroxy-7,6-borazarothieno[3,2-*c*]pyridine was obtained according to NMR analysis. NMR (CD_3COCD_3): δ 7.53 and 8.03 (H-2 and H-3 or H-3 and H-2, J_{23} 5.0 Hz). [Found: C 25.87; H 2.06; Br 34.20; N 12.04; S 13.63. Calc. for $C_5H_4BBrN_2OS$ (230.9): C 26.01; H 1.74; Br 34.61; N 12.13; S 13.89.]

4-Bromo-7-hydroxy-6-methyl-7,6-borazarothieno[3,4-c]pyridine (IIIb). In the same way as described above 1.7 g (70 %) of the title compound, m.p. 92.0 – 94.0 °C after recrystallization from 35 – 40 ml (30 %) of aqueous acetic acid, was obtained from 1.66 g (0.010 mol) of 7-hydroxy-6-methyl-7,6-borazarothieno[3,4-*c*]-pyridine,[11] and 1.70 g (0.011 mol) of bromine in pyridine and carbon tetrachloride. NMR (CD_3SOCD_3): δ 7.97 (H-1), 8.47 (H-3, J_{13} 2.8 Hz), 9.00 (OH), 3.42 (NCH_3). [Found: C 29.35; H 2.86; Br 33.10; N 11.19; S 12.84. Calc. for $C_6H_6BBrN_2OS$ (244.9): C 29.43; H 2.47; Br 32.63; N 11.48; S 13.08.]

1-Bromo-4-hydroxy-3-methyl-4,3-borazaroisoquinoline. (Vb). From 1.60 g (0.010 mol) of 4-hydroxy-3-methyl-4,3-borazaroisoquinoline [4] and 1.70 g (0.011 mol) of bromine in pyridine-carbon tetrachloride, 1.85 g (77 %) of the title compound, m.p. 99.5 – 100 °C (141.0 – 142.0 °C after resolidification and remelting) was obtained after recrystallization from 30 % aqueous acetic acid in the same way as described above. NMR (CD_3COCD_3): δ 7.83 (m, arom.), 3.58 (NCH_3). [Found: C 39.94; H 3.26; Br 33.21; N N 11.49. Calc. for $C_8H_8BBrN_2O$ (238.9): C 40.22; H 3.38; Br 33.45; N 11.73.] Oxidation of the title compound with potassium permanganate in alkaline solution yielded benzoic acid (25 %) having the same IR spectrum as an authentic sample.

Attempted bromination of 3,4-dimethylisoquinoline. When 0.79 g (0.0050 mol) of 3,4-dimethylisoquinoline [7] and 0.85 g (0.0065 mol) of bromine in pyridine-carbon tetrachloride were reacted as described above, only starting material was detected by GLC. The same was true when 2,4,6-trimethylpyridine was used instead of pyridine as cosolvent.

4,6-Dibromo-2,3-dimethyl-5-ethyl-3,2-borazaropyridine (If). From 2.52 g (0.0185 mol) of 2,3-dimethyl-5-ethyl-3,2-borazaropyridine [14] in 25 ml of anhydrous carbon tetrachloride, and 9 ml of anhydrous pyridine and 8.8 g (0.055 mol) of bromine in 15 ml of anhydrous carbon tetrachloride, 4.1 g (75 %) of the title compound, b.p. 84 – 86 °C/0.5 mmHg was obtained, when the procedure given for IIc was followed. it had the same spectroscopic properties as a previously described sample.[1]

Bromination of 5-ethyl-3-hydroxy-2-methyl-3,2-borazaropyridine. When 1.38 g (0.010 mol) of 5-ethyl-3-hydroxy-2-methyl-3,2-borazaropyridine [14] was reacted with 1.70 g (0.11 mol) of bromine in pyridine-carbon tetrachloride and the product worked up as described for IIc, 1.6 g of crude product was obtained, which according to NMR and GLC consisted of a mixture of starting material and of mono- and dibromo derivatives. The compounds could not be separated.

4,6-Dibromo-3-hydroxy-2-methyl-3,2-borazaropyridine (Xd). When 1.38 g (0.010 mol) of 5-ethyl-3-hydroxy-2-methyl-3,2-borazaropyridine [14] and 3.2 g (0.020 mol) of bromine in

pyridine-carbon tetrachloride were reacted in the usual way, the crude product did not crystallize. Purification was achieved by sublimation *in vacuo*. At the cold finger 2.0 g (68 %) of the title compound collected as a colourless, very viscous syrup. NMR ($CDCl_3$): δ 1.13 (CH_3), 2.83 (CH_3), 3.55 (NCH_3). [Found: C 25.87; H 3.14; Br 52.34; N 9.15. Calc. for $C_6H_9BBr_2N_2O$ (295.8): C 24.36; H 3.07; Br 54.03; N 9.47.]

Attempted bromination of 2,3,4-trimethylpyridine and 2,3,5-trimethylpyridine. 1.21 g (0.010 mol) of 2,3,4-trimethylpyridine [12] and the same amount of 2,3,5-trimethylpyridine [13] were each reacted with 1.70 g (0.011 mol) of bromine in pyridine and carbon tetrachloride for 24 h and worked up as described above. More than 90 % of unchanged starting material was recovered, and no bromo derivatives were detected by GLC.

6,7-Dimethyl-4-iodo-7,6-borazarothieno[3,2-c]-pyridine (IVf). To a solution of 1.64 g (0.010 mol) of 6,7-dimethyl-7,6-borazarothieno[3,2-*c*]-pyridine [14] in 15 ml of anhydrous acetonitrile (distilled from phosphorus pentoxide) and 6 ml of anhydrous pyridine, 1.63 g (0.010 mol) of iodine chloride in 15 ml of anhydrous acetonitrile was added dropwise with stirring and the mixture refluxed for 6 h. GLC analysis (BDS, 10 %, 200 °C) showed that about 60 % of the starting material had reacted. The mixture was therefore allowed to stand overnight, an additional 1.63 g (0.010 mol) of iodine chloride in 15 ml of anhydrous acetonitrile was added, and the mixture refluxed for 3 h. The red solution was allowed to cool and was evaporated to one fourth of its original volume. Four volumes of ether were added and the pyridinium hydrochloride which precipitated was filtered off and washed with ether. The combined ether phases were washed twice with thiosulfate solution, twice with water, dried and evaporated. Recrystallization of the residue from 80 % aqueous ethanol yielded 2.66 g (92 %) of the title compound in colourless crystals, m.p. 80.0 – 81.0 °C. NMR ($CDCl_3$): δ 7.37 and 7.69 (H-2 and H-3 or H-3 and H-2, J_{23} 4.8 Hz), 3.78 (NCH_3), 0.92 (BCH_3). [Found: C 28.89; H 2.78; B 3.72; I 43.84; N 9.65. Calc. for $C_7H_8BIN_2S$ (289.9): C 28.99; H 2.78; B 3.68; I 43.76; N 9.66.]

2,3-Dimethyl-5-ethyl-4-iodo-3,2-borazaropyridine (Ih). To a solution of 1.00 g (0.0074 mol) of 2,3-dimethyl-5-ethyl-3,2-borazaropyridine [14] in 15 ml of anhydrous acetonitrile and 4 ml of anhydrous pyridine, 2.4 g (0.0147 mol) of iodine chloride in 15 ml of anhydrous acetonitrile was added dropwise with cooling. The mixture was then refluxed for 30 min. GLC analysis (BDS, 10 %, 90 – 200 °C), showed no trace of starting material, but only monoiodinated and about 5 % of diiodinated product (mass spectra). The mixture was worked up as described above, yielding 1.72 g of crude product, which upon distillation *in vacuo* gave 1.15 g (60 %) of the title compound, b.p. 122 – 124 °C/10 mmHg. The reaction was also carried out with carbon tetrachloride instead of acetonitrile as solvent giving the same result. NMR ($CDCl_3$): δ 7.57 (H-6), 1.15 (CH_3), 2.70 (CH_2), 3.75 (NCH_3), 0.87 (BCH_3). [Found: C 32.15; H 4.69; B 4.08; I 48.51. Calc. for $C_7H_{12}BIN_2$ (261.9): C 32.10; H 4.62; B 4.13; I 48.45.]

4,6-Diiodo-2,3-dimethyl-5-ethyl-3,2-borazaropyridine (Ii). To a solution of 1.0 g (0.0074 mol) of 2,3-dimethyl-5-ethyl-3,2-borazaropyridine [14] in 15 ml of anhydrous acetonitrile and 5 ml of anhydrous pyridine, 3.58 g (0.0221 mol) of iodine chloride in 15 ml of anhydrous acetonitrile was added with stirring and the mixture refluxed for 2 h. GLC analysis showed that about 50 % mono- and 50 % diiodo derivatives had been formed. Therefore, 1.2 g (0.074 mol) of iodine chloride was added and the mixture refluxed for 1 h. The mixture was worked up as described above, yielding a yellow oil which crystallized on standing overnight. Recrystallization from 95 % ethanol at – 30 °C gave 2.1 g (74 %) of the title compound as colourless crystals, m.p. 37.5 – 38 °C. NMR ($CDCl_3$): δ 1.13 (CH_3), 3.03 (CH_2), 3.72 (NCH_3), 0.87 (BCH_3). [Found: C 21.72; H 2.90; B 2.80; I 65.37; Calc. for $C_7H_{11}BI_2N_2$ (287.8): C 21.68; H 2.86; B 2.79; I 65.45.]

2,3-Dimethyl-4-ethyl-6-iodo-3,2-borazaropyridine (Ig). To a solution of 1.0 g (0.0074 mol) of 2,3-dimethyl-4-ethyl-3,2-borazaropyridine [14] in 15 ml of acetonitrile and 4 ml of pyridine, 2.4 g (0.0147 mol) of iodine chloride was added and the mixture refluxed for 1.5 h and worked up as described above, yielding 1.05 g (55 %) of the title compound, b.p. 122 – 123 °C/10 mmHg. NMR ($CDCl_3$): δ 7.23 (H-5), 1.10 (CH_3), 2.50 (CH_2-4), 3.68 (NCH_3), 0.67 (BCH_3). [Found: C 32.03; H 4.67; B 4.21; I 48.38. Calc. for $C_7H_{12}BIN_2$ (261.9): C 32.10; H 4.62; B 4.13; I 48.45.]

GLC analyses were carried out on a Varian model 1400 or a Perkin-Elmer 900 gas chromatograph. NMR spectra were recorded on a Varian A 60 NMR spectrometer and mass spectra were obtained with an LKB A-9000 mass spectrometer. Elemental analyses were carried out by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mülheim/Ruhr.

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1,3-Addition of *t*-Butylmagnesium Chloride to Ethyl Cinnamate: an Unprecedented Course of Reaction

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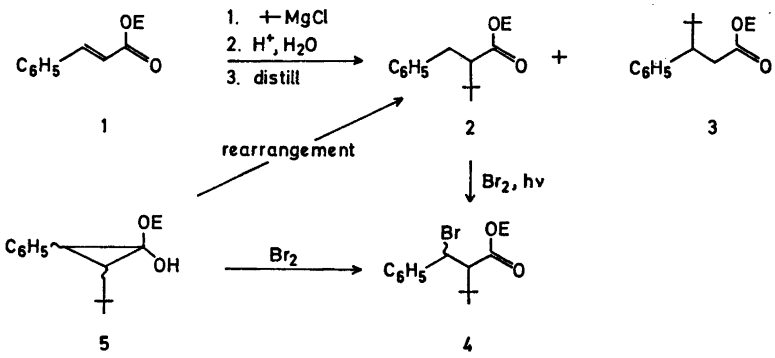
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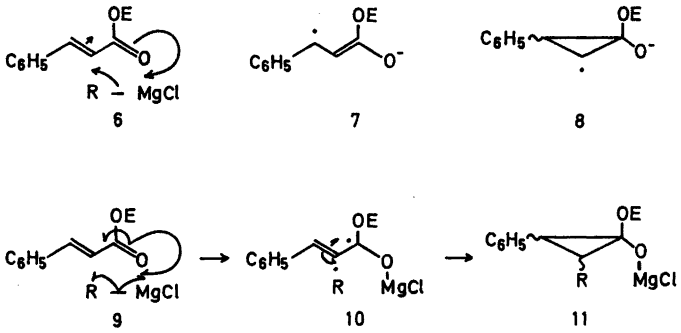
The distilled reaction mixture, resulting from addition of *t*-butylmagnesium chloride to ethyl cinnamate, contains, in addition to ethyl 3-*t*-butylhydrocinnamate (the expected 1,4-addition product) a considerable proportion of the isomeric 2-*t*-butylhydrocinnamate. A cyclopropanone hemiketal is suggested as the initial reaction product undergoing thermal rearrangement to the α-substituted ester. The reaction seems without precedent in addition reactions of the present type.

Alkyl cinnamates undergo unexceptional reactions (1,2- and 1,4-addition, dimerisation) with primary alkylmagnesium halides [1] whereas non-identifiable products,[2] or, after saponification, a low yield of β-*t*-butylhydrocinnamic acid,[3] constitute the only reported outcome of the reaction with *t*-butylmagnesium chloride. In contrast to the ester, cinnamic acid undergoes 1,4-addition with the tertiary reagent under similar conditions.[4,5]

In the present work, the distilled ester fraction, resulting from addition of *t*-butylmagnesium chloride to ethyl cinnamate in ether solution, was found to consist of a 2:3 mixture of ethyl 2-*t*-butylhydrocinnamate *2* and ethyl 3-*t*-butylhydrocinnamate *3* (total yield 70 %), as apparent from ^{1}H NMR spectra and from the selective removal of the latter ester on controlled alkaline hydrolysis, followed by conversion of the former into 2-*t*-butylhydrocinnamic acid [6] on prolonged alkali treatment. Magnesium purity, so important for the course of reaction in similar additions,[1] was of no decisive significance in the present case.

^{1}H NMR-Inspection of the original reaction mixture, after protonation but prior to distillation, reveals the presence of only small amounts of *2*. Singlets, positioned close to that arising from the *t*-butyl group of *2* and apparently converted into the latter upon distillation, may be attributed to dimeric, or polymeric species, but may arise also from one or more of the four cyclopropanone hemiketal racemates, collectively represented as *5*. Though still unproven, the intermediacy of *5* in the production of *2* is consonant with the finding that simple bromina-





tion of the non-distilled reaction mixture yields one of the diastereoisomeric 2-*t*-butyl-3-bromohydrocinnamic esters *4* in crystalline form.[6,7] Significantly, ethyl 2-*t*-butylhydrocinnamate *2* is virtually unsusceptible to bromination under these conditions. Subjected to photobromination, however, it readily affords a product mixture from which a bromoester *4*, identical with that prepared above, may be crystallized.

The ability of tertiary Grignard reagents to react by way of radical species, formed either by single electron transfer [8,9] or by induced homolysis,[10] may be reflected in the product composition of the present reaction, so different from that of similar reactions involving primary Grignard reagents.[1] A concerted mechanism, as indicated in *6*, is therefore hardly likely. A single electron transfer mechanism may be invoked to explain the formation of the *β*-*t*-butyl adduct *via* the radical *7*, but the production of *11* by a similar single electron transfer would involve the supposedly far less stable species *8*. For these reasons, the α-alkylation is rather believed to involve attack of a *t*-butyl radical, formed by induced homolysis of the Grignard reagent as formulated by *9*. The *t*-butyl radical may subsequently attack the α-carbon of an other ethyl cinnamate molecule (chain reaction), or react directly with the α (or *β*) carbon of the ketyl as shown in *10*. The main driving force in the initial step of this reaction sequence is believed to be the remarkably low C – Mg bond energy of *t*-butylmagnesium chloride [10] paired with the high energy of the O – Mg bond in the adduct.

EXPERIMENTAL

Proton magnetic resonance spectra were recorded on a Varian A-60 instrument using approx. 25 % solutions in $CDCl_3$. Chemical shift values are given in ppm on the *δ*-scale. Boiling points and melting points are uncorrected.

Ethyl 2- and 3-t-butylhydrocinnamate. (*2* and *3*) Ethyl cinnamate (20 mmol) was added at once with stirring to 60 mmol of *t*-butylmagnesium chloride [11] in ether (100 ml) at 5 °C. The cooling bath was removed and the temperature rose to 22 °C within 15 min. The suspension was poured into ice, acidified, and the aqueous phase was extracted with $CHCl_3$. The combined extracts were dried ($MgSO_4$) and distilled to give a yellow oil (3.24 g, 80 – 112 °C/0.5 mmHg). NMR analyses revealed it to be a mixture of ethyl cinnamate (0.28 g), ethyl 2-*t*-butylhydrocinnamate (1.19 g), and ethyl 3-*t*-butylhydrocinnamate (1.80 g). The yields and product composition were practically unaltered by using triply sublimed magnesium. NMR data for the two *t*-butylated esters were obtained from a mixture of the two esters and from the α-butylated ester *2*, isolated below; *δ*-values for the isomer *3* are given in parentheses: *t*-butyl 1.06 (0.87); methyl 0.99 (0.94); ABC system between 2.23 and 3.18; methylene 3.96 (3.91); phenyl 7.21.

2- and 3-t-Butylhydrocinnamic acid. The above procedure may be modified for larger scale synthesis and for preparation of the acids: the Grignard reagent (410 ml, 1.48 M by acid titration) was cooled to −8 °C and the ester (35.2 g) added rapidly with stirring. The cooling bath was removed after 2 min and the temperature rose to 25 °C within 10 min. After a total of 20 min, the mixture was worked up as above to give a mixture of the esters (32.9 g, containing less than 3 % of ethyl cinnamate). Reflux of the esters in a mixture of water (10 ml), ethanol (33 ml), and NaOH (5.7 g) for 1 h followed by addition of water, extraction with $CHCl_3$, and distillation gave crude ethyl 2-*t*-butylhydrocinnamate (12.43 g, 80 – 87 °C/0.5 mmHg. Saponi-

fication of the latter ester in methanolic sodium hydroxide (10 g in 40 ml) for 17 days gave crude 2-*t*-butylhydrocinnamic acid (5.2 g from petroleum ether, m.p. 68 – 70 °C). Recrystallization from a mixture of water (10 ml), acetic acid (10 ml), and ethanol (7 ml) gave colourless crystals, m.p. 69 – 71 °C; reported:[5] 70 °C.

The aqueous phase from the extraction of ethyl 2-*t*-butylhydrocinnamate (above) was acidified and extracted with $CHCl_3$ to give 16.1 g of crude acid. Recrystallization from a mixture of ethanol (18 ml), acetic acid (16 ml), and water (32 ml) gave 14.5 g of 3-*t*-butylhydrocinnamic acid, m.p. 113 – 115 °C; reported [4] 115 – 116 °C.

Ethyl 2-t-butyl-3-bromohydrocinnamate 4. Bromine (0.7 ml) was added to ethyl 2-*t*-butylhydrocinnamate (2.32 g crude, above) in CCl_4 (10 ml). No reaction took place in the dark. Two hours in the sun (evolution of HBr) and distillation gave crude ethyl 2-*t*-butyl-3-bromohydrocinnamate (2.66 g, 80 – 112 °C/0.5 mmHg). NMR analysis disclosed a 4 to 1 content of two similar, most likely diastereoisomeric compounds. Trituration with petroleum ether gave crystals (1.17 g) and an oil (1.48 g, isomer ratio *ca* 2:1). Recrystallization from ethanol (5 ml) gave colourless crystals (746 mg, m.p. 48 – 49 °C). The product was recrystallized from ethanol for analysis (Found: C 57.70; H 6.76; Br 25.46. Calc. for $C_{15}H_{21}BrO_2$: C 57.52; H 6.76; Br 25.51). NMR: δ 0.78 (*t*-butyl); 1.35 (methyl, triplet); 3.32 (C^{α} H, doublet, $J = 12.0$ Hz); 4.30 (methylene, quartet); 5.43 (C^{β} H; doublet, $J = 12.0$ Hz); *ca.* 7.45 (multiplet, phenyl). NMR on the crude product showed also a singlet at δ 1.35 and two doublets (δ 3.27 and 5.21, $J = 11$ Hz).

The same bromoester was also obtained from the adduct above (from 20 mmol ethyl cinnamate and 60 mmol of the Grignard reagent). The suspension was cooled to −50 °C and poured into excess ice with vigorous swirling. Acidification with hydrochloric acid, immediate bromination (20 mmol of Br_2 in 10 ml of CCl_4), and thorough shaking for about 1 min gave two phases. The aqueous phase was set aside (see below). The organic phase was washed with cold, aqueous ammonia, dried and distilled. A fraction (720 mg, 100 – 120 °C/0.4 mmHg) contained approximately equal amounts of ethyl 3-*t*-butylhydrocinnamate and the bromoester (the same isomer as isolated above, according to NMR). Less than 10 % of ethyl 2-*t*-butylhydrocinnamate was present. Crystallization as above gave crystals, m.p. 47 – 49 °C.

The bromination was modified as follows: Bromine (20 mmol) in CCl_4 (10 ml) was added to a mixture of ethyl 2-*t*-butylhydrocinnamate (2.34 g), ether (100 ml), ice and the aqueous phase (from the bromination, above). The suspension was stirred thoroughly for 15 min and worked up as above. The distillate contained less than 1.5 % of ethyl 3-bromo-2-*t*-butylhydrocinnamate (integration of the two relevant resonances from *t*-butyl groups).

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Preparation of Modified Agarose Gels Containing Thiol Groups

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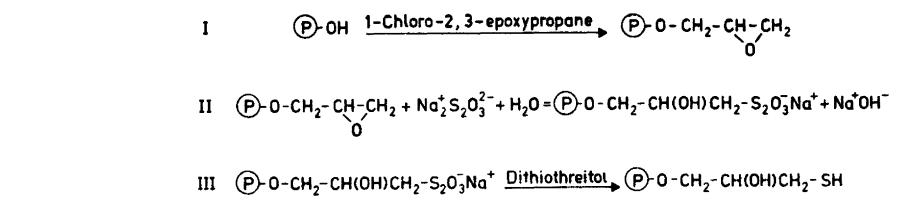
A simple and efficient route for preparation of thiol-agarose derivatives of various degrees of substitution is described. The technique for introducing thiol-groups in the agarose gel network involves the following steps: (a) introduction of epoxide groups; (b) treatment by sodium thiosulfate to convert epoxide groups into *S*-alkyl-thiosulfate structures; (c) reductive cleavage of the alkyl thiosulfate structures by dithiothreitol.

Chemists concerned with biochemical research are to an increasing extent making use of immobilized biological activity as a means of conversion and separation. Many adsorbents for bioaffinity chromatography are based on beaded agarose gels [1] and enzymes have been immobilized by their attachment to such gels.[2] Thiol-group containing agarose gels would be of particular interest in this connection on account of the variety of reactions thiol groups may undergo even under mild conditions in water solution. Products containing S – C, S – S, S – O, and S – metal bonds may thereby be formed.

Thiolated agarose gel beads have been prepared by Cuatrecasas [3] from ω-amino alkyl agarose by reaction with homocysteine thiolactone. The ω-aminoalkyl derivatives were prepared by the cyanogen bromide technique. The thiolated products were, upon further derivatization, used as adsorbents for bioaffinity chromatography. Brocklehurst *et al.*[4] prepared thiolated agarose by direct coupling of glutathione to cyanogen bromide activated agarose gel without protecting the thiol group. The glutathione gel was derivatized with 2,2′-dipyridyl-disulfide and the disulfide derivative used for the preparation of fully active papain from dried powder of papaya latex. The chromatographic procedure used involved specific formation of a disulfide linkage between the thiol gel and the active site thiol-group of papain and subsequent breaking of the disulfide by means of a low-molecular weight thiol. The technique was described as covalent chromatography. Subsequently, Carlsson *et al.* demonstrated the usefulness of glutathione-agarose for the immobilization of urease.[5] In this connection it should be mentioned that Eldjarn *et al.*[6] prepared a thiolated Sephadex derivative which, after mercuration, was used to selectively isolate thiol-proteins from proteins not containing thiol groups. The thiolated Sephadex was also used to prevent autooxidation of biologically important thiols in biological materials.[7]

We wish to report a simple and efficient route for the preparation of thiol-agarose derivatives of various degrees of substitution. The thiolated agarose gels described above have a thiol content of < 50 μmol/g dried polymer. With the presented procedure, substitution degrees of 800 – 900 μmol/g are obtained. The technique is mild enough not to destroy the gel beads and involves chemical cross-linking of the gel to make it more stable to leakage. The attachment of thiol groups *via* extended hydrocarbon chains can be performed. The method is based on the reactions given in Scheme 1.

The initial epoxidation step was performed essentially according to Sundberg and Porath.[8] As reagents 1-chloro-2,3-epoxypropane (epichlorohydrin) or, in order to get an extended arm, 1,4-bis(2,3-epoxypropoxy)butane have been applied. Introduction of epoxide structures was governed by varying the amount of reagent. The epoxide gel was then treated with a sodium thiosulfate solution to transform the epoxide structures to *S*-alkyl-thiosulfate structures.[9] The reaction is accompanied by a release



Scheme 1.

of OH^-, which can be analytically utilized for determining the degree of substitution by titration with hydrochloric acid (Table 1). Finally the thiosulfate ester gel was reduced by dithiothreitol.[10] The products were analyzed for sulfur and thiol groups (Table 1).

While the thiosulfate ester gels are stable, the thiol gels oxidize when stored in water suspension with the formation of S—S-structures. The oxidation is particularly rapid when the gel is highly substituted with thiol groups. Such gels, having partially lost their original content of thiol groups can become fully restored to the original thiol group content by reduction with dithiothreitol.

Experiments show that the thiol groups of the thiolated agarose gels are so reactive that the reactions with thiol groups which are known to occur in solution are possible to perform even on the gel and under such mild conditions that the fragile gel structure is preserved. The thiolated gels have been utilized in this laboratory as a medium for sorption processes based on thiol-disulfide interchange reactions and on metal-complex formation reactions. They have been applied as electron exchangers and as water-insoluble metal-complexing agents. The possibility of attaching ligands *via* stable thioether bonds to the gel carrier has been utilized to prepare adsorbents for bioaffinity chromatography. The experimental details will be given in separate communications.

In this paper we want to exemplify the use of the thiolated agarose by immobilizing urease according to the technique of Carlsson *et al.*[5] The thiol gel is transferred to a 2-pyridyl-disulfide gel by means of 2,2′-dipyridyl-disulfide. The content of the reactive 2-pyridyl-disulfide structures was determined by Kjeldahl analyses (Table 2). A solution of a urease preparation was passed through a column of the disulfide gel. An enzyme conjugate of high protein content and high catalytic activity was obtained (Table 2). The column with immobilized enzyme could then be used for continuous hydrolysis of urea. During the immobilization step the urease preparation was purified by a factor of about 8.

Table 1. Preparation of thiolated agarose gels (Sepharose 6B). Beads of agarose were activated to various degrees of substitution with 1-chloro-2,3-epoxypropane or 1,4-bis(2,3-epoxypropoxy)-butane by variation of the amount of reagent. The epoxide-activated gels were treated with sodium thiosulfate solution and the obtained *S*-alkyl-thiosulfate structures were finally reduced with dithiothreitol. Analytical data.

	"Epoxide-activation" (ml reagent/3 g gel)	Epoxide gel; μmol epoxide structure/g dried product	Thiol gel; μmol S/g dried product	Thiol gel; μmol SH groups/g dried product
1-Chloro-2,3-epoxy-propane	(0.45	670	690	660
	(0.15)	310	220	170
	(0.075)	70	55	50
1,4-Bis(2,3-epoxy-propoxy)butane	(5)	1050	850	860
	(1.2)	460	370	410
	(0.3)	120	105	110

Table 2. Chemical fixation of urease to thiolated agarose (Sepharose 2B) by activation with 2,2′-dipyridyl-disulfide.

Carrier	Thiol-Sepharose
Thiol content, μmol/g	440
Content of 2-Py−S−S−, μmol/g	270
Content of urease in conj., mg/g conj.	320
Activity, μmol NH_3/min mg	
free urease	84
bound urease	695

METHODS*

Preparation of epoxide-activated agarose gel beads.[8] *(a) By 1-chloro-2,3-epoxypropane.* Sepharose 6B (Pharmacia Fine Chemicals, Uppsala, Sweden) was washed on a glass filter with distilled water and sucked free from interstitial water. The gel beads (3 g) were suspended in 2.4 ml 1 M sodium hydroxide solution. 1-chloro-2,3-epoxypropane (various amounts; see Table 1) was added slowly under stirring at room temperature, whereupon the temperature was increased to 60 °C and maintained for 2 h. The activated gel was washed with distilled water on a glass filter until neutral. The product is not stable enough to store.

(b) By 1-4-bis(2,3-epoxypropoxy)butane. Sepharose 6B (3 g), washed as above, was suspended in 1.6 ml 2.5 M sodium hydroxide solution, and 12 mg sodium borohydride was added. 1,4-bis(2,3-epoxypropoxy)butane (EGA-Chemie, Steinheim, Switzerland) (various amounts; see Table 1) was slowly added under stirring. Reaction time 6 h at room temperature. The activated gel was washed with distilled water until neutral, with 20 ml acetone and finally with water. The product is not stable enough to store.

Epoxide group analyses. The reaction between epoxide groups and sodium thiosulfate which proceeds with release of OH^- can be used to determine the amount of epoxide structures in the gel. An amount of activated gel corresponding to 10 − 100 μmol of epoxide groups was suspended in water to a volume of 10.0 ml suspension. The pH of the suspension was corrected to 7.0. The suspension was stirred and 1.00 ml was transferred to a plastic titration vessel by means of an Oxford pipette with plastic tip. One ml of 2 M sodium thiosulfate solution (pH 7) was added to the gel suspension. The pH was kept constant by addition of 50 mM HCl by means of a pH-stat. The reaction mixture was stirred. The time for titration was 30 − 120 min. The dry weight content of the gel suspension was determined by transferring 5.00 ml gel suspension to a small glass filter and washing with water and water/acetone mixtures of increasing concentration of acetone. The gel was dried over P_2O_5 at 100 °C *in vacuo* for 20 h and weighed.

Preparation of S-alkyl-thiosulfate agarose gel beads. Epoxide activated Sepharose 6B (3 g) was washed on a glass filter with 0.5 M phosphate buffer (4.1 g $NaH_2PO_4.H_2O$ + 2.8 g $Na_2HPO_4.2H_2O$ dissolved in 100 ml distilled water, pH = 6.3). The gel was sucked free from interstitial buffer and suspended in the same buffer to a final volume of 6 ml. A solution of 2 M sodium thiosulfate (3 ml) was added and the reaction mixture was shaken for 6 h at room temperature. The gel was washed free from sodium thiosulfate with distilled water. The thiosulfate ester gel, suspended in distilled water, is suitable for storage.

Reduction of S-alkyl-thiosulfate gel. The thiosulfate ester gel (3 g) was suspended in 0.1 M. sodium bicarbonate solution. Total volume 6 ml. Dithiothreitol was used in at least twofold excess. The value of epoxide content could thereby be used. Dithiothreitol (*threo*-1,4-dimercapto-2,3-butandiol, Aldrich Chem. bo., Milwaukee, Wisc., USA) was dissolved in 3 ml 1 mM EDTA-solution. The reaction time was 30 min at room temperature. The gel was washed on a glass filter with 30 ml 0.1 M sodium bicarbonate solution (1 M in sodium chloride and 1 mM in EDTA) and finally with 100 ml 1 mM EDTA-solution.

The thiolated gel was normally used directly. For a couple of days the gel can be stored in 0.01 M deaerated sodium acetate buffer of pH 4 (1 mM in EDTA). Upon storage of a highly substituted thiol gel under these conditions for 1 month, a decrease of the thiol content by 80 % was found. Upon renewed reduction by dithiothreitol the original thiol content was obtained.

Thiol group analyses. Thiol content was determined by means of 2,2′-dipyridyl-disulfide according to Brocklehurst *et al.*[4] but 0.1 M sodium bicarbonate buffer was used instead of Tris buffer. The results are given in Table 1. The thiol content could also be determined by means of Ellman's reagent. The sulfur content of the gel was determined according to Gustavsson.[11]

Preparation of 2-pyridyl-disulfide-agarose gel beads. Thiol agarose gel beads were prepared from Sepharose 2B according to the procedures described above by means of 1-chloro-2,3-epoxypropane (0.15 ml reagent per 3 g gel). The thiol gel (3 g) was washed on the glass filter with 60 % acetone-40 % 0.05 M sodium bicarbonate solution (1 mM in EDTA). The gel was suspended in the same solvent to a total volume of 5 ml. A 0.3 M solution of 2,2′-dipyridyl-disulfide (10 ml) in the above-mentioned solvent system was prepared. The suspension of thiol was rapidly added to the solution of 2,2′-

* The preparations described have also been performed on a 10-fold larger scale.

dipyridyl-disulfide. The mixture was stirred during the reaction; reaction time 1 h at room temperature. The product was washed with 60 % acetone in water and finally with 1 mM EDTA solution. The degree of substitution was determined by nitrogen determination according to Kjeldahl. The product is stable to storage.

Immobilization of urease. A reactor column was prepared by pouring a suspension of 2-pyridyl-disulfide-Sepharose 2B into a Perspex tube (⌀ 10 mm). The height of the packed gel bed was 22 mm. The gel was washed for 4 h with 0.1 M Tris-HCl buffer of pH 7.2 (0.1 M in potassium chloride and 1 mM in EDTA), flow rate 5.1 ml/h. The tube was connected to a peristaltic pump. A solution of urease (Sigma type IV) (150 mg) in 50 ml of the above Tris buffer system was prepared and passed through the column. The column was washed with the buffer until A_{280} and A_{343} had decreased to the base line level.

The activities of free and bound urease were determined.[5] The protein content of the gel and the protein concentration of the solutions of free urease were determined by amino acid analyses (Table 2).

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Free Radicals in γ-Irradiated Single Crystals of Trehalose Dihydrate and Sucrose Studied by Electron Paramagnetic Resonance

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The γ-induced free radicals in single crystals of trehalose dihydrate and sucrose have been studied by Electron Paramagnetic Resonance (EPR). In trehalose dihydrate a free radical conversion was observed. Interpretation of the EPR spectra leads to the proposal that the primary radicals are formed by hydrogen abstraction at particular positions in the monosaccharide units, and the secondary radicals are formed by water elimination from the primary radicals. The EPR spectra from the sucrose single crystals may be assigned to free radicals similar to the secondary radicals in trehalose dihydrate.

The free radicals induced by ionizing radiation in disaccharides have been the subject of several Electron Paramagnetic Resonance (EPR) studies in the past.[1–6] In the present investigation we have studied the radiation-induced free radicals in single crystals of the disaccharides trehalose dihydrate and sucrose. In the trehalose dihydrate crystals a 1:1 free radical conversion takes place upon storage at room temperature. Interpretation of the EPR spectra before and after this conversion gave us enough information to propose mechanisms both for the initial radical formation and the subsequent conversion reaction. To our knowledge these free radicals have not been previously identified.

Irradiated sucrose was one of the first substances studied in single crystal form by the EPR method.[1–3] In spite of this the nature of the free radicals has never been adequately explained. Similarities in the EPR spectra from the secondary free radicals in trehalose dihydrate and the free radicals observed in sucrose has led us to conclude that the mechanism of radical formation may be similar in both crystals but that the radical conversion takes place instantaneously in the irradiated sucrose crystal.

EXPERIMENTAL

Single crystals of trehalose dihydrate and sucrose were obtained by dissolving the carbohydrates in H_2O or D_2O followed by addition of ethanol and subsequent storage of the solution at 4 °C until suitable single crystals could be harvested. The solutions had the following compositions: 2.5 g trehalose dihydrate in 31 ml H_2O and 72 ml ethanol or 28 ml D_2O and 53 ml ethanol (corresponding to a ratio of dissociable [D] and [H] of [D]/[H]$\simeq$3:1); 20 g sucrose in 17 ml H_2O and 25 ml ethanol.

The disaccharide materials and their radiation degradation have previously been described.[7] The crystals were irradiated in a ^{60}Co γ-source at 77 K or at room temperature with doses in the range $5 \times 10^{19} - 15 \times 10^{19}$ eV/g.

Figs. 1 and 2 show molecular structures and schematic crystal shapes for trehalose dihydrate and sucrose, respectively. Crystal structures are known both for trehalose dihydrate,[8–10] and sucrose.[11–13] For trehalose dihydrate the space group is $P2_12_12_1$, and 4 formula units are contained in each orthorhombic unit cell with dimensions $a = 17.90$ Å, $b = 12.21$ Å, and $c = 7.59$ Å. For sucrose the space group is $P2_1$, and 2 formula units are contained in each monoclinic unit cell with dimensions $a = 10.86$ Å, $b = 8.70$ Å, and $c = 7.76$ Å. The unit cell axes directions were determined by X-ray crystallography for both disaccharide crystals. Orthogonal sets of axes, *abc* for trehalose dihydrate, and *a*bc* for sucrose, are indicated in Figs. 1 and 2.

For the room temperature studies the crystals

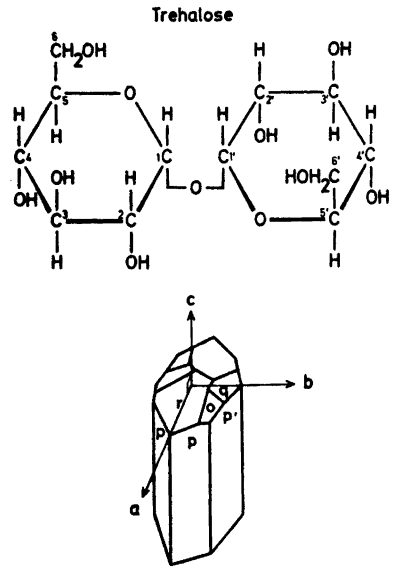


Fig. 1. Molecular structure and schematic crystal shape of the trehalose dihydrate single crystal. The orthogonal *a*, *b*, and *c* directions of the orthorhombic unit cell are indicated.

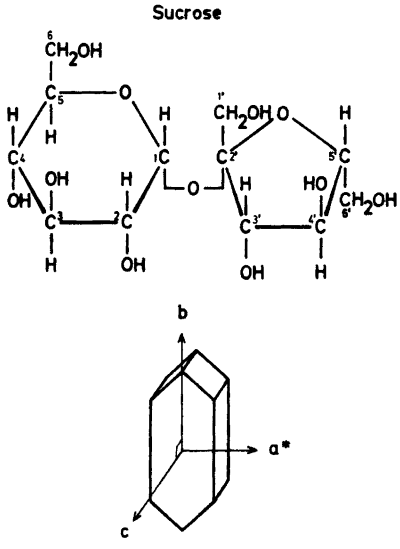


Fig. 2. Molecular structure and schematic crystal shape of the sucrose single crystal. The orthogonal *a**, *b*, and *c* directions of the monoclinic unit cell are indicated.

were mounted in a simple goniometer permitting rotation in a plane perpendicular to the plane of rotation of the spectrometer magnet. For a low temperature recording the sample was simply placed in an EPR tube with a specific direction perpendicular to the magnet plane. The EPR spectra were recorded on a Varian V-4502 EPR spectrometer equipped with 100 kHz field modulation, a 30 cm magnet and a V-4533 cylindrical cavity, around which the spectrometer magnet could be rotated. A modified microwave bridge permitted studies at low microwave power.

EPR spectra at room temperature were recorded in various directions in the main crystallographic planes of the single crystals. Any chosen direction of the magnetic field *H versus* the crystal could be obtained by rotating the goniometer and the spectrometer magnet. In all experiments the microwave power level was chosen as high as possible without causing noticeable microwave power saturation.

All spectra were recorded as first derivatives of the absorption lines. For the quantitative analyses in trehalose dihydrate double integrations were performed on the spectra from polycrystalline samples. This was done by an IBM 360-75 computer, which was fed digitized spectra on paper-tape from a Time-Averaging-Computer (TMC-CAT-1024) connected to the spectrometer and with an attached paper-tape punch.

Dihedral angles in the molecules were calculated by using an IBM 1800 and a Varian 620-i computer.

RESULTS AND DISCUSSION

Trehalose dihydrate, radical A. Fig. 3 shows an EPR spectrum of a single crystal of trehalose $2D_2O$ at 77 K after γ-irradiation at 77 K. The

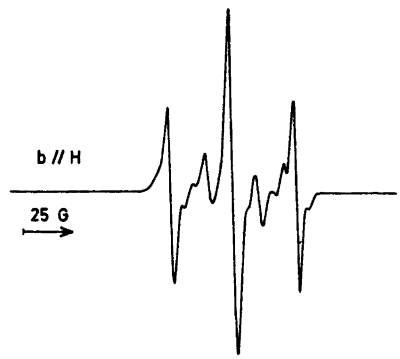
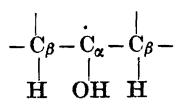


Fig. 3. The EPR spectrum recorded with H||b of a single crystal of trehalose.$2D_2O$ at 77 K after γ-irradiation at 77 K. The field modulation was 0.9 G and the microwave power was 3 μW.

degree of deuteriation was estimated to 75 atom % from the relative amounts of dissociable [D] and [H] in the solutions from which the crystals were precipitated. The partial substitution of dissociable hydrogens with deuterium does not change the EPR spectrum, except for a sharpening of the lines and the diminishing of the weak satellite lines ("shoulders"), which are seen in irradiated non-deuteriated carbohydrates and are probably due to forbidden transitions.[14] The main feature of the spectrum is a 1:2:1 triplet structure with a spacing of 32 G, which is almost isotropic when the sample orientation in the magnetic field is varied. Single crystals irradiated and studied at room temperature yield similar spectra immediately after the irradiation.

The isotropic triplet structure in the EPR spectrum indicates interaction between the unpaired electron and two equivalent protons in β positions (α denotes the carbon atom carrying the unpaired electron, and β its nearest neighbours). The radical A is consistent with the EPR spectrum.



Radical A

From the magnitude of the isotropic coupling it is possible to estimate the dihedral angle θ between the z-direction of the $2p_z$-orbital of the unpaired electron and the $C_\beta - H_\beta$ bond, projected perpendicularly to the $C_\alpha - C_\beta$-bond. Formula (1) has been proposed.[15]

$$A(H_\beta) = B_0 + B_2 \cos^2\theta \qquad (1)$$

where B_0 is small (0 – 3 G) and B_2 is approximately 50 G.[16] $A(H_\beta) = 32$ G corresponds to $|\cos\theta_A| = 0.80 - 0.76$ and $\theta_A \simeq 37 - 40°$ (using $B_0 =$ 0 or 3 G, and $B_2 = 50$ G).

Trehalose dihydrate, radical B. When the irradiated trehalose dihydrate crystal was kept at room temperature for 3 months, a free radical conversion took place in the sample. This was indicated by a complete change of the EPR spectrum. However, double integration of spectra from polycrystalline samples at various times after the irradiation showed that the spin concentration was constant during the reaction. The rate of radical conversion could be increased by warming the sample. The secondary radicals were stable for at least a year at room temperature (as long as the crystal was kept).

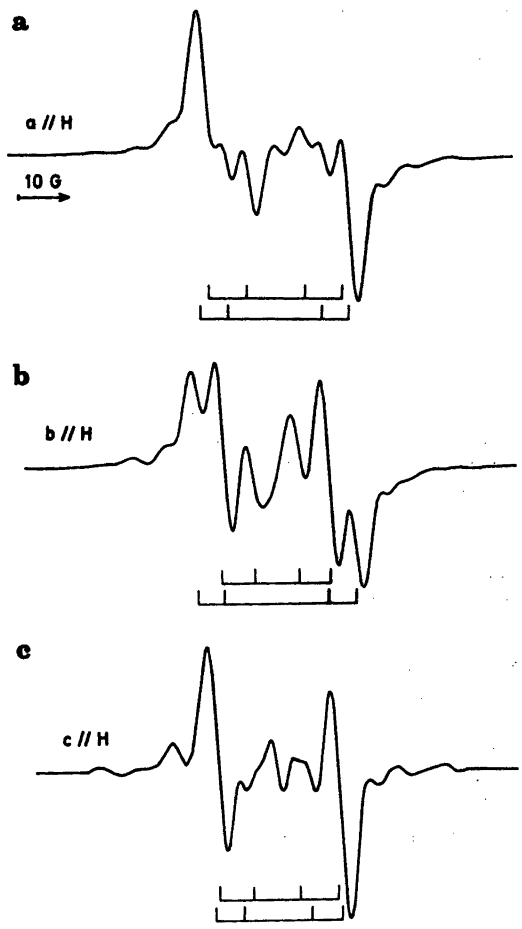


Fig. 4. EPR spectra of a single crystal of trehalose.$2D_2O$ at room temperature after γ-irradiation at 77 K and storage at +40 °C for 3 days. The field modulation was 0.6 G and the microwave power was 30 μW. (a) $H||a$; (b) $H||b$; (c) $H||c$.

The EPR spectrum after the storage was narrower, and of a more anisotropic nature. Fig. 4 shows spectra after the conversion, which were obtained with the magnetic field of the spectrometer in the a, b and c directions (defined in Fig. 1). In these directions all four molecules in the unit cell are magnetically equivalent. In other directions the spectra are generally less

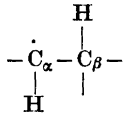
well-resolved, and show considerable asymmetry.

The spectra of Fig. 4 are almost, but not quite, symmetric. We have found it difficult to assign them to one single radical species, responsible for the line pattern in all three directions. Since trehalose dihydrate is composed of two almost identical monosaccharide units, we instead propose that the spectra should be explained as composed of two similar spectral components present in equal amounts, one from each glucopyranose unit of the disaccharide. These two units are magnetically inequivalent also in the directions of the crystallographic axes. Each unit should contribute a quartet of lines, arising from a large anisotropic hyperfine coupling from a hydrogen nucleus in α position and a smaller isotropic one from a hydrogen nucleus in β position. Stick spectra in agreement with this interpretation are inserted below the spectra of Fig. 4.

The isotropic hyperfine coupling $A(H_\beta)$ is approximately 6 G, whereas the anisotropic $A(H\alpha)$ varies, and takes values as low as 14 G and as high as 25 G. (14 G and 25 G can be estimated from Fig. 4b, where the two components are easy to distinguish.)

The magnitude of $A(H_\alpha)$ and its variation is in good agreement with what is usually observed for hydrogen nuclei in α positions. From the magnitude of $A(H_\beta)$ one can estimate the dihedral angle θ in the same way as for radical A [see eqn. (1)]: $A(H_\beta) \simeq 6$ G corresponds to $|\cos\theta_B| = 0.35$ and $\theta_B = 70°$ (using $B_0 = 0$ and $B_2 = 50$ G). Because $A(H_\beta)$ is small, the influence of a small positive value of B_0 in eqn. (1) is comparatively large, and another estimate is $|\cos\theta_B| = 0.25$ and $\theta_B = 75°$ (using $B_0 = 3$ G and $B_2 = 50$ G).

This interpretation of the EPR spectra of radical B leads to the following radical model:



Radical B

The g-values of both radicals A and B are close to 2, and show only a small anisotropy, as expected for carbon-centered free radicals.

Radical formation scheme in trehalose dihydrate. The initial formation of radical A can be simply explained by hydrogen abstraction from one of the carbon atoms in the ring. Radical B shows a H_α hyperfine coupling. Thus the radical conversion from A to B must involve more than a conformational change. The elimination of water from radical A would yield secondary radicals with one H_α and one H_β hyperfine coupling, as observed for radical B. Furthermore, the elimination of water is a plausible radical conversion mechanism. This mechanism has been found to explain the radiomimetic oxidation of monosaccharides in aqueous solu-

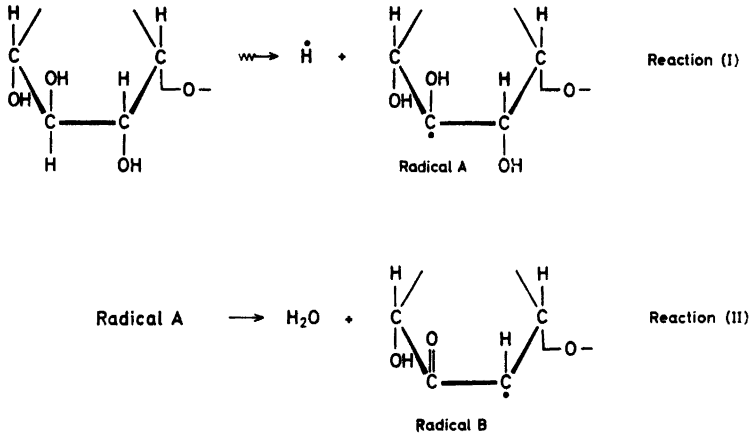


Fig. 5. Schematic model of free radical formation by hydrogen abstraction (I) and conversion by water elimination (II) in the trehalose dihydrate crystal.

Table 1. Calculated values of $|\cos\theta|$, where θ is the dihedral angle, in the trehalose dihydrate and sucrose crystals under assumption of various sites of radical formation. The crystal structures were defined by the atomic coordinates given in Ref. 10 for trehalose dihydrate and in Ref. 13 for sucrose. The calculations were performed in two different ways (a) and (b), assuming different molecular conformations illustrated in Fig. 6 and described in the text. The configurations best corresponding to the experimental $|\cos\theta|$-values ($|\cos\theta_A| = 0.76 - 0.80$ and $|\cos\theta_B| = 0.25 - 0.35$) are set in italics.

Crystal	Site of radical A	H_β	$\|\cos\theta\|$ Calc. (a)	Calc. (b)	Site of radical B	H_β	$\|\cos\theta\|$ Calc. (a)	Calc. (b)
Trehalose dihydrate	C(2)	H(1)	0.03	0.38				
		H(3)	0.85	0.69	C(3)	H(4)	0.89	0.48
	C(3)	*H(2)*	*0.81*	*0.76*	*C(2)*	*H(1)*	*0.03*	*0.24*
		H(4)	*0.89*	*0.71*	C(4)	H(5)	0.89	0.48
	C(4)	H(3)	0.87	0.70	C(3)	H(2)	0.81	0.48
		H(5)	0.89	0.71				
	C(2′)	H(1′)	0.10	0.47				
		H(3′)	0.85	0.72	C(3′)	H(4′)	0.89	0.47
	C(3′)	*H(2′)*	*0.81*	*0.71*	*C(2′)*	*H(1′)*	*0.10*	*0.28*
		H(4′)	*0.89*	*0.70*	C(4′)	H(5′)	0.86	0.52
	C(4′)	H(3′)	0.87	0.71	C(3′)	H(2′)	0.81	0.48
		H(5′)	0.86	0.73				
Sucrose	C(2)	H(1)	0.15	0.51				
		H(3)	0.83	0.70	C(3)	H(4)	0.85	0.48
	C(3)	H(2)	0.83	0.74	*C(2)*	*H(1)*	*0.15*	*0.32*
		H(4)	0.85	0.73	C(4)	H(5)	0.85	0.51
	C(4)	H(3)	0.84	0.71	C(3)	H(2)	0.83	0.49
		H(5)	0.85	0.71				
	C(3′)	H(4′)	0.97	0.71	*C(4′)*	*H(5′)*	*0.70*	*0.50*

tion,[17] and also the radiation-induced chain reactions in crystalline α-lactose monohydrate.[18]

From these considerations we propose a model, summarized in Fig. 5, for the radiation-induced free radical formation and conversion in solid trehalose dihydrate. In Fig. 5 C(2) and C(3) have been chosen as sites of radicals A and B, respectively. The reasons for this assignment will be given later. Reactions (I) and (II) should occur with equal probability in each of the two monosaccharide units.

Because of the complexity of the anisotropic spectrum of radical B we found it difficult to estimate the directions of the principal axes of the $A(H_\alpha)$ tensors and in this way determine which carbon atoms carry the unpaired electrons in radicals A and B. However, the values of $A(H_\beta)$ are related to the molecular configuration *via* eqn. (1). The experimentally estimated values of the dihedral angle θ can be compared with calculated values of θ based on the fractional atomic coordinates given in Ref. 10. Since the conformational changes accompanying free radical formation are unknown, and probably depend also on the hydrogen bonding in the structure, this can only be done approximately and be used to confirm numerically the results from visual inspection of molecular models. Only carbon atoms C(2), C(3), and C(4) and the corresponding C(2′), C(3′), and C(4′) are possible sites of radical A, if the proposed mechanism of radical formation and conversion is valid. Initial radical formation at C(1) and C(1′) would not give the proper configuration

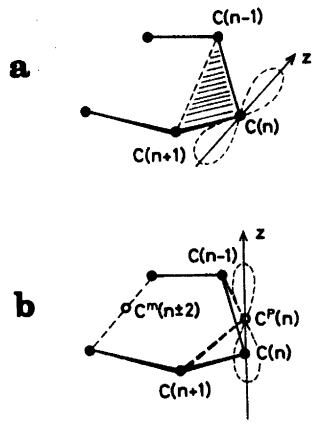


Fig. 6. Schematic configurations of a free radical at C(n). The direction of the z orbital axis of the unpaired electron in radical A is indicated.
(a) The monosaccharide ring system is assumed unchanged.
(b) C(n) is assumed to move into a new position $C^P(n)$ in a plane defined by C($n+1$), C($n-1$) and the mean value $C^m(n\pm2)$ of C($n+2$) and C($n-2$). Radical B assumed to be positioned at C($n+1$) or C($n-1$), has its radical plane defined by C($n-2$), C($n+2$), and C($n-1$) or C($n+1$), respectively.

for radical A. C(5) and C(5′) do not have the OH group necessary for the water elimination mechanism.

As a start the fractional atomic coordinates were transformed to space coordinates in Ångström units. The directions of the orthorhombic unit cell axes were used as an orthogonal coordinate system. The calculations were then performed in two ways, tentatively chosen after building molecular models (Fig. 6): (a) We assumed that the configuration of the monosaccharide ring was intact, and that the z orbital axis of the unpaired electron at C(n) ($n = 2, 3, 4, 2', 3'$ or $4'$) was perpendicular to the plane defined by C($n+1$), C(n) and C($n-1$). (b) We assumed that the initial site of radical formation C(n) was twisted into a new position $C^P(n)$ in a radical plane defined by C($n+1$), C($n-1$) and the mean value of C($n+2$) and C($n-2$), denoted $C^m(n\pm2)$. (The notation C($n+2$) or C($n-2$) may also denote the ring oxygen atom). After the conversion reaction, when C($n+1$) or C($n-1$) is the site of the unpaired electron, the radical plane was assumed to

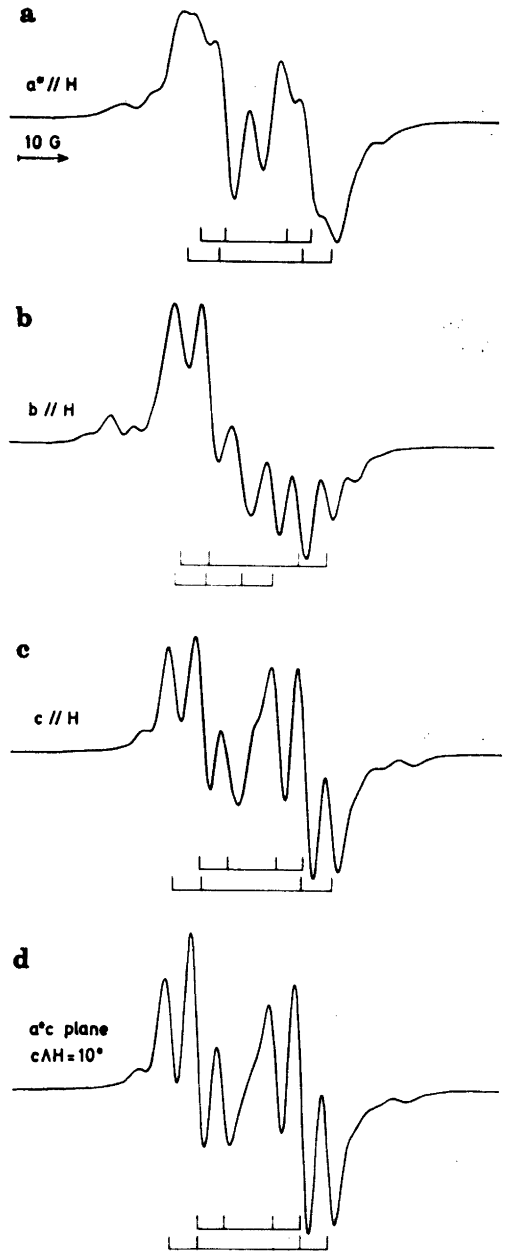


Fig. 7. EPR spectra of a single crystal of sucrose at room temperature after γ-irradiation at room temperature. The field modulation was 1.7 G and the microwave power was 0.3 mW.
(a) $H \| a^*$; (b) $H \| b$; (c) $H \| c$; (d) H in the a^*c plane, and the angle between the c axis and H is 10°.

remain approximately the same, and was defined by C($n+2$), C($n-2$) and C($n-1$) or C($n+1$), or the corresponding atoms in the second monosaccharide ring.

The results of these calculations, both yielding similar results, are summarized in Table 1. A comparison with the experimentally estimated values of $\cos\theta_A = 0.76-0.80$ and $\cos\theta_B = 0.25-0.35$ indicates that the most probable sites of radical A are C(3) and C(3′), which after the elimination of water yield radicals B positioned at C(2) and C(2′). This is also in agreement with what one can guess after inspection of molecular models. It can be observed that calculation (b) seems to account better for the experimental situation than does calculation (a).

The stable radical in sucrose. Fig. 7a–c shows the EPR spectra in the a^*, b, and c directions of a sucrose single crystal, γ-irradiated at room temperature. Similar spectra are observed from crystals irradiated and investigated at 77 K,[3] and from crystals grown in deuteriated media.[2] The spectra remain essentially stable for years. Because of the crystal symmetry, the two molecules in each unit cell should be magnetically equivalent in the a^*-c plane and in the b direction. However, the presented EPR spectra (which are similar to, but better resolved than the spectra shown in Ref. 1 for the corresponding directions) are clearly asymmetric. This must be due to g-value anisotropy and at least two contributing radicals with different orientations. The spectrum of Fig. 7c in the c direction is almost identical to the trehalose radical B spectrum of Fig. 4b. The best resolved spectrum in the a^*c plane, shown in Fig. 7d, was obtained in a direction at 10° angle from the c axis. This spectrum is also very nearly symmetric.

The symmetric spectrum agrees with the earlier postulated radical [2,3] in sucrose which involves hyperfine couplings to one H_α and two identical H_β's with small couplings. The EPR spectrum should then be a double 1:2:1 triplet. However, that model does not explain the observed anisotropies in the spectra.

Instead, in view of the similarities between the stable sucrose EPR spectrum and that of the stable radical B of trehalose dihydrate, we propose that the stable sucrose radicals are similar to the radicals B of trehalose dihydrate. Thus there should be one radical component with one H_α and one H_β hyperfine coupling in each of the two monosaccharide units, which are magnetically inequivalent also in the a^*c-plane and in the b direction. Stick spectra according to this interpretation are inserted in Fig. 7. It is reasonable to assume that the reaction routes have been similar in the two disaccharides, but that the radical A stage is much less stable in sucrose, so that only the water elimination radical is observed.

The value of A(H_β) is practically identical to that found for radical B in trehalose dihydrate. Thus we obtain $|\cos\theta_B| \simeq 0.25-0.35$. Calculations of dihedral angles θ based on the atomic coordinates given in Ref. 13 were performed in the same way as was done for trehalose dihydrate. Since the sucrose unit cell is monoclinic, the orthogonal coordinate system for the space coordinates was chosen as a^*bc.

The results of the calculations are shown in Table 1. In the glucopyranose ring the calculated values are similar to those from trehalose dihydrate, and C(2) is indicated as the site of the unpaired electron. In the fructofuranose ring there is only one possibility for the proposed mechanism to work, *i.e.* carbonyl formation at C(3′) and the stable radical centered at C(4′). However, the calculated value of $\cos\theta$ seems too large to agree with the experimentally estimated value. The reason could be a more fundamental conformational change than the one we have considered.

In studies of the radical processes in frozen aqueous solutions of sucrose and D-glucose, Baugh *et al.*[19] observed two types of radicals originating from the solutes. One was described as a 1:2:1 triplet with spacing of 29.5 G, the other one as a 1:1 doublet with a spacing of 20.5 G. These values of coupling constants suggest that these powder spectra correspond to radicals similar to those described as radicals A and B in the present study. In a powder spectrum one does not expect the small H_β coupling of radical B to be resolved.

The assignment of radicals to both monosaccharide units in sucrose is in agreement with a recent ENDOR study,[20] in which evidence is put forward that protons from both units are involved in forming the so called matrix ENDOR signal in the irradiated samples.

Comparison with radiochemical data. The present interpretation of the radical structures in trehalose dihydrate and sucrose is in line

with previously reported chemical data. The result that both monosaccharide moieties harbour radicals complies with the fact that both monosaccharide moieties in sucrose are radiation-damaged to a similar extent.[7] The result that the radicals are associated with C(3), C(3′), C(2), and C(2)′ is in accord with the chemical analysis showing that not more than a small part of the radiation induced degradation is linked with breakage of the glycosidic bond in the investigated disaccharides.[21] It is also in accord with the result that the yield of acids is small compared with the total degradation.[7,22] Formation of neutral carbonyl compounds which is indicated in the present study has also been observed in chemical analyses.[23,24]

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Mass Spectra of 1,2,5-Selenadiazoles and their Oxygen and Sulfur Analogues

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The mass spectra of a series of 1,2,5-selenadiazoles and some of their oxygen and sulfur analogues are reported and discussed. Their main fragmentation modes, inferred from results of metastable defocusing and high resolution mass measurements, are compared. A close analogy to thermal and photochemical reactions was found.

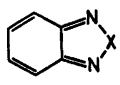
Although the mass spectra of heterocyclic oxygen and sulfur compounds have been intensively investigated (*cf.* Ref. 1), only little attention has been directed towards the study of heterocyclic selenium compounds,[2–14] and only few comparisons have been made with the analogous sulfur and oxygen compounds.[2,6]

The present paper is concerned with the study of the mass spectra of compounds *1* – *11* (Table 1). Although the spectra of some of these compounds have been reported earlier (*1*,[15] *4*,[16] *6*,[5] *9*,[17] and *11*[3,4]) no detailed investigation of the fragmentations was made.

Table 1. Compounds *1* – *11*.



1 – *8*



9 – *11*

	R^1	R^2	X
1	C_6H_5	C_6H_5	O
2	C_6H_5	C_6H_5	S
3	C_6H_5	C_6H_5	Se
4	H	H	O
5	H	H	S
6	H	H	Se
7	CH_3	CH_3	Se
8	C_6H_5	CH_3	Se
9	–	–	O
10	–	–	S
11	–	–	Se

RESULTS AND DISCUSSION

Compounds 1 – 3. The major fragmentation modes for compounds *1* – *3* are shown in Fig. 1. In order to minimize thermal decompositions prior to ionization (compounds *1* [18,19] and *3* [20] are known to decompose to benzonitrile upon heating) the spectra were recorded at the lowest possible ion source temperatures with direct sample insertion into the ion source. At higher ion source temperatures the relative abundances of the benzonitrile ion (*m/e* 103) were observed to increase drastically. In all cases reproducible mass spectra were obtained.

Direct loss of the heteroatom X (O, S, Se) from the molecular ions was not observed in any case. However, the reported difference [6] in behaviour of analogous sulfur and selenium compounds, *i.e.* the easier elimination of the selenium atom is demonstrated in the decomposition of the $[M-C_6H_5CN]^+$ ion, *a*. Application of the metastable defocusing technique revealed that elimination of O, S and Se takes place from ion *a* with formation of *m/e*

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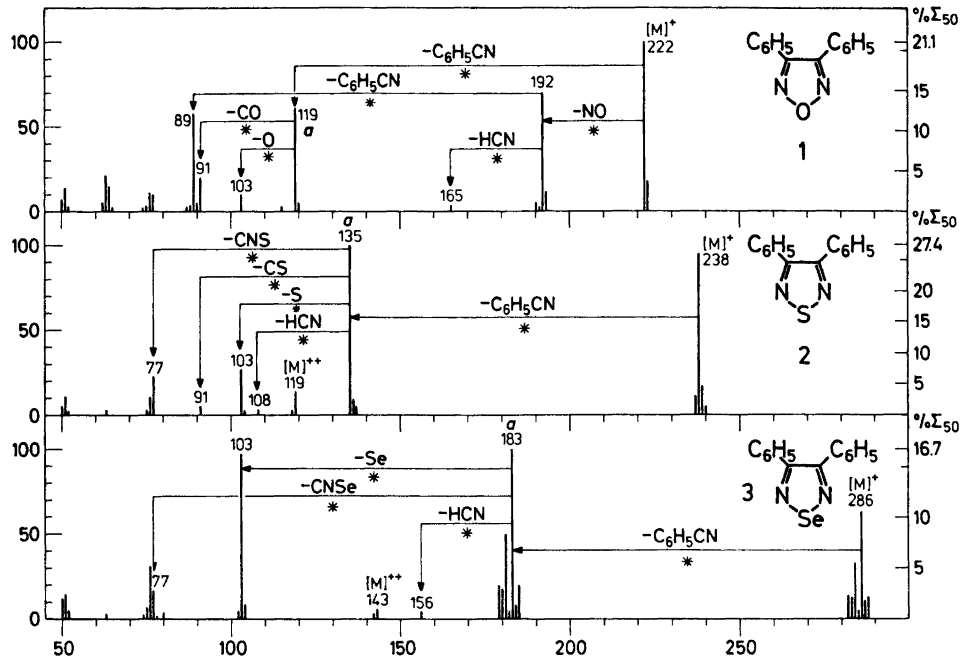


Fig. 1. MS of compounds *1 – 3*.

103. Since this process appears to be the predominant mode for formation of this ion (a metastable transition of very low intensity for direct formation of *m/e* 103 from the molecular ions was also detected by metastable defocusing) the abundance of *m/e* 103 in the three spectra may reflect the ease of elimination of X (Se > S > O).

However, these observations may not be due only to a different ability of initially formed $[C_6H_5CNX]^+$ ions (*a*) to perform a simple N – X bond cleavage. Other fragmentation processes observed for these ions (loss of CX and HCN) indicate that they undergo rearrangement involving a migration of the heteroatom X. A possible structure for the rearranged ion may correspond to the molecular ions of C_6H_5XCN or C_6H_5NCX. While the fragmentations of *a* are clearly different from the fragmentations of C_6H_5XCN (X = O,[21] X = S,[22] and X = Se [23]) similarities are found when comparisons are made with the spectra of C_6H_5NCX (X = O,[21] X = S,[24],* and X = Se*). This suggests that *a* rearranges to some extent to the latter structure (*cf.* the discussion of the phenylthiacylnitrenium ion, $[C_6H_5C(S)N]^+$ [25]). However, the internal energy distribution may be quite different for a molecular ion and a fragment ion of the same structure and the abundances of their decomposition products may as well be quite different. Thus it is not possible to determine to what extent the rearrangement may depend on the nature of X and to what extent the elimination of X takes place from the two structures in question, C_6H_5CNX and C_6H_5NCX. However, the difference in ability to eliminate X (Se > S > O) is much more pronounced in the spectra of C_6H_5NCX than observed for *a* in Fig. 1. (In the spectrum of phenylisoselenocyanate the $[M - Se]^+$ ion is twice as abundant as the molecular ion. Loss of oxygen was not observed in the spectrum of phenylisocyanate [21]).

Compounds 4 – 8. The mass spectra of the parent compounds *4 – 6* are shown in Fig. 2.

* The most important features in the spectra of phenylisothiocyanate and phenylisoselenocyanate are the following: Phenylisothiocyanate, *m/e* 135 (M^+, 100 %), *m/e* 108 ($M^+ - HCN$, 3 %), *m/e* 103 ($M^+ - S$, 4 %), *m/e* 91 ($M^+ - CS$, 4 %), and *m/e* 77 ($M^+ - NCS$, 58 %). Phenylisoselenocyanate, *m/e* 183 ($M^+(^{80}Se)$, 48 %), *m/e* 156 ($M^+ - HCN$, 3 %), *m/e* 103 ($M^+ - Se$, 96 %) and *m/e* 77 ($M^+ - NCSe$, 100 %).

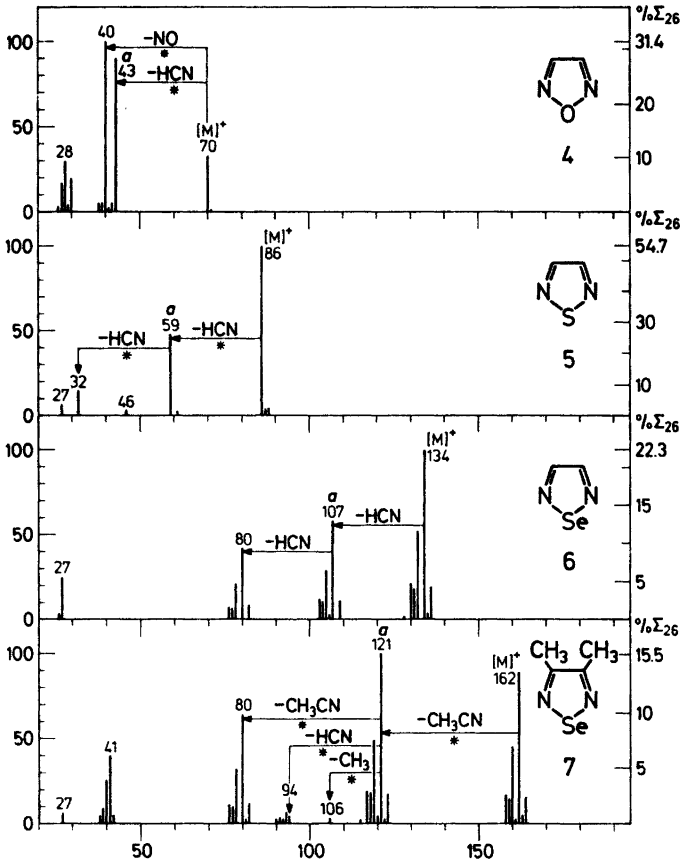
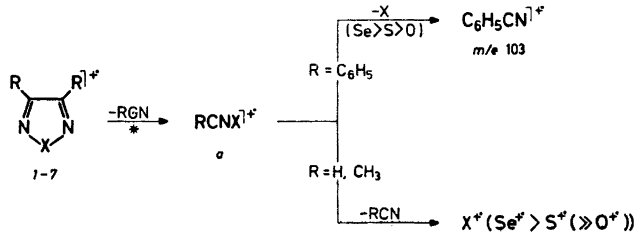


Fig. 2. MS of compounds *4 – 7*.

The loss of HCN from the molecular ions is a parallel to the loss of C_6H_5CN in the spectra of *1 – 3*. However, the further fragmentation of the $[M-RCN]^+$ ion is clearly affected by the nature of R as demonstrated by a comparison between the mass spectra (Figs. 1 and 2) of the selenadiazoles *3* and *6*. In *3* the elimination of selenium takes place with charge retention preferentially on the phenyl containing moieties leading to very low abundances of the selenium ions (*m/e* 80: 4 %). In the case of *6* reversed charge retention leads to the formation of abundant Se^+ ions and elimination of HCN. The total abundance of the selenium ions is approximately three times as high as that of $[HCN]^+$.

Corresponding differences are found in the spectra of the thiadiazoles *2* and *5*. However, the sulfur ion is less abundant in *5* than the



Scheme 1.

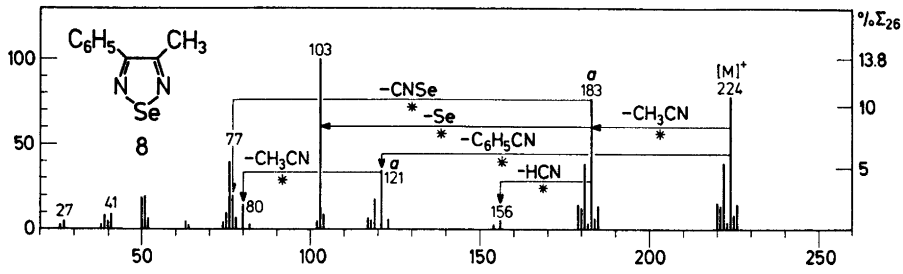


Fig. 3. MS of compound *8*.

selenium ions in *6* and in the spectrum of 1,2,5-oxadiazole (*4*) *m/e* 16 is negligible.

3,4-Dimethyl-1,2,5-selenadiazole (*7*) exhibits similar fragmentation modes as 1,2,5-selenadiazole (*6*). The selenium ions and the $[RCN]^+$ ion are formed in approximately the same mutual ratio in both spectra.

Except for the loss of NO the more important differences in the fragmentation modes of compounds *1* to *7* are summarized in Scheme 1.

Both fragmentation routes are observed in the spectrum (Fig. 3) of 4-methyl-3-phenyl-1,2,5-selenadiazole (*8*). One is initiated by the loss of CH_3CN from the molecular ion, yielding the *m/e* 183 ion, the further decomposition of which is identical to that observed for *a* in the spectrum of *3*. The other involves the initial elimination of C_6H_5CN with generation of *m/e* 121 which then decomposes as ion *a* in the spectrum of *7*. The former route, giving rise to a sequence of ions involving the phenyl group, is more pronounced than the latter (*cf.* Fig. 3).

Compounds 9 – 11. The spectra and the predominant fragmentation modes of compounds *9 – 11* are shown in Fig. 4. The stability of NO *versus* NS and NSe shows a marked influence on the fragmentation of these compounds. Thus, the only major fragmentation of the molecular

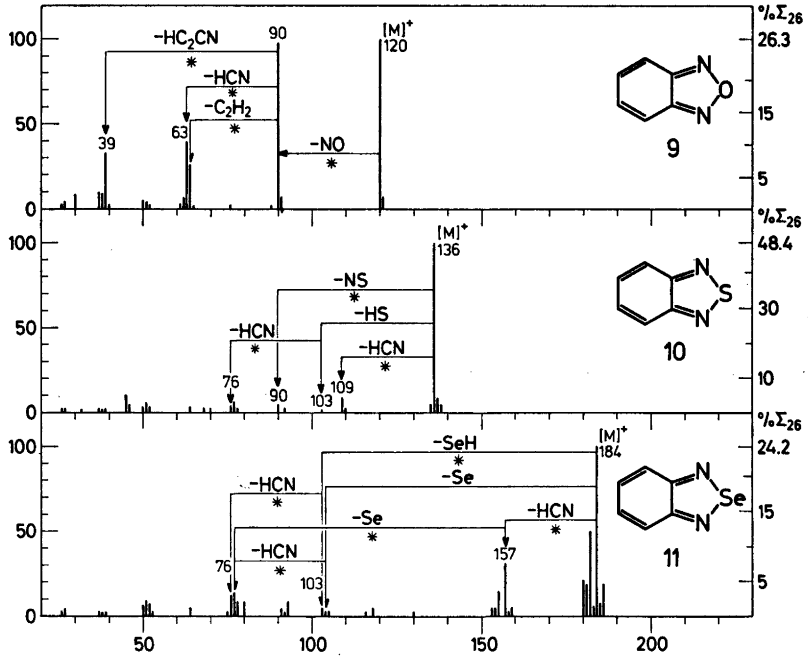


Fig. 4. MS of compounds *9 – 11*.

ions of *9* is elimination of NO. The corresponding NS-loss is much less important in *10* and NSe-loss does not occur in *11*.

The *m/e* 76 ion in the spectrum of benzoselenadiazole (*11*) has previously been suggested[3] to be the benzyne ion. However, the metastable defocusing technique showed that *m/e* 103 ($M^+ - XH$) in the spectra of *10* and *11* was the progenitor for *m/e* 76 and exact mass measurements established its elemental composition as C_5H_2N. Similarly, the composition of *m/e* 77 and 78 was found to be C_5H_3N and C_5H_4N, respectively.

Comparison with thermal and photochemical reactions. With the exception of the fragmentation mode initiated by loss of NO from the molecular ion of *1*, the primary electron impact induced cleavages of compounds *1*, *2*, and *3* into C_6H_5CN and C_6H_5CNX (with charge retention preferably on the latter moiety) show a close analogy to their thermal and photochemical behaviour. Thermolysis of diphenylfurazan (*1*) was reported [18,19] to give a mixture of benzonitrile, phenylisocyanate and diphenyl-1,2,4-oxadiazole. These findings strongly suggest that the primary reaction is cleavage to benzonitrile and benzonitrile *N*-oxide. Similarly, it has been found that the primary products in the photolysis of *1* are benzonitrile and its *N*-oxide.[15,26]

Thermolysis of *3* and photolysis at room temperature of *2* and *3* give benzonitrile.[20] However, low temperature photolysis (85 K) of compound *3* gives, besides benzonitrile, an intermediate assigned as benzonitrile *N*-selenide. This intermediate is converted to benzonitrile upon irradiation or by raising the temperature above 110 K.[20]

Irradiation of benzofurazan (*9*) was reported to give 4-cyanobutadiene-1-carbonitrile *N*-oxide as the primary product.[17,27] This double cleavage reaction is analogous to the photochemistry of diphenylfurazan (*1*).[15,26]

EXPERIMENTAL

Materials. All compounds were prepared by previously described methods (*1*,[18] *2*,[28] *3*, *6* and *7*,[29] *4*,[16] *5*,[30] *8* by a procedure analogous to that given for compound *3* in Ref. 29, *9*,[31] *10*,[32] and *11*[33]).

The mass spectra were recorded on an AEI MS-902 mass spectrometer operating at 70 eV. Compounds *4* – *7*, *9* – *11*, phenylisothiocyanate, and phenylisoselenocyanate were introduced through the all-glass heated inlet system (temperatures 25 – 80 °C). The remaining compounds were introduced through the direct insertion lock, and the temperature of the water-cooled source was kept as low as possible. Typical sample temperatures were 50 °C for *1*, 60 °C for *2*, and 90 °C for *3*. When necessary, the elemental composition of an ion was determined by high resolution mass measurements (± 5 ppm). Peaks of relative abundance lower than 2 % are omitted in the spectra.

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Indirect Electrolysis. Reaction between Alkyl Halides and Electrolytically Generated Anion Radicals

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The reaction between alkyl halides and electrolytically generated anion radicals of aromatic hydrocarbons has been studied by cyclic voltammetry and controlled potential electrolysis. The results may be interpreted to show that the reaction proceeds through an electron transfer to the alkyl halide. The aliphatic radical, probably after loss of a halide ion, may react with an aromatic anion radical in two ways; the unpaired electron on each of the two reactants may form a bond between the reactants (coupling) or one electron is transferred from the aromatic anion radical to the aliphatic radical. The outcome of this competition depends on the structure of the aliphatic halide and is discussed in terms of electron affinities of the reactants. In some cases the two-electron product, the dianion, may be considered as the electron-donating species and its reaction with an alkyl halide would be of interest in connection with the mechanism of the S_N2-reaction.

From a preparative point of view, an advantage of the electrocatalytic reduction of alkyl halides is that it takes place with much less overvoltage than is required for the direct reduction at the electrode; the coupling reaction has the disadvantage that it seldom leads to a single product, but more often to a complex mixture. This disadvantage is, however, sometimes compensated by the fact that otherwise inaccessible compounds may be formed.

This paper describes an investigation on the reaction between aromatic anion radicals and aliphatic halides as a part of a more general study [1,2] of indirect reductions and oxidations by means of electrolytically generated anion and cation radicals.

The reaction between aromatic anion radicals, formed by reaction between an alkali metal and an aromatic hydrocarbon, and an alkyl halide has been the subject of many studies;[3–9] sodium dihydronaphthylide (sodium naphthalene, $NaC_{10}H_8$) has been a favoured reactant, and several types of alkyl halides, including primary alkyl fluorides,[9] have been included in these studies. The conclusion has been that the following scheme describes the reactions between alkyl halides and radicals from difficultly reducible aromatic hydrocarbons.

$$NaAr + RX \rightarrow Ar + NaX + R\cdot$$

$$R\cdot + NaAr \begin{cases} \rightarrow \alpha Ar + \alpha NaR \xrightarrow{H^+} \alpha RH \\ \rightarrow (1-\alpha)RArNa \end{cases}$$

$$RArNa \begin{cases} \xrightarrow{H^+} RArH \\ \xrightarrow{RX} RArR \end{cases}$$

The reaction between anion radicals and alkyl halides depends on many parameters, *e.g.* the electron affinities of the reactants, the solvent, and ion pairing.[10–12] In the present investigation *N*,*N*-dimethylformamide (DMF) has been used as solvent; as supporting electrolyte has been employed tetrabutylammonium iodide (TBAI). The ion pairing of the anion radical with the quaternary ammonium cation in DMF is much less pronounced than with lithium or sodium ions in dimethoxyethane or tetrahydrofuran.

The systems have been studied primarily by cyclic voltammetry and controlled potential electrolysis.

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RESULTS AND DISCUSSION

Catalytic reactions. The results will be discussed on the basis of the following reaction scheme, where A represents a compound forming a stable anion radical and BX is a compound forming an unstable anion radical; the reactions (1) and (7) are heterogeneous reactions involving the cathode, whereas the other reactions are homogeneous ones; (2) and (3) may be concerted.

$$A + e^- \rightleftharpoons A^{\cdot -} \quad (1)$$
$$A^{\cdot -} + BX \rightleftharpoons A + BX^{\cdot -} \quad (2)$$
$$BX^{\cdot -} \rightarrow B^{\cdot} + X^- \quad (3)$$
$$A^{\cdot -} + B^{\cdot} \rightleftharpoons A + B^- \quad (4)$$
$$A^{\cdot -} + B^{\cdot} \rightarrow AB^- \quad (5)$$
$$B^{\cdot} + B^{\cdot} \rightleftharpoons B - B \quad (6)$$
$$B^{\cdot} + e^- \rightleftharpoons B^- \quad (7)$$
$$B^{\cdot} + HS \rightleftharpoons BH + S^{\cdot} \quad (8)$$
$$B^- + H^+ \rightleftharpoons HB \quad (9)$$
$$AB^- + H^+ \rightleftharpoons ABH \quad (10)$$
$$AB^- + BX \rightarrow AB_2 + X^- \quad (11)$$
$$A^{\cdot -} + A^{\cdot -} \rightleftharpoons A + A^{2-} \quad (12)$$
$$A^{2-} + BX \rightarrow [A^{\cdot -} - -BX^{\cdot -}]$$
$$\rightarrow AB^- + X^- \quad (13)$$
$$A^{2-} + BX \rightarrow AB^- + X^- \quad (14)$$
$$A^{2-} + H^+ \rightleftharpoons AH^- \quad (15)$$
$$AH^- + BX \rightarrow ABH + X^- \quad (16)$$
$$AH^- + H^+ \rightleftharpoons AH_2 \quad (17)$$

Cyclic voltammetry. The reactivities of organic halides, proton donors and olefins in DMSO towards electrochemically generated radical ions have recently been studied [13] by cyclic voltammetry, but the electrocatalytic nature of the reactions involving halides seems not to have been appreciated.

A typical electrocatalytic reaction follows (1), (2), (3), (4), and (9). In Fig. 1 is shown cyclic voltammograms of a system (phenanthrene, chlorobenzene) which follows that sequence;[1] no coupling products (5) have been isolated. From Fig. 1 it is seen that the peak-height of phenanthrene grows steadily with the concentration of chlorobenzene. Note also, that no wave appears at about -2.6 V (aq. SCE; -2.1 V *vs* Ag/AgI) between the first peak and that of chlorobenzene at about -2.8 V (aq. SCE; -2.3 V *vs* Ag/AgI), and that the oxidation wave of the anion radical disappears on addition of chlorobenzene.

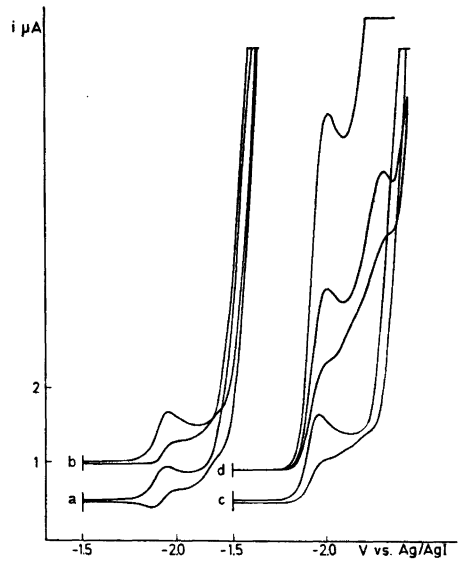


Fig. 1. Cyclic voltammograms at a stationary mercury electrode of phenanthrene (8×10^{-4} M) in presence of an increasing concentration of chlorobenzene; medium DMF/0.1 M TBAI, v = 10 mV/s. Concentration of chlorobenzene: a, 0; b, 1.2×10^{-4} M; c, 6×10^{-4} M; d, 4.8×10^{-3} M; d is shown with two sensitivities.

Cyclic voltammograms of a system, in which some coupling (5) occurs, is shown in Fig. 2, where biphenyl is studied in the presence of an increasing concentration of *tert*-butyl chloride. The following features will be discussed: The peak height of the first wave, the disappearance of the second wave and the appearance of a third wave (III) between the first and the second wave.

In contradistinction to the purely catalytic wave the height of the first peak does not continue to grow with increasing concentration of the BX compound, but reaches a limit (compare also Figs. 3 and 4). This may be explained by the occurrence of the coupling reaction (5) which removes the "catalyst" $A^{\cdot -}$ from the recycling by the formation of AB^-. There is a competition between (4) and (5), but when the concentration of BX is high enough all A eventually ends up as AB^- (ABH), even if some of the A molecules have recycled several times.

This also explains why the second wave (the wave of $A^{\cdot -}$) disappears; the anion radical does

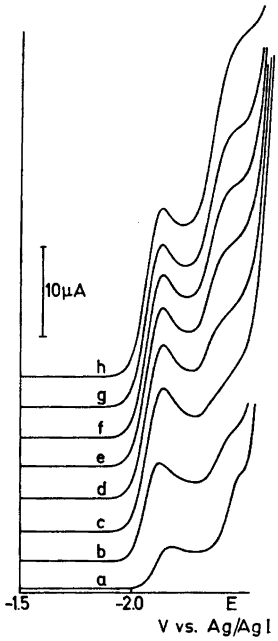


Fig. 2. Cyclic voltammograms at a stationary mercury electrode of biphenyl (5.2×10^{-3} M) with an increasing concentration of *tert*-butyl chloride; medium DMF/0.1 M TBAI; v = 10 mV/s. Concentration of *tert*-butyl chloride: a, 0; b, 4×10^{-3} M; c, 8×10^{-3} M; d, 1.2×10^{-2} M; e, 1.6×10^{-2} M; f, 2.0×10^{-2} M; g, 2.4×10^{-2} M; h, 2.8×10^{-2} M.

not survive in the presence of an excess BX-compound, and there is thus no anion radical left to give the peak.

The appearance of wave III between the two original peaks may be due to the reduction of ABH, in the present case a phenylcyclohexadiene; the reduction potential of such a compound would be expected to fall at about the same potential as that of peak III. Some ABH are shown below in the preparative part, where III, VII, VIII, and XI exemplify such compounds. ABH may undergo similar reactions [(1) to (17)] as A, and as the difference in reduction potential between *tert*-butyl chloride and ABH is smaller than between biphenyl and *tert*-butyl chloride the rate of the electron exchange (2) is greater for ABH than for A. The peak heights of I and III are thus not directly comparable, and a certain peak height of III would be caused by a much smaller

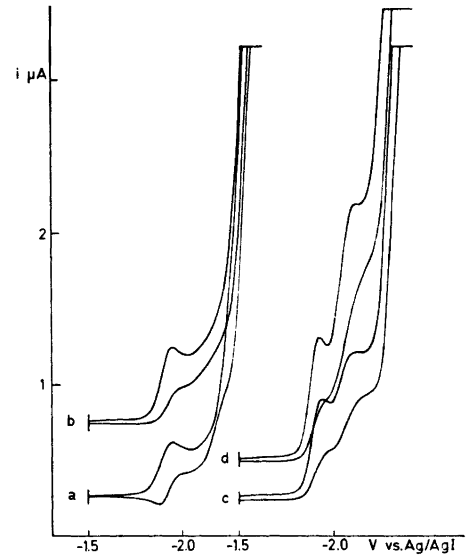


Fig. 3. Cyclic voltammograms at a stationary mercury electrode of phenanthrene (8×10^{-4} M) in the presence of an increasing concentration of *tert*-butyl chloride; medium DMF/0.1 M TBAI; v = 10 mV/s. Concentration of *tert*-butyl chloride: a, 0; b, 1.5×10^{-4} M; c, 7.5×10^{-4} M; d, 6×10^{-3} M.

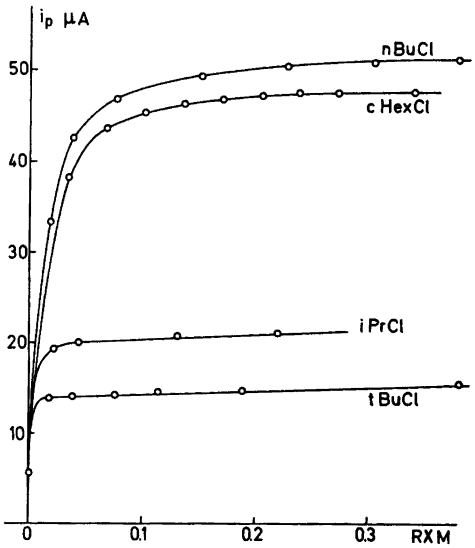


Fig. 4. Variation of the peak-height in cyclic voltammograms of naphthalene (5.5×10^{-3} M) at a stationary mercury electrode in dependence of the concentration of various halides (abscissa); medium DMF/0.1 M TBAI, v = 10 mV/s. The following halides were used: *tert*-butyl chloride, isopropyl chloride, cyclohexyl chloride, and butyl chloride.

concentration of ABH than would be required of A to give a similar peak height of I.

The wave III could, however, also be considered to be a prewave to the second wave in a similar way as described below for the first wave. The occurrence of this wave would then imply a fast reaction (*e.g.* proton abstraction, electron transfer) between the dianion and the alkyl halide.

In Fig. 3 the voltammograms of phenanthrene in the presence of *tert*-butyl chloride illustrates the constant peak height of wave I at higher concentrations of BX and the disappearance of wave II and the oxidation wave of $A^{\cdot -}$ at low concentrations of BX. The existence of coupled, partly hydrogenated products is suggested by the occurrence of a peak between I and II (the latter partly covered by the background current); peak III can be interpreted from its potential to be caused by a compound having a naphthalene or biphenyl nucleus intact.

The fact that the peak III occurs at about the same potential for BX being *tert*-butyl and *n*-butyl chloride supports the suggested explanation that peak III is caused by an ABH-compound. This, combined with a rather large difference (about 280 mV) between peak III and peak II makes it less likely that III is a "prepeak" to peak II.

When a coupling (5) takes place the peak height reaches a limit or a very slow growth on increasing the concentration of the BX-compound, the value of this limit may be interpreted as being dependent on the competition between the "catalyst-neutral" reactions [(4), (6), (7), (8)] and the "catalyst-removing" reaction, the coupling reaction (5). In Fig. 4 is depicted the dependence of the peak height of the first peak of naphthalene on the concentration of the following BX-compounds; *tert*-butyl chloride, isopropyl chloride, cyclohexyl chloride, and butyl chloride.

The limiting values of the peak heights depend on the structure of the BX-compound; the lower the limit the more effectively (5) competes with the other reactions. The results depicted in Fig. 4 thus predict that the current yield of coupling products with *tert*-butyl chloride is much higher than that for butyl chloride in the reaction with naphthalene anion radical.

This prediction was tested in the following experiment; four similar reductions of naphthalene in DMF/TBAI were made in the presence of one of the four above mentioned aliphatic chlorides; a quantity of electricity, 2 F/mol, calculated on the basis of the concentration of the hydrocarbon, was passed and the reaction mixture analyzed by NMR after isolation of the mixture of hydrocarbons. The results, which are shown in Table 1, indicate a good yield (~70 %) of coupled product for *tert*-butyl chloride and a much lower yield (22 %) for butyl chloride with the secondary chlorides giving intermediate yields (40 – 45 %). Besides the radical coupling (5) the S_N2-reaction of AH^- (16) may be responsible for the formation of some of the butylated products. The magnitude of, *e.g.*, the *tert*-butyl signals compared to the aromatic protons of the partly hydrogenated ring shows that the *tert*-butyl chloride has not to any appreciable degree functioned as a proton donor, as only traces, if any, of tetraline and dihydronaphthalenes could be detected in the mixture.

Table 1. Relative yields of unreacted naphthalene and coupled products in a 2 F/mol reduction of naphthalene in the presence of a 5-fold excess of alkyl chloride (BCl).

B	Naphthalene/ coupled products
Butyl	3.6
Cyclohexyl	1.35
Isopropyl	1.2
tert-Butyl	0.44

In the cyclic voltammetric experiments (Fig. 4) cyclohexyl chloride behaves more like butyl chloride than like isopropyl chloride, whereas in the preparative electrolysis (Table 1) the two secondary chlorides behave more alike.

These results indicate, that the ratio $R^* = i_p^1/i_p^o$, where i_p^1 is the peak height of A in the presence of high concentrations of BX and i_p^o is the height in the absence of BX, is dependent on B. If B is aromatic or benzylic no limiting value has been observed in the concentration region employed; primary alkyl halides give a high ratio R*, whereas tertiary alkyl halides give a low ratio, with secondary alkyl halides lying in between.

This may be rationalized on the basis of considerations on electron affinity of the reac-

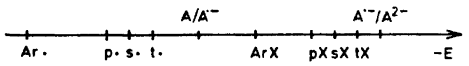


Fig. 5. Schematical representation of a possible distribution of half-wave potentials of some reactants in electron transfer reactions. ArX = aromatic halide; pX = primary aliphatic halide; sX = secondary aliphatic halide; tX = tertiary aliphatic halide; Ar·, p·, s·, t· = aromatic, primary, secondary, and tertiary aliphatic radicals, respectively.

tion partners. Only few measurements have been made on the redox potential of aliphatic radicals,[13–17] but it seems reasonable to assume the following order of electron affinity: Ar· > RCH_2· > RR′CH· > RR′R″C·. In Fig. 5 the redox potentials of the different reaction partners are shown schematically on a potential axis. The rate of electron transfer from an aromatic anion radical to a primary alkyl radical is thus faster than the transfer to a tertiary alkyl radical. If it is also assumed, that for aliphatic radicals the rate of the coupling reaction (5) is less dependent on structure than (4), it follows that the coupling reaction would be favoured for tertiary alkyl halides compared to primary ones.

The ratio R* (i_p^1/i_p^o) is, for a given BX compound, independent of the concentration of A as shown in Fig. 6. Further experiments are

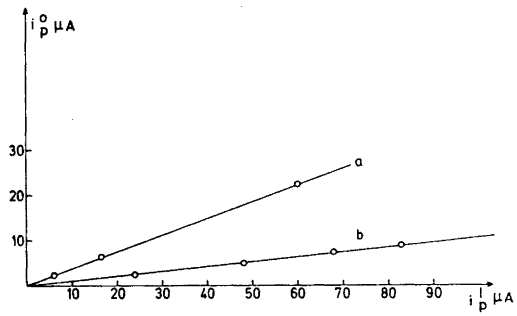


Fig. 6. Cyclic voltammetric results of different concentrations of naphthalene at a stationary mercury electrode in presence of an excess of alkyl halide. Medium: DMF/0.1 M TBAI; scan rate 10 mV/s. Ordinate: Peak currents of naphthalene at different concentrations; abscissa: Peak currents of naphthalene at the same concentrations in presence of excess of the following halides: a, *tert*-butyl chloride; b, butyl chloride.

planned to elucidate the influence of solvent, temperature, and other parameters on the ratio R*. From the concentration independence of R* it seems likely that the second order dimerization (6) is less important for the fate of B· than the electron exchange reaction (4).

If the competition between (4) and (5) is the deciding factor for R*, then the reactants $A^{\cdot -}$ and B· react with each other in such a way that a constant product ratio occurs. A possible interpretation of this is, that the radical reactants form a complex, in which there is a constant probability for the two unpaired electrons either to form a bond (5) or to be localized at one of the reactants (4). The concept of a complex seems attractive, but the available data cannot exclude other interpretations.

In Fig. 7 is shown the dependence of the ratio R* on the peak potential (easily related to the redox potential) of the aromatic A-compound; *tert*-butyl chloride, isopropyl chloride and butyl chloride are included as BX-compounds. The trend of the data is clear, the more negative the redox potential, the higher is the ratio R*, in accordance with the explanation, that a high value of R* is the result of a high rate of an electron exchange reaction.

In Fig. 8 are shown cyclic voltammograms of *trans*-stilbene in the presence of an increasing concentration of *tert*-butyl bromide. *trans*-Stilbene alone gives two one-electron steps of which the first one is reversible. On addition of a small amount of *tert*-butyl bromide a prepeak appears; the peak height of the second peak of stilbene decreases and so does the anodic peak of the reoxidation of the stilbene anion radical. On further addition of *tert*-butyl bromide these trends continue until the prepeak and the first peak of stilbene merge, and the second one-electron step and the anodic one disappear completely. This happens at a concentration of *tert*-butyl bromide which is considerably smaller than that necessary for reaching the limiting value of the peak height.

Such a prepeak could be observed if a species, more easily reduced than the A-compound, were formed in a fast follow-up reaction (or reactions) of the anion radical $A^{\cdot -}$. In the present case such reactions could be (2) and (3) with formation of the easily reducible radical B·. Electron transfer to this radical and/or the re-formed A would be responsible for the observed prepeak,

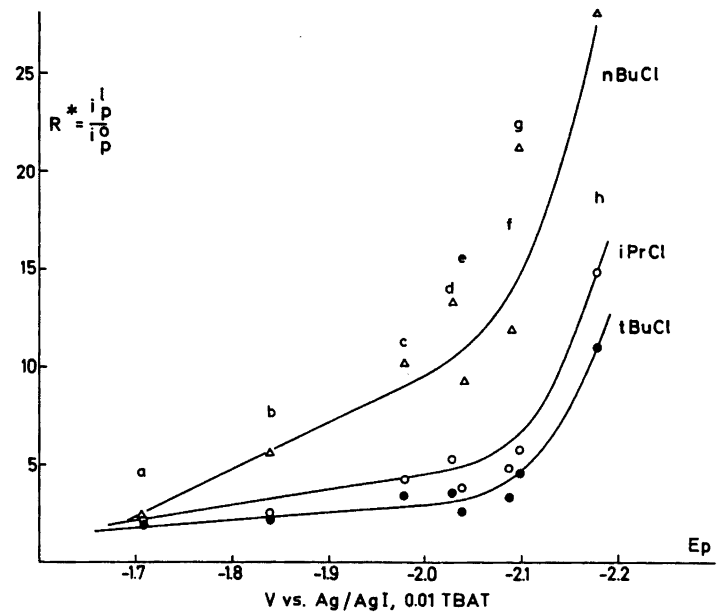


Fig. 7. Dependence of the ratio $R^* = i_p^l/i_p^o$ of different A-compounds on their peak-potentials for different aliphatic halides in DMF/TBAI, a, *trans* stilbene; b, *p*-terphenyl; c, phenanthrene; d, 1-methylnaphthalene; e, naphthalene; f, 2-methylnaphthalene; g, biphenyl; h, acenaphthene. Aliphatic halides: Butyl chloride; isopropyl chloride; *tert*-butyl chloride.

the height of which would be limited by the concentration of BX.

The prepeak could, however, also be formed by a mechanism akin to the one observed during the anodic pyridination of 9-phenylanthracene; such prepeaks were studied by means of simulation technique.[18] It was found that a prepeak could be obtained as well in an *ece*-reaction as in an *ecec*-reaction (electron transfer – chemical reaction – electron transfer – chemical reaction), provided certain parameters were of the right magnitude and certain conditions met. The prepeak depended on many factors such as the rate constants of the reactions involved, the concentrations of the reacting molecules, their diffusion coefficients, and the sweep rate. The essential feature of the phenomenon in a cathodic process is that there in the reaction scheme is a rapid, practically irreversible reaction which removes the reduced product; the removal of the reduction product induces a further reduction of the substrate in order to retain the concentration ratio required by the Nernst equation. The reduction thus occurs at a less negative potential than in the reversible case. Both reactants are removed in the irreversible reaction, and the height of the prepeak is limited by the depletion of the reactant present in lowest concentration. In the present case the peak height might be limited by the concentration of the B-radical, which in turn is dependent on, among other things, the BX-concentration.

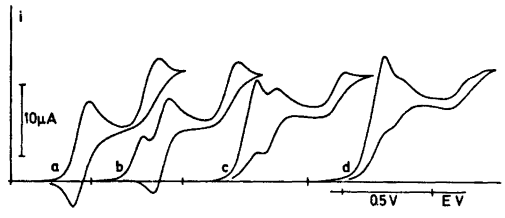


Fig. 8. Cyclic voltammograms at a stationary mercury electrode of *trans*-stilbene (5.1×10^{-3} M) in DMF/0.1 M TBAI containing different concentrations of *tert*-butyl bromide. Starting potential -1.3 V *vs.* Ag/AgI; sweep rate 10 mV/s. Concentration of *tert*-butyl bromide: a, 0; b, 3.5×10^{-3} M; c, 1.08×10^{-2} M; d, 1.5×10^{-2} M.

A prewave has not been observed for aromatic BX-compounds; aromatic B-radicals do not

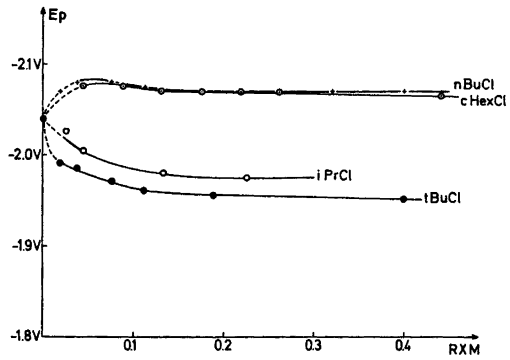


Fig. 9. Dependence of the peak potential of the first wave of naphthalene on the concentration of the following aliphatic halides: *tert*-butyl chloride, isopropyl chloride, cyclohexyl chloride, and butyl chloride.

couple with $A^{\cdot-}$. For aromatic BX-compounds the reaction sequence (1), (2), (3), (4) is followed, and the reaction responsible for the prepeak cannot be sought among these reactions. Aliphatic BX-compounds couple to a higher or lower degree (5). If thus (2) and (4) behave reversibly in this context and (5) for all practical purposes behave irreversibly, the occurrence of the prepeak in the present case could be explained in a way akin to that employed for the voltammetric pyridination of 9-phenylanthracene.[18]

In Fig. 9 is shown the dependence of the peak potential of the first wave of naphthalene in dependence of the concentration of *tert*-butyl chloride, isopropyl chloride, cyclohexyl chloride, and butyl chloride. The halides, which are assumed (Fig. 4, Table 1) to give a high current yield of coupling products, shift the peak potentials to more positive potentials, whereas a slight shift to more negative values is observed in the presence of good electron acceptors, such as aromatic BX-compounds and, to a lesser degree, butyl chloride.

The positive shift of the peak potential has the same background as the prewave, as it is due to an irreversible process which removes the reduced product, thereby bringing about a further reduction of the substrate in order to retain the concentration ratio required by the Nernst equation. When the prepeak merges with the original peak the positive shift is observed. However, as a purely catalytic peak is shifted [19] to more negative potentials on increasing the concentration of BX, there are at least two opposing factors operating. If the coupling reaction dominates, a positive shift is found, but when the electron exchange dominates, a shift to more negative potentials is observed.

In some cases electron-transfer from a dianion A^{2-} of an aromatic compound to a BX-compound may be possible.[20] Addition of highly active alumina to DMF removes proton donors and makes it possible to observe two reversible one-electron steps for many compounds;[21] using that technique the reaction between A^{2-} and BX has been studied.[22] The dianions are much stronger bases (and nucleophiles) than the anion radicals; perylene dianion thus exchanges electrons with dichlorobenzene, but its reaction with butyl chloride is a proton abstraction or substitution reaction rather than an electron exchange reaction involving a catalytic reduction of butyl chloride. In this connection it could also be mentioned that a reaction with the dianion is only observed when the reaction between $A^{\cdot-}$ and BX is so slow that BX is not preferentially removed by this reaction.

For compounds forming less basic dianions or where ion-pairing diminishes the tendency to proton abstraction the two-electron reduction product may undergo electron exchange with a suitable aliphatic BX-compound. The reaction of a dianion with a BX-compound could be formulated as a classical S_N2-reaction (transfer of two electrons simultaneously) or as an electron exchange resulting in two anion radicals, which then could couple; the coupling could possibly be between $A^{\cdot-}$ and $B^{\cdot}$, the latter formed by decomposition of $BX^{\cdot-}$. The S_N2 reaction has been shown to proceed in certain cases [23,24] *via* a primary electron exchange; experiments with other types of substrates than used here are in progress to investigate the possible use of electrochemical methods in elucidating certain aspects of the S_N2 reaction.

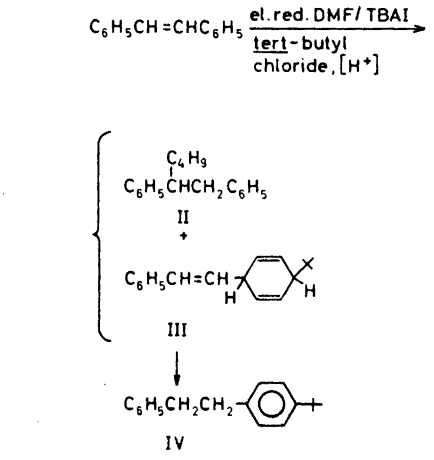
The results from the cyclic voltammetric investigation are in agreement with the findings by earlier methods [3–9] that the coupling between an anion radical from an aromatic hydrocarbon ($A^{\cdot-}$) and an alkyl halide occurs between $A^{\cdot-}$ and the aliphatic radical $B^{\cdot}$. The cyclic voltammetric data often makes it possible to foretell the relative importance of the possible reactions; sometimes merely the reduction potentials of

the reactants involved may suffice, but often a proper cyclic voltammetric investigation is needed to make a prediction.

Preparative electrolysis. The preparative electrolysis of the systems described in this investigation has two aspects, the catalytic reduction [25,26] and the reductive coupling reaction. The catalytic reduction of an alkyl halide by means of an anion radical can be performed at a much less negative potential than that required by a direct reduction at the electrode; in effect, the overvoltage is drastically reduced. The electron transfer between an aromatic anion radical and an aliphatic alkyl halide (possibly within a collision complex) is thus not inhibited by a similar high reaction barrier as the heterogeneous electron transfer between the mercury electrode and the alkyl halide. The activation energy in the reduction of alkyl chlorides at a vitreous carbon electrode [27] seems to be smaller than that connected with the reduction at a mercury electrode, but both of the heterogeneous reactions mentioned have apparently larger activation energies than the homogeneous reaction. Possibly the electrode does not act only as an electron source; the electrode material may play a role in the process.

Besides aliphatic hydrocarbons, preparative electrolysis of aromatic hydrocarbons in the presence of an alkyl halide gives, in general, rise to the formation of alkylated, partly hydrogenated compounds. Some examples will be mentioned.

Reduction of stilbene in DMF/TBAI in the presence of *tert*-butyl chloride at −2.3 V (aq. SCE) yielded a 3:2 mixture of the known [28] 3,3-dimethyl-1,2-diphenylbutane (II) and the hitherto unreported 1-phenyl-2-(4′-*tert*-butylphenyl)ethane (IV). No dialkylated products were observed; presumably the anion [AB^-, reaction (5)] would rather abstract a proton than react with *tert*-butyl chloride in a substitution reaction.



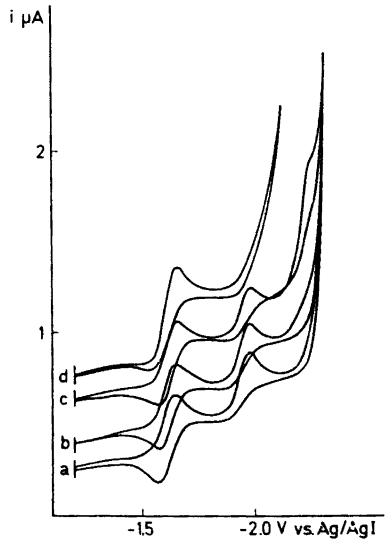
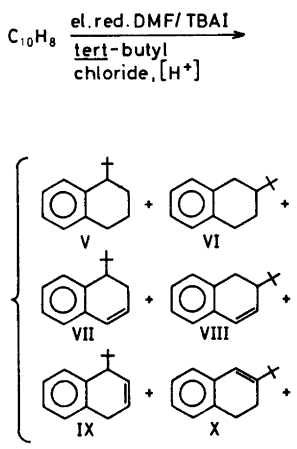


Fig. 10. Cyclic voltammograms at a stationary mercury electrode of *trans*-stilbene (7.5×10^{-4}M) in presence of different concentrations of *tert*-butyl chloride; medium DMF/0.1 M TBAI, v = 10 mV/s. Concentrations of *tert*-butyl chloride: a, 0; b, 1.5×10^{-4} M; c, 7.5×10^{-4} M; d, 1.2×10^{-2} M.

III is assumed to be an intermediate on the basis of the cyclic voltammetric curves (Fig. 10); these indicate the formation of a compound which is reducible just prior to the reduction of TBAI. As neither II nor IV are reducible at that potential, the styrene derivative III has been suggested here as the compound responsible for that wave.

Reduction of naphthalene under similar conditions gave a reaction mixture which unsuccessfully was sought separated by preparative GLC. Only one fraction was sufficiently pure for identification by ^{1}H NMR; the others contained three or more compounds. By ^{13}C NMR the mixtures could be analyzed, and the following products were found and characterized by their ^{13}C NMR-spectra:

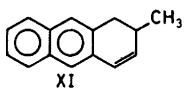


The coupling products formed are probably primarily the difficultly reducible 1,4-dihydronaphthalenes; these tautomerize under influence of the base created at the cathode into the 1,2- or 3,4-dihydro derivative which is reducible at a potential about 0.2 V more negative than that of naphthalene. These dihydronaphthalenes are thus examples of ABH-compounds suggested above to be responsible for the occurrence of the so-called wave III in the cyclic voltammetric experiments. Possibly some isomerization occurs during the work-up. The reaction mixture was aromatized by treatment with sulfur and the resulting products consisted of a 1:3 mixture of 1- and 2-*tert*-butylnaphthalene.

Anthracene produced on reduction in presence of methyl chloride mono- and dimethylated dihydroanthracenes. On column chromatography only one of the minor components was separated from the major components, which from the ^{1}H NMR-spectrum and mass spectrum seemed to be mainly a mixture of 9-methyl- and 9,10-dimethyl-9,10-dihydroanthracenes. The isolated minor component was 2-methyl-1,2-dihydroanthracene (XI), which is another example of a reducible ABH-compound.

The dialkylated compounds may be formed in a classical S_N2-reaction (11) between AB^-, formed in (5), and alkyl chloride.

Reduction of anthracene in the presence of



tert-butyl chloride yielded much more products alkylated in the 1- and 2-positions (60 %) than did the reaction with methyl chloride. Steric factors may be responsible for the lowered tendency of the *tert*-butyl radicals to attack the 9-position.

From a preparative point of view such reactions are not attractive due to the complexity of the reaction mixtures formed; however, this complexity makes it probable, that many of the possible isomers are present in the mixture, and if the separation problem can be solved, the reaction may be used to prepare otherwise inaccessible compounds. 1-*tert*-Butylpyrene, which has been sought prepared in several conventional ways without success, is thus the main product on electrolytic reduction of pyrene in DMF/TBAI in the presence of *tert*-butyl chloride.[29]

EXPERIMENTAL

Apparatus. For electrolysis and cyclic voltammetry was used apparatus previously described;[30] a Hewlett Packard 7045A $x-y$ recorder was used for recording the cyclic voltammograms.

Reduction of anthracene. Anthracene (1 g) was reduced in DMF/TBAI at -2.0 V (SCE) in the presence of methyl chloride, $n = 7-10$. The reduction completed, the DMF was removed *in vacuo*, the residue treated with water and diethyl ether, and the ether layer washed three times with water. After drying the ether was evaporated; ^{1}H NMR of the residue suggested a mixture of mono- and dimethylated 9,10-dihydroanthracenes (85 %) and about 15 % 1- or 2-methyldihydroanthracene. Attempted separation of the mixture by column chromatography on silica with light petroleum as eluent met only limited success, as only one of the components was separated from the remaining mixture, this compound was identified as 2-methyl-1,2-dihydroanthracene from the ^{1}H NMR-spectrum ($CDCl_3$): δ 1.06 (d, 3 H, J 6.5 Hz), 2.0–3.3 (m, 3 H), 5.91 (pair of d, 1 H, J_1 10 Hz, J_2 2.7 Hz), 6.55 (broadened d, 1 H, J_1 10 Hz, $J_2 \sim 1$ Hz), 7.1–7.9 (m, 6 H).

Reduction of anthracene under similar conditions with *tert*-butyl chloride replacing methyl chloride gave a different product distribution ($n = 1.9$), as compounds alkylated in the 1- or 2-positions were responsible for about 60 % of the reaction mixture. This estimation was made on basis of a comparison of the integration of the protons in the region of ethylenic protons with that of the aromatic and aliphatic protons.

Reduction of stilbene and tert*-butyl chloride.* *trans*-Stilbene (1 g) was reduced at -2.3 V (aq.

SCE) in DMF/0.1 m TBAI in the presence of 10 ml *tert*-butyl chloride. The solution turned green, fading at the end of the reduction, $n = 2.6$ F/mol; the n-value indicates a low degree of catalytic reduction of *tert*-butyl chloride. The reduction completed, most of the DMF was removed *in vacuo*, the remnant treated twice with water and distilled light petroleum. The light petroleum was washed with water, dried and evaporated. The ^{1}H NMR-spectrum indicated a mixture of approximately 60 % 3,3-dimethyl-1,2-diphenylbutane and 40 % 1-phenyl-2-(4′-*tert*-butylphenyl)ethane. The mixture was separated by preparative GLC on a 15 % SE 30 at a programmed column temperature 100 to 210 °C and a flow rate of 200 ml/min. The fractions were recrystallized from a small volume of ethanol and sublimed. 3,3-Dimethyl-1,2-diphenylbutane, m.p. 40 °C (36 – 37.5 °C);[28] ^{1}H NMR-spectrum ($CDCl_3$): δ 0.95 (s, 9 H), 2.5 – 3.3 (m, 3 H), 6.9 – 7.2 (m, 10 H). (Found: C 90.75; H 9.26. Calc. for $C_{18}H_{22}$: C 90.70; H 9.30). 1-Phenyl-2-(4-*tert*-butylphenyl)ethane, m.p. 45 °C; ^{1}H NMR-spectrum ($CDCl_3$): δ 1.30 (s, 9 H), 2.89 (s, 4 H) 7 – 7.4 (m, 9 H). (Found: C 90.49; H 9.22. Calc. for $C_{18}H_{22}$: C 90.70; H 9.30).

Reduction of naphthalene (1). 1 g of naphthalene was reduced in DMF/TBAI in the presence of excess BX [(a) 10 ml *tert*-butyl chloride; (b) 10 ml butyl chloride; (c) 12 ml cyclohexyl chloride; (d) 8 ml isopropyl chloride]; 2 F/mol naphthalene were passed using a constant current of 2.5 mA/cm^2. The solvent was removed *in vacuo*, and water and distilled light petroleum added to the residue. The organic layer was washed 3 times with water, dried, the solvent evaporated, and the residue analyzed by ^{1}H NMR ($CDCl_3$). (a) δ 0.85 – 0.95 (two main s, 9 H) 1.8 – 2.3 (m, 3 H), 5.8 – 6.65 (two sets of m, 2 H), 7.0 – 7.2 (two main s, 4 H, dihydronaphthalenes) 7.3 – 7.9 (two sets of m, 8 H, naphthalene). Yield of coupling products 70 %. The results reported in Table 1 are evaluated in a similar way.

Reduction of naphthalene (2). Naphtalene (2 g) was reduced in DMF/TBAI at −2.5 V (aq. SCE) in the presence of 10 ml *tert*-butyl chloride, $n = 3.3$ F/mol. The catholyte was diluted with water, acidified with a small volume of hydrochloric acid, and extracted with light petroleum twice. The organic layer was washed three times with water, dried, and the solvent removed *in vacuo*, residue 2.5 g. The residue was separated in three fractions by preparative GLC on a 15 % SE 30 at a column temperature of 140 °C with a flow of 120 ml/min. The components (V to X) in the fractions were identified by ^{13}C NMR.[31]

Acknowledgement. Financial assistance to this research from the NATO Research Grants Programme is gratefully acknowledged.

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Oxidation of Two 2,6-Di-*tert*-butyl-substituted Phenols with Quaternary Alkylammonium Hexacyanoferrate(III) Salts

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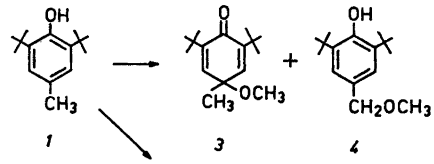
A method is described for the oxidation of phenols with tetrabutylammonium hexacyanoferrate(III). The oxidant was tested with two 2,6-di-*tert*-butyl-substituted phenols. For comparison, the same phenols were oxidized in methanol with hydrogen hexacyanoferrate(III). With the latter oxidant, quinones and quinol ethers were formed. Oxidation with the former, less acid, oxidant gave products that were formed *via* quinone methide intermediates. The results suggest that a change in mechanism from radical to ionic occurs when going from a less acid to a more acid system.

In a recent report Taimr and Pospíšil [1] pointed out the possibility of using hydrogen hexacyanoferrate(III) for the oxidation of some phenols. The use of this oxidant is limited by its insolubility in organic solvents other than alcohols and by its high acidity. In order to find a less acid system which would be soluble in non-polar solvents the acid was converted to its tetrabutylammonium salt by ion-pair extraction.[2] The usefulness of this salt as an oxidant was tested on some sterically hindered alkyl phenols.

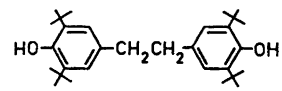
RESULTS

The ion-pair extraction yielded a chloroform-soluble, light green powder with the formula $(Bu_4N)_3Fe(CN)_6$ indicated by iodometric titration. No oxidation was observed when 2,6-di-*tert*-butyl-4-methylphenol (*1*) was treated with this compound in chloroform solution at room temperature even for several days. *p*-Toluenesulfonic acid was added to the solution in order to alleviate the steric hindrance of the bulky tetraalkylammonium groups and to provide counter-ions for the tetravalent hexacyanoferrate(II) anion. Oxidation of *1* started when two equivalents of *p*-toluenesulfonic acid had been added. Solutions containing $(Bu_4N)_3Fe(CN)_6$ and *p*-toluenesulfonic acid in a molar ratio of 1:2, were found to be useful for the oxidation of phenols. The oxidant in these solutions was taken to have the composition $Bu_4NH_2Fe(CN)_6$. Compound *1* in chloroform was oxidized to 3,3′,5,5′-tetra-*tert*-butyl-4,4′-dihydroxybibenzyl (*2*).

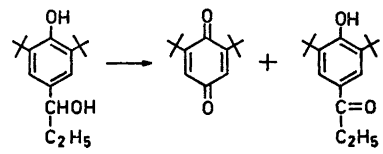
The oxidation of *1* was repeated in methanol solution to compare the new oxidant with the more acidic hydrogen hexacyanoferrate(III). In the oxidation with $H_3Fe(CN)_6$ we were able to reproduce the results of Taimr and Pospíšil:[1] in a combined yield of 30 %, the quinol ether *3*



1 *3* *4*



2



5 *6* *7*

and the benzyl methyl ether *4* were formed roughly in the proportions 9 to 1. The formation of the quinol ether *3* is kinetically controlled: extensive isomerization of *3* to *4* occurred when the oxidation time was extended from 1 to 25 h. This isomerization reaction is the subject of further investigation.

In the oxidation with $Bu_4NH_2Fe(CN)_6$ the same two compounds were formed but in inverse proportions, the benzyl methyl ether *4* being the main product. A similar change in reaction path with change in acidity was observed with the substituted benzyl alcohol *5*. High acidity leads to loss of the side-chain and formation of the 1,4-quinone *6*, lower acidity favours the formation of the ketone *7*.

DISCUSSION

Although there is at present insufficient evidence to confirm the mechanism of these reactions, the change in product composition with change in acidity suggests two different mechanisms for the two cases. Waters has recently suggested [3] that the pH of the reaction medium can influence the reaction mechanism in phenol oxidations. Thus a low pH favours the formation of phenoxonium ions instead of the phenoxy radicals which predominate in neutral and alkaline solutions.

Application of this concept to the present reactions would give the following two mechanisms.

At high acidity the phenol is oxidized to a phenoxonium ion which reacts with the nucleophilic solvent to form a cyclohexadienone (Scheme 1). This reaction has been observed in the anodic oxidation of *1* in acetonitrile containing methanol,[4] and has been shown to be a two-electron oxidation.[5] In the case of the benzyl alcohol *5*, the intermediate quinol ether will be unstable under acid conditions, the side-chain splitting off as propionic aldehyde.[6] The resulting *p*-methoxyphenol has a lower redox potential than the starting material [7] and is rapidly oxidized further to 2,6-di-*tert*-butyl-*p*-benzoquinone.

At low acidity the formation of phenoxonium ion is retarded in favour of the disproportionation of the phenoxy radical. Phenoxy radicals from sterically hindered phenols having benzylic hydrogens tend to disproportionate to a quinone methide and the parent phenol. This has been demonstrated for the phenoxy radical from *1*.[8,9] The decay of the radical has been studied kinetically,[8] and the results are consistent with a mechanism which involves the reaction between two phenoxy radicals. Products formed *via* a quinone methide have been identified.[8c,9] When the radical from *1* disproportionates in methanol the quinone methide intermediate adds methanol to form the benzyl methyl ether *4*.[9] A similar disproportionation of the phenoxy radical from *5* would give a quinone methide which is a tautomer of the ketone *7* (Scheme 2).

The implication of these results is that the concept [3] of a competition between radical and ionic mechanisms offers a partial explanation for the often confusing variety of products encountered in phenol oxidations.

EXPERIMENTAL

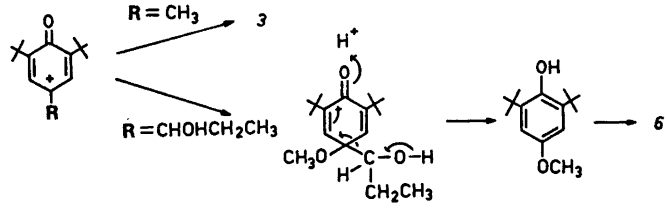
NMR-spectra. 60 MHz in $CDCl_3$, tetramethylsilane as internal standard, δ-values.

Preparative TLC. The products were separated on 2 mm precoated TLC-plates (Merck PSC F 254) with ethyl acetate-cyclohexane (1:15) as eluent.

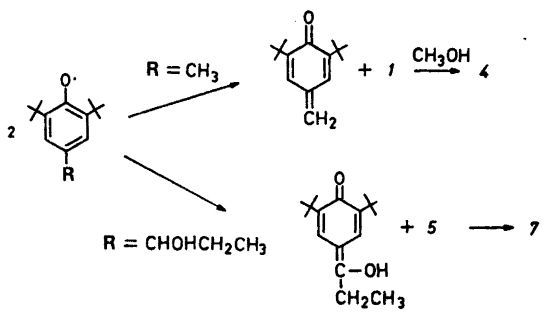
Materials

2,6-Di-tert-*butyl-4-methylphenol.* Fluka AG.

2,6-Di-tert-*butyl-4-(1-hydroxypropyl)phenol (5).* Prepared from ethyl magnesium bromide



Scheme 1.



Scheme 2.

and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde,[10] m.p. 74–76 °C (Lit.[11] 77 °C).

Hydrogen hexacyanoferrate(III). The acid was precipitated from an aqueous solution of potassium hexacyanoferrate(III) with an excess of concentrated hydrochloric acid.[1]

Tri-(tetrabutylammonium)hexacyanoferrate-(III). An aqueous solution of hydrogen or potassium hexacyanoferrate(III) was made alkaline with an excess of tetrabutylammonium hydroxide and extracted with chloroform. The chloroform extract was washed with water and dried ($CaSO_4$). Evaporation yielded a pale green powder for which iodometric titration gave an equivalent weight of about 960 (Calc. for $C_{54}H_{108}N_9Fe$: 938)

Tetrabutylammonium dihydrogen hexacyanoferrate(III). The above preparation was dissolved in chloroform or methanol, and *p*-toluenesulfonic acid (2 equiv.) was added. The solution was used as such for oxidations.

Oxidations

Compound 1 *in chloroform.* 1.97 g (2.1 mmol) of tetrabutylammonium hexacyanoferrate(III) and 4.2 mmol of *p*-toluenesulfonic acid was added to 220 mg (1 mmol) of *1* dissolved in dry chloroform. After standing overnight at room temperature diethyl ether and water were added, the ether layer was washed with water and dried. Evaporation left 276 mg of product which yielded 36 mg pure 3,3′,5,5′-tetra-*tert*-butyl-4,4′-dihydroxybibenzyl (*2*) on preparative TLC. M.p. 170–171 °C (Lit.[8] 170.0–170.5 °C). NMR ($CDCl_3$): δ 1.42 (36 H, s, *t*-Bu), 2.83 (4 H, s, Ar–CH_2–), 5.00 (2 H, s, ArOH), 6.99 (4 H, s, ArH).

Compound 1 *in methanol with* $H_3Fe(CN)_6$. The procedure described by Taimr and Pospíšil was followed. Separation of the reaction mixture by preparative TLC gave 20–30 % of 2,6-di-*tert*-butyl-4-methyl-4-methoxy-2,5-cyclohexadienone (*3*) m.p. 90–91 °C (Lit.[1] 93 °C). NMR CCDCl$_3$: δ 1.25 (18 H, s, *t*-Bu), 1.37 (3 H, s, CH_3), 3.12 (3 H, s, OCH_3), 6.45 (2 H, s, ring protons). In addition, between 2 and 3 % of *4* was isolated in the different runs.

Compound 1 *in methanol with* $Bu_4NH_2Fe(CN)_6$. To 1 mmol of *1* in methanol was added 2.1 mmol of tetrabutylammonium hexacyanoferrate(III) and 4.2 mmol of *p*-toluenesulfonic acid. After 1 h at room temperature the products were precipitated with water, extracted with ether and separated on preparative TLC. 39–42 % of unreacted *1* was recovered. The rest was a mixture of *3* and *4* in proportions (determined from NMR-spectra and by weighing the TLC fractions) which varied between 1:6 and 1:10 in different runs, the main product being 2,6-di-*tert*-butyl-4-(methoxymethyl)-phenol (*4*). NMR ($CDCl_3$): δ 1.44 (18 H, s, *t*-Bu), 3.34 (3 H, s, OCH_3), 4.29 (2 H, s, $ArCH_2$), 5.14 (1 H, s, ArOH), 7.1 (2 H, s, ArH).

Compound 5 *in methanol with* $H_3Fe(CN)_6$. To 264 mg (1 mmol) of *5* in methanol was added 450 mg (2.1 mmol) of $H_3Fe(CN)_6$ in methanol. After 1 h at room temperature the products were precipitated with water and extracted with ether. Preparative TLC gave two products: 2,6-di-*tert*-butyl-1,4-benzoquinone (56.4 mg), m.p. 66–67 °C (Lit.[12] 66.5–68 °C) and 3,5-di-*tert*-butyl-4-hydroxyphenyl ethyl ketone (*7*) (4.0 mg). The ketone was identified by comparison with material synthesized by oxidation of *5* with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, m.p. 137 °C (Lit.[11] 137 °C).

Compound 5 *in methanol with* $Bu_4NH_2Fe(CN)_6$. To 52.8 mg (0.2 mmol) of *5* in methanol was added 394.8 mg (0.4 mmol) of tetrabutylammonium hexacyanoferrate(III) and 145.5 mg (0.8 mmol) of *p*-toluenesulfonic acid. After 1 h at room temperature TLC showed an almost quantitative conversion to 3,5-di-*tert*-butyl-4-hydroxyphenyl ethyl ketone (*7*). NMR ($CDCl_3$): δ 1.18 (3 H, t, H_β), 1.43 (18 H, s, *t*-Bu), 2.92 (2 H, q, H_α), 5.70 (1 H, s, AtOH), 7.81 (2 H, s, ArH). $J_{\alpha\beta} = 7.0$ Hz.

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2,3-Epoxyindanone. Synthesis and Reactions

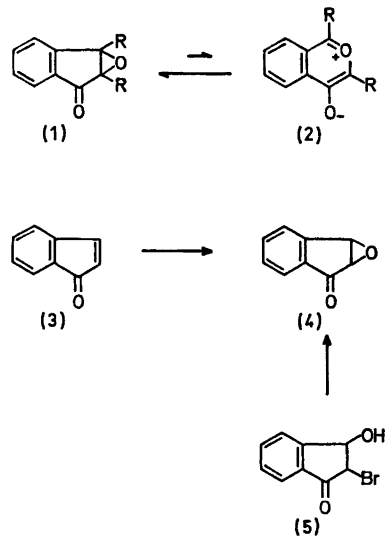
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2,3-Epoxyindanone has been synthesised by the bromohydrin-epoxide reaction from 2-bromo-3-hydroxy-1-indanone by means of silver oxide; both the intermediates and the epoxide are sensitive to alkali. Direct epoxidation of 1-indenone was less satisfactory. Both irradiation and Lewis acid catalysis resulted in rearrangement of the epoxide to isocoumarin. 2-Hydroxy-1-indanone was almost quantitatively oxidised to 1,2-indanedione by *N*-bromosuccinimide.

In a recent paper we have described a synthesis of *N*-substituted 1a,6a-dihydroindeno[1,2-*b*]azirin-6(1*H*)-ones and the photochemical transformation of these compounds into the valence isomeric isoquinolinium-4-oxides.[1] In this report we describe a synthesis and some reactions of 1a,6a-dihydroindeno[1,2-*b*]oxiren-6-one (*4*) which is the oxygen analogue. Disubstituted derivatives of *4* are known in the literature as indanone oxides;[2] in this work they are named indenone epoxides. Most attention has been devoted to 2,3-diphenylindanone epoxide (*1*, R=Ph) which exists in equilibrium with the valence isomeric benzopyrylium-4-oxide (*2*) or ylide;[2,3] the equilibrium position varies with the conditions but is far over towards the epoxide form. The compound readily undergoes 1,3-dipolar cycloadditions *via* a carbonyl ylide. Valence isomerisation of the epoxide (*4*) would yield the parent benzo[*c*]-pyrylium-4-oxide (*2*, R=H) which in the absence of stabilising phenyl groups is expected to be unstable; this postulate is also in accordance with recent experience for the thio analogue.[4]

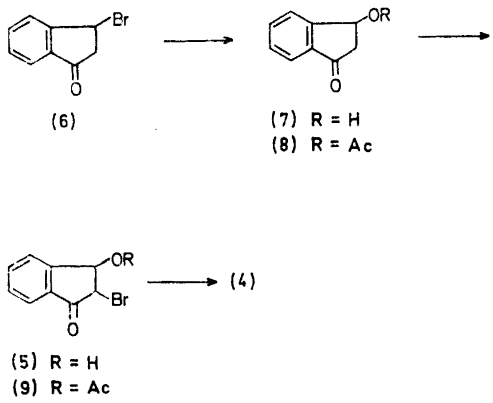
The desired epoxide *4* can in principle be prepared by oxidation of 1-indenone (*3*). A satisfactory synthesis of the latter has been developed,[1] but indenone polymerises very readily under acidic or alkaline conditions.[1,5] A slow peracid oxidation (electrophilic) is likely



Scheme 1.

to result in extensive polymerisation besides competitive Baeyer-Villiger oxidation as reported for related [6,7] systems. Instead a Michael type oxidation [8] was employed using *t*-butylhydroperoxide in benzene solution in the presence of catalytic amounts of Triton-B as base. Some of the desired epoxide *4* was obtained by this method but the major product was polymeric. With phenyl group stabilisation epoxidation proceeds readily in alkaline hydrogen peroxide.[9] Since the direct epoxidation of indenone was preparatively unsatisfactory, the synthesis of the epoxide was directed through the vicinal bromohydroxy indanone (*5*). The synthesis of the latter is shown in Scheme 2. Free radical bromination of indanone readily yields 3-bromo-1-indanone (*6*).[1] Heating of the latter with silver acetate in acetic acid furnished the

acetate *8*. During our work an alternative synthesis of *8* was reported which consists in reductive acetylation of 1,3-indanedione.[10] Ionic bromination of *8* furnished the 2-bromo derivative *9*. In the ensuing ester hydrolysis, alkaline conditions are to be avoided to prevent ready elimination to the indenone which would polymerise. Hydrolysis of the acetate group in *9* was therefore carried out under acidic conditions. We prefer, however, to carry out ester hydrolysis before bromination; the ester *8* is suspended in 3 N HCl in the cold for one day to yield 3-hydroxyindanone (*7*). Less satisfactory was the direct preparation of *7* by heating of the bromo derivative *6* in aqueous acetone. Ionic bromination as above then furnished *5*.



Scheme 2.

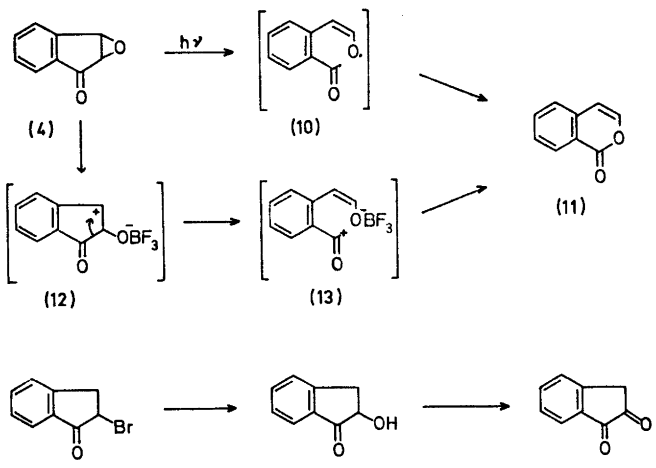
The vicinal bromo and hydroxy substituents in *5* must have the *trans* arrangement for cyclisation to the epoxide *4*. Bromination of the enolised ketone *7* is expected to yield the *trans* isomer for steric reasons; furthermore, ready epimerisation is likely to take place through the reversible enolisations. In the cyclisation reaction alkaline conditions are to be avoided. The reaction was therefore carried out with freshly prepared silver oxide[11] suspended in hexane/benzene under a nitrogen atmosphere; a "push and pull mechanism" is visualised. The absence of any 1,3-diketone in the reaction product excludes any reaction from the *cis* form.[12]

The NMR spectrum of the epoxide shows the methine protons as doublets at τ 5.5 (H-3) and 6.1 (H-2), J 2.5 Hz. The vinical coupling is less than for a simple epoxide but agrees with previous observations that fusion of a three-membered heterocyclic ring to another cyclic system decreases the vicinal coupling.[1,13]

The base peak in the mass spectrum at *m/e* 118 was due to [M − CO]. The only other important fragments are due to a further loss of CO (*m/e* 90, 40 %) and HCO (*m/e* 89, 35 %); the molecular ion (*m/e* 146) intensity was 40 %. The synthetic precursor for the epoxide, 2-bromo-3-hydroxyindanone, showed no molecular ion; ready bromine expulsion instead gave the base peak at *m/e* 147. The relative intensity for the mass number (146) corresponding to the epoxide was less than 2 %, which virtually excludes any pyrolytic cyclisation of *5* with HBr liberation. In fact the spectrum is almost identical with the spectrum of the acetate *9*. The latter by analogy to many acetates has ketene expulsion as the totally dominating initial fragmentation; the additional bromine expulsion gave the base peak at *m/e* 147.

The behaviour of the epoxide towards light was studied by illumination of a 0.02 M deoxygenated solution in benzene under nitrogen using a medium pressure Hg-lamp. The UV absorptions recorded during the illumination showed the appearance of a transient spectrum which disappeared on prolonged illumination with the precipitation of a polymeric material. The intermediate product has been identified as isocoumarin by comparison with authentic material prepared according to the literature.[14] Illumination of a solution of isocoumarin under the above conditions also led to polymer formation. With the use of a pyrex filter the maximum yield of isocoumarin was obtained after 30 min; without this filter the reaction should be stopped after 15 min.

Aza analogues of *4*, however, are transformed on photolysis to the valence isomeric isoquinolinium-4-oxides.[1] Substituted indanone epoxides such as 2,3-diphenylindanone epoxide exist in equilibrium with its benzopyrylium valence isomer *2*; the equilibrium position on irradiation is dependent on the wavelength of the light used.[2,15] Prolonged illumination led to dimerisation and formation of 3,4-diphenylisocoumarin. Substitution of the 2-phenyl group in the indanone with a methyl group leads to more rapid dimerisation.[16] The quantum yield for the



Scheme 3.

isomerisation of substituted indanone epoxides to benzo[c]pyrylium-4-oxides decreases when less bulky substituents are attached to the epoxide ring;[15] in the case of *4* no pyrylium betaine was detected.

The ylide nature of substituted pyrylium oxides has been widely used in cyclo-addition reactions.[2,3] Addition of dipolarophiles to solutions of the epoxide *4* followed by illumination failed to yield any adducts. The trapping experiment in itself, however, may not exclude pyrylium formation as it has been found that the thio analogue dimerises preferentially in the presence of added dipolarophiles.[4] No dimeric material from *4* was isolated; isocoumarin rather than pyrylium formation was favoured. Isocoumarin was also formed on Lewis acid isomerisation of *4* by treatment with boron trifluoride etherate. The incipient carbonium ion during the rearrangement is visualised in the benzylic position (Scheme 3). The usual facile migration of an acyl group in this case did not occur presumably because of four-ring formation. Hydride shift with carbonyl formation would lead to 1,2-indanedione in the product but was not seen in chromatographic comparisons with authentic 1,2-dione. The latter was prepared in almost quantitative yield by oxidation of 2-hydroxy-1-indanone with *N*-bromosuccinimide. This oxidation method is superior to the reported chromic acid oxidation which easily proceeds further to homophthalic acid.[17]

EXPERIMENTAL

NMR spectra were recorded with a Varian A-60 A instrument, the UV spectra with a Cary 14 spectrophotometer and the MS spectra with an AEI-902 mass spectrometer.

3-Acetoxy-1-indanone (8). A solution of 3-bromo-1-indanone[1] (10.8 g, 0.051 mol) and silver acetate (8.4 g, 0.051 mol) in acetic acid (300 ml) was heated under reflux for 24 h. The precipitated silver bromide was removed by filtration of the cold reaction mixture and the filtrate evaporated. The residual oil was purified by distillation; b.p. 89 °C/0.15 mmHg, yield 6.2 g (64 %). (Found: C 69.34; H 5.19. Calc. for $C_{11}H_{10}O_3$: C 69.40; H 5.26); $\tau(CCl)_4$ 8.0 (Me), 7.0 and 7.5 (2 H-2, ABX, J_{AB} 19 Hz, J_{AX} 7Hz, J_{BX} 3 Hz), 3.8 (H-3, ABX), 2.3 – 2.9 (4 H-arom.); λ_{max} (CH_2Cl_2) 240 (log ε 4.00), 280 (3.14), 289 nm (3.16).

3-Hydroxy-1-indanone (7). (a) A suspension of 3-acetoxy-1-indanone (6.0 g, 0.033 mol) in 3 N HCl (400 ml) was stirred at room temperature for 24 h. The reaction mixture was next extracted with chloroform, the extracts dried and evaporated, and the residual oily material crystallised from ether/petroleum at about 0 °C; yield 4.4 g (90 %), m.p. 51 °C (Found: C 72.65; H 5.40. Calc. for $C_9H_8O_2$: 72.90; H 5.40).

(b) 3-Bromo-1-indanone (7.0 g, 0.033 mol) was dissolved in water (500 ml) and acetone (300 ml). The solution was refluxed for 3 h, most of the acetone was evaporated, water was added and the title compound extracted into chloroform and isolated as above; yield 2.5 g (51 %).

2-Bromo-3-hydroxy-1-indanone (5). (a) 3-Hydroxy-1-indanone (2.0 g, 0.014 mol) was dissolved in anhydrous ether (400 ml) and bromine (2.2 g, 0.014 mol) addition carried out in the following way: A drop of bromine was added to the solution at room temperature. The solution

was then cooled to about 5 °C and the bromine added dropwise at such a rate that the solution was decolorised before addition of the next drop of bromine. In this way selective mono-bromination is achieved. The decolorised solution was next washed with water, dried and the solvent evaporated. The residual oil was chromatographically homogeneous and was used further as such; yield 2.9 g (93 %). For analytical purposes a part of the material was crystallised from $CHCl_3/CCl_4$/petroleum at 0 °C, m.p. 84–86 °C. (Found: C 47.24; H 3.08. Calc. for $C_9H_7BrO_2$: C 47.58; H 3.11); τ($CDCl_3$) 5.5 (H-2, $J_{2,3}$ 3.5 Hz), 4.6 (H-3), 1.9–2.5 (4 H-arom.); λ_{max} (CH_2Cl_2) 249 (log ε 4.03), 285 nm (3.28).

(b) 3-Acetoxy-2-bromo-1-indanone (2.0 g, 0.007 mol) in a solution of methanol (100 ml) and 3 N HCl (80 ml) was kept at room temperature for 24 h. Addition of water followed by chloroform extraction furnished the title compound as above; yield 1.4 g (86 %).

3-Acetoxy-2-bromo-1-indanone (9) was prepared as *5* above from 3-acetoxy-1-indanone; yield 87 %, m.p. 97 °C (ether/petroleum). (Found: C 48.56; H 3.50. Calc. for $C_{11}H_9BrO_3$: C 48.80; H 3.34); τ($CDCl_3$) 7.8 (Me), 5.4 (H-2, $J_{2,3}$ 3 Hz), 3.6 (H-3), 2.2–2.5 (4 H-arom.); λ_{max} (CH_2Cl_2) 255 (log ε 4.24), 288 nm (3.25).

2,3-Epoxyindanone (4). 2-Bromo-3-hydroxy-1-indanone (2.9 g, 0.013 mol) was dissolved in a mixture of benzene (250 ml) and hexane (500 ml), and the solution was deoxygenated by bubbling nitrogen through. Activated silver oxide[11] was then added and the stirred suspension heated under reflux in a nitrogen atmosphere for 24 h. The insoluble material from the cold reaction mixture was removed by filtration and the solvent was evaporated at reduced pressure. The residual oil was extracted with, warm hexane (500 ml), the hexane evaporated under reduced pressure and the residual oil crystallised from hexane; yield 1.1 g (60 %), m.p. 62 °C. (Found: C 74.10; H 4.25. Calc. for $C_9H_6O_2$: C 73.97; H 4.11); τ($CDCl_3$) 6.1 (H-2, $J_{2,3}$ 2.5 Hz), 5.5 (H-3), 1.8–2.8 (4 H-arom); λ_{max} (CH_2Cl_2) 261 (log ε 3.79), 299 nm (3.04).

Photochemical isomerisations; Isocoumarin (11). A solution of 2,3-epoxyindanone (290 mg, 0.002 mol) in anhydrous benzene (100 ml) was deaerated by bubbling nitrogen gas through the solution. The solution was next illuminated at room temperature using a Hanovia medium pressure Hg-lamp with a pyrex filter. The illumination time was 30 min. The solution was then evaporated and the residual material chromatographed on a silica column. The major reaction product, isocoumarin, was eluted with benzene/ethyl acetate (4:1); yield 89 mg (33 %). The identity of the product was verified by comparison with authentic material prepared after the literature.[14]

Isocoumarin (11) by Lewis acid isomerisation. Boron trifluoride etherate (1 ml, 0.007 mol) was added to a solution of 2,3-epoxyindanone (250 mg, 0.0017 mol) in anhydrous ether (20 ml). The resultant solution was left for 15 min at room temperature. The solution was then washed with water, dried and evaporated. The residual material was chromatographed as above; the yield of isocoumarin was 39 % (96 mg).

1,2-Indanedione (16). 2-Hydroxy-1-indanone[17] was prepared from 2-bromo-1-indanone according to the literature.[17,18] The former (1.0 g, 0.007 mol), *N*-bromosuccinimide (1.2 g, 0.007 mol) and catalytic amounts of benzoyl peroxide were heated together in refluxing carbon tetrachloride (200 ml) for 20 min. The precipitated succinimide was removed from the cold reaction mixture at about 0 °C and the solvent evaporated. The residual solid was recrystallised from carbon tetrachloride; yield 1.0 g (99 %), m.p. 95–115 °C. The identity of the material was verified by comparison with an authentic sample otherwise prepared.[19]

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Synthesis of Methyl 2,6-Dideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside ("Methyl α-Axenoside")

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The synthesis of methyl 2,6-dideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside and of the corresponding free sugar is described. The physical constants agree with those previously reported for methyl α-axenoside and axenose, respectively.

In 1973, Arcamone and co-workers described the isolation of a new branched sugar, axenose, from hydrolysates of axenomycin B, one of three antibiotics produced by *Streptomyces lysandri n.sp.* The sugar was obtained in crystalline form, as were the derived methyl α- and β-glycopyranosides and identified as 2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexose.[1]

In the present paper we describe the synthesis of this sugar and its methyl α-L-glycopyranoside. The physical constants of the synthetic products confirm the structural assignment of axenose. Korte, Claussen and Snatzke have reported the synthesis of racemic 2,6-dideoxy-3-*C*-methyl-DL-*xylo*-hexose.[2] Howarth, Szarek and Jones have reported the synthesis of 2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-D-*xylo*-hexose (D-arcanose) thereby confirming the structure of the naturally occurring, corresponding L-enantiomer.[3] However, neither D-axenose nor its glycosides were synthesised at that time, since axenose was yet to be discovered in Nature. In designing the present synthesis, we preferred to start from L-fucose, which is more readily available than L-galactose, the required starting material for making axenose using a similar synthetic pathway to that described for the synthesis of D-arcanose.

L-Fucose was converted into 3,4-di-*O*-acetyl-L-fucal.[4,5] This glycal, upon treatment with acidic methanol afforded an anomeric mixture which was separated by column chromatography on silica gel. Deacetylation of each of the pure anomers of methyl 3,4-di-*O*-acetyl-2,6-dideoxy-L-*lyxo*-hexopyranoside afforded the crystalline glycosides Ia and Ib. Methyl 2,6-dideoxy-α-L-*lyxo*-hexopyranoside (Ia) was obtained in 51 % yield from 3,4-di-*O*-acetyl-L-fucal. Our constants for this compound (m.p. 58 – 59 °C, $[\alpha]_D^{20}$ −173°, chloroform) do not agree with those described by Brimacombe and Portsmouth [6] for the D-enantiomer (m.p. 70 – 72 °C, $[\alpha]_D^{16}$ +122°, chloroform) and the reason for this is not clear. The glycoside Ia was benzoylated in the 3-position in 64 % yield by treatment with benzoyl chloride and pyridine in chloroform at −5 to −10 °C. Surprisingly, partial benzoylation with *N*-benzoylimidazole in chloroform using the method described by Chittenden,[7] gave almost equal amounts of the 3- and 4-benzoates of Ia. This difference may indicate a difference in conformational equilibrium of Ia in the two experiments (−5 to −10 °C as compared to 62 °C using *N*-benzoylimidazole). The 3-*O*-benzoyl derivative II was characterized by NMR. The position of the benzoyl group is verified by the H-3 octet having the highest chemical shift (δ) of all the ring protons, as expected for a hydrogen atom attached to a benzoyloxy carbon. The spectral assignments were corroborated by spin decoupling experiments (Table 1). The 3-benzoyl derivative II was benzylated with benzyl trifluoromethanesulfonate as devised by Lemieux and Kondo.[8] The product III was debenzoylated to give the 4-benzyl ether IV in 58 %

Table 1. First-order chemical shifts and coupling constants of Ia-VIII (60 MHz NMR) Solvents: For sext, sextet; oct,

Substance	Chemical shifts (δ) downfield from TMS and multiplicity								
	H-1	H-2	H-2′	H-3	H-4	H-5	H-6	$OCOCH_3$	OCH_3
Ia 3,4-acetate [a]	4.87t	1.8–2.2m	1.8–2.2m	5.2–5.5m	5.1–5.3m	4.03q	1.13d	1.97s 2.15s	3.33s
Ia	4.87t	1.83±0.02m	1.83±0.02m	3.6–4.3m	3.6–4.3m	3.6–4.3 m	1.22d	–	3.35s
II [b]	4.83q	1.9–2.45m	1.9–2.45m	5.40 oct	3.92d	3.97q	1.27d	–	3.35s
III	4.92q	2.17m or 2.40m	2.40m or 2.17m	5.52 oct	3.80d	4.02q	1.23d	–	3.35s
IV	~4.75	1.87±0.02m	1.87±0.02m	3.8–4.2m	3.48d	3.92q	1.25d	–	3.28s
V	5.08q	3.14q	2.37sext	–	3.47t	4.13oct	1.32d	–	3.33s
VIa	4.82q	2.05q	1.58sext	–	3.03	4.28q	1.23d	–	3.35s
VIb	4.7	1.7–1.9m	1.7–1.9m	–	3.10	3.98 oct	1.28d	–	3.28s
VIIa	4.82q	1.4–2.2m	1.4–2.2m	–	3.13s	4.35q	1.25d	–	3.38s
VIIb	4.77q	1.7–2.0m	1.7–2.0m	–	3.20s	4.00q	1.28d	–	3.33s
VIII	7.39d	5.43q	–	–	3.55q	4.48 oct	1.48d	–	

[a] Confirmed by adding $Eu(DPM)_3$. [b] Confirmed by spin decoupling experiments.

yield from II. Ruthenium tetroxide oxidation [9] of IV afforded the 3-ulose derivative V in 97 % yield. Treatment of V with methylmagnesium iodide gave two main products, (yield 75 %) VIa and VIb in a ratio of 1:2.4. These were hydrogenated to give VIIa and VIIb, respectively. The stereochemical arrangement of these products at C-3 was demonstrated by strong intramolecular hydrogen bonding (C-3 OH to OCH_3) of VIIa and by the change in optical rotation of VIIa and VIIb upon the addition of cuprammonium hydroxide ("Cupra B"),[10] which caused a shift in molecular rotation at 436 nm of +1746° for VIIb (*lyxo* configuration). The corresponding shift for VIIa (*xylo* configuration) was +32°. The glycoside VIIa has physical constants identical to those of methyl α-axenoside.[1] Furthermore, hydrolysis of VIIa affords the corresponding free sugar with physical constants in agreement with those previously reported for the natural sugar.[1]

The reaction of the uloside V with methylmagnesium iodide thus gave a mixture of two products, the predominant one having the *lyxo*-configuration (VIb). This is in contradistinction to the results obtained when methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*threo*-hexopyranosid-3-ulose was similarly treated to give the corresponding 3-*C*-methyl-*xylo*-hexoside [3] in high yield. This difference probably reflects the stereochemical rigidity conferred by the 4,6-*O*-benzylidene group in the latter derivative. The NMR parameters for V are indicative of a skew-boat conformation or a dynamic conformational equilibrium averaging this conformation. In this conformation (Scheme 1) attack from either side of the pyranose ring is about equally hindered.

In preliminary experiments, treatment of V with the 1,3-dithiane anion [11] gave products having both the *xylo* and the *lyxo* configuration in a ratio of 1:1. The overall yield of VIa was, however, not improved and this route offers little, if any, advantage over the one shown in Scheme 1. Treatment of V with methyllithium afforded an olefinic product with NMR parameters suggesting structure VIII.

substance Ia D_2O, for all others $CDCl_3$. Observed multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; octet; m, multiplet.

				Coupling constants (Hz)									
CCH_3	$PhCH_2$	OH	Aromatic	$J_{1,2}$	$J_{1,2'}$	$J_{2,2'}$	$J_{2,3}$	$J_{2',3}$	$J_{2',4}$(?)	$J_{2,4}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
–	–	–	–	2.5	2.5				–	–		<1	6.5
–	–		–	2.5	2.5				–	–			6.5
–	–	2.27s	7.1–7.5m	2 or	3.5 or		6 or	11.5 or	–	–	3	<1	6.5
			7.8–8.1m	3.5	2		11.5	6					
–	4.7m		7.1–7.5m	1.5 or	4 or		5 or	11 or	–	–	3	<1	6.5
			7.9–8.1m	4	1.5		11	5					
–	~4.75 ABq		7.2–7.7m								3	<1	6.5
–	4.38d 4.61d $J_{H,H}$12		7.33	4.5	1.5	14	–	–	1.5	0	–	2	6.5
1.25s	4.67s	4.25s	7.35	4	1.5	14	–	–	1.5	0	–	<1	6.5
1.38s	4.7s	2.53s	7.35				–	–	–		–	1	6.5
1.23s	–	2.4,3.9 broad s	–	4	1.5				–	–	–	<1	6.5
1.38s	–	3.0 broad s	–	4	2	–	–	–	–	–	–	<1	6.5
	4.53d 4.83d $J_{H,H}$12		7.37	6	–	–	–	–	–	1.5	–	2	6.5

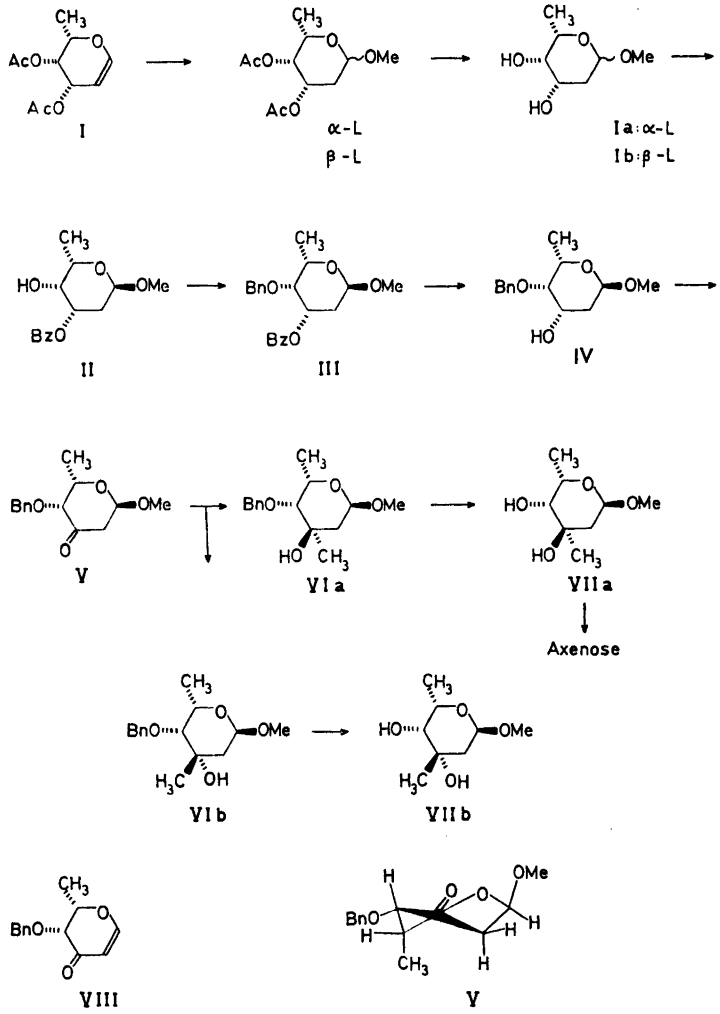
EXPERIMENTAL

General methods were the same as those described in a recent paper.[5] Column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck).

Methyl 2,6-dideoxy-α-L-lyxo-hexopyranoside (Ia). 3,4-Di-*O*-acetyl-1,2,6-trideoxy-L-*lyxo*-hex-1-enopyranose ("di-*O*-acetyl-L-fucal") (4.80 g)[4,5] was dissolved in analytical grade methanol (200 ml). Acetyl chloride (1.94 ml) was added to provide approximately 0.5 % hydrochloric acid. After standing at room temperature overnight, the mixture was neutralized with silver carbonate (10 g), filtered and concentrated.[12] The mixture was reacetylated with pyridine and acetic anhydride (2:1) at room temperature overnight, concentrated and repeatedly co-distilled with toluene, yielding a syrup (5.70 g) which (TLC, toluene-ethyl acetate, 4:1) contained one major (R_F ~0.45) and two minor components (slightly lower R_F). GLC (150 °C) showed the presence of three components whose peak areas gave a ratio of 77:10:13. Chromatography using a column of silica gel (250 g) eluted with toluene, followed by a gradient containing up to 20 % ethyl acetate in toluene gave first the main fraction (3,4-acetate of Ia) (3.20 g) as a colourless syrup, a second component (0.28 g, β anomer) and a fraction (0.35 g) which was not characterized. Deacetylation of the major component with 3 % ammoniacal methanol (100 ml) at room temperature overnight and concentration afforded chromatographically pure, crystalline Ia (1.95 g) which, after recrystallization from diisopropyl ether had m.p. 58–59 °C, $[\alpha]_D$ −173° (*c* 0.5, chloroform) (Found: C 51.8; H 8.7. $C_7H_{14}O_4$ requires: C 51.8; H 8.7).

Similar deacetylation and work-up of the second component (methyl 3,4-di-*O*-acetyl-2,6-dideoxy-β-L-*lyxo*-hexopyranoside, Ib, 0.28 g) gave chromatographically pure crystals (0.15 g) which on recrystallization from di-isopropyl ether gave methyl 2,6-dideoxy-β-L-*lyxo*-hexopyranoside m.p. 108–109 °C, $[\alpha]_D$ +48° (*c* 0.5, chloroform). (Found: C 51.7; H 8.8. $C_7H_{14}O_4$ requires: C 51.8; H 8.7).

Methyl 3-O-benzoyl-2,6-dideoxy-α-L-lyxo-hexopyranoside (II). Methyl 2,6-dideoxy-α-L-*lyxo*-hexopyranoside (Ia) (3.55 g) was dissolved in dry chloroform (20 ml). Benzoyl chloride (3.08 g), dissolved in dry chloroform (20 ml) containing pyridine (10 ml) was added dropwise while the reaction mixture was maintained at −5 to −10 °C. After standing overnight at 0 °C and then for a further 24 h at room temperature, TLC (toluene-ethyl acetate, 3:2) showed the presence of II (major spot, R_F about 0.6) in addition to minor amounts of the dibenzoate, 4-benzoate, and starting material. The mixture was diluted with chloroform (100 ml) and the solution shaken sequentially with



Scheme 1. Bz = Ph – CO, Bn = Ph – CH_2.

water, ice-cold 2 M sulfuric acid, saturated aqueous sodium hydrogen carbonate, and water. The chloroform solution was dried over magnesium sulfate, filtered and concentrated giving a syrup, from which pure II (3.75 g) was obtained by crystallization from diethyl ether-hexane, m.p. 88–89 °C, $[\alpha]_D$ –156° (*c* 0.5, chloroform). (Found: C 63.1; H 6.7. $C_{14}H_{18}O_5$ requires: C 63.1; H 6.8).

*Methyl 4-O-benzyl-2,6-dideoxy-α-*L*-lyxo-hexopyranoside (IV).* A solution of the above monobenzoate II (3.21 g) and 2,6-lutidine (1.94 g) in dry chloroform (10 ml) was added dropwise, to a stirred solution (–60 °C) of benzyl trifluoromethanesulfonate prepared as described by Lemieux and Kondo [8] from benzyl alcohol (3.93 g), 2,6-lutidine (3.89 g), and trifluoromethanesulfonic anhydride (10.24 g) in chloroform (30 ml). After 48 h at –60 to –70 °C, more 2,6-lutidine (1.94 g) was added and the mixture poured into ice-cold, saturated aqueous sodium hydrogen carbonate (200 ml). The mixture was extracted with chloroform; the combined chloroform phases were shaken with, in turn, ice-cold 2 M sulfuric acid, aqueous sodium hydrogen carbonate and then water. The chloroform solution was then dried over magnesium sulfate, filtered and concentrated to a syrup containing one major product in addition to some unreacted II which had a lower mobility on TLC (toluene-ethyl acetate, 8:2). An aliquot of this syrup was purified by TLC (above solvent) to give a substance which gave an NMR spectrum (including decoupling experiments)

consistent with the substance being methyl 4-*O*-benzyl-3-*O*-benzoyl-2,6-dideoxy-α-L-*lyxo*-hexopyranoside (III). The syrup was dissolved in methanol (500 ml), barium oxide (2.0 g) was added and the mixture was refluxed for 45 min. Neutralization with solid carbon dioxide and concentation gave a syrup which was taken up in diethyl ether, filtered to remove salts and concentrated. Column chromatography on silica gel (200 g) using hexane-diethyl ether (1:9) as eluent afforded chromatographically pure crystalline IV (1.76 g). Recrystallization from diisopropyl ether-hexane gave material with m.p. 82 °C $[\alpha]_D$ −149° (*c* 0.6, chloroform). (Found: C 66.7; H 8.1. $C_{14}H_{20}O_4$ requires C 66.6; H 8.0).

*Methyl 2,6-dideoxy-3-*C*-methyl-α-*L*-*xylo*-hexopyranoside (VIIa).* The 4-*O*-benzyl ether IV (700 mg) was stirred with a mixture of potassium periodate (3.82 g), ruthenium dioxide (50 mg), and potassium carbonate (700 mg) in ethanol-free chloroform (15 ml) and water (15 ml) at room temperature. TLC (hexane-diethyl ether, 2:8) indicated that no starting material remained after 6 h; therefore, propan-2-ol (1 ml) was added and the mixture stirred at room temperature for another 15 min. Then, the mixture was filtered through Celite, the aqueous layer extracted with chloroform and the combined chloroform extracts dried over magnesium sulfate, filtered and concentrated to give syrupy V (0.67 g). The structure of V was verified by IR [strong absorption at 1730 cm^{-1} (film)] and by NMR. The ulose V (670 mg) in dry benzene (10 ml) was added dropwise to a cooled (0 °C), stirred solution of methylmagnesium iodide (prepared from magnesium (1.010 g) and iodomethane (3.44 ml) in dry diethyl ether (50 ml)). The reaction mixture was allowed to stand at room temperature overnight, poured into ice-water (100 ml) and the aqueous phase was extracted with chloroform. The combined chloroform extracts were dried over magnesium sulfate, filtered and concentrated to a syrup (0.77 g). GLC (160 °C) gave three peaks in an approximate ratio of 2:5:14. Column chromatography on silica gel (200 mg), packed in toluene and eluted with toluene containing gradually increasing concentrations of ethyl acetate (up to 40 % in toluene) gave a fast-moving, unidentified, minor fraction (40 mg) and then VIa (160 mg) as a colourless syrup. The last compound to be eluted, VIb, (380 mg) was also obtained as a colourless syrup. Debenzylation of VIa (160 mg) in absolute ethanol (25 ml) by catalytic hydrogenation at room temperature and atmospheric pressure for 3 h in the presence of a catalytic amount of 10 % palladium on charcoal, filtration and concentration afforded crystalline VIIa (100 mg), m.p. 102 – 103 °C (from di-isopropyl ether), $[\alpha]_D$ −151° (*c* 0.5, chloroform) (Arcamone and co-workers [1] give m.p. 101 – 103 °C, $[\alpha]_D$ −142° (chloroform) for methyl α-axenoside, derived from the natural sugar). IR: 3580 cm^{-1} (sharp), 3535 cm^{-1} (broad) (*c* < 0.005 M in carbon tetrachloride).

*Methyl 2,6-dideoxy-3-*C*-methyl-α-*L*-lyxo-hexopyranoside (VIIb)* The above benzyl ether VIb (380 mg) was hydrogenated as described above for IVa. Recrystallization of the product from diisopropyl ether afforded VIIb (0.198 g), m.p. 77 – 78 °C, $[\alpha]_D$ −161° (*c* 0.5 in chloroform). IR: 3575 cm^{-1} (*c* 0.005 M in carbon tetrachloride). (Found: C 54.7; H 9.2. $C_8H_{16}O_4$ requires: C 54.5; H 9.2).

*2,6-Dideoxy-3-*C*-methyl-*L*-xylo-hexopyranose (axenose).* The above glycoside VIIa (28.4 mg) was hydrolyzed in 1 M aqueous hydrochloric acid at room temperature for 4 h. The solution was neutralized with silver carbonate, filtered and concentrated. TLC (ethyl acetate) showed the presence of a single component with a lower mobility than that of VIIa. Crystals (20.3 mg) were obtained from ethyl acetate. One recrystallization from ethyl acetate-hexane afforded crystals (15.3 mg) m.p. 103 – 106 °C. Further recrystallisation from the same solvents afforded material (13.1 mg), m.p. 106 – 108 °C, $[\alpha]_D$ −27° (*c* 0.32, water). Arcamone and co-workers report m.p. 111 – 112 °C, $[\alpha]_D$ −28.5° (water) for axenose.

Treatment of methyl 4-O-*benzyl-2,6-dideoxy-α-*L*-threo-hexopyranosid-3-ulose with methyllithium.* The uloside V (50 mg) was dissolved in dry diethyl ether and the solution was cooled to −20 °C. Methyllithium in diethyl ether (1 ml 1 M solution) was added at −20 °C and the solution was allowed to stand, first at this temperature for 20 min and then for a further 20 min at room temperature. Water was added and the mixture was extracted with chloroform. The combined chloroform solutions were shaken with water. Then chloroform solution was dried over magnesium sulfate, filtered and concentrated to a chromatographically homogeneous syrup (30 mg) (TLC, toluene-ethyl acetate 6:4). The NMR spectrum is consistent with the structure VIII. IR (chloroform) 1680 cm^{-1}.

Repetition of the above experiment at −78 °C and examination of the reaction mixture by TLC showed that VIII was formed together with minor amounts of VIb.

Optical rotations in cuprammonium hydroxide.[10] The optical rotations of the two glycosides VIIa and VIIb were measured at 436 nm in "Cupra B" (*c* 0.1 – 0.4) containing more than a ten-fold excess of copper. The optical rotations at the same wave-length of VIIa and VIIb in ammonium hydroxide was also determined. The results are given in the general part.

Acknowledgements. We are indebted to Professor Bengt Lindberg for his interest and to the Swedish Natural Science Research Council for financial support.

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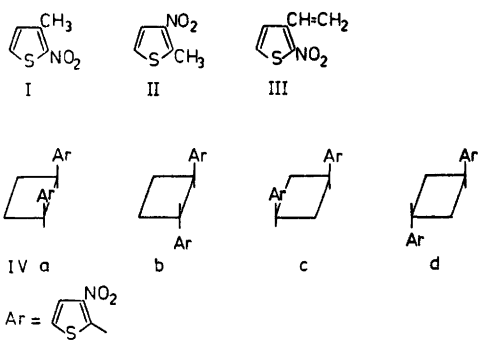
Acta Chemica Scandinavica B 29 (1975) 513—523

On the Base-catalyzed Reaction of Some Methyl Nitrothiophenes with Aldehydes. An Unexpected Cyclobutane Formation

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The methyl group in the *o*-methylnitrothiophenes shows varying reactivity towards aldehydes under base catalysis. 3-Methyl-2-nitrothiophene reacts to give vinyl compounds,[5,6] while 3-methyl-4-nitrothiophene does not react at all. 2-Methyl-3-nitrothiophene, with formaldehyde in methanol/water, gives, in addition to 3-nitro-2-vinylthiophene (V), a bis(3-nitro-2-thienyl)cyclobutane (IV), probably *trans*-1,2-bis(3-nitro-2-thienyl)- or *cis*-1,3-bis(3-nitro-2-thienyl)cyclobutane. 3-Nitro-2-vinylthiophene was also prepared from 1-(3-nitro-2-thienyl)-ethanol (XVI). 2-Isopropenyl-3-nitrothiophene (XXII), prepared *via* bis(2-isopropenyl-3-thienyl)iodonium chloride (XXI), gave with triethyl phosphite 6-methylthieno[3,2-*b*]pyrrole (XXIV), indicated by NMR and mass spectroscopy. 3-Nitro-2-vinylthiophene and 2-nitro-3-vinylthiophene gave no thienopyrrole upon treatment with triethyl phosphite.



In connection with our work on heteroaromatic boron compounds (*cf.* Ref. 1), *ortho*-nitrovinylthiophenes were desired as starting materials for the synthesis of thieno-fused borazarenes. For this reason we became interested in studying the condensation of *ortho*-methylnitrothiophenes with different aldehydes. Wesslén [2] and Bakke [3,4] have shown that *o*-nitrotoluene can be condensed with formaldehyde as paraformaldehyde, using potassium *t*-butoxide in dimethyl sulfoxide as catalyst. With equimolar amounts of *o*-nitrotoluene and formaldehyde, 2-(2-hydroxyethyl)nitrobenzene was obtained as the main product. However, all of our attempts to condense 3-methyl-2-nitrothiophene (I), 2-methyl-3-nitrothiophene (II), and 5-methoxycarbonyl-2-methyl-3-nitrothiophene under the above-mentioned conditions, or under Knoevenagel reaction conditions, were unsuccessful. While this work was in progress, a short communication appeared [5] in which a facile condensation was observed when I was heated under reflux with 40 % formalin in methanol containing catalytic amounts of pyrrolidine and acetic acid, yielding 2-nitro-3-vinylthiophene (III) in 73 % yield. Similar reactions were observed with benzaldehyde and *p*-anisaldehyde. The yields of styryl derivatives were between 32 and 35 %.[6] We could confirm the formation of III, and we also found that *o*-nitrotoluene did not react under these conditions.

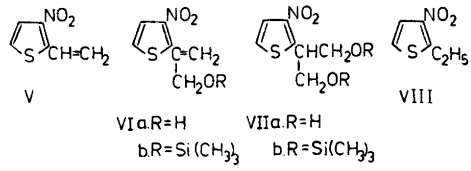
When II was reacted with formalin under the same conditions as I, a product (IV), m.p. 141.0 – 142.5 °C, was obtained with a molecular weight of 310 (mass spectrum). Its NMR spectrum showed, in addition to a thiophenic AB quartet (δ 7.13 and 7.54, J 5.6 Hz), complex signals at δ 4.5 and 2.5 with relative intensities 1:2. These data indicate that the product is one of the four possible bis(3-nitro-2-thienyl)-cyclobutanes (IVa – d). We supposed that de-

coupling of the signals centered at δ 4.5 should convert the methylene signals to an AA'BB' spectrum (for IVa–c) or an A_4 spectrum (for IVd), since cyclobutanes are known to invert rapidly.[7a] In the case of an AA'BB' spectrum it should be possible to determine the NMR parameters and consequently to determine whether the cyclobutane is 1,2- or 1,3-disubstituted. Upon attempted decoupling, however, the appearance of the methylene signals did change but not to a symmetrical pattern. The spectrum was too complex for further interpretation. Attempts to brominate IV in the benzylic positions with *N*-bromosuccinimide in carbon tetrachloride or ethylene dibromide, or with elemental bromine under basic conditions, failed. A benzylic dibrominated derivative of IV should give an AA'BB' NMR spectrum without decoupling.

It has been demonstrated by Dodson and Zielske [8] that *trans*-1,2-diphenylcyclobutane is the thermodynamically more stable isomer, since *cis*-1,2-diphenylcyclobutane obtained by hydrogenation of 1,2-diphenylcyclobutane was isomerized to the *trans* isomer by treatment with potassium *t*-butoxide. Less than 1 % of the *cis* isomer remained. In order to establish whether IV was thermodynamically controlled we attempted deuterium exchange in monodeuteriomethanol-deuterium oxide with pyrrolidine as base. Reflux for 63 h followed by work-up gave a sample which was analyzed on the mass spectrometer *via* GLC inlet. The spectrum of monomerized IV (see below) showed that 38 % of the molecules had been deuteriated. Thus, an eventual thermodynamical control cannot be carried out by proton exchange in IV. On the other hand deuterium exchange in the benzylic positions occurred within 50 min at 60 °C in hexadeuteriodimethyl sulfoxide-deuterium oxide containing catalytic amounts of pyrrolidine as could be followed by NMR spectroscopy. Treatment of IV in dimethyl sulfoxide-water with pyrrolidine at 67 °C for 1.5 h gave after work-up a 64 % recovery of material, which according to IR spectrum was starting material.

It has been found that in 1,3-disubstituted cyclobutanes the *cis* isomers generally are thermodynamically favored over the *trans* isomers.[8b] Furthermore, Lambert and Roberts found that for a substituent on a cyclobutane ring the equatorial position is favored over the axial position.[8c] These facts together with the finding by Dodson and Zielske make us believe that IV is either *trans*-1,2-bis(3-nitro-2-thienyl)cyclobutane (IVb) or *cis*-1,3-bis(3-nitro-2-thienyl)-cyclobutane (IVc). An X-ray diffraction investigation of compound IV is under progress.

Mass spectral analysis has been used for structure determination of cyclobutanes.[7b] Gross and Wilkins reported that the two isomeric 1,2-diphenylcyclobutanes show loss of ethylene from their molecular ions.[9] Schauble *et al.* took such a loss as evidence for the structure of their photodimer of 4-nitrostyrene to be a 1,2-disubstituted cyclobutane.[10] However, the mass spectrum of *trans*-1,3-diphenylcyclobutane also shows an ion at *m/e* 180 (relative abundance 0.1 %), corresponding to loss of fragment *m/e* 28 from the molecular ion (2.6 %).[11] The fragment ion is a member of a cluster centered at *m/e* 178 as is the case of the two 1,2-diphenylcyclobutanes.[9] The abundance of 0.2 % of the fragment ion at *m/e* 282 in the mass spectrum of IV therefore seems too low to allow conclusions to be drawn about the structure of IV. On the other hand, one can argue that in the case of IV there is a competing fragmentation due to interaction between the nitro group and its *ortho* alkyl group, giving rise to a loss of OH. This *ortho* effect has been found for *ortho* alkylnitrobenzenes [12] and, in the thiophene series, for 3-nitro-4,5-dihydro-5-methyl-6H-cyclopenta[*b*]thiophene-6-one [13] and 3-methyl-4-nitrothiophene.[14] The same effect is observed for compounds II, III, V, VIII, and XXII in this paper.



In order to ascertain that the formation of IV was not photochemically induced, the reaction was carried out in the dark. Also in this case the dimer was formed. NMR spectral analysis of the product, however, showed, along with signals from II and IV, vinylic protons, which were assigned to 3-nitro-2-vinylthiophene (V) by

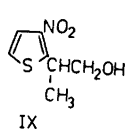
comparison with an authentic sample (*cf.* below). The dimer and the monomer had been formed in a ratio of 1.1:1. Also the spectrum of a reaction mixture performed in the light showed, before separation of components, the presence of compounds II, IV, and V. The reaction was not reversible: after heating of pure IV for 96 h in methanolic solution no V could be found by NMR analysis. Monomerization occurred, however, on heating to 180 °C for 30 min in dimethyl sulfoxide. Complete monomerization of IV occurred in the gas chromatograph. This is in accordance with the finding by Overberger *et al.* that the two 1,2-diphenylcyclobutanes undergo partial decomposition to styrene upon passage through the gas chromatograph.[15]

According to the Woodward-Hoffman rules, a concerted thermal $(2\pi+2\pi)$-cycloaddition is allowed only if it is suprafacial-antarafacial (*cf.* Ref. 16). Such an addition is less probable due to steric factors, as it necessitates a rotation of the carbon-carbon double bonds. Such a cycloaddition between olefins has hitherto not been observed with certainty. (For some other cases of ${}_{\pi}2_{s}+{}_{\pi}2_{a}$ cycloadditions, *cf.* Refs. 17–18 and refs. therein). It therefore appears reasonable to us that, if IV is formed by dimerization of V, the dimerization is not concerted, but involves ionic or radical intermediates. A way of deciding this matter would be a study of the eventual dimerization of *cis*-1-deuterio-2-(3-nitro-2-thienyl)ethene.

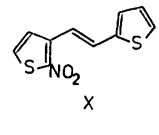
In the literature, many dimerizations of styrene derivatives are described.[19–22] In most cases in which styrene has been thermally or photochemically dimerized, cyclobutane derivatives have been obtained only in low yields, along with polymeric products. These reactions were carried out under non-polar conditions. In the thiophene field, the thermal polymerization of 2-vinylthiophene has been studied. It was found [23] that a dimer, 4-(2-thienyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene, was involved in the initiation of the polymerization (analogous to the polymerization of styrene). The photochemically induced dimerization of β-nitrostyrene to cyclobutane derivatives has been studied.[24]

The different reaction paths of 3-methyl-2-nitrothiophene and 2-methyl-3-nitrothiophene are interesting, but not without precedence. Thus, while 3-(3-thienyl)propyl-trimethylammonium iodide gave 3-(3-thienyl)-1-propene upon treatment with sodium amide, the corresponding 2-isomer gave 2-(cyclopropyl)thiophene.[25]

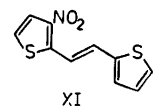
When II was condensed with formaldehyde in *N*,*N*-dimethylformamide, the reaction product was more complex. GLC-mass spectral analyses indicate that besides V or its dimer IV, appreciable amounts of a compound with a highest *m/e* of 185 were also formed. Silylation with *N*,*O*-bis(trimethylsilyl)acetamide prior to GLC-mass spectral analysis gave two peaks with molecular weights of 257 and 347, respectively. From the molecular weights and fragmentation patterns, it follows that these two compounds are 2-(3-nitro-2-thienyl)-3-(trimethylsilyloxy)-propene (VIb) and 1,3-bis-(trimethylsilyloxy)-2-(3-nitro-2-thienyl)propane (VIIb), respectively. The compound with a molecular weight of 185 formed in the condensation is thus 3-hydroxy-2-(3-nitro-2-thienyl)propene (VIa). If silylation is assumed to be complete, it follows that 1,3-dihydroxy-2-(3-nitro-2-thienyl)propane (VIIa) must have also been formed and that this compound either does not pass through the GLC column or is dehydrated to VIa. We have made no attempts to obtain the different reaction products in pure form. 3-Nitro-2-ethylthiophene (VIII) did not react with formaldehyde in refluxing methanol. Slowly in refluxing butanol, but faster in *N*,*N*-dimethylformamide at 110–115 °C, 2-(3-nitro-2-thienyl)propanol (IX) was



IX



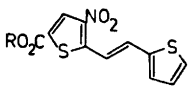
X



XI

formed. The structure followed from its mass and NMR spectra.

If the condensation of I and 2-thiophene aldehyde was carried out according to Srinivasan *et al.*,[6] only a low yield of *trans*-1-(2-nitro-3-thienyl)-2-(2-thienyl)ethene (X) was obtained, together with a great deal of tars. Compound II and 2-thiophene aldehyde reacted very slowly under these conditions to yield 1-(3-nitro-2-thienyl)-2-(2-thienyl)ethene (XI). However, by reacting 1 mol of I of II with 2 mol of the aldehyde with piperidine as catalyst and without

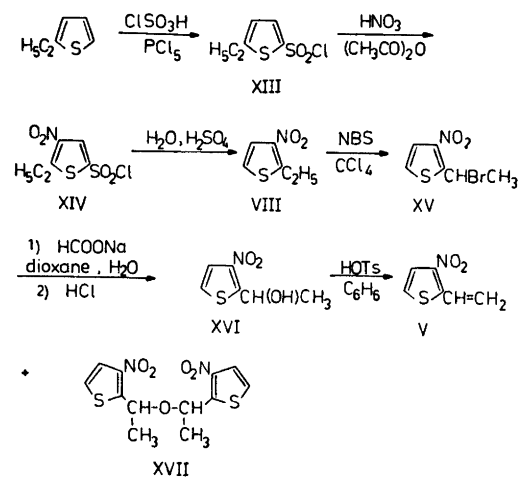


XII a R=CH_3
b R=H

solvent at about 100 °C, good yields of X or XI were obtained. 5-Methoxycarbonyl-2-methyl-3-nitrothiophene also reacted under these conditions to give *trans*-1-(5-methoxycarbonyl-3-nitro-2-thienyl)-2-(2-thienyl)ethene (XIIa). This compound has previously been prepared by Campaigne *et al.*[26] from the same reactants but under other conditions. XI was also obtained from XII by ester hydrolysis and subsequent decarboxylation. The facile condensation of 5-methoxycarbonyl-2-methyl-3-nitrothiophene and 3,5-dinitro-2-methylthiophene with benzaldehyde has been observed by Rinkes.[27]

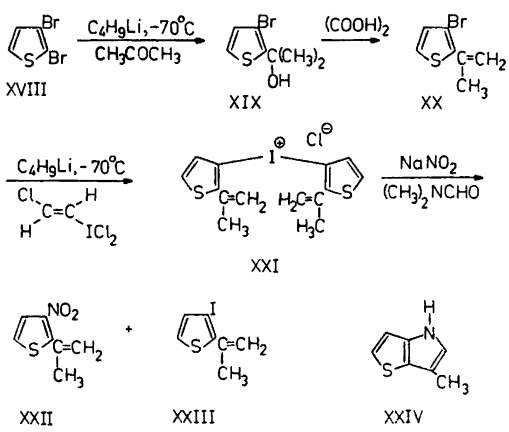
We also attempted condensation of 3-methyl-4-nitrothiophene [28] with formaldehyde, and with 2-thiophene aldehyde. However, no reaction was observed. This is perhaps expected from the well-known fact that the 4-position in 3-substituted thiophenes is not a normal *ortho* position.[29] Resonance stabilization of the benzylic carbanion by the nitro group is not possible, without including a resonance structure with ten electrons around the sulfur, or with additional charge separation.

2-Methyl-3-nitrothiophene (II) was prepared by metalation of 2-methylthiophene with butyllithium and subsequent carbonation, followed by nitration of the 2-methyl-5-thiophenecarboxylic acid according to Campaigne *et al.*[30] Decarboxylation with copper bronze and quinoline gave a better yield of II than the method used previously.[31] 2-Ethyl-3-nitrothiophene (VIII) was prepared by the same route. Alternatively, 2-ethylthiophene was chlorosulfonated to 2-ethyl-5-thiophenesulfonyl chloride (XIII) with phosphorus pentachloride and chlorosulfonic acid,[32] nitrated with a mixture of fuming nitric acid and acetic anhydride, and the resulting 2-ethyl-3-nitro-5-thiophenesulfonyl chloride (XIV) hydrolyzed and desulfonated to VIII. The latter method is preferable, as yields (based on 2-ethylthiophene) of 43 % were obtained, compared to only 14 % with the first-mentioned method. Benzoyl peroxide-catalyzed side-chain bromination of VIII with *N*-bromosuccinimide (NBS) in carbon tetrachloride gave 2-(α-bromoethyl)-3-nitrothiophene (XV) in high yield. The bromo derivative was transformed to 1-(3-nitro-2-thienyl)ethanol (XVI) by reaction with sodium formate in water-dioxane (*cf.* Scheme 1). Reaction in aqueous ethanol gave the ethyl ether in addition to the desired alcohol. Dehydration of XVI caused some difficulties. Dehydration with oxalic acid [33] or by boiling in dimethyl sulfoxide [34] failed. In benzene solution using *p*-toluenesulfonic acid, dehydration occurred easily. However, besides V (58 %), a mixture of racemic and meso 1,1′-bis(3-nitro-2-thienyl)diethyl ether (XVII) (42 %) was obtained, according to NMR analysis. The NMR spectrum showed two methyl doublets. This disturbing facile ether formation from thenyl alcohols has been observed previously.[35] By means of column chromatography V could be obtained pure.



Scheme 1.

In this connection we wish to report another approach to vinylnitrothiophene in which the nitro group is introduced after the vinyl group. Gronowitz and Holm [28] have shown that dithienyliodonium salts, prepared from thienyllithium derivatives and *trans*-chlorovinyliodoso dichloride, react with sodium nitrite to give nitrothiophenes. This approach was used for the synthesis of 2-isopropenyl-3-nitrothiophene (*cf.* Scheme 2). Reaction of 2,3-dibromothiophene (XVIII) with butyllithium followed by acetone gave 2-(3-bromo-2-thienyl)-2-propanol (XIX), which was dehydrated to 3-bromo-2-isopropenylthiophene (XX) by refluxing with oxalic acid at



Scheme 2.

reduced pressure. Halogen-metal exchange at −70 °C with butyllithium followed by reaction with *trans*-chlorovinyliodoso dichloride gave bis(2-isopropenyl-3-thienyl)iodonium chloride (XXI), which upon reaction with sodium nitrite gave 2-isopropenyl-3-nitrothiophene (XXII) and 2-isopropenyl-3-iodothiophene (XXIII), which were separated by recrystallization and chromatography on an aluminium oxide column.

On treatment with triethyl phosphite, XXII gave, along with other products, 6-methylthieno[3,2-*b*]pyrrole (XXIV), a thiophene analogue of skatole, in a way similar to that by which Srinivasan *et al.* obtained thienopyrroles from 1-(nitrothienyl)-2-aryl ethenes.[6] Although XXIV was not obtained in pure form, its NMR and mass spectra were recorded and confirmed the structure. The mass spectrum of the second abundant peak on the GLC showed a molecular ion at *m/e* 273, which underwent two successive losses of *m/e* 28 (ethylene) and a subsequent loss of water. Such a fragmentation has been reported for diethyl *N*-phenylphosphoramidate.[36] Therefore, the present substance seems to be one of the four possible diethyl thieno[3,2-*b*]pyrrolylphosphonates. A similar compound from the reaction between *o*-nitrostilbene and triethyl phosphite has been reported.[37] Unsuccessful attempts to obtain XXIV from 6-methylthieno-[3,2-*b*]pyrrole-5-carboxylic acid have been reported.[38] Thieno[3,2-*b*]pyrrole is unstable in air,[31] and so is the product obtained in the present work. The use of the same approach to prepare the parent thienopyrroles from III and V failed. Thieno[3,2-*b*]pyrrole was prepared by Snyder *et al.* from thieno[3,2-*b*]pyrrole-5-carboxylic acid.[31] Thieno[2,3-*b*]pyrrole was recently prepared by Farnier *et al.* from thieno[2,3-*b*]pyrrole-5-carboxylic acid.[39]

EXPERIMENTAL

All melting points are uncorrected. The NMR spectra were recorded on a Varian A-60. A JEOL MH 100 NMR spectrometer was used for the double resonance experiment. The mass spectra were recorded on a LKB A 9000 mass spectrometer. GLC analyses were carried out on a Perkin-Elmer 900 gas chromatograph and the chromatograms were integrated with a Varian Aerograph Model 480.

2-Methyl-3-nitrothiophene (II) was prepared according to Hörnfeldt,[40] who modified the synthesis by Snyder.[31] 225 g of dry quinoline, 38 g of copper bronze and 56.0 g of 2-methyl-3-nitro-5-thiophenecarboxylic acid[30] were introduced into a round-bottomed flask. The mixture was heated slowly on an oil-bath, and when it was liquid vacuum distillation at 11 mmHg was started. The temperature of the oil was then raised to 150 °C during a period of 4 h 45 min. The distillate containing quinoline and the title compound was mixed with ice and water, whereupon conc. hydrochloric acid was added in portions until pH 1. The remaining organic layer was taken up in ether and the acid water phase was extracted with ether. The combined ether phases were washed with sodium carbonate solution and water and yielded after evaporation 29.9 g (70 %) of the title compound. Recrystallization from low boiling petroleum ether gave 27.3 g (64 %), m.p. 43.8–45.0 °C, lit.[41] m.p. 44–45 °C. MS (direct inlet): Found *m/e* = 142 (89 %, M^+), 126 (98 %, loss of OH), the 100 % peak was at *m/e* = 45. Calc. for $C_5H_5NO_2S$: *m/e* = 143.

5-Ethyl-2-thiophenecarboxylic acid. 85.0 g (0.76 mol) of 2-ethylthiophene was dissolved in 200 ml of dry ether. With stirring and under a stream of nitrogen, 1000 ml of 0.84 M butyllithium was added at such a rate that the solution refluxed gently. After all butyllithium had been added, the reaction mixture was refluxed for an additional 2 h. The solution was then poured onto dry ice and was left over night. 5 M sodium hydroxide was added until no solid remained, and the phases were separated. The ether phase was washed with sodium hydroxide solution. The combined sodium hydroxide solutions were acidified with concentrated hydrochloric acid. The solid was collected, washed with water and dried. The yield was 109.2 g (92 %) of the title compound, m.p. 70 °C. lit.[42] m.p. 71 °C. The product was used in the next step without further purification.

2-Ethyl-3-nitro-5-thiophenecarboxylic acid was prepared as described by Gol'dfarb *et al.*[43] by nitration with nitric acid in sulfuric acid. The yield of crude product was 46 %.

5-Ethyl-2-thiophenesulfonyl chloride (XIII). This compound was prepared according to a patent by Siedel and Sturm.[32] 50 ml of chlorosulfonic acid was added dropwise with stirring and cooling with ice-water to 62 g of phosphorus pentachloride. To the resulting solution, 33.6 g (0.30 mol) of 2-ethylthiophene was added dropwise over a period of 30 min. The temperature was kept at 15 – 20 °C utilizing the ice-water bath. Immediately after the addition was finished, the reaction mixture was added from a dropping funnel to a beaker containing ice-water. After extraction with ether, the ether phase was washed with sodium hydrogen carbonate solution, dried and evaporated. The residue was distilled *in vacuo*, yielding 32.9 g (52 %) of the title compound, b.p. 95 – 100 °C/0.6 – 0.7 mmHg, lit.[32] 70 – 72 °C/0.1 mmHg. The product is pure according to GLC (SE 30 1.5 %, 2 m).

2-Ethyl-3-nitro-5-thiophenesulfonyl chloride (XIV). This nitration was carried out under the conditions used by Leandri *et al.*[44] for nitration of 5-bromo-2-thiophenesulfonyl chloride. 450 ml of nitric acid ($d = 1.52$) at a temperature of 0 – 10 °C was added dropwise with stirring to 490 ml of acetic anhydride. A solution of 182.5 g (0.870 mol) of 5-ethyl-2-thiophenesulfonyl chloride in 490 ml of acetic anhydride was added dropwise at 3 – 5 °C during a period of 50 min. The temperature was then raised and kept at 13 – 15 °C for 3 h. The mixture was poured onto crushed ice. The solution was extracted with ether, and the ether phase washed with sodium carbonate solution until neutral. After drying and evaporation, 215.9 g (98 %) was obtained. GLC (SE 30 1.5 %, 2 m) showed only one peak. The product was used directly in the next step.

2-Ethyl-3-nitrothiophene (VIII). A. From 2-ethyl-3-nitro-5-thiophenecarboxylic acid. The reaction was carried out in the same manner as described above for 2-methyl-3-nitrothiophene. The yield of the crude product was 33 %. and after recrystallization from petroleum ether 25 %, m.p. 38.1 – 41.0 °C.

B. From 2-ethyl-3-nitro-5-thiophenesulfonyl chloride (XIV). The method by Leandri *et al.* for the preparation of 2-bromo-3-nitrothiophene was used.[44] 215.9 g of XIV was boiled with 1500 ml of water for 3.5 h. A solution of 1000 ml of conc. sulfuric acid in 1200 ml of water was introduced into a 5 l three-necked round-bottomed flask equipped for steam distillation. Steam distillation was started using a heating mantle and superheated steam. The sulfonic acid solution was slowly introduced into the distillation flask *via* a dropping funnel. 9 l was distilled. The distillate was left over night and was then extracted with ether. The ether layer was washed with sodium hydrogen carbonate solution until neutral reaction, dried and evaporated. The crude product weighed 108.7 g (82 %). GLC (NPGS 5 %, 2 m) showed the product to be almost pure. The analytical sample of 2-ethyl-3-nitrothiophene was obtained by two recrystallizations from light petroleum ether, m.p. 41.2 – 42.0 °C. [Found: C 46.1; H 4.73; N 8.96; S 20.3. Calc. for $C_6H_7NO_2S$ (157.2): C 45.9; H 4.49; N 8.91; S 20.4.] NMR ($CDCl_3$): δ 7.53 (4), 7.05 (5), 3.26 (CH_2, q, 2 H), 1.35 (CH_3, t, 3 H), J_{45} 5.5 Hz, $J_{CH_2CH_3}$ 7 Hz. Mass spectrum (direct inlet): Found *m/e* 157 (78 %, M^+), 140 (100 %, loss of OH).

2-Nitro-3-vinylthiophene (III). 18.5 g (0.129 mol) of 3-methyl-2-nitrothiophene[45] was dissolved in 325 ml of methanol, 65 g of 34 % formaldehyde solution in water, 1.3 ml of acetic acid and 2.2 ml of pyrrolidine were added and the solution was refluxed for 46 h. After cooling, methanol was evaporated until about 100 ml of solution remained. Crystals were obtained (12.1 g; 60 %). Recrystallization from methanol gave 10.2 g (51 %) of the title compound, m.p. 47 – 48 °C, lit.[5] m.p. 49 – 50 °C. A second crop could be obtained from the combined filtrate and mother liquor. NMR ($CDCl_3$): δ 7.40 (5), 7.25 (4), 7.56 (α), 5.63 (β), 5.86 (β'). J_{45} 5.7 Hz, $J_{\alpha\beta}$ 10.1 Hz, $J_{\alpha\beta'}$ 17.9 Hz, $J_{\beta\beta'}$ 1.0 Hz. The β and β' quartets overlapped with each other, resulting in 6 signals. H_α and H_β are *cis* to each other. Mass spectrum (*via* GLC): Found $m/e = 155$; calc. for $C_6H_5NO_2S$: $m/e = 155$. The first fragment occurs at $m/e =$ 138, indicating loss of OH.

Bis(3-nitro-2-thienyl)cyclobutane (IV). 10.0 g (0.070 mol) of 2-methyl-3-nitrothiophene, 40.0 g of 34 % formaldehyde solution in water, 0.35 ml of acetic acid and 0.60 ml of pyrrolidine were dissolved in 200 ml of methanol, and the solution was boiled. After 21 h. an additional 0.35 ml of acetic acid and 0.60 ml of pyrrolidine were added. The reaction was followed by GLC (NPGS 5 %, 2 m). After 72 h the heating was interrupted. On immediate scratching precipitation occurred. When cooled to room temperature the solid was filtered off and washed carefully with 50 % methanol. The weight after drying was 5.9 g, m.p. 141.0 – 142.5 °C. From the solution, an additional 0.6 g was obtained, to make a total yield of 60 %. Two recrystallizations from methanol gave the analytical sample, m.p. 142.0 – 142.5 °C. [Found: C 46.4; H 3.21; N 9.00; S 20.7. Calc. for $C_{12}H_{10}N_2O_4S_2$ (310.4): C 46.4; H 3.25; N 9.03; S 20.7.] NMR ($CDCl_3$): δ 7.54 (4), 7.13 (5), 4.5 (CH, m, 2 H), 2.5 (CH_2, m, 4 H). J_{45} 5.6 Hz. A spectrum of a solution in pentadeuteriopyridine was run at 100 MHz. In this spectrum the multiplet at δ 2.5 was divided into two. Upon saturation of the signals at δ 4.5 these two multiplets changed but no AA'BB' pattern could be obtained.

The mass spectrum *via* GLC is very like the spectrum of 3-nitro-2-vinylthiophene (see below) with the highest *m/e* at 155, correspond-

ing to $C_6H_5NO_2S$. Mass spectrum *via* direct inlet: Found $m/e = 310$ (2.2 %); 293 (0.5 %, loss of OH); 282 (0.2 %, loss of C_2H_4); 280 (0.2 %, loss of NO); 278 (0.5 %); 266 (1.9 %); 265 (2.6 %); 264 (16 %); 155 (100 %).

The reaction was repeated in the dark starting from 0.5 g of 2-methyl-3-nitrothiophene. Boiling was interrupted after 62 h. The methanol was evaporated and benzene was added to dissolve the organic residue. Acetic acid and pyrrolidine were removed, and after drying the benzene was evaporated. NMR ($CDCl_3$) showed the mol ratio [V]:[IV] = 1.1, *i.e.* 65 % of the vinyl compound appeared as cyclobutane.

Deuterium exchange in bis(3-nitro-2-thienyl)-cyclobutane (IV). A. In a mixture of 5 ml of CH_3OD and 1 ml of deuterium oxide 0.2 g of IV, 2 drops of pyrrolidine and 1 drop of acetic acid were dissolved. The solution was refluxed for 63 h. After evaporation of the methanol, the residue was dissolved in chloroform, washed with 1.2 M hydrochloric acid and sodium hydrogen carbonate solution. The solution was analyzed by GLC-MS, which showed the following values (*m/e*, peak intensity): 155, 9.5; 156, 6.6; 157, 1.2. This gives 38 % of mono-deuterated compound.

B. A small amount of IV was dissolved in a mixture of $CDCl_3$ and DMSO-d_6. A few drops of D_2O were added, and the 60 MHz NMR spectrum was run at 60 °C. (At lower temperature solid IV precipitated.) After adding a drop of pyrrolidine to the NMR tube, the solution was maintained at 60 °C for 50 min. The signals at δ 4.5 had now disappeared.

Equilibration of bis(3-nitro-2-thienyl)cyclobutane (IV) in dimethyl sulfoxide-water. Bis(3-nitro-2-thienyl)cyclobutane, 290 mg, was dissolved in a mixture of 1 ml of water and 9 ml of dimethyl sulfoxide. The solution was heated to 67 °C under nitrogen and 3 drops of pyrrolidine were added. After 1.5 h at 67 °C the heating was interrupted and the solution poured into ice-water. The mixture was extracted with ether, and the ether phase was washed with 1.2 M hydrochloric acid, sodium hydrogen carbonate solution, water and was dried. Evaporation gave 187 mg of material. Its IR spectrum was identical with that of IV with some minor additional absorptions. Recrystallization from methanol gave 111 mg of material, m.p. 141.0 – 143.1 °C. Its IR spectrum was identical to that of IV.

Attempt to monomerize bis(3-nitro-2-thienyl)-cyclobutane (IV) in methanolic solution. 0.18 ml of acetic acid and 0.30 ml of pyrrolidine were dissolved in 100 ml of methanol. 0.5 g of IV was dissolved in 21 ml of this solution and 10 drops of formaldehyde solution were added. After boiling for 96 h and removal of solvent and catalysts, the NMR spectrum showed signals only from IV.

Monomerization of bis(3-nitro-2-thienyl)-cyclobutane (IV) in DMSO. 0.5 g of IV was dissolved in 5 ml of dimethyl sulfoxide, dried over calcium hydride. The solution was heated on an oil bath to 180 °C for 30 min under a stream of nitrogen. The solution was poured into water and extracted with ether. After evaporation 0.2 g remained. Integration of the NMR spectrum showed the mol ratio [V]:[IV] = 3.4.

Reaction between 2-methyl-3-nitrothiophene and formaldehyde in dimethylformamide. 1.0 g (7.0 mmol) of 2-methyl-3-nitrothiophene, 35 mg of acetic acid and 51 mg of pyrrolidine were dissolved in 20 ml of freshly distilled DMF. 4.0 g of formalin was added and the solution was heated to 110 – 115 °C for 138 h. The red-brown solution was poured into water and was extracted with ether. The extract was washed twice with 1.2 M hydrochloric acid, twice with 1 M sodium carbonate, and twice with water. GLC analysis (OV 17 3 %, 2.5 m) showed two peaks, one of which corresponded to IV or V. The other component showed a mass spectrum with highest $m/e = 185$, corresponding to $C_7H_7NO_3S$ (VIa). The ether solution was treated with *N,O*-bis(trimethylsilyl)acetamide, and the GLC now showed two peaks in addition to the one from IV or V. Mass spectra gave molecular weights of 257 and 347, corresponding to $C_{10}H_{15}NO_3SSi$ (VIb) and $C_{13}H_{25}NO_4SSi_2$ (VIIb), respectively.

trans-*1-(Methoxycarbonyl-3-nitro-2-thienyl)-2-(2-thienyl)ethene (XIIa).* This synthesis is analogous to Rinkes' synthesis of 1-(5-methoxycarbonyl-3-nitro-2-thienyl)-2-phenylethene.[27] 20.1 g (0.100 mol) of 5-methoxycarbonyl-2-methyl-3-nitrothiophene, obtained by esterification of 2-methyl-3-nitro-5-thiophenecarboxylic acid[30] with methanol and hydrogen chloride,[27] was dissolved with slight heating in 22.4 g of freshly distilled 2-thiophene aldehyde. The solution was heated with stirring on an oil-bath to 120 °C, 30 drops of piperidine were added and the solution became blue. After 15 min an additional 20 drops of piperidine were added, and after another 15 min period the heating was interrupted. The mixture was immediately poured into a mortar and left to solidify. After cooling to room temperature, the solidified reaction mixture was transferred to a suction funnel, and the excess aldehyde and the catalyst were removed by suction. The crystals were washed thoroughly with ethanol. The yield of the title compound was 85 %, m.p. 134 – 138 °C. A sample recrystallized twice from methanol melted at 135.8 – 138.0 °C, lit.[26] 139 – 140 °C. The crude product was used in the next step.

trans-*1-(5-Carboxy-3-nitro-2-thienyl)-2-(2-thienyl)ethene (XIIb).* 20.0 g (0.068 mol) of 1-(5-methoxycarbonyl-3-nitro-2-thienyl)-2-(2-thienyl)ethene was dissolved with heating in 275 ml of acetic acid. 50 ml of concentrated sulfuric acid dissolved in 67 ml of water was added. The solution was boiled for 2 h. After standing over night, the resulting brown crystallized compound was filtered with suction and

washed with a little acetic acid. The yield of the title compound was 16.1 g (84 %). The acid could be recrystallized from ethanol. Sublimation gave a sample melting at 230–235 °C. IR (KBr disc): Broad absorption at 2560–3100 cm^{-1} (carboxylic acid), 1670 cm^{-1} (aromatic carboxylic acid).

trans-*1-(3-Nitro-2-thienyl)-2-(2-thienyl)-ethene (XI)*. A. Decarboxylation of 1-(5-carboxy-3-nitro-2-thienyl)-2-(2-thienyl)ethene. 95 g of dry quinoline was heated to 75 °C on an oil-bath. 15 g of copper bronze and 33.0 g (0.117 mol) of the acid XIIb were added with stirring. The reaction was followed by IR of the solution, observing the carbonyl absorption. The temperature was raised to 180 °C during a period of 70 min, after which the carbonyl band had disappeared. After cooling, ether was added and the copper was removed by filtration. The filtrate, water and crushed ice were introduced into a separatory funnel, concentrated hydrochloric acid was added in small portions with shaking between the additions. When the water phase had reached pH 1, the layers were separated. The water layer was extracted once with ether. The combined ether phases were washed with sodium hydrogen carbonate solution until neutral. Drying and evaporation gave 24.4 g (88 %) of the title compound. Treatment with charcoal combined with recrystallization from ethanol yielded 15.2 g (55 %), m.p. 67 °C. The analytical sample, obtained by one further recrystallization from ethanol, was of red color and melted at 65.8–67.2 °C. [Found: C 50.8; H 3.11; N 5.78; S 27.1. Calc. for $C_{10}H_7NO_2S_2$ (237.3): C 50.6; H 2.97; N 5.90; S 27.0.] NMR ($CDCl_3$): Signals in the region δ 6.90–8.00 were recorded. One half of the AB spectrum of the olefinic protons occurs at largest δ and shows a coupling constant of 16.0 Hz, indicative of two *trans* hydrogens. Further, one half of a thiophene AB quartet was distinguished with a coupling constant of 5.7 Hz.

B. From 2-methyl-3-nitrothiophene and 2-thiophene aldehyde. This method gives a purer product than A. 1.14 g (8.0 mmol) of 2-methyl-3-nitrothiophene was dissolved in 2.2 g of freshly distilled 2-thiophene aldehyde and 8 drops of piperidine were added. The mixture was kept at 120 °C for 2 h. GLC (OV 17 3 %, 2.5 m) showed that II had disappeared and a new compound had been formed. After cooling, the solution was dissolved in ethanol and added with stirring to a saturated solution of sodium hydrogen sulfite. After stirring for 30 min, the hydrogen sulfite adduct was filtered off by suction. The filtrate was extracted with ether. The hydrogen sulfite adduct was thoroughly washed with ether until all orange substance included had dissolved. After drying and evaporation, the residue was recrystallized from ethanol, yielding 0.8 g (43 %) of the title compound as glittering crystals, m.p. 68.1–68.8 °C. The compound is according to GLC and IR identical with that obtained above. The mother liquor was evaporated, and the residue was recrystallized from ethanol, yielding 0.2 g, m.p. 66.6–67.8 °C.

trans-*1-(2-Nitro-3-thienyl)-2-(2-thienyl)-ethene (X)*. 14.3 g (0.100 mol) of 3-methyl-2-nitrothiophene was dissolved in 22.0 g of freshly distilled 2-thiophene aldehyde. 2.5 ml of piperidine was added and the solution was heated on an oil-bath to 90–100 °C. After 2 h the reaction was complete according to GLC (OV 1 3 %, 3 m). After cooling, the mixture crystallized on scratching. The substance was filtered off and thoroughly washed with ethanol until no smell of 2-thiophene aldehyde remained. After drying, the crystals weighed 14.1 g (60 %), m.p. 101–103 °C. Two recrystallizations from ethanol gave the analytical sample, m.p. 103.0–104.1 °C. [Found: C 50.6; H 3.00; S 27.2. Calc. for $C_{10}H_7NO_2S_2$ (237.3): C 50.6; H 2.97; S 27.0.] NMR ($CDCl_3$): The spectrum is complex, showing signals at δ 6.9–8.0. The two signals at lowest field are separated by 16.0 Hz, indicating that the olefinic hydrogens are *trans* to each other.

3-Methyl-4-nitrothiophene was a gift from B. Holm.[28]

Attempted reaction between 3-methyl-4-nitrothiophene and formaldehyde. 0.4 ml of acetic acid and 0.7 ml of pyrrolidine were dissolved in 50 ml of methanol. To 5 ml of this solution, 1.1 g of formalin was added. In 0.5 ml of this mixture, 6.9 mg of 3-methyl-4-nitrothiophene was dissolved. 0.03 ml portions were enclosed in capillary tubes which were then heated in an oil-bath to 80 °C. Samples were analyzed on GLC (NPGS 5 %, 2 m, 180 °C). Even after 40 h no new compound was detected.

Attempted reaction between 3-methyl-4-nitrothiophene and 2-thiophene aldehyde. In 0.15 ml of freshly distilled 2-thiophene aldehyde, some crystals of 3-methyl-4-nitrothiophene were dissolved. The solution was heated on an oil-bath to 100–110 °C for 1 h and then the temperature was raised slowly to 140 °C during a period of 70 min. GLC (OV 1 3 %, 3 m) showed that the main compound was still starting material. Four minor components were analyzed by mass spectroscopy; none of them was the expected ethylene.

2-(α-Bromoethyl)-3-nitrothiophene (XV). 7.4 g (0.047 mol) of 2-ethyl-3-nitrothiophene was dissolved in 75 ml of dry carbon tetrachloride. 8.4 g (0.047 mol) of *N*-bromosuccinimide (recrystallized from water and dried over sulfuric acid) and a small amount of dibenzoyl peroxide (dried over phosphorus pentoxide) were added. The mixture was boiled with stirring for several hours. Since nothing had happened after this time, a drop of water was added and after 75 min all of the heavy NBS had been replaced by floating succinimide. The solid was filtered off and the solvent was removed. The residue weighed 11.1 g. The product decomposed on the GLC column. Its NMR showed, however, that

only monobromination had occurred. Recrystallization from light petroleum gave 8.7 g (82 %). One further recrystallization, including treatment with aluminium oxide, gave the analytical sample, m.p. 59.0 – 59.6 °C. [Found: C 30.5; H 2.48; Br 33.9; N 5.88; S 13.7. Calc. for $C_6H_6BrNO_2S$ (236.1): C 30.5; H 2.56; Br 33.9; N 5.93; S 13.6.] NMR ($CDCl_3$): δ 7.57 (4), 7.28 (5), 6.25 (CH, q, 1 H), 2.14 (CH_3, d, 2 H), J_{45} 5.7 Hz, J_{CHCH_3} 7.0 Hz, J_{CH-5} 0.5 Hz. The assignments of the thiophene proton chemical shifts were confirmed by their ^{13}C couplings (J_{CH-5} 189 Hz, J_{CH-4} 175 Hz, *cf.* Ref. 46).

1-(3-Nitro-2-thienyl)ethanol (XVI). 11.8 g (0.050 mol) of 2-(α-bromoethyl)-3-nitrothiophene was dissolved in 160 ml of peroxide-free dioxane. 10 g of sodium formate dissolved in 110 ml of water was added. The mixture was boiled for 5.5 h, whereupon 14 ml of conc. hydrochloric acid was added and boiling was continued for 4 h. After cooling, the solution was neutralized with sodium hydrogen carbonate. As much as possible of the dioxane was evaporated and the residue (two liquid phases) was extracted twice with ether. The ether phase was washed with sodium bisulfite to remove possibly formed peroxides. Integrated GLC (NPGS 5 %, 2 m) showed the product to be 98 % pure. The crude product weighed 7.6 g. Two recrystallizations from carbon tetrachloride gave 4.7 g (54 %) of the title compound, m.p. 55.5 – 56.7 °C. The solvent dioxane-water was superior to ethanol-water, in which 2-(α-ethoxyethyl)-3-nitrothiophene was formed as by-product. [Found: C 41.8; H 4.24; N 8.12; S 18.7. Calc. for $C_6H_7NO_3S$ (173.2): C 41.6; H 4.07; N 8.09; S 18.5.] NMR ($CDCl_3$): δ 7.56 (4), 7.22 (5), 5.74 (CH, q, 1 H), 1.61 (CH_3, d, 3 H), 3.75 (OH, s, 1 H), J_{45} 5.7 Hz, J_{CHCH_3} 6.5 Hz.

3-Nitro-2-vinylthiophene (V). 2.5 g (0.0145 mol) of 1-(3-nitro-2-thienyl)ethanol was dissolved in 400 ml of dry benzene. 30 ml of a warm saturated benzene solution of *p*-toluenesulfonic acid was added and the mixture was poured into a round-bottomed flask, which was then equipped with a Soxhlet extractor, the thimble containing molecular sieves. The solution was refluxed, the condensed liquid dropping into the thimble and thereby being dried. After refluxing over night, GLC (OV 17 3 %, 2.5 m) showed that the alcohol had disappeared. The benzene solution was shaken with water, sodium hydrogen carbonate solution, and water; it was then dried and evaporated. The residue weighed 2.2 g. NMR of the crude product showed it to consist of 3-nitro-2-vinylthiophene (V), bis(3-nitro-2-thienyl)cyclobutane (IV), and 1,1′-bis(3-nitro-2-thienyl)diethyl ether (XVII) in the mol ratio 7.0:1.7:1. The components were separated on a silica gel column using dry benzene as eluent. They occur in the eluate in the order V, IV, and XVII. From 2.0 g of the crude product 1.1 g of V, 0.2 g of IV, and 0.2 g of XVII were obtained.

The fraction containing V was dissolved in a few ml of benzene; the solution was filtered through glass wool, a few ml of methanol was added and the solution was evaporated at room temperature almost to dryness. The crystals were filtered off, washed with methanol and dried. The main part melted at 41 – 43 °C, but the remaining part did not melt below 60 °C and probably consisted of IV. [Found: C 46.5; H 3.18; N 9.05; S 20.6; Calc. for $C_8H_6NO_2S$ (155.2): C 46.4; H 3.25; N 9.03; S 20.7]. NMR ($CDCl_3$): δ 7.52 (4), 7.10 (5), 7.57 (α), 5.82 (β), 5.50 (β′), J_{45} 5.6 Hz, $J_{5\alpha}$ 0.7 Hz, $J_{\alpha\beta}$ 17.3 Hz, $J_{\alpha\beta'}$ 10.8 Hz, $J_{\beta\beta'}$ 0 Hz. MS (*via* GLC): Found $m/e = 155$ (molecular ion, 100 %), 139, 138 (loss of OH), 126. NMR ($CDCl_3$) of XVII: The material consists of diastereomers in the ratio 2:1. Major component: δ 7.55 (4), 7.23 (5), 5.46 (CH, q), 1.60 (CH_3, d), J_{45} 5.6 Hz, J_{CHCH_3} 6.2 Hz. Minor component: δ 7.51 (4), 7.14 (5), 3.9 (CH, q), 8.64 (CH_3, d), J_{45} 5.5 Hz, J_{CHCH_3} 6.1 Hz. Mass spectrum of XVII: Found: $m/e = 172$. Calc. for $C_{12}H_{12}N_2O_5S_2$ $m/e = 328$. The observed value corresponds to cleavage of the C – O bond. A similar fragmentation is known in, *e.g.* dibenzyl ether.[47]

2-(3-Bromo-2-thienyl)-2-propanol (XIX). 242.0 g (1.00 mol) of 2,3-dibromothiophene was dissolved in 400 ml of dry ether. The solution was placed in a three-necked flask swept with nitrogen. Under a stream of nitrogen, the solution was cooled to −70 °C with dry ice-ethanol. 650 ml of 1.70 M butyllithium in hexane was added dropwise with stirring. After stirring for an additional 15 min, 90 g of dry acetone in 200 ml of dry ether was introduced at such a rate that the temperature did not increase. After an additional 30 min the cooling bath was removed, and when the temperature was +10 °C, water was added dropwise to the solution, cautiously at first. The phases were separated and the water phase was extracted twice with ether. The combined ether layers were washed with water and dried. Evaporation gave 230.9 g of solid substance, which was used directly in the next step. Two recrystallizations from petroleum ether gave 2-(3-bromo-2-thienyl)-2-propanol, which according to GLC (NPGS 5 %, 2 m) was pure, m.p. 59.6 – 62.8 °C. [Found: C 38.0; H 4.10; Br 36.1; S 14.2. Calc. for C_7H_9BrOS (221.1): C 38.0; H 4.10; Br 36.1; S 14.5.] NMR ($CDCl_3$): δ 7.03 (5), 6.88 (4), 2.97 (OH), 1.70 (CH_3), $J_{45} = 5.3$ Hz.

3-Bromo-2-isopropenylthiophene (XX). The crude 2-(3-bromo-2-thienyl)-2-propanol prepared above was melted and 25 g of anhydrous oxalic acid was added. The melt was then refluxed at 17 mmHg using an oil-bath as heat source. The temperature of the oil was raised from 77 to 107 °C over a period of 2 h. Heating was interrupted, and after cooling the reaction mixture was dissolved in ether, shaken twice with water, then with sodium hydrogen carbo-

nate solution until neutral reaction. After drying and evaporation, the residue was distilled, yielding 152.7 g of 3-bromo-2-isopropenylthiophene (75 % from 2,3-dibromothiophene), b.p. 96 – 98 °C/16 mmHg. [Found: C 41.0; H 3.48; Br 38.9; S 15.5. Calc. for C_7H_7BrS (203.1): C 41.4; H 3.47; Br 39.3; S 15.8.] NMR ($CDCl_3$): δ 7.00 (5), 6.84 (4), 5.48 (β, m, 1 H), 5.19 (β', quint, 1 H), 2.13 (CH_3, q, 3 H), J_{45} 5.3 Hz, $J_{\beta\beta'} = J_{\beta'\text{-}CH_3}$ 1.6 Hz, $J_{\beta\text{-}CH_3}$ 0.8 Hz. The coupling constants suggest that H_β is *trans* to the methyl group.[48]

Bis-(2-isopropenyl-3-thienyl)iodonium chloride (XXI). This synthesis was carried out in dry flasks, swept with nitrogen. 55.0 g (0.271 mol) of 3-bromo-2-isopropenylthiophene in 250 ml of dry ether was cooled to – 70 °C and 0.30 mol of butyllithium in ether or hexane was added dropwise with stirring. The stirring was continued for 30 min, and the solution was then poured through a PVC tube into a stirred solution of 39.0 g (0.15 mol) of *trans*-chlorovinyliodoso dichloride [49] dissolved in 180 ml of toluene cooled to – 70 °C. After stirring at this temperature for 3 h, the cooling bath was removed, and when the temperature had increased to about 10 °C water was added, at first cautiously. The precipitate was filtered off on a Büchner funnel, which was troublesome because of the two liquid phases. The precipitate was washed with ether, and after drying in vacuum it was washed with water and then with a small amount of acetone, yielding after drying 28.2 g (51 %) of the title compound.

2-Isopropenyl-3-nitrothiophene (XXII) and 3-iodo-2-isopropenylthiophene (XXIII). 72.4 g (0.177 mol) of bis-(2-isopropenyl-3-thienyl)-iodonium chloride and 65.2 g (0.48 mol) of sodium nitrite were stirred in 720 ml of *N*,*N*-dimethylformamide for 3.5 h at 120 – 130 °C. The solution was poured into water and extracted with ether. The ether phase gave on drying and evaporation 58.1 g of crude product mixture, which was dissolved in 1300 ml of boiling hexane, treated with neutral aluminum oxide and the hot solution filtered and then cooled to – 70 °C. The mother liquor was decanted from the crystals, which were then washed three times with 65 ml portions of hexane at – 70 °C. The solid melted on warming to room temperature. GLC (NPGS 5 %, 2 m) showed that the material contained XXII and XXIII in the ratio 11:1. The recrystallization procedure was repeated, using 380 ml of hexane, and the solid was washed once at – 70 °C. GLC showed a ratio of 142, that is more than 99 % of XXII. Yield of 2-isopropenyl-3-nitrothiophene: 14.2 g (48 %). Two recrystallizations from hexane at – 18 °C gave m.p. 34.0 – 34.8 °C. [Found: C 49.9; H 4.20; N 8.21; S 18.9. Calc. for $C_7H_7NO_2S$ (169.2): C 49.7; H 4.17; N 8.28; S 19.0.] NMR ($CDCl_3$): δ 7.18 (5), 7.57 (4), 5.22 (β, m), 5.33 (β', quint), 2.13 (CH_3), J_{45} 5.6 Hz, $J_{\beta\beta'} = J_{\beta'\text{-}CH_3}$ 1.5 Hz, $J_{\beta\text{-}CH_3} = 0.9$ Hz. MS (*via* GLC): Found $m/e = 169$ (M^+), 152 (loss of OH), 126, 124. The combined hexane solutions were evaporated and the residue was chromatographed on neutral aluminium oxide, the column diameter being 6.7 cm and its height 19 cm. Elution with petroleum ether gave 24.5 g (55 %) of 3-iodo-2-isopropenylthiophene. Elution with ether: petroleum 30:70 gave 6.5 g of material, the GLC of which showed [XXII]:[XXIII] = 3.5. XXIII had a b.p. 69 °C/0.5 mmHg. [Found: C 34.0; H 2.91. Calc. for C_7H_7IS (250.1): C 33.6; H 2.82.] NMR ($CDCl_3$): δ 7.03 (5), 6.94 (4), 5.40 (β, m), 5.21 (β', quint) 2.11 (CH_3, q), J_{45} 5.2 Hz, $J_{\beta\beta'} = J_{\beta'\text{-}CH_3}$ 1.4 Hz, $J_{\beta\text{-}CH_3}$ 0.8 Hz. Mass spectrum (*via* GLC): Found: 250. Calc. for C_7H_7IS: 250.

6-Methylthieno[3,2-b]pyrrole (XXIV). 4.0 g of 2-isopropenyl-3-nitrothiophene was dissolved in 50 ml of triethyl phosphite, and under a stream of nitrogen the solution was warmed to 130 °C. GLC (OV 17 3 %, 2.5 m) showed that the starting material disappeared and other compounds were formed. The main product (52 %) was, according to its mass spectrum, 6-methylthieno[3,2-b]pyrrole (XXIV). The second abundant peak (38 %) on the GLC had the highest *m/e* 273 and showed consecutive losses of fragments 28, 28, and 18. After 2 h the starting material had disappeared. Excess triethyl phosphite was removed at 12 mmHg, followed by distillation of the residue under nitrogen. 4.5 g of almost pure triethyl phosphate was obtained as a first fraction. At 90 – 130 °C/0.35 mmHg 0.7 g of material distilled, which according to NMR consisted of 55 % 6-methylthieno[3,2-*b*]pyrrole and 45 % triethyl phosphate. The distillation residue weighed 4.2 g. NMR ($CDCl_3$): δ 6.98 (2, q), 6.81 (3, d), 6.64 (5, m), 8.2 (4, broad), 2.22 (CH_3, d), J_{23} 5.2 Hz, J_{25} 1.3 Hz, $J_{5\text{-}CH_3}$ 1.1 Hz. Spectrum of triethyl phosphate: δ 4.09 (CH_2, quint), 1.29 (CH_3, t). MS (*via* GLC): Found $m/e = 137$ (80 %, M^+), 136 (100 %), 110, 109. The compound has an unpleasant odour, and becomes dark on standing. The red-brown picrate of XXIV was obtained from ethanol solution, m.p. *ca.* 115 °C (dec.).

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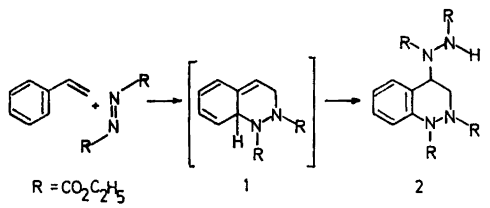
Short Communications

The Reaction between Diethyl Azodicarboxylate and Styrene in the Presence of a Radical Inhibitor*

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The ene reaction is generally considered to be a six-centred cyclic process, the double bond shift being concerted with transfer of allylic hydrogen.[1] However, certain ene reactions involving azodicarboxylates show a radical behavior.[1,2] In an earlier paper in this series, the addition of diethyl azodicarboxylate to cyclohexene was shown to take place *via* radical intermediates.[3] An additional example of such behavior is presented here.



The 2:1 adduct *2* between azodicarboxylate and styrene is formed *via* an ene reaction of the initial Diels-Alder 1:1 adduct *1*.[4] Qualitative kinetic studies now show that the formation of the adduct *2* is strongly inhibited when a radical inhibitor (*t*-butylresorcinol) is present in the reaction mixture. In fact, an aged mixture of styrene and *t*-butylresorcinol even failed to give the adduct *2* with diethyl azodicarboxylate. A kinetic study of the formation of the adduct *2* showed the reaction to be first order in styrene and first order in azodicarboxylate. The disappearance rate of styrene is unaffected by the presence of *t*-butylresorcinol in the reaction mixture. Thus, the formation of the intermediate *1* is the rate-determining step.* Furthermore, the effect of the radical inhibitor indicates that the subsequent ene reaction takes place to a significant extent *via* a radical chain mechanism. A related process seems to be the self-induced free radical polymerisation of styrene. In that case, radicals are generated in the reaction between styrene and the Diels-Alder type dimer of styrene,[5] a compound very similar to the intermediate *1*.

Experimental. The kinetic experiments were run in ether solution at 25.0 ± 0.5 °C. The reaction flask was equipped with a rubber septum through which samples were withdrawn with a syringe at suitable intervals. Styrene was quantitatively determined by GLC and diethyl azodicarboxylate by UV measurements at 400 nm. GLC was done on a Varian 1200 gas chromatograph equipped with a Varian 480 integrator.

UV Spectra were determined with a Beckman Dk2-spectrophotometer.

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* Attempts to isolate the intermediate *1* have been unsuccessful.

* Cycloaddition Reactions, Part 7. Par t 6: Ahlgren, G. and Åkermark, B. *Tetrahedron Lett.* (1974) 987.

Animal Carotenoids. 10*. Chirality of Astaxanthin of Different Biosynthetic Origin

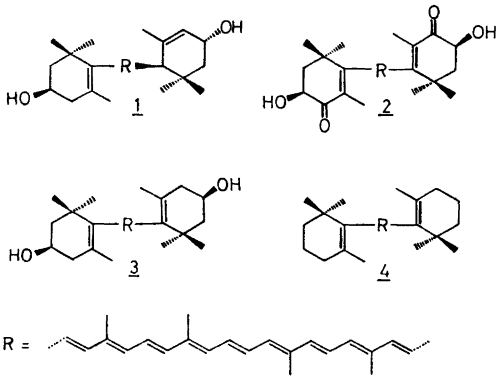
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Hitherto no carotenoids of identical constitution have been encountered in Nature with different chirality,[1,2] a phenomenon which may reflect general biosynthetic routes.

Astaxanthin has a wide-spread occurrence. Its biosynthesis in goldfish has been considered to occur from lutein,[3] a result recently questioned on the basis of the absolute configuration of lutein (*1*) [4,5] and astaxanthin (*2*).[6] A precursor role of zeaxanthin (*3*) or β-carotene (*4*) is better compatible with the chirality of astaxanthin. More recently an alternative biosynthetic route from β-carotene (*4*) to astaxanthin (*2*) has been reported for goldfish,[7,8] similar to findings for Crustaceae.[9–12]

Studies by Veerman [13–15] suggest that astaxanthin in spider mites is synthesized from β-carotene (*4*) of plant origin. It was considered of interest to examine the chirality of astaxanthin resulting from mite metabolism.



Astaxanthin diesters (λ_{max} 500 nm in CS_2) were isolated from an unsaponified extract of *Schizonobia sycophanta* Womersley [16] by TLC on plates according to Bjørnland [17] (prepared from 30 g kieselgel, 9 g MgO, 12 g $Ca(OH)_2$, 3 g $CaSO_4$ and 93 ml H_2O) using 30 % acetone in petroleum ether for development. The CD spectrum in EPA (diethyl ether:isopentane: ethanol 5:5:2) solution (Fig. 1) was in the 230–340 nm region consistent with that of

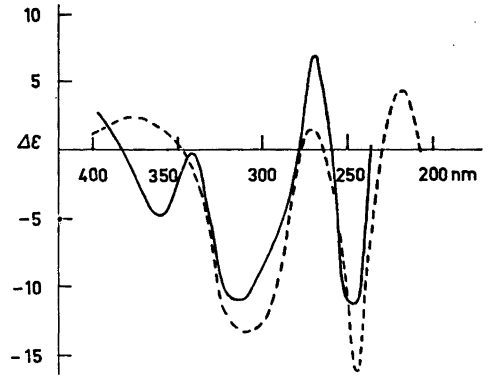


Fig. 1. CD spectra of – astaxanthin diester *ex S. sycophanta* and - - - astaxanthin monoester *ex* lobster in EPA solution. $\Delta\varepsilon$ values valid only for ———.

astaxanthin (*2*) monoester *ex* lobster,[6] demonstrating the 3*S*,3′*S*-chirality also of astaxanthin (*2*) produced by spider mite.

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Dicarboxylic Acids Produced by Oxygen-Alkali Treatment of Birch Xylan

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In recent years great interest has been focussed on processes in which wood pulp and wood are delignified by oxygen in alkaline media. Unfortunately, the carbohydrates are attacked and give rise to soluble carboxylic acids. 4-*O*-Methylglucuronoxylan is a major constituent in birch and the formation of monocarboxylic acids from this material was recently studied.[1] The present paper deals with the formation of dicarboxylic acids. Under the severe conditions applied, the yield of the dicarboxylic acid fraction was 21 % compared to 35 % for the non-volatile monocarboxylic acids and 18 % for the non-electrolytes.

The use of trimethylsilyl (Me_3Si) derivatives permitted analyses of the dicarboxylic acids by gas chromatography (Fig. 1). Similarly, excellent separations of the acids were obtained by anion exchange chromatography. The amount of individual acids and their retention data are given in Table 1.

C-(2-Hydroxyethyl)tartronic acid is predominant among the dicarboxylic acids and its formation in 12 % yield makes it a major product from xylan. The structure indicates that it is formed from terminal, oxidized xylose units by β-elimination at C-4 and subsequent benzilic acid rearrangement (Fig. 2). It is noteworthy that the corresponding acid with six carbon atoms, *C*-(2,3-dihydroxypropyl)tartronic acid, is the major dicarboxylic acid formed from

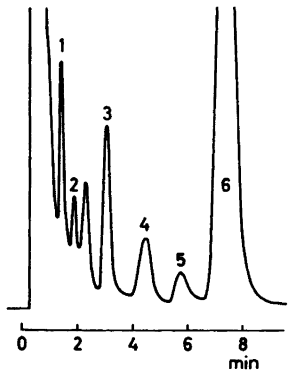


Fig. 1. Gas chromatographic separation on QF-1 at 120 °C of the dicarboxylic acids as Me_3Si derivatives. Identified acids: 1. Oxalic. 2. *C*-Methyltartronic. 3. Tartronic. 4. Deoxytetraric and *C*-(hydroxymethyl)tartronic. 5. *O*-Methylerythraric. 6. *C*-(2-Hydroxyethyl)tartronic.

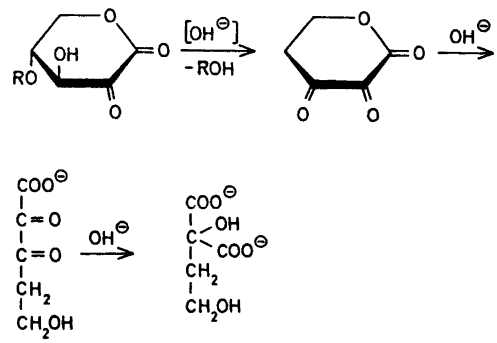


Fig. 2. Schematic representation of the formation of *C*-(2-hydroxyethyl)tartronic acid.

Table 1. Chromatographic data and amounts of dicarboxylic acids formed during oxygen-alkali treatment of xylan.

Acids	Yield g [a]	r [b] OV-1 160 °C	OV-17 160 °C	QF-1 120 °C	D_v [c]
C-(2-Hydroxyethyl)tartronic	11.9	0.252	0.444	0.479	15.7
C-(Hydroxymethyl)tartronic	0.3	0.154	0.261	0.275	22.6
O-Methylerythraric	0.6	0.130	0.303	0.374	11.0
Deoxytetraric	0.7	0.106	0.211	0.281	8.7
C-Methyltartronic	0.3	0.056	0.104	0.110	24.7
Tartronic	1.5	0.065	0.148	0.188	33.4
Oxalic	0.7	0.017	0.044	0.075	–

[a] Obtained from 100 g xylan. [b] Retention of Me_3Si derivatives in GLC relative to the glucitol derivative. [c] Values in ion exchange chromatography, 0.3 M NaOAc + 2 M HOAc.

cellulose [2] and cellobiose.[3] Evidently, the two acids are formed in analogous reactions. These observations suggest that 2-ulosonic acid end-groups or ulosono-1,5-lactone end-groups, related in structure to the ascorbic acids are formed as precursors. The formation of the lactones can be envisaged as oxidation of an enediol grouping at C-1 to C-2 of aldosulose end-groups in the pyranose form. Since aldosulose end-groups are formed as intermediates,[1] the reaction path *via* ulosono-1,5-lactone end-groups appears to be most likely. Oxidative cleavage may explain the formation of oxalic acid and contribute to the large production of 3-hydroxypropanoic acid.[1]

On the average, every tenth xylopyranose unit in birch xylan is substituted at carbon atom 2 by a 4-*O*-methylglucuronic acid unit. Appreciable amounts of 4-*O*-methylglucuronic acid were detected in the solution after oxygen-alkali treatment of birch xylan carried out under such conditions that the alkali was consumed.[1] Under conditions comparable to those applied in the present work, the liberated 4-*O*-methylglucuronic acid is decomposed rapidly [3] and as expected only a very small amount (less than 0.1 % of the xylan) could be traced in the monocarboxylic acid fraction obtained in the present work. *O*-Methylerythraric and deoxytetraric acids are the most abundant dicarboxylic acids formed during oxygen-alkali treatment of 4-*O*-methylglucuronic acid. Obviously *O*-methylerythraric acid (Table 1) originates from liberated 4-*O*-methylglucuronic acid and most likely deoxytetraric acid is at least in part derived from the same source.

Experimental. Water (160 ml) was heated to 98 °C in a bubbling column made of Teflon. Xylan isolated from birch meal [4] was added, oxygen was bubbled through the column, and a sodium hydroxide solution injected. The concentration of xylan was 1.3 % and of sodium hydroxide 1.1 %. After a 4 h treatment at a gauge pressure of 0.6 MPa and an oxygen flow rate of 1.3×10^{-2} mol cm^{-2} min^{-1}, the solution was analysed. The dicarboxylic acids were separated by anion exchange chromatography [2] from non-volatile monocarboxylic acids and non-electrolytes. They were analysed by gas chromatography as acyclic Me_3Si derivatives [5] and by anion exchange chromatography (30 °C) with 0.3 M sodium acetate in 2 M acetic acid as eluent.[6]

C-(2-Hydroxyethyl)tartronic acid was identified by mass spectrometry of its Me_3Si derivative.[3] To confirm the structure, a sample of the mixture of dicarboxylic acids was evaporated with 3 M hydrochloric acid and reduced with potassium borohydride. The major product was shown by gas chromatography-mass spectrometry to be the anticipated 3-deoxy-2-*C*-(hydroxymethyl)tetronic acid. *O*-Methylerythraric and *C*-(hydroxymethyl)tartronic acids were identified by comparison of the retention data (Table 1) and the mass spectra with those of acids identified in previous work from this laboratory. The identification of the other acids by gas chromatography and ion exchange chromatography was confirmed by a comparison of their mass spectra with those of commercial samples.

The quantitative determination of *C*-(2-hydroxyethyl)tartronic acid was made from the peak area recorded on automatic analysis by chromic acid oxidation of the eluate from ion exchange chromatography.[7] The other acids were determined by gas chromatography assuming that the peak areas were proportional to the weight of the derivatives. The quantities determined for oxalic and tartronic acids may be somewhat low because their Me_3Si derivatives decompose easily.

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Berichtigung zu »Reaktionen von Trichlormethansulfenylchlorid mit Stickstoffverbindungen. IV. Substituenteneffekte in 5-Aryl-1,3,4-oxathiazol-2-onen»*

ALEXANDER SENNING und
JAKOB SCHMIDT RASMUSSEN

Chemisches Institut der Universität Aarhus, DK-8000 Århus C, Dänemark

Auf Seite 2170, Zeile 18 muss es statt «0,056« richtig »5,6 × 10^{-5}» heissen.

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Tobacco Chemistry. 32. The Absolute Configuration of Norsolanadione, (5*S*)-5-Isopropyl-3*E*-nonen-2,8-dione, a Nor-thunberganoid of *Nicotiana tabacum* L.

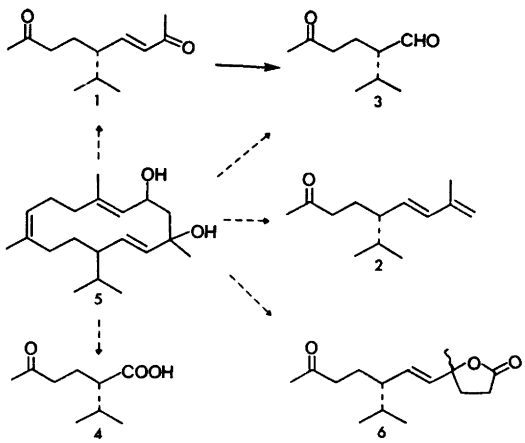
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Synthetic, racemic norsolanadione, (±)-5-isopropyl-3*E*-nonen-2,8-dione (*1*), was first described in 1965 as an air oxidation product of synthetic solanone, (±)-2-methyl-5-isopropyl-1,3*E*-nonadien-8-one (*2*), by Johnson and Nicholson who had isolated the dextrorotatory form of the latter from aged Burley tobacco.[1] The absolute configuration of solanone was later established ** as *S* by Fukuzumi *et al.*[2] by correlation with (2*S*)-2-isopropyl-5-oxohexanoic acid (*4*). In 1971 norsolanadione (*1*) was identified by Shigematsu *et al.*[3] as a component of tobacco smoke, and it was subsequently also found to be a constituent of Burley,[4,5] Turkish [6] and Greek tobacco leaves.[7]

Since norsolanadione (*1*), solanone (2) and other structurally related tobacco constituents can be viewed as degradation products of thunbergane diterpenoids,[8] determination of remaining chiral centres offers a possibility of checking the assumed interrelationships. The absolute configuration of norsolanadione (*1*) was elucidated by converting it and solanone (*2*), both isolated from Greek tobacco,[9] to a common degradation product, (2*S*)-5-oxo-2-isopropylhexanal (*3*), by ozonolysis and reductive work-up. In view of their identical absolute configurations and since solanone decomposes to norsolanadione when subjected to air oxidation,[1] and the β-4,8,13-duvatrien-1,3-diol (*5*),[10] commonly encountered in tobacco, is known to yield solanone on irradiation,[11] it seems plausible that norsolanadione (*1*) is formed by a similar pathway in the tobacco plant.

The absolute configurations *** of the eleven thunbergane diterpenoids encountered in tobacco are presently unknown,[12] although it is probable that cembrene, detected in tobacco only under the influence of enzyme-blocking agents,[13] possesses the same absolute configuration, *S*, as cembrene [(1*S*)-2*E*,4*Z*,7*E*,11*E*-thunbergatetraene] isolated from pine-trees of the subgenus *Haploxylon*.[14,15] Since it has been demonstrated [13] that ^{14}C-labelled cembrene enzymatically is converted to duvatrienols in the tobacco plant, and that all tobacco compounds of known chirality and assumed to be derived from thunbergane diterpenoids, possess the *S*-configuration – *i.e.* (2*S*)-2-isopropyl-5-oxohexanoic acid (*4*), (7*S*)-10-oxo-4ξ-methyl-7-isopropyl-5*E*-undecen-4-olide (*6*),[16] solanone (*2*),[2] and norsolanadione (*1*) – it is reasonable to postulate that all known tobacco thunberganoids possess *S*-configuration.†



Scheme 1. The absolute configuration of norsolanadione (*1*), established by conversion to (2*S*)-5-oxo-2-isopropylhexanal (*3*), and of other tobacco constituents assumed to be derived from diterpenoids of the thunbergane-type, *e.g.* *5*.

Experimental. NMR, IR, and mass spectra were recorded on Varian XL-100, Digilab FTS-14, and LKB 9000 instruments, respectively. Rotations were measured on a Perkin-Elmer 141 instrument. Norsolanadione (*1*) was isolated from fraction B7 [9] by preparative gas chromatography performed on a 3 m × 3.2 mm glass column packed with 5 % Carbowax 20 M on Chromosorb G.

Norsolanadione. (*1*). MS: 196 (M^+, 1), 43 (100), 97 (54), 95 (40), 41 (24), 93 (18), 126 (18), 55 (17), 111 (15), 135 (14), 120 (14), 121 (14), 123 (13); δ($CDCl_3$): 0.88 (3 H, d, *J* 6.5 Hz), 0.93 (3 H, d, *J* 6.5 Hz), 2.12 (3 H, s), 2.26 (3 H, s), *ca.* 2.37 (2 H, distorted t, *J ca.* 7 Hz), 6.03 (1 H, d, *J* 16 Hz), 6.56 (1 H, dd, *J* 9 and 16 Hz); lit.[1] δ(CCl_4): 0.92 (6 H, 'two superimposed doublets'), 2.04 (3 H, s), 2.15 (3 H, s), 2.35 (2 H, t), AB-part of an ABX-system "centred at δ 6.25 (*J* 8 and 16 Hz); ν_{max}(film): 2960 (s), 2931 (m), 2876 (m), 1717 (s), 1676 (s), 1626 (w), 1367 (s), 1258 (s), 1168 (m), 989 (m); lit.[1] ν_{max}: 1715, 1675, 1624, 990; $[\alpha]^{20}$ −2.2° (589 nm), −2.2° (578), −2.2°

** The *R*/*S*-convention was erroneously applied in the original paper [2] by attributing the *R*-configuration to solanone.

*** *Note added in proof*: By ozonolytic degradation we have recently established that the tobacco thunberganoids possess the 1*S*-configuration.

† In all these compounds the units linked to the asymmetric centre, C(1), are the same, *i.e.* −H, $-CH_2-$, −CH=CH−, and $-CH(CH_3)_2$.[17,18]

(546), −0.3° (436), (*c* 0.67, $CHCl_3$).

Ozonolysis of norsolanadione. Norsolanadione (*1*, 30 mg) dissolved in CH_2Cl_2 (15 ml) and pyridine (0.1 ml) was treated with excess ozone at −65 °C. Zinc powder (500 mg) and acetic acid (2 ml) were added and the mixture stirred 35 min during which the temperature was slowly raised to 20 °C. The solution was decanted, the reaction vessel with its residue was washed with CH_2Cl_2, and the combined solutions washed four times with water. Removal of the solvent left a slightly yellow oil which was chromatographed on silica gel furnishing pure (2*S*)-5-oxo-2-isopropylhexanal (*3*, 10 mg). NMR, IR, and mass spectra were indistinguishable from those of (2*S*)-5-oxo-2-isopropylhexanal (*3*) prepared previously by ozonolysis of solanone. $[\alpha]^{20}$ +47.8° (589 mn), +50.7° (578), +61.4° (546), +146.6° (436), +400.8° (365), (*c* 0.73, $CHCl_3$); lit.[16] $[\alpha]^{20}$ +43.7° (589 nm), +45.9° (578), +55.6° (546), +133.3° (436), +364.8° (365) (*c* 0.54, $CHCl_3$).

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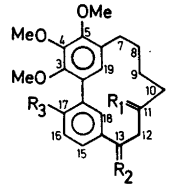
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Porson, a New [7,0]-Metacyclophane from *Myrica gale* L.

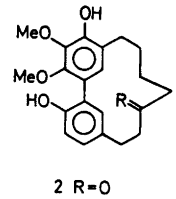
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[a] Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH and [b] University of Trondheim, Department of Chemistry, NLHT-Rosenborg, N-7000 Trondheim, Norway

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2 R=O
3 R=H,OH

The most prominent features of the NMR spectrum of *1* are the presence of resonances due to three methoxy groups (δ 3.83, 3.91 and 3.95) and the signals of four aromatic protons [δ 6.46 (s, H-19), ABX pattern: 6.65 ($J \approx 2$ Hz, H-18), 6.88 ($J \approx 8$ Hz, H-16), 7.02 ($J \approx 2$ and 8 Hz, H-15)]. Two hydroxy resonances are also visible; one phenolic (δ 7.82) and one due to a secondary aliphatic hydroxy group ($\delta \sim 3$), the carbinyl proton being located at δ 4.35 as a doublet of doublets ($J = 2$ and 6 Hz). The relative low field resonance of this proton is indicative of its position at C-13, close to the aromatic nucleus. Irradiation at δ 4.35 collapses a multiplet at δ 2.95 and *vice versa* thus indicating the resonance position of the C-12 methylene group. In the same region of the spectrum (δ 2.5–3.0) is also located the resonances of four more protons, probably 2H-7 and 2H-10. Moreover, the resonance position of the H-12

(546), −0.3° (436), (*c* 0.67, $CHCl_3$).

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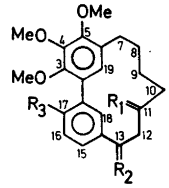
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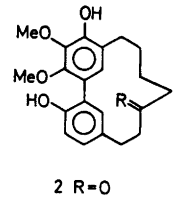
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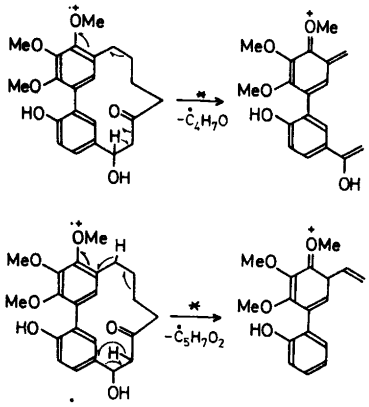


2 R=O
3 R=H,OH

The most prominent features of the NMR spectrum of *1* are the presence of resonances due to three methoxy groups (δ 3.83, 3.91 and 3.95) and the signals of four aromatic protons [δ 6.46 (s, H-19), ABX pattern: 6.65 ($J \approx 2$ Hz, H-18), 6.88 ($J \approx 8$ Hz, H-16), 7.02 ($J \approx 2$ and 8 Hz, H-15)]. Two hydroxy resonances are also visible; one phenolic (δ 7.82) and one due to a secondary aliphatic hydroxy group ($\delta \sim 3$), the carbinyl proton being located at δ 4.35 as a doublet of doublets ($J = 2$ and 6 Hz). The relative low field resonance of this proton is indicative of its position at C-13, close to the aromatic nucleus. Irradiation at δ 4.35 collapses a multiplet at δ 2.95 and *vice versa* thus indicating the resonance position of the C-12 methylene group. In the same region of the spectrum (δ 2.5–3.0) is also located the resonances of four more protons, probably 2H-7 and 2H-10. Moreover, the resonance position of the H-12

protons indicates that their neighbour is the keto group. Consequently the keto group must be located at C-11. The resonances of the remaining four hydrogens in the seven membered carbon chain (2H-8 and 2H-9) are located between δ 1.5 and 2.0.

The mass spectrum of porson has a very large molecular ion peak, more than ten times the second most abundant peak. However, characteristic peaks due to cleavages in the seven-membered carbon chain are also of diagnostic value. Thus peaks at *m/e* 315 (6 %) and *m/e* 287 (7 %) may be explained as indicated in Scheme 1.



Scheme 1.

On acetylation porson yields a diacetate (*4*) (m.p. 168 – 169 °C, ν_{max}($CHCl_3$) 1765, 1740, 1715 cm^{-1}). The most significant features of its NMR spectrum are the appearance of the resonances of two acetoxy groups (δ 2.09, 2.23), the downfield shift of the methine proton at C-13 (δ 5.30) and an upfield shift of one of the methoxy resonances (δ 3.63). This upfield shift accords with one reported for one of the OCH_3 groups in myricanone diacetate [2] and it seems reasonable that the phenolic hydroxy group (C-17) and one of the methoxy groups (C-3) have a similar relative position in the two compounds.

The sterically hindered position of the phenolic hydroxy group is probably also the reason for its unwillingness to be methylated with diazomethane. Oxydation of porson with Jones reagent furnished an oily diketone (*5*).

The IR spectrum ($CHCl_3$) of this diketone reveals the presence of one aliphatic (1715 cm^{-1}) and one aromatically conjugated (1695 cm^{-1}) keto group. Due to the conjugation the band at 1615 has also gained considerably in intensity. This β-diketone does not seem to be enolized to any extent. Enolization is probably unfavourable due to steric reasons. In the NMR spectrum of *5* the resonance of the methine proton is absent.

When the secondary hydroxy group of porson (*1*) is acetylated or oxidized, there are only minor changes in the chemical shift of the methoxy groups. This indicates that the secondary hydroxy group is located at C-13 and not at C-7.

Oppenauer oxydation of porson (acetone/Al *tert*-butoxide) yielded 10 % of an isomeric ketol (*6*) in which the keto group is conjugated to the aromatic nucleus (ν_{max}($CHCl_3$) 1695, 1615 cm^{-1}).

The chemical and spectroscopic properties of porson supports the constitution *1*. However, conclusive evidence for its correctness has not yet been provided. X-Ray studies of porson are under way.

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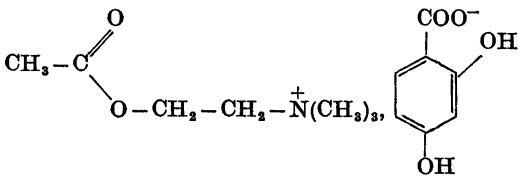
The Crystal Structure of Acetylcholine β-Resorcylate

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Acetylcholine β-resorcylate, $C_{14}H_{21}NO_6$, crystallizes in space group $P2_1/c$ with a unit cell having dimensions $a = 14.562(6)$, $b = 14.109(4)$, $c = 15.096(6)$ Å, and $\beta = 106.90(3)°$. $Z = 8$. The structure was determined by direct methods from diffractometer collected three-dimensional X-ray data and refined by full-matrix least-squares techniques to an R index of 0.078. Layers of β-resorcylate ions alternate with layers of acetylcholine ions. The β-resorcylate ions are linked together in infinite chains by strong hydrogen bonds while no hydrogen bonds involve the acetylcholine ions.

The crystal structure determination of acetylcholine β-resorcylate was taken up as part of conformational studies of choline derivatives in



the solid state. The crystal structures of a large number of salts of choline derivatives are known. Among these the carbamoylcholine salts [1-2] seem to be exceptional, showing an apparently greater flexibility than found for most other choline derivatives. This is in agreement with the PCILO calculations of Pullman and Courière.[3] However, it is noticed, that in all known crystal structures of carbamoylcholine salts the two possible hydrogen bonds N—H···X are actually found, and the possibility is not precluded that the formation of these hydrogen bonds is of importance for the observed conformational changes. Among the salts of other choline derivatives, of which the crystal structures have been determined, only very few have any possibility of formation of hydrogen bonds. It therefore seems to be of interest to examine salts of choline derivatives with hydroxy acids and other potential hydrogen bond donors, especially in view of the actions of acetylcholine in biological media.

EXPERIMENTAL

Acetylcholine β-resorcylate was prepared from an aqueous solution of acetylcholine chloride and excess of freshly precipitated silver salt of β-resorcylic acid. After stirring for 1 h the precipitate was filtered off, and the solution evaporated *in vacuo*. Colourless prisms of acetylcholine β-resorcylate were obtained by recrystallization from ethanol. The crystals used for the X-ray examination were grown by diffusion at room temperature of dimethoxymethane into a solution of the compound in ethanol containing 5 % water.

Crystal data. Acetylcholine β-resorcylate, $C_{14}H_{21}NO_6$, M = 299.33. M.p. 144.5 – 147 °C (decomp.). Monoclinic, $a = 14.562(6)$, $b = 14.109(4)$, $c = 15.096(6)$ Å. $\beta = 106.90°$, $U = 2967.6$ Å^3. $D_m = 1.33$ g cm^{-3}, $Z = 8$, $D_c = 1.34$ g cm^{-3}. Linear absorption coefficient for X-rays [λ(Mo$K\alpha$) = 0.7107 Å], $\mu = 1.1$ cm^{-1}. $F(000) = 1280$. Systematically absent reflections: $h0l$ when l is odd, $0k0$ when k is odd: space group $P2_1/c$.

The unit-cell parameters were refined by least-squares techniques from the diffractometer-measured θ angles observed for 33 reflections. The crystal density was measured by flotation in a mixture of chlorobenzene and bromobenzene. The melting point was determined on a Leitz hot stage microscope.

Data collection. Three-dimensional diffraction data were measured at room temperature (20 – 22 °C) on a Nonius three-circle automatic dif-

Table 1. Atomic parameters with estimated standard deviations, referring to the last significant figure, in parenthesis. Positional parameters are $\times 10^4$. Thermal parameters are $\times 10^2$. The temperature factor is defined by
$\exp[-2\pi^2(U_{11}h^2a^{*2}+\cdots+2U_{12}hka^*b^*+\cdots)]$

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(11)	2234(4)	1723(4)	0104(3)	4.0(3)	5.0(3)	3.1(3)	0.3(2)	1.4(2)	−0.2(2)
C(12)	2197(4)	2395(4)	0775(4)	4.1(3)	4.9(3)	3.9(3)	−0.2(3)	0.8(2)	−0.5(3)
C(13)	2200(4)	2100(4)	1665(3)	5.3(3)	4.9(3)	2.6(3)	−0.4(3)	1.1(2)	−0.5(2)
C(14)	2221(4)	1155(4)	1865(4)	4.6(3)	5.6(4)	3.4(3)	0.3(3)	0.7(2)	0.4(3)
C(15)	2258(4)	0477(4)	1209(4)	5.1(3)	4.2(3)	4.7(3)	0.3(3)	1.1(3)	−0.4(3)
C(16)	2262(4)	0772(4)	0334(4)	4.7(3)	5.6(4)	3.7(3)	0.2(3)	1.2(3)	−0.4(3)
C(17)	2262(4)	2037(5)	−0836(4)	4.4(3)	6.5(4)	3.9(3)	0.3(3)	1.0(3)	−0.3(3)
O(171)	2318(3)	1436(3)	−1417(3)	8.9(3)	7.3(3)	4.4(2)	0.0(3)	3.0(2)	−0.7(2)
O(172)	2221(3)	2933(3)	−0990(3)	8.9(3)	6.5(3)	4.4(2)	0.1(2)	3.1(2)	0.9(2)
O(12)	2183(3)	3342(3)	0604(3)	8.5(3)	4.7(2)	4.8(2)	−0.3(2)	2.9(2)	0.1(2)
O(14)	2215(3)	0856(3)	2727(3)	8.7(3)	6.0(3)	3.4(2)	−0.1(2)	1.9(2)	1.1(2)
C(21)	7143(4)	3098(4)	1060(3)	4.0(3)	4.8(3)	2.9(3)	−0.4(2)	1.4(2)	−0.4(2)
C(22)	7330(4)	2476(4)	0403(4)	4.0(3)	3.6(3)	4.0(3)	−0.2(2)	1.6(2)	−0.4(2)
C(23)	7359(4)	2827(4)	−0456(4)	5.1(3)	4.8(3)	3.7(3)	0.2(3)	2.0(3)	−0.2(3)
C(24)	7209(4)	3780(4)	−0652(4)	5.0(3)	4.8(3)	3.1(3)	−0.1(3)	1.4(2)	−0.1(3)
C(25)	7016(4)	4406(4)	−0014(4)	6.1(4)	4.7(3)	3.7(3)	0.2(3)	1.2(3)	−0.2(3)
C(26)	6992(4)	4046(4)	0830(4)	4.9(3)	5.0(3)	3.7(3)	0.0(3)	1.2(3)	−0.5(3)
C(27)	7145(4)	2751(5)	1994(4)	4.6(3)	6.2(4)	3.2(3)	−0.8(3)	1.3(3)	−0.4(3)
O(271)	6932(4)	3302(3)	2543(3)	10.1(4)	7.1(3)	4.0(2)	1.0(3)	3.6(2)	0.1(2)
O(272)	7388(3)	1876(3)	2177(3)	9.2(3)	5.1(3)	4.4(2)	0.1(2)	3.0(2)	0.6(2)
O(22)	7484(3)	1542(3)	0586(3)	8.7(3)	3.8(2)	4.8(2)	0.4(2)	2.7(2)	0.4(2)
O(24)	7232(3)	4144(3)	−1479(3)	9.6(3)	5.0(2)	4.1(2)	0.4(2)	3.2(2)	0.6(2)
C(31)	4600(5)	3500(5)	−0424(5)	7.4(5)	8.5(5)	5.2(4)	0.2(4)	2.3(3)	−0.5(4)
C(32)	4637(4)	2957(5)	0436(5)	4.8(4)	6.6(5)	5.7(4)	0.5(3)	1.7(3)	−1.5(3)
O(33)	4716(4)	2108(4)	0526(3)	9.6(4)	5.9(3)	7.9(3)	0.8(3)	3.8(3)	−1.5(3)
O(34)	4566(3)	3532(3)	1123(3)	6.8(3)	5.9(3)	5.0(2)	0.1(2)	2.2(2)	−0.9(2)
C(35)	4559(5)	3070(4)	1976(4)	7.0(4)	5.3(4)	5.5(4)	−0.2(3)	2.5(3)	−0.6(3)
C(36)	4108(4)	3741(4)	2515(4)	4.9(3)	5.8(4)	5.0(3)	−0.6(3)	1.6(3)	−0.7(3)
N(37)	4777(3)	4470(3)	3109(3)	4.7(3)	5.1(3)	3.9(2)	0.3(2)	1.4(2)	0.1(2)
C(38)	5193(5)	5106(5)	2524(5)	8.5(5)	6.1(4)	5.0(4)	−1.4(4)	2.6(4)	0.6(3)
C(39)	5581(5)	4016(5)	3842(4)	5.7(4)	7.4(4)	4.1(3)	0.6(3)	0.7(3)	0.8(3)
C(310)	4177(5)	5050(5)	3571(5)	6.8(4)	7.1(5)	6.1(4)	1.2(4)	2.4(3)	−1.2(4)
C(41)	9718(5)	3575(6)	5182(5)	7.3(5)	9.9(6)	7.7(5)	0.6(4)	3.5(4)	1.3(5)
C(42)	9708(5)	3090(6)	4312(6)	5.2(4)	7.9(5)	8.8(5)	0.2(4)	2.9(4)	2.5(5)
O(43)	9704(5)	2249(5)	4201(5)	12.3(5)	8.2(4)	12.5(5)	0.7(4)	5.4(4)	1.8(4)
O(44)	9701(4)	3707(4)	3638(3)	10.2(4)	8.3(4)	6.3(3)	−2.4(3)	2.1(3)	1.1(3)
C(45)	9687(8)	3275(7)	2744(6)	17.1(10)	8.9(6)	6.7(5)	−4.4(6)	2.0(6)	0.2(5)
C(46)	9419(7)	3919(7)	2040(7)	14.2(8)	9.6(7)	9.3(7)	−3.4(6)	3.4(6)	−0.2(6)
N(47)	10107(4)	4685(4)	1885(4)	5.7(3)	6.3(3)	6.0(3)	0.4(3)	2.6(3)	0.1(3)
C(48)	10426(6)	5292(6)	2712(6)	11.3(7)	7.6(5)	7.7(5)	−2.9(5)	2.2(5)	−1.2(4)
C(49)	10915(6)	4326(8)	1580(6)	8.1(6)	19.1(10)	9.6(7)	5.8(6)	4.4(5)	2.6(7)
C(410)	9454(6)	5239(7)	1101(6)	8.6(6)	14.1(8)	7.0(5)	5.0(6)	1.6(4)	1.7(5)

fractometer using graphite monochromated Mo$K\alpha$ radiation. The ω scan technique with a scan speed of 1.2° min^{-1} was employed, and the scan angle was 1.2°. Background counts were taken for half the scanning time at each of the scan range limits. One standard reflection was measured after each 25 reflections. All the data were measured from a single crystal of irregular shape. The approximate dimensions were 0.20 × 0.36 × 0.48 mm. The crystal was mounted with [001] along the ϕ-axis of the goniostat. Out of the 5435 reflections measured in the range $2.5° \leq \theta \leq 25°$, 2509 had $I_{net} \geqq 2.5\ \sigma(I)$, where $\sigma(I)$ is the standard deviation from counting

statistics. These were regarded as observed reflections. Lorentz and polarization corrections were applied, but no absorption corrections were made owing to the low absorption coefficient.

Structure determination and refinement. The structure was solved by direct methods in a straight forward way using the programs of the X-RAY system.[4] In an *E*-map based on 299 *E(hkl)*'s with $|E(hkl)| \geq 1.7$ the 29 highest peaks were found in positions corresponding to the heavier atoms of the two β-resorcylate ions and to seven atoms [C(31) – N(37)] of one of the acetylcholine ions. Seven more of the higher peaks were found at correct atomic positions [corresponding to C(38) – C(310) and C(41) – O(44)]. From an electron density map calculated using the phases from the contributions of the above mentioned 29 atoms the positions of the remaining 13 atoms in the asymmetric unit could be deduced. Individual atomic parameters of all 42 carbon, nitrogen, and oxygen atoms were refined using the full-matrix least-squares method. Owing to the great number of variables the refinements were performed in a few big blocks. A three-dimensional difference synthesis calculated at the stage of convergence of the refinement using individual isotropic temperature factors ($R \sim 0.14$) showed maxima at the calculated positions for 19 of the 42 hydrogen atoms, namely the hydrogen atoms bonded to the benzene rings and 13 of the 16 hydrogen atoms of one of the acetylcholine ions, III. The 19 hydrogen atoms were introduced in the refinement as a fixed contribution in the calculated positions and with arbitrary temperature factors. After refinement of the heavier atoms using individual anisotropic temperature factors a difference synthesis based only on reflections for which $\sin\theta/\lambda \leq 0.35$ was examined, but from this only the positions of the four OH-hydrogen atoms could further be postulated. Nevertheless all 42 hydrogen atoms were now introduced as a fixed contribution in the refinement, in an attempt to reduce the very strong "thermal motion" observed (*cf.* Table 1). The three hydrogen atoms of each of

Table 2. Atomic parameters used for the hydrogen atoms. Positional parameters are × 10^4. Thermal parameters are × 10^2.

Atom	x/a	y/b	z/c	U	P.P.	Atom	x/a	y/b	z/c	U	P.P.
H(13)	2177	2577	2147	5.1		H(391)	5304	3626	4244	7.6	
H(15)	2285	−0214	1362	5.1		H(392)	5962	3625	3533	7.6	
H(16)	2269	0284	−0141	5.1		H(393)	6015	4512	4234	7.6	
H(12)	2200	3450	0000	7.6		H(3101)	3659	5365	3095	7.6	
H(14)	2200	1370	3200	7.6		H(3102)	3916	4624	3963	7.6	
						H(3103)	4628	5524	3964	7.6	
H(23)	7507	2384	−0920	5.1							
H(25)	6898	5094	−0160	5.1		H(4113)	9138	3333	5339	10.1	0.5
H(26)	6836	4490	1281	5.1		H(4123)	10309	3357	5644	10.1	0.5
H(22)	7350	1430	1240	7.6		H(4133)	9698	4282	5256	10.1	0.5
H(24)	7350	3820	−1960	7.6		H(4114)	10303	3978	5380	10.1	0.5
						H(4124)	9131	3994	5056	10.1	0.5
H(3113)	5182	3340	−0595	7.6	0.5	H(4134)	9707	3123	5680	10.1	0.5
H(3123)	4014	3242	−0913	7.6	0.5	H(451)	10420	3075	2821	10.1	
H(3133)	4522	4211	−0511	7.6	0.5	H(452)	9334	2667	2658	10.1	
H(3114)	3957	3854	−0630	7.6	0.5	H(461)	9152	3582	1391	10.1	
H(3124)	5123	3975	−0300	7.6	0.5	H(462)	8807	4293	2086	10.1	
H(3134)	4630	3086	−0952	7.6	0.5	H(481)	9860	5636	2794	10.1	
H(351)	4161	2484	1836	7.6		H(482)	10731	4894	3247	10.1	
H(352)	5222	2912	2357	7.6		H(483)	10905	5782	2629	10.1	
H(361)	3589	4094	2058	7.6		H(491)	11235	3770	2032	10.1	
H(362)	3839	3356	2928	7.6		H(492)	10615	3937	0954	10.1	
H(381)	5576	4707	2200	7.6		H(493)	11376	4735	1498	10.1	
H(382)	4672	5437	2051	7.6		H(4101)	9209	4862	0524	10.1	
H(383)	5637	5587	2916	7.6		H(4102)	8911	5546	1268	10.1	
						H(4103)	9938	5734	1030	10.1	

Table 3. Least squares planes. The equations of the planes are in direct (unit cell) space. Distances (Å) to atoms defining the plane are marked with an asterisk.

Plane I	$13.8613x + 0.4643y + 0.2239z = 3.1780$
Plane II	$13.2396x + 2.4566y + 1.4735z = 10.3725$
Plane III	$13.7909x + 1.3358y + 0.2758z = 6.8000$
Plane IV	$13.9762x - 0.0328y - 0.1564z = 13.4899$

I		II	
C(11)	0.001*	C(21)	0.002*
C(12)	−0.005*	C(22)	−0.001*
C(13)	0.006*	C(23)	−0.002*
C(14)	−0.004*	C(24)	0.005*
C(15)	0.000*	C(25)	−0.004*
C(16)	0.001*	C(26)	0.001*
C(17)	0.034	C(27)	0.056
O(171)	0.070	O(271)	−0.009
O(172)	0.015	O(272)	0.190
O(12)	0.016	O(22)	0.002
O(14)	−0.007	O(24)	0.002
III		**IV**	
C(31)	−0.000*	C(41)	−0.000*
C(32)	0.001*	C(42)	0.001*
O(33)	−0.001*	O(43)	−0.000*
O(34)	−0.000*	O(44)	−0.000*
C(35)	−0.048	C(45)	−0.005
C(36)	−0.565	C(46)	−0.371
N(37)	0.471	N(47)	0.591
C(38)	1.114	C(48)	1.022
C(39)	1.539	C(49)	1.726
C(310)	−0.267	C(410)	−0.311

the two acetyl groups were introduced as two times six atoms with occupancy-factor 0.5. On convergence of the refinement using anisotropic temperature factors the R-value was 0.078. The quantity minimized was $\sum w(|F_o| - |F_c|)^2$, where weights were taken as a unity. A weight analysis showed this not to be too unsatisfactory and in view of the limited amount of data and the expence in computer time further refinement was judged not to be justified. In all calculations the contribution from unobserved reflections were included in the refinement, when $|F_c| \geq |F_o|$. All the atoms were treated as uncharged, and the form factors were taken from *International Tables for X-Ray Crystallography*.[5] The calculations were performed on a GIER computer and an IBM 360/75 computer using *INDIFF*,[6] a local version of *The N.R.C. 2A Picker Data Reduction Program*,[7] *The X-Ray System*,[4] and *ORTEP*.[8]

The observed and calculated structure factor data are available from the author upon request.

The final atomic parameters of carbon, nitrogen and oxygen atoms are listed in Table 1. In Table 2 the parameters used for the hydrogen atoms are given. Bond lengths and angles are shown in Fig. 1. The parameters of a number of planes calculated through parts of the ions are given in Table 3.

RESULTS AND DISCUSSION

The structure is built up of alternating negatively and positively charged puckered layers roughly parallel to (100) and with an *inter*layer distance of approximately $x/4$, *cf.* Fig. 2. Layers consisting of both of the crystallographically independent β-resorcylate ions I and II are found about $x \sim 1/4$ and $x \sim 3/4$. A layer consisting of acetylcholine ions III only is found about $x \sim \frac{1}{2}$, and a layer of acetylcholine ions IV is situated about $x \sim 1.0$. The layers are held together by a great number of relatively weak *inter*ionic contacts. The resorcylate ions within each layer are linked together in infinite chains in the c direction by strong hydrogen bonds O(x4)−H···O(x72). Chains consisting of β-resorcylate ions I alternative with chains con-

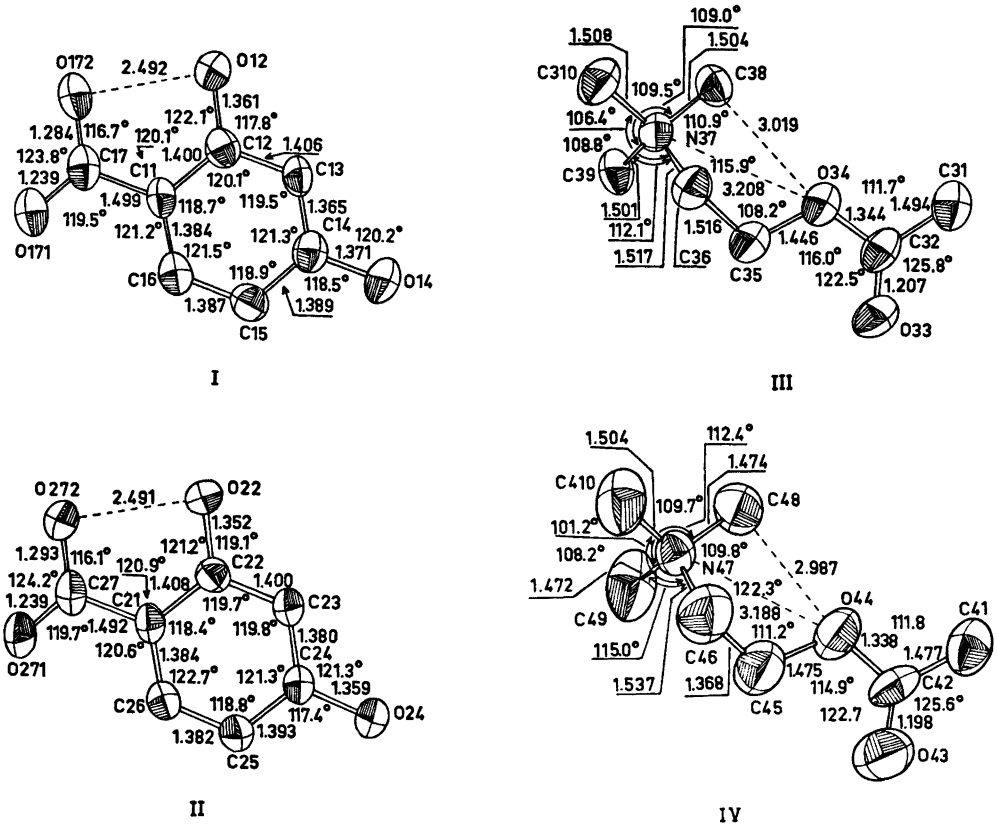


Fig. 1. Numbering system, bond distances and bond angles of β-resorcylate ion I, β-resorcylate ion II, acetylcholine ion III, and acetylcholine ion IV. All ions are viewed along the a^*-axis. The *intra*ionic hydrogen bonds of the β-resorcylate ions are indicated, and so are the distances O(x4)···N(x7) and O(x4)···C(x8) of the acetylcholine ions. The estimated standard deviations in bonds and angles are 0.008 Å and 0.5°, respectively, for dimensions of the β-resorcylate ions, while the corresponding figures for dimensions of the acetylcholine ions are in the range 0.008 – 0.013 Å and 0.4 – 0.8°, respectively.

sisting of β-resorcylate ions II. The O(x4)··· O(x72) distance is 2.580 Å when x = 1, and 2.550 Å when x = 2. No close contacts are found between the chains. The angle between (100) and the ring plane of β-resorcylate ion I is 2.1°, while the corresponding angle for β-resorcylate ion II is 11.5°. The angle between the ring planes I and II is 9.5°.

Within the layer consisting of acetylcholine ions III only very few contacts between the ions are observed, *cf.* Fig. 3a. The angle between the best plane through the atoms C(31), C(32), O(33) and O(34) and the ring plane of resorcylate ion I is 3.6°, and the corresponding angle to the ring plane of resorcylate ion II is 6.5°. The five atoms C(31) to C(35) are squeezed in between the resorcylate ions $I_{(x,y,z)}$ and $II_{(x,y,z)}$. The distances of the five atoms to the planes of the two rings are in the range 3.3 to 3.5 Å, the shorter *inter*atomic distances being of this magnitude too. A number of rather weak contacts are seen between the cationic head of the acetylcholine ion III and atoms, mostly oxygen atoms, of resorcylate ions in the two adjacent layers, *cf.* Fig. 3a.

The situation within the layer consisting of acetylcholine ions IV is very similar to that described for the layer of acetylcholine ions III. Only few contacts between the acetylcholine ions are found, *cf.* Fig. 3b. The angle between

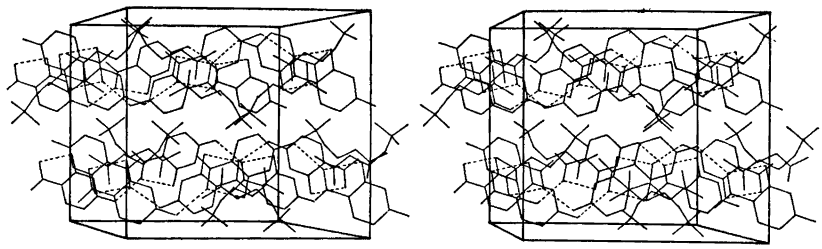


Fig. 2. Stereo diagram illustrating the packing. The *b*-axis is ↑, the *c*-axis is →, and a^* is pointing into the paper. Hydrogen bonds are drawn with broken lines.

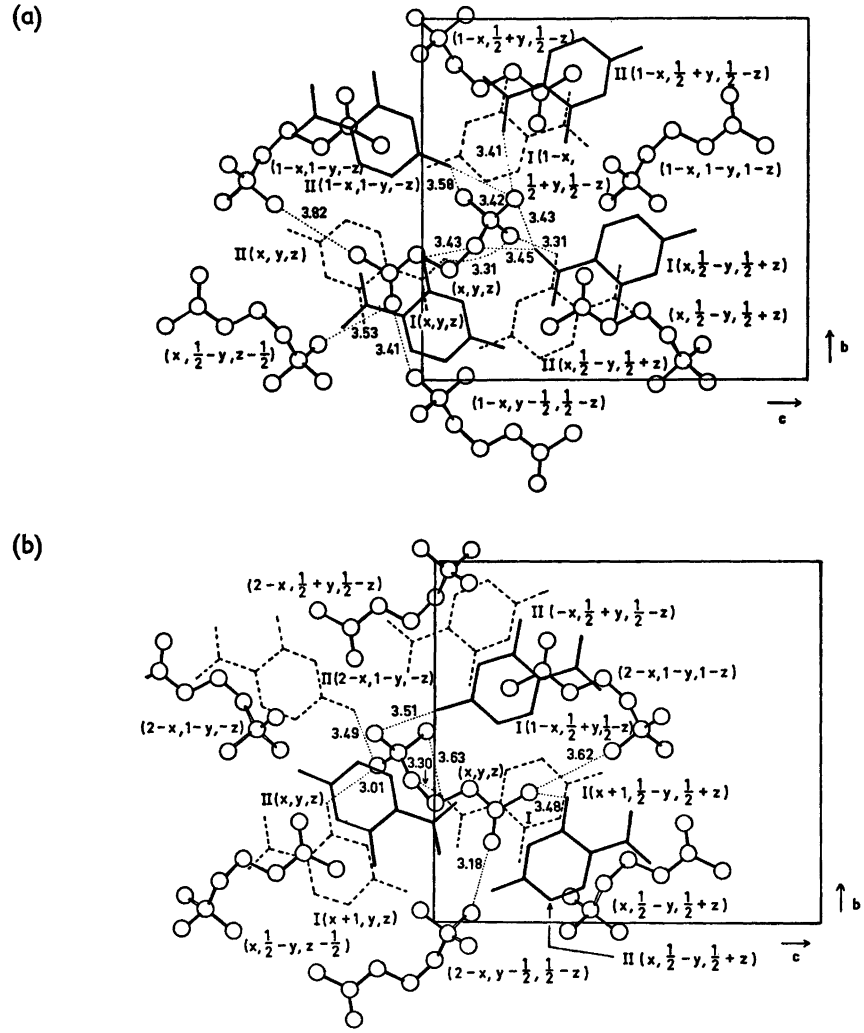


Fig. 3. Projection along a^* of (a) the packing around acetylcholine-ion III and (b) the packing around acetylcholine-ion IV.

the best plane through the atoms C(41), C(42), O(43), and O(44) and the ring plane of resorcylate ion $I_{(x+1,\frac{1}{2}-y,\frac{1}{2}+z)}$ is 2.3°. The distances of the five atoms C(41) to C(45) to the plane of the above mentioned adjacent ring are all ≥3.5 Å. No resorcylate ion is found directly on top of the atoms of the ester group of acetylcholine ion IV. A number of contacts between the cationic head and resorcylate ions in the adjacent layers are observed. One of them, C(49)···$O(14)_{(x+1,y,z)}$ is remarkably short, 3.01 Å. The shortest contacts to C(45) are C(45)···$C(27)_{(x,y,z)}$ 3.62 Å, and C(45)···$O(171)_{(x+1,\frac{1}{2}-y,\frac{1}{2}+z)}$, 3.69 Å.

The β-resorcylate ions. As visualized in Fig. 1 the dimensions of the two resorcylate ions I and II are very similar, and no significant differences between corresponding figures are found. As in the crystal structure of salicylic acid [9] and of nicotinyl salicylate [10] there is a strong hydrogen bond between the *ortho*-hydroxyl and a carboxylate oxygen atom. The distance O(x2)··· O(x72) is 2.49 Å. The corresponding distances in salicylic acid and in nicotinyl salicylate are 2.620 and 2.544 Å, respectively. The carbon – oxygen distances C(x7) – O(x71) and C(x7) – O(x72), 1.239 and 1.284 Å for x = 1, and 1.239 and 1.293 Å for x = 2, are significantly different. This difference is associated to the fact that O(x72) is acceptor for two strong hydrogen bonds, while no hydrogen bond involve O(x71). The distance C(x2) – O(x2), 1.361 Å and 1.352 Å for the ions I and II, respectively, is of the same magnitude as the corresponding bond (1.358 Å) in salicylic acid, and it thus seems doubtful whether the shortening of the corresponding bond (1.318 Å) observed in the salicylate ion [10] can be ascribed to an effect of the strength of the *intra*ionic hydrogen bond. The carboxylate group is out of the plane of the benzene ring by 2 and 6° for resorcylate ion I and II, respectively. These figures do not describe a torsion around the bond C(x1) – C(x7), *cf.* Table 3.

The acetylcholine ions. Rather pronounced differences are observed between the dimensions of the two crystallographically independent acetylcholine ions III and IV, *cf.* Fig. 1. Due to the extremely large "thermal motion" of acetylcholine ion IV these differences can, however, not be interpreted as significant differences in real bond lengths and angles. The bond lengths and angles of acetylcholine ion III are not significantly different from those found by Herdklotz and Sass [11] in the crystal structure of acetylcholine chloride. The conformations of both of the acetylcholine ions are approximately the same and equal to that found in crystals of acetylcholine chloride, while differences from the conformation found by Canepa, Pauling and Sörum [12] are observed. The pertinent torsion angles are listed in Table 4. The distances O(x4)···N(x7) and O(x4)···C(x8) are 3.21 and 3.02 Å when x = 3 and 3.19 and 2.99 Å when x = 4.

Table 4. Torsion angles (°) of the acetylcholine-ions III and IV.

	III x = 3	IV x = 4
C(x1) – C(x2) – O(x4) – C(x5)	±178	180
C(x2) – O(x4) – C(x5) – C(x6)	±159	±163
O(x4) – C(x5) – C(x6) – N(x7)	±84	±77
C(x5) – C(x6) – N(x7) – C(x8)	±61	±60

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Studies on Organophosphorus Compounds. XI.* Oxidation of Aromatic Compounds with Sulfur in Hexamethylphosphoric Triamide (HMPA). A New Method for Preparation of *N,N*-Dimethylthiocarboxamides

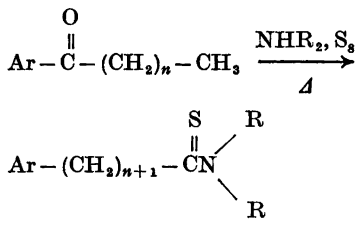
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Elemental sulfur in HMPA at elevated temperatures has unique oxidizing properties. Alkyl aryl ketones are oxidized to aromatic *N,N*-dimethylthiocarboxamides in reasonable yields. Benzaldehydes give *N,N*-dimethylthiobenzamides and *E*-stilbenes. *N,N*-Dimethylthiobenzamide is demethylated by S_8 in HMPA at about 200 °C. Benzylic compounds $PhCH_2X$ (X = OH, Cl, SH, NMe_2, OEt, COOEt, $CSNMe_2$) all yield *N,N*-dimethylthiobenzamide. α- and γ-Picolines produce the corresponding *N,N*-dimethylthioamides in good yields, while β-picoline does not react under the same conditions. The reaction between S_8 and HMPA is quite complex but the following products have been isolated:

$Me_2NCSSCH_2SCSNMe_2$, $HCSNMe_2$, $Me_2NCSNHMe$, and $Me_2NCS_2NH_2Me_2$.

It is a known fact, that by heating sulfur and certain amines with compounds containing oxidizable groups thiocarboxamides are produced. Depending upon reaction conditions also carboxamides and/or carboxylic acids are often isolated.[1] The most well-known reaction of that type is the Willgerodt-Kindler reaction:[2,3]



* Part X. H. Kolind-Andersen and S.-O. Lawesson, *Acta Chem. Scand. B 29* (1975) 430.

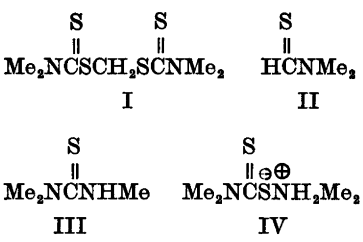
Good reviews are also available.[4,5] A drawback is, that working with low-boiling amines the preparation has to be performed in an autoclave in order to reach optimal and quite high reaction temperatures; often also a very high pressure from H_2S is developed.[2] The synthetic utility of the method is thus limited, if specific amides (thioamides) are wanted. *N,N*-Dimethylformamide has also been used as source of dimethylamine in synthesis of *N,N*-dimethylthiocarboxamides, but due to the low boiling-point of the starting material long reaction times are needed [6,7] or again an autoclave.[8] As it has been found, that hexamethylphosphoric triamide (HMPA) at high temperatures can act as a precursor of dimethylamine,[9–12] we felt prompted to study the behaviour of elemental sulfur in HMPA at elevated temperatures and with different substrates present. This paper reports about oxidation of some aldehydes, ketones, benzylic derivatives, and methylpyridines.

Reaction of HMPA with S_8. It has been shown earlier that certain bases open up the S_8-ring and give a sulfur anion:[1]

As HMPA is a rather strong base,[13] its reaction with S_8 is of great potential interest to the organic chemist. Recently Chivers and Drummond [14] claimed that a blue solution is formed when sulfur is dissolved in anhydrous HMPA,

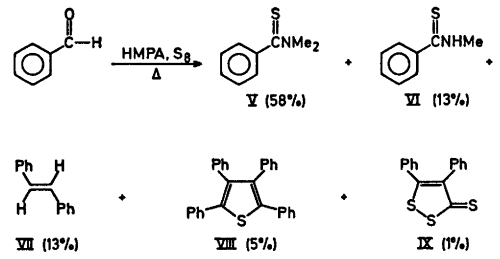
and based on spectroscopic evidence and electrical conductivity measurements they suggested the presence of the species S_3^-. However, we have shown, that HMPA, purified according to Brandsma [15] and kept over molecular sieves, does not give the blue colour or the reported IR absorption at 620 nm with elemental sulfur. But addition of traces of dimethylamine to the HMPA + S_8 solution produces the reported findings.[14] Earlier results [14] have thus to be dealt with with great doubt.

The reaction of HMPA with S_8 at 190 °C is rather complex and within 4 h 2 mol of sulfur (per mol of HMPA) are consumed under development of H_2S. Besides unreacted HMPA the products, I – IV, are isolated, however, only in minor amounts.



The presence of IV strongly indicates the formation of carbon disulphide during the reaction. The products show that oxidation and oxidative demethylation take place on the dimethylamino groups.

Oxidation of benzaldehydes. It is already known that aromatic aldehydes when refluxed with sulfur and a secondary amine, yield the corresponding thioamide in high yields.[4,16,17] No attempts have been made to isolate other products. The reaction of benzaldehyde with S_8 in HMPA at 205 °C gives a series of products, V – IX, with the thioamide, V, as main product.



o-Methoxybenzaldehyde also produces the *N*,*N*-dimethylthiobenzamide and the corresponding *E*-stilbene. The *N*-demethylated thioamide was not found in this reaction.

An interesting paper [7] reports that oxidation of benzaldehydes with S_8 in *N*,*N*-dimethylformamide produces *N*,*N*-dimethylbenzamides in considerable yields besides *N*,*N*-dimethylthiobenzamides. In an experiment with benzaldehyde, S_8 and HMPA, performed at 185 °C, *N*,*N*-dimethylbenzamide was formed, but obviously at higher temperatures it reacted further to the thioamide.

Oxidations of alkyl aryl ketones. Acetophenone (X, R = CH_3, R′ = H), when heated in HMPA with great excess of S_8, gave *N*,*N*-dimethylthiobenzamide, XI, in appreciable yields and also a small yield of the normal Kindler product, XII. Small amounts of 2,5-diphenylthiophene are produced, too. (For other results, see Table 1). In the Willgerodt reaction with great excess of sulfur and quite high reaction temperatures only non-degraded products are produced.[18] Under

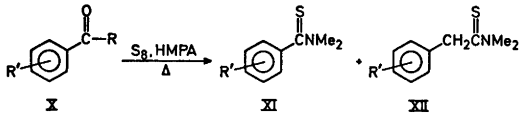


Table 1. Oxidations of alkyl aryl ketones. In all experiments 0.1 mol of ketone was used.

R′	R	S_8 (mol)	HMPA (ml)	React. time (h)	React. temp. (°C)	XI (%)	XII (%)
H	Me [a]	0.6	25	12	172	71	10
p-Me	Me	0.6	25	12	170	64	11
H	Et [b]	0.6	25	10	172	57	19
H	CH_2CO_2Et	1.0	50	12	155	55	5

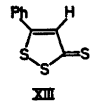
[a] 2,5-Diphenylthiophene was isolated. [b] 5-Phenyl-3*H*-1,2-dithiole-3-thione was isolated in 10 % yield.

Table 2. Oxidation of benzylic derivatives. 0.1 mol of benzylic derivative was used in all experiments.

$$PhCH_2X \xrightarrow[\Delta]{HMPA,\ S_8} PhC(=S)NMe_2$$

X	S_8 (mol)	HMPA (ml)	React. time (h)	React. temp. (°C)	V (%)
OH	0.3	25	2	185	75
SH	0.3	25	3.5	173	82
Cl	0.3	25	0.25	190 – 240	80
NMe_2	0.3	25	0.75	185	81
OEt	1.0	30	8	200	47 [a]
COOEt	1.0	50	7	188	37 [b]
$CSNMe_2$	0.4	30	12	170	78

[a] 16 % of PhCSNHMe isolated. [b] 37 % of $PhCH_2CSNMe_2$ was formed.



XIII

almost the same conditions but with HMPA present instead of a free amine the main product, XI, is due to degradation of X. Apparently another mechanism has to be considered. Compound XII might be one of the possible intermediates as it is degraded to XI when heated with sulfur in HMPA. No attempts have been made to get optimal yields of XI, but greater excess of sulfur, possibly also longer reaction times, should decrease the yield of XII and increase that of XI.

Propiophenone (X, R = Et, R′ = H) produced the degradation product XI in 57 % yield, and surprisingly also XII, meaning that a C_3-side-chain has been shortened by one carbon. From the same reaction also the non-degraded 5-phenyl-3*H*-1,2-dithiol-3-thione, XIII, was isolated.

Oxidation of ethyl benzoylacetate gave, besides XI and XII, also *N*,*N*-dimethylthioacetamide. As the same thioamide also is formed by oxidizing ethyl phenylacetate with S_8 in HMPA it might be possible that the ethyl group is responsible for this compound. A separate paper [19] on oxidation of esters will be published in due time.

Oxidation of benzylic derivatives. A series of derivatives, $PhCH_2X$, where X = OH, SH, Cl, NMe_2, OEt, COOEt and $CSNMe_2$, was oxidized by sulfur in the presence of HMPA, and the main product, V, was isolated in quite high yields (Table 2). No attempts were made to reach optimal yields.

Also other benzylamines than *N*,*N*-dimethylbenzylamine have been investigated.

$$PhCH_2NH_2 \xrightarrow[\Delta]{HMPA,S_8} Ph-C(=S)NHCH_2Ph\ (90\ \%)$$

$$PhCH_2NHEt \xrightarrow[\Delta]{HMPA,S_8} PhC(=S)NHEt\ (65\ \%) + V\ (7\ \%)$$

N-Benzylthiobenzamide has earlier been produced without the presence of HMPA.[20] From the second reaction it is concluded that transamidation does not seem to occur to any great extent.

Benzyl alcohols yield *N*,*N*-dimethylbenzylamines when heated at about 230 °C with HMPA.[12] Reaction in the presence of sulfur at 185 °C gives V in quite high yields. Benzyl mercaptan also yields V. However, under the same reaction conditions without sulfur present no reaction between benzyl mercaptan and HMPA occurred. At higher temperatures dibenzylsulfide was formed. These results indicate that *N*,*N*-dimethylbenzylamine is not initially formed in these reactions.

Oxidation of benzyl ethyl ether required rather severe reaction conditions, but when all

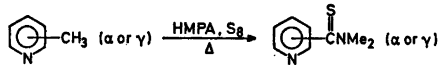
the ether had been reacted three compounds could be isolated:

$$PhCH_2OCH_2CH_3 \xrightarrow[\Delta]{HMPA,S_8} V + VI + \underset{XIV}{CH_3\overset{S}{\overset{\|}{C}}NMe_2}$$

V and XIV are supposed to be formed first by oxidation of the ether to two different thion-acids, or derivatives thereof, and subsequent reaction with HMPA to the corresponding thio-amides. VI is formed from V as has been shown in a separate experiment. When *N,N*-dimethyl-thiobenzamide was heated with sulfur in HMPA for 2 hours at 225 °C about 20 % of *N*-methyl-thiobenzamide was isolated, besides non-reacted starting material. Compound VI was recognized as minor product (by NMR) in most of the reaction mixtures. Thus it is noted that long reaction times and high reaction temperatures cause an appreciable demethylation of V to give VI.

Ethyl phenylacetate, after reaction with sulfur in HMPA, gave 37 % yield of V and the same yield of phenyl-*N,N*-dimethylthioacetam-ide. That means that an ester can be directly converted to a thiocarboxamide.[19]

Oxidation of picolines. Both α- and γ-picolines are converted into the corresponding *N,N*-di-methylthiocarboxamides when heated at 130–160 °C for 20–22 h with DMF in the presence of sulfur. With HMPA the same results were obtained after only one hour's reflux.



The β-picoline was recovered unchanged after reflux in HMPA for 20 h, which is in agreement with earlier observed reactivities of the methyl groups of picolines in oxidation with sulfur.[1]

Conclusion. The results presented in this paper show that S_8 in HMPA is a rather strong oxidizing reagent, which easily converts many different types of aromatic compounds into aromatic *N,N*-dimethylthiocarboxamides. A preparative advantage is that all reactions can be run in open vessels contrary to previously described reactions with dimethylamine. It is foreseen that elemental sulfur+HMPA at elevated temperatures should comprise unique reactivities, which now are being studied on a whole range of different substrates.

EXPERIMENTAL

NMR spectra were recorded at 60 MHz on a Varian A-60 spectrometer. TMS was used as internal reference standard. The chemical shifts are expressed in δ values (ppm) (s = singlet, d = doublet, t = triplet, m = multiplet). IR spectra were recorded on a Beckman IR-18 spectrophotometer. Mass spectra were recorded on a CEC 21–104 operating at 70 eV, using direct inlet. Ion source temperature 250 °C. Column chromatography was carried out with silicagel 60 (Merck), using light petroleum as eluent and then stepwise raising the polarity by addition of ether. Commercial HMPA dried over molecular sieves (3A) was used. Elemental analyses were performed by Novo Industry A/S, Copenhagen. M.p's and b.p's are uncorrected.

Oxidation of HMPA with sulfur. HMPA (17.9 g) was heated with S_8 (6.4 g) at 190 °C for 4 h. After cooling to room temperature the mixture was dissolved in 100 ml of hot CH_2Cl_2. On addition of ether a gum precipitated. The solution was decanted and the solvent evaporated. Column chromatography yielded: I. 140 mg of *methylene dimethyldithiocarbamate* (I), m.p. 150–151 °C (lit.[21] m.p. 150–151 °C). (Found: C 32.97; H 5.63; N 11.03; S 49.92. $C_7H_{14}N_2S_4$ requires: C 33.07; H 5.51; N 11.02; S 50.39). NMR ($CDCl_3$): 3.4 (d, 12 H); 5.4 (s, 2 H). MS: *m/e* 254 (M^+, ≃1 %); 164 ($Me_2NCSSCH_2^+$, 60 %); 88 (Me_2NCS^+, 100 %). II. 1.1 g of N,N-*dimethylthioformamide* (II), b.p. 96–98 °C/11 mmHg (lit.[22] b.p. 95–100 °C/10 mmHg). An NMR spectrum was in accordance with literature.[22] III. 2.2 g of N,N,N′-*trimethylthiourea* (III), m.p. 84 °C (lit.[23] m.p. 87 °C). NMR ($CDCl_3$): 3.1 (d, 3 H); 3.3 (s, 6 H); ≃6.0 (m, 1 H). IR(KBr): 1550, 2940 and 3280 cm^{-1}. Further elution with CH_3CN yielded some very hygroscopic crystals which could not be purified for identification. Finally elution with MeOH afforded a mixture of products, which after distillation (b.p. 60–66 °C/0.3 mmHg) yielded 3.8 g of HMPA. From a condenser a crystalline material was collected, dissolved in $CHCl_3$ and on addition of ether 400 mg of *dimethylammonium dimethyldithiocarbamate* (IV), m.p. 130–131 °C was obtained (lit.[22] m.p. 130–132 °C). NMR (D_2O): 2.7 (s, 6 H); 3.5 (s, 6 H); 4.7 (s, 2 H).

In all the following experiments a common working-up procedure was used: When cooled down to room temperature the reaction mixtures were poured into 250 ml of brine and 100 ml of ether. Non-reacted sulfur was filtered off. After ether extraction (3 × 100 ml) (except for the picolines where extraction was performed with benzene) and washing with brine (2 × 50 ml)

the combined ether phases were dried ($CaSO_4$), the ether evaporated and the crude organic phases were further purified according to the procedures below.

Benzaldehydes

Benzaldehyde (10.6 g) was heated with 9.6 g of S_8 in 25 ml of HMPA at 205 °C for 75 min. Column chromatography of the organic phase yielded: I. 1.2 g (13 %) of E-*stilbene* (VII), m.p. 122–123 °C (lit.[24] m.p. 124 °C). NMR ($CDCl_3$): 7.1 (s, 2 H); 7.3 (m, 10 H). IR (CCl_4): 960, 1490, 1600 and 3030 cm^{-1}. II. 500 mg (5 %) of *tetraphenylthiophene* (VIII), m.p. 181–183 °C (lit.[25] m.p. 183–184 °C). (Found: C 86.54; H 5.13; S 8.47. $C_{28}H_{20}S$ requires: C 86.56; H 5.19; S 8.25). NMR (CS_2): 7.0 (m). MS: *m/e* 388 (M^+, 100 %); 311 (M–Ph, 13 %); 121 ($PhCS^+$, 5 %); 77 (5 %). III. 200 mg (1 %) of orange-red *4,5-diphenyl-3H-1,2-dithiole-3-thione*, m.p. 158–159 °C (lit.[26] m.p. 159 °C). (Found: C 62.40; H 3.41; S 33.31. $C_{15}H_{10}S_3$ requires: C 62.90; H 3.52; S 33.58). NMR (CS_2): 7.2 (m). IR ($CHCl_3$): 830, 1040, 1150, 1510, 3000 cm^{-1}. MS: *m/e* 286 (M^+, 88 %); 285 (100 %); 178 (PhCCPh, 36 %); 165 (M–PhCS, 32 %); 145 (PhC_3S^+, 44 %); 121 ($PhCS^+$, 54 %); 77 (46 %). IV. 9.2 g (55 %) of N,N-*dimethylthiobenzamide*, m.p. 67 °C (lit.[7] m.p. 67 °C). NMR ($CDCl_3$): 3.1 (s, 3 H); 3.5 (s, 3 H); 7.3 (m, 5 H). A mass spectrum was in accordance with literature.[27] V. 2.0 g (13 %) of N-*methylthiobenzamide*, m.p. 77–78 °C (lit.[28] m.p. 79 °C). NMR ($CDCl_3$): 3.2 (s, 1.5 H); 3.3 (s, 1.5 H); 7.5 (m, 5 H); 8.0 (m, 1 H). A mass spectrum was in accordance with literature.[27]

In another reaction of benzaldehyde run at 185 °C for 2 h the organic phase yielded after column chromatography besides the products found above 2.7 g (18 %) of N,N-*dimethylbenzamide*, m.p. 40–42 °C (lit.[28] m.p. 43 °C). NMR ($CDCl_3$): 3.0 (s, 6 H); 7.4 (m, 5 H). IR (CCl_4): 1075, 1380, 1630, 2900 and 3020 cm^{-1}.

o-Methoxybenzaldehyde (13.6 g) was heated with 9.6 g of S_8 in 25 ml of HMPA at 200 °C for 30 min. Column chromatography of the organic phase first yielded 1.5 g of a mixture of products, which could not be further separated. Then 1.3 g (11 %) of *2,2'-dimethoxy*-E-*stilbene* was isolated, m.p. 135–136 °C (lit.[30] m.p. 140 °C). NMR ($CDCl_3$): 3.8 (s, 6 H), 7.3 (m, 10 H). IR ($CHCl_3$): 970, 1230, 1450, 1480, 1590, 2920 and 3050 cm^{-1}. Finally 13.6 g (70 %) of o-*methoxy*-N,N-*dimethylthiobenzamide* was eluted, m.p. 84 °C. (Found: C 61.39; H 6.72; N 7.18; S 16.49. $C_{10}H_{13}NOS$ requires: C 61.54; H 6.67; N 7.18; S 16.41). NMR ($CDCl_3$): 3.1 (s, 3 H); 3.6 (s, 3 H); 3.8 (s, 3 H); 7.1 (m, 4 H).

Alkylarylketones

Reaction conditions are given in Table 1.

Acetophenone. Column chromatography of the organic phase yielded as the first fraction 2.6 g of a mixture of products. Distillation (until 200 °C/0.3 mmHg) followed by separation on preparative TLC (silica gel, elution with 15 % ether in light petroleum) yielded 160 mg of *2,5-diphenylthiophene*, m.p. 148–150 °C (lit.[2] m.p. 150.5 °C). NMR ($CDCl_3$): 7.3 (m). A mass spectrum was in accordance with literature.[31] Further elution of the organic phase yielded 13.4 g of a mixture of N,N-*dimethylthiobenzamide* (XI) (71 %) and *phenyl*-N,N-*dimethylthioacetamide* (XII) (10 %). The yields are calculated according to NMR. Authentic phenyl-*N,N*-dimethylthioacetamide was prepared.[8] NMR ($CDCl_3$): 3.2 (s, 3 H); 3.5 (s, 3 H); 4.3 (s, 2 H); 7.3 (m, 5 H).

p-*Methylacetophenone*. Column chromatography of the organic phase gave first 2.0 g of a mixture of products, which was not further purified. Then 13.6 g of a mixture of two thioamides was eluted. Crystallization of the mixture twice from ether yielded pure p-*methyl*-N,N-*dimethylthiobenzamide*, m.p. 49–50 °C (lit.[32] m.p. 49–49.5 °C). NMR ($CDCl_3$): 2.3 (s, 3 H); 3.1 (s, 3 H); 3.5 (s, 3 H); 7.1 (m, 4 H). From the mixture p-*methylphenyl*-N,N-*dimethylthioacetamide* was recognized according to an NMR-spectrum ($CDCl_3$): 2.3 (s, 3 H); 3.1 (s, 3 H); 3.4 (s, 3 H); 4.2 (s, 2 H); 7.1 (m, 4 H).

Propiophenone. Column chromatography of the organic phase yielded: I. 2.2 g (10 %) of *5-phenyl-3H-1,2-dithiole-3-thione*, m.p. 121–123 °C (lit.[33] m.p. 126 °C). (Found: C 51.44; H 2.86; S 45.08. $C_9H_6S_3$ requires: C 51.39; H 2.88; S 45.73). NMR (CS_2): 7.2 (s, 1 H); 7.5 (m, 5 H). II. 12.9 g of a mixture of N,N-*dimethylthiobenzamide* (57 %) and *phenyl*-N,N-*dimethylthioacetamide* (19 %).

Ethyl benzoylacetate. Column chromatography of the organic phase yielded: I. 5.9 g of a mixture of products which were not further purified. II. 10.0 g of a mixture of N,N-*dimethylthiobenzamide* (55 %) and *phenyl*-N,N-*dimethylthioacetamide* (5 %). III. 2.1 g (20 %) of N,N-*dimethylthioacetamide*. M.p. 72 °C (lit.[34] m.p. 73–74 °C). NMR ($CDCl_3$): 2.7 (s, 3 H); 3.3 (s, 3 H); 3.5 (s, 3 H).

Benzylic derivatives

In most reactions the organic phase was simply distilled twice, yielding pure *N,N*-dimethylthiobenzamide. Reaction conditions and yields are given in Table 2. The following are exceptions to the general procedure.

Benzyl ethyl ether. Column chromatography of the organic phase yielded 7.8 g (47 %) of N,N-*dimethylthiobenzamide* and 2.4 g (16 %) of N-

methylthiobenzamide. Further elution yielded 600 mg (6 %) of N,N-*dimethylthioacetamide*.

Ethyl phenylacetate. The reaction mixture was very stiff, when cooled down, so it was dissolved in boiling CH_2Cl_2, cooled to room temperature and filtered. Filtration through a silica gel column (eluted with CH_2Cl_2) followed by column chromatography yielded 12.8 g of an approximately 1:1 mixture of N,N-*dimethylthiobenzamide* and *phenyl*-N,N-*dimethylthioacetamide*. Further elution yielded 2.3 g of a mixture of N,N-*dimethylthioformamide* (~80 %) and N,N-*dimethylthioacetamide* (~20 %).

Benzylamine (10.7 g) was heated with 9.6 g of S_8 in 25 ml of HMPA at 175 °C for 45 min. After the general working-up procedure, the organic phase was recrystallized from light petroleum–ether (1:1), yielding 10.2 g (90 %) of N-*benzylthiobenzamide*, m.p. 82–83 °C (lit.[30] m.p. 84–85 °C). NMR ($CDCl_3$): 4.8 (d, 2 H); ~7.5 (m, 11 H).

Benzylethylamine (13.5 g) was heated with 9.6 g of S_8 in 25 ml of HMPA at 185 °C for 45 min. Distillation of the organic phase yielded a fraction (11.9 g) with b.p. 135–143 °C/0.25 mmHg. An NMR-spectrum showed that it contained N,N-*dimethylthiobenzamide* (7 %) and N-*ethylthio*benzamide (65 %) (lit.[35] b.p. 148–150 °C/1 mmHg). NMR ($CDCl_3$): 1.3 (t, 3 H); 3.8 (m, 2 H); 7.3 (m, 3 H); 7.7 (m, 2 H); 7.9 (m, 1 H).

Reaction of HMPA with benzylmercaptan. Benzylmercaptan (12.4 g) was heated in 25 ml of HMPA at 172 °C for 3 ½ h. Distillation of the organic phase yielded benzylmercaptan almost quantitatively. Another reaction performed at 205 °C for 3 h afforded a very complex reaction mixture. However, column chromatography of the organic phase yielded a mixture of products, containing dibenzylsulfide. Gentle distillation gave 2.4 g (22 %) of pure *dibenzylsulfide*, m.p. 48–49 °C (lit.[37] m.p. 49 °C). NMR ($CDCl_3$): 3.5 (s, 4 H); 7.2 (m, 10 H).

Picolines

General working-up procedure: After cooling to room temperature the reaction mixtures were poured into 250 ml of $NaHCO_3$-saturated H_2O and 100 ml of benzene. Non-reacted S_8 was filtered off. After benzene extraction (4 × 100 ml) the combined benzene phases were washed with 50 ml of $NaHCO_3$-saturated H_2O, dried ($CaSO_4$) and the benzene evaporated.

α-Picoline (9.3 g) was refluxed in 25 ml of HMPA with 12.8 g of S_8 for 50 min. Distillation of the worked-up reaction mixture twice yielded 8.8 g (53 %) of N,N-*dimethyl-α-thiopicolinamide*, b.p. 119–122 °C/0.2 mmHg (lit.[6] 129–133 °C/0.55 mmHg). NMR ($CDCl_3$): 3.2 (s, 3 H); 3.6 (s, 3 H); 7.5 (m, 3 H); 8.4 (m, 1 H).

γ-Picoline (9.3 g) was refluxed in 25 ml of HMPA with 12.8 g of S_8 for an hour. Distillation of the worked-up reaction mixture, followed by recrystallization from light petroleum–ether (4:1) yielded 7.9 g (48 %) of N,N-*dimethylthioisonicotinamide*, m.p. 62–64 °C (lit[6]. m.p. 64–65 °C). NMR ($CDCl_3$): 3.2 (s, 3 H); 3.6 (s, 3 H); 7.2 (m, 2 H); 8.5 (m, 2 H).

β-Picoline (9.3 g) was refluxed in 25 ml of HMPA with 12.8 g of S_8 for 20 h. Distillation of the worked-up reaction mixture gave 7.6 g of picoline.

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The Crystal Structure of 5,6-Epoxy-4-hydroxy-2-methoxy--4,6-di-*t*-butylcyclohex-2-enone

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The crystal structure of 5,6-epoxy-4-hydroxy-2-methoxy-4,6-di-*t*-butylcyclohex-2-enone, $C_{15}H_{24}O_4$, has been determined by single-crystal X-ray diffraction techniques. The unit cell is triclinic, space group $P\bar{1}$, with dimensions $a=12.833$, $b=11.695$, $c=10.861$ Å, $\alpha=104.49$, $\beta=94.62$, $\gamma=96.83°$. There are four molecules in the unit cell. Data were collected on an computer-controlled Philips PW 1100 diffractometer. The structure was determined by direct methods and refined by full-matrix least-squares; the final value of the conventional R index is 0.062.

The title compound may be prepared by autooxidation of 4,6-di-*t*-butylguaiacol in alkaline solution. The two molecules in the asymmetric unit are joined together by two hydrogen bonds, one from the hydroxyl oxygen of each molecule to the epoxide oxygen of the other. With the atoms of one molecule designated by primer, the distances O′(15)···H(O14) and O(15)···H(O′14) are 1.83 and 1.97 Å, respectively, and the angles O(15)···H(O′14) − O′(14) and O′(15)···H(O14) − O(14) are 171 and 167°, respectively. Since the hydroxyl group and the epoxide ring are found to be *cis* to each other,

Table 1. Starting phases obtained from the convergence map. The correct phases are those without parentheses.

h			ϕ_h	$\lvert E_h\rvert$
0	3	1	0	3.58
7	−4	6	0	3.55
5	8	−7	0	3.02
3	7	−7	0,(π)	3.65
3	−12	5	0,(π)	3.22
4	7	−8	0,(π)	3.04
6	−9	5	0,(π)	2.73
9	−8	−4	(0),π	2.46

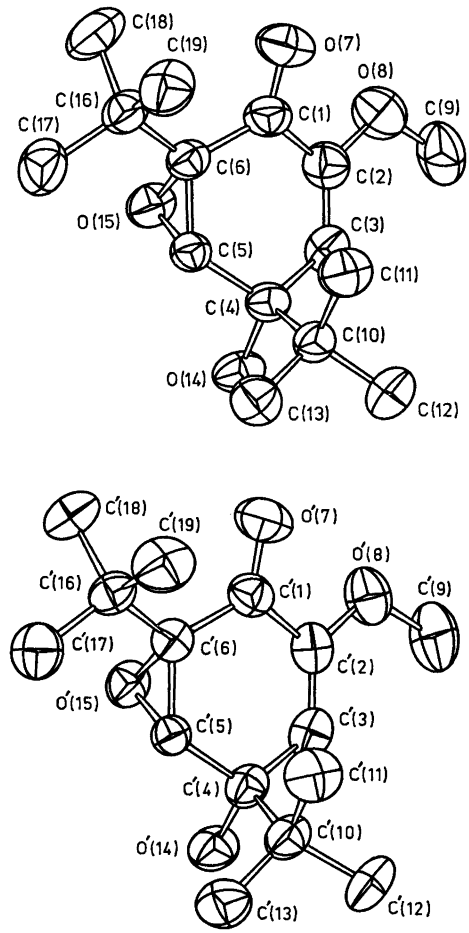


Fig. 1. Perspective views of the two separate molecules in the asymmetric unit.

Table 2. Positional and anisotropic thermal parameters of the non-hydrogen atoms. The β-values refer to the temperature factor expression $\exp[-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+hk\beta_{12}+hl\beta_{13}+kl\beta_{23})]$. Estimated standard deviations are given in parentheses. Values are $\times 10^4$.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	2482 (3)	8337 (3)	2389 (3)	79 (3)	91 (3)	75 (3)	35 (5)	−5 (5)	42 (5)
C(2)	3262 (3)	8875 (3)	1678 (3)	59 (3)	96 (3)	97 (4)	13 (4)	−10 (5)	30 (5)
C(3)	2967 (3)	9224 (3)	639 (3)	60 (2)	91 (3)	90 (4)	−7 (4)	19 (4)	35 (5)
C(4)	1841 (3)	9153 (3)	114 (3)	61 (2)	62 (3)	78 (3)	12 (4)	10 (4)	43 (4)
C(5)	1098 (3)	9071 (3)	1123 (3)	62 (2)	63 (3)	75 (3)	26 (4)	11 (4)	34 (4)
C(6)	1379 (3)	8648 (3)	2267 (3)	73 (3)	62 (3)	75 (3)	25 (4)	22 (4)	38 (4)
O(7)	2735 (2)	7677 (3)	3033 (3)	106 (3)	168 (4)	148 (4)	90 (5)	15 (4)	178 (6)
O(8)	4268 (2)	8880 (3)	2190 (3)	65 (2)	162 (3)	137 (3)	24 (4)	−24 (4)	74 (5)
C(9)	5090 (3)	9322 (4)	1543 (5)	58 (3)	162 (5)	172 (6)	46 (6)	6 (6)	27 (8)
C(10)	1526 (3)	8106 (3)	−1128 (3)	66 (2)	69 (3)	75 (3)	22 (4)	18 (4)	33 (4)
C(11)	1594 (4)	6910 (3)	−823 (4)	120 (4)	70 (3)	107 (4)	34 (5)	20 (6)	25 (5)
C(12)	2278 (3)	8252 (4)	−2121 (4)	95 (3)	115 (4)	91 (4)	19 (5)	48 (5)	27 (6)
C(13)	405 (3)	8105 (3)	−1696 (4)	83 (3)	97 (4)	103 (4)	21 (5)	−19 (5)	10 (6)
O(14)	1708 (2)	10228 (2)	−260 (2)	103 (2)	65 (2)	81 (2)	6 (3)	10 (3)	47 (3)
O(15)	1416 (2)	9909 (2)	2347 (2)	96 (2)	64 (2)	85 (2)	23 (3)	28 (3)	29 (3)
C(16)	539 (3)	8133 (3)	2994 (3)	86 (3)	87 (3)	91 (4)	33 (5)	53 (5)	66 (5)
C(17)	−577 (3)	8566 (4)	2768 (4)	94 (3)	126 (4)	153 (5)	43 (6)	105 (6)	106 (7)
C(18)	917 (4)	8540 (5)	4424 (4)	145 (5)	171 (6)	89 (4)	50 (8)	91 (7)	87 (8)
C(19)	373 (4)	6774 (4)	2537 (4)	114 (4)	92 (4)	163 (5)	13 (6)	93 (7)	106 (7)
C′(1)	4257 (3)	13041 (3)	2299 (3)	60 (3)	81 (3)	99 (4)	20 (4)	17 (5)	45 (5)
C′(2)	4288 (3)	12516 (3)	3423 (3)	62 (2)	65 (3)	97 (4)	12 (4)	−16 (5)	39 (5)
C′(3)	3547 (3)	12596 (3)	4205 (3)	81 (3)	73 (3)	85 (3)	6 (4)	−8 (5)	66 (5)
C′(4)	2555 (3)	13133 (3)	4048 (3)	61 (2)	70 (3)	75 (3)	−9 (4)	15 (4)	42 (5)
C′(5)	2382 (3)	13348 (3)	2734 (3)	55 (2)	67 (3)	73 (3)	5 (4)	8 (4)	32 (4)
C′(6)	3204 (2)	13356 (3)	1858 (3)	56 (2)	58 (3)	77 (3)	4 (4)	10 (4)	35 (4)
O′(7)	5033 (2)	13157 (3)	1761 (3)	71 (2)	218 (4)	168 (4)	82 (5)	73 (4)	188 (7)
O′(8)	5181 (2)	12023 (2)	3519 (3)	79 (2)	97 (3)	147 (3)	55 (3)	−20 (4)	74 (4)
C′(9)	5357 (4)	11555 (4)	4584 (5)	107 (4)	141 (5)	182 (6)	83 (7)	−52 (7)	143 (9)
C′(10)	2538 (3)	14322 (3)	5098 (3)	78 (3)	79 (3)	76 (3)	−4 (4)	18 (4)	24 (5)
C′(11)	3339 (3)	15316 (3)	4893 (4)	96 (3)	77 (3)	134 (5)	−21 (5)	31 (6)	−1 (6)
C′(12)	2814 (4)	14123 (4)	6428 (4)	121 (4)	132 (4)	75 (4)	13 (6)	10 (6)	24 (6)
C′ (13)	1432 (3)	14703 (4)	5036 (4)	94 (3)	103 (4)	114 (4)	32 (3)	44 (6)	20 (6)
O′(14)	1676 (2)	12304 (2)	4182 (2)	81 (2)	85 (2)	97 (3)	−35 (5)	37 (3)	44 (4)
O′(15)	2393 (2)	12328 (2)	1672 (2)	72 (2)	67 (2)	82 (2)	−22 (3)	6 (3)	29 (3)
C′(16)	3155 (3)	14021 (3)	797 (3)	74 (3)	83 (3)	93 (3)	23 (4)	32 (3)	80 (5)
C′(17)	2042 (3)	14298 (4)	528 (4)	103 (4)	131 (4)	128 (5)	69 (6)	24 (6)	147 (7)
C′(18)	3456 (3)	13237 (4)	−424 (4)	107 (4)	128 (4)	85 (4)	35 (6)	41 (5)	75 (6)
C′(19)	3906 (4)	15199 (4)	1227 (4)	111 (4)	95 (4)	151 (5)	−13 (6)	50 (7)	102 (7)

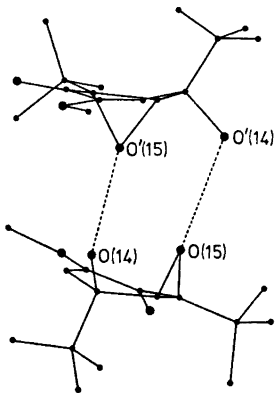


Fig. 2. The hydrogen-bonding system in the dimer molecule.

these hydrogen bonds are probably responsible for the boat conformation of the cyclohexene ring.

As part of a series of investigations on oxidation products of phenols, the crystal structure of the autooxidation product of 4,6-di-*t*-butyl-guaiacol in alkaline solution has been determined.

Butylated hydroxyanisoles (BHA) are used extensively as antioxidants in food.[1,2] The aim of this investigations was to establish the

Table 3. Positional parameters (× 10^3) of the hydrogen atoms.

	x	*y*	*z*		*x*	*y*	*z*
H(C3)	353	954	18	H(C'3)	356	1228	493
H(C5)	34	906	81	H(C'5)	175	1382	259
H1(C9)	499	1027	158	H1(C'9)	480	1096	463
H2(C9)	507	882	66	H2(C'9)	532	1210	548
H3(C9)	571	921	197	H3(C'9)	598	1126	465
H1(C11)	137	628	−156	H1(C'11)	306	1553	406
H2(C11)	240	693	−53	H2(C'11)	338	1596	561
H3(C11)	111	679	−23	H3(C'11)	397	1501	488
H1(C12)	208	759	−291	H1(C'12)	275	1482	707
H2(C12)	229	893	−234	H2(C'12)	359	1401	659
H3(C12)	303	813	−190	H3(C'12)	230	1348	660
H1(C13)	31	868	−186	H1(C'13)	88	1402	508
H2(C13)	27	761	−249	H2(C'13)	120	1488	413
H3(C13)	15	800	−115	H3(C'13)	141	1543	575
H(O14)	189	1093	46	H(O'14)	163	1155	346
H1(C17)	−85	833	189	H1(C'17)	184	1489	128
H2(C17)	−103	834	323	H2(C'17)	159	1365	39
H3(C17)	−142	932	304	H3(C'17)	203	1468	−24
H1(C18)	160	811	467	H1(C'18)	418	1312	−37
H2(C18)	49	834	485	H2(C'18)	341	1350	−117
H3(C18)	104	947	487	H3(C'18)	299	1246	−76
H1(C19)	22	655	175	H1(C'19)	386	1564	61
H2(C19)	102	644	264	H2(C'19)	370	1574	201
H3(C19)	14	639	280	H3(C'19)	451	1517	147

structure and stereochemistry of the epoxide formed upon oxidation of 4,6-di-*t*-butylguaiacol to gain information concerning the mechanism of formation of the epoxide.[1-3]

EXPERIMENTAL

Intensities were measured with monochromated Cu$K\alpha$ radiation by a computer-controlled Philips PW 1100 diffractometer. Unit-cell dimensions were determined by measurement of the angular positions of 25 accurately centered reflexions found by the "Peak Hunting Program". The unit cell and orientation parameters were obtained from these angular coordinates by the method of least squares. Crystal data are: space group $P\bar{1}$, $a = 12.833(2)$, $b = 11.695(2)$, $c = 10.861$ (2) Å, $\alpha = 104.49$ (2), $\beta = 94.62$ (2), $\gamma = 96.83$ (2)°, $V = 1299$ Å^3, $Z = 4$, $D_c = 1.371$ g cm^{-3}.

The $\theta - 2\theta$ scan technique was employed to

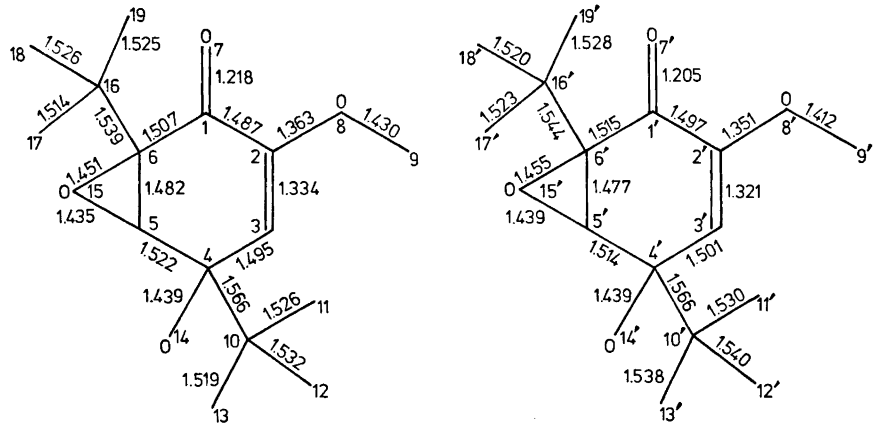


Fig. 3. The interatomic bond distances.

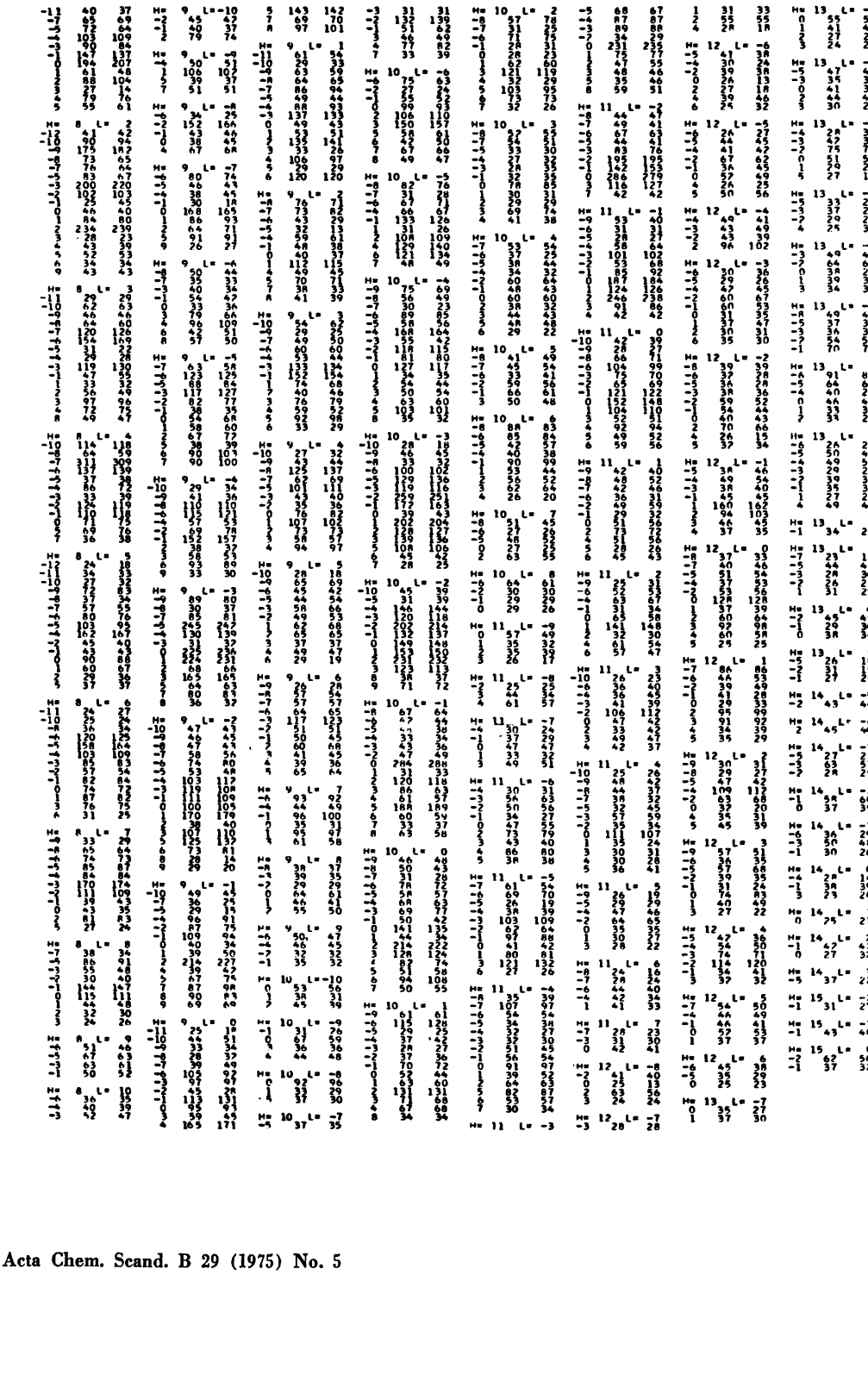
Table 4. Observed and calculated structure amplitudes. The columns use the running index k, $10|F_o|$, and $10|F_c|$, respectively.

Table 4. Continued.

Table 4. Continued.



Table 4. Continued.



measure 5274 reflexions out to $\theta = 65°$ at a scan speed of 0.026 degrees per second. Backgrounds were estimated by stationary counting at $\pm 0.75°$ 2θ from the peak maxima. Three monitor reflexions were measured every 90 min. Lorentz and polarization corrections were applied to the data but no correction for absorption was made. The 3546 reflexions for which $\sigma(I)/I \leq 0.25$, where I is the net intensity and $\sigma(I)$ its estimated standard deviation, based on counter statistics, were used in refinement of the structure.

STRUCTURE DETERMINATION AND REFINEMENTS

The absolute scale and overall temperature factor for calculation of normalized structure factor magnitudes, $|E|$, were estimated by Wilson's method.[4] The structure was solved by a modified version of the MULTAN direct phase determination procedure [5] from the 358 reflexions with $|E|$ values ≥ 1.85. The three origin specifying reflexions and five reflexions used as variables in the starting set of phases are given in Table 1. An E map computed with the signs of the most reliable set showed all the non-hydrogen atoms.

The positional parameters and isotropic temperature factors of all the 38 non-hydrogen atoms were subjected to four cycles of full-matrix least-squares refinement ($R = 0.164$) by a modified version of the program LALS.[6] A Hughes'[7] weighting scheme was used in the refinement. One cycle of refinement of the non-hydrogen atoms with anisotropic temperature factors reduced R to 0.095. A difference Fourier synthesis computed at this point revealed the positions of all the hydrogen atoms. Additional cycles of least-squares calculations with hydrogen atoms included with fixed positional and thermal ($B = 3.5$ Å^2) parameters reduced R to 0.062 for 3546 reflexions. Freeman's [8] scattering factors for oxygen and carbon and that of Stewart, Davidson and Simpson [9] for hydrogen, were used throughout the analysis.

The final positional and thermal parameters of the non-hydrogen atoms, together with their standard deviations, are listed in Table 2 and those of the hydrogen atoms in Table 3. A list of the observed and calculated structure amplitudes is given in Table 4.

RESULTS AND DISCUSSION

The autooxidation product of 4,6-di-*t*-butylguaiacol in alkaline solution was established to be 5,6-epoxy-4-hydroxy-2-methoxy-4,6-di-*t*-butylcyclohex-2-enone with the hydroxyl group and epoxide ring *cis* to each other. The molecular structure is shown in Fig. 1; the two molecules are identically numbered, with the numbers of one distinguished by primes.

Table 5. Bond distances (Å) between non-hydrogen atoms, with estimated standard deviations in parentheses.

C(1)–C(2)	1.487 (4)	C′(1)–C′(2)	1.497 (4)
C(1)–C(6)	1.507 (4)	C′(1)–C′(6)	1.515 (4)
C(1)–O(7)	1.218 (4)	C′(1)–O′(7)	1.205 (4)
C(2)–C(3)	1.334 (4)	C′(2)–C′(3)	1.321 (4)
C(2)–O(8)	1.363 (4)	C′(2)–O′(8)	1.351 (4)
C(3)–C(4)	1.495 (4)	C′(3)–C′(4)	1.501 (4)
C(4)–C(5)	1.522 (4)	C′(4)–C′(5)	1.514 (4)
C(4)–C(10)	1.566 (4)	C′(4)–C′(10)	1.566 (4)
C(4)–O(14)	1.439 (3)	C′(4)–O′(14)	1.439 (4)
C(5)–C(6)	1.482 (4)	C′(5)–C′(6)	1.477 (4)
C(5)–O(15)	1.435 (3)	C′(5)–O′(15)	1.439 (3)
C(6)–O(15)	1.451 (3)	C′(6)–O′(15)	1.455 (3)
C(6)–C(16)	1.539 (4)	C′(6)–C′(16)	1.544 (4)
O(8)–C(9)	1.430 (5)	O′(8)–C′(9)	1.412 (5)
C(10)–C(11)	1.526 (4)	C′(10)–C′(11)	1.530 (5)
C(10)–C(12)	1.532 (5)	C′(10)–C′(12)	1.540 (5)
C(10)–C(13)	1.519 (5)	C′(10)–C′(13)	1.538 (5)
C(16)–C(17)	1.514 (5)	C′(16)–C′(17)	1.523 (5)
C(16)–C(18)	1.526 (5)	C′(16)–C′(18)	1.520 (5)
C(16)–C(19)	1.525 (5)	C′(16)–C′(19)	1.528 (5)

Table 6. Interatomic angles (°), with estimated standard deviations in parentheses.

C(2)–C(1)–C(6)	116.8 (3)	C′(2)–C′(1)–C′(6)	116.7 (3)
C(2)–C(1)–O(7)	120.9 (3)	C′(2)–C′(1)–O′(7)	120.4 (3)
C(6)–C(1)–O(7)	122.3 (3)	C′(6)–C′(1)–O′(7)	122.9 (3)
C(1)–C(2)–C(3)	122.0 (3)	C′(1)–C′(2)–C′(3)	122.5 (3)
C(1)–C(2)–O(8)	110.7 (3)	C′(1)–C′(2)–O′(8)	110.0 (3)
C(3)–C(2)–O(8)	127.1 (3)	C′(3)–C′(2)–O′(8)	127.4 (3)
C(2)–C(3)–C(4)	123.8 (3)	C′(2)–C′(3)–C′(4)	125.6 (3)
C(3)–C(4)–C(5)	111.3 (3)	C′(3)–C′(4)–C′(5)	111.2 (3)
C(3)–C(4)–C(10)	111.6 (3)	C′(3)–C′(4)–C′(10)	113.0 (3)
C(3)–C(4)–O(14)	109.1 (3)	C′(3)–C′(4)–O′(14)	108.2 (3)
C(5)–C(4)–C(10)	111.8 (3)	C′(5)–C′(4)–C′(10)	109.6 (3)
C(5)–C(4)–O(14)	106.8 (3)	C′(5)–C′(4)–O′(14)	108.0 (3)
C(10)–C(4)–O(14)	105.9 (2)	C′(10)–C′(4)–O′(14)	106.6 (3)
C(4)–C(5)–C(6)	123.4 (3)	C′(4)–C′(5)–C′(6)	124.4 (3)
C(4)–C(5)–O(15)	114.9 (3)	C′(4)–C′(5)–O′(15)	115.6 (3)
C(6)–C(5)–O(15)	59.6 (2)	C′(6)–C′(5)–O′(15)	59.8 (2)
C(1)–C(6)–C(5)	114.8 (3)	C′(1)–C′(6)–C′(5)	116.1 (3)
C(1)–C(6)–O(15)	110.2 (3)	C′(1)–C′(6)–O′(15)	109.2 (2)
C(1)–C(6)–C(16)	119.3 (3)	C′(1)–C′(6)–C′(16)	118.7 (3)
C(5)–C(6)–O(15)	58.5 (2)	C′(5)–C′(6)–O′(15)	58.8 (2)
C(5)–C(6)–C(16)	122.4 (3)	C′(5)–C′(6)–C′(16)	122.4 (3)
O(15)–C(6)–C(16)	115.2 (3)	O′(15)–C′(6)–C′(16)	115.2 (3)
C(2)–O(8)–C(9)	115.8 (3)	C′(2)–O′(8)–C′(9)	116.7 (3)
C(4)–C(10)–C(11)	110.2 (3)	C′(4)–C′(10)–C′(11)	110.1 (3)
C(4)–C(10)–C(12)	109.6 (3)	C′(4)–C′(10)–C′(12)	109.1 (3)
C(4)–C(10)–C(13)	110.7 (3)	C′(4)–C′(10)–C′(13)	110.4 (3)
C(11)–C(10)–C(12)	109.1 (3)	C′(11)–C′(10)–C′(12)	109.7 (3)
C(11)–C(10)–C(13)	108.7 (3)	C′(11)–C′(10)–C′(13)	108.8 (3)
C(12)–C(10)–C(13)	108.7 (3)	C′(12)–C′(10)–C′(13)	108.7 (3)
C(5)–O(15)–C(6)	61.8 (2)	C′(5)–O′(15)–C′(6)	61.4 (2)
C(6)–C(16)–C(17)	112.5 (3)	C′(6)–C′(16)–C′(17)	110.7 (3)
C(6)–C(16)–C(18)	108.8 (3)	C′(6)–C′(16)–C′(18)	109.2 (3)
C(6)–C(16)–C(19)	109.5 (3)	C′(6)–C′(16)–C′(19)	109.6 (3)
C(17)–C(16)–C(18)	108.3 (3)	C′(17)–C′(16)–C′(18)	108.5 (3)
C(17)–C(16)–C(19)	107.5 (3)	C′(17)–C′(16)–C′(19)	108.3 (3)
C(18)–C(16)–C(19)	110.2 (3)	C′(18)–C′(16)–C′(19)	110.5 (3)

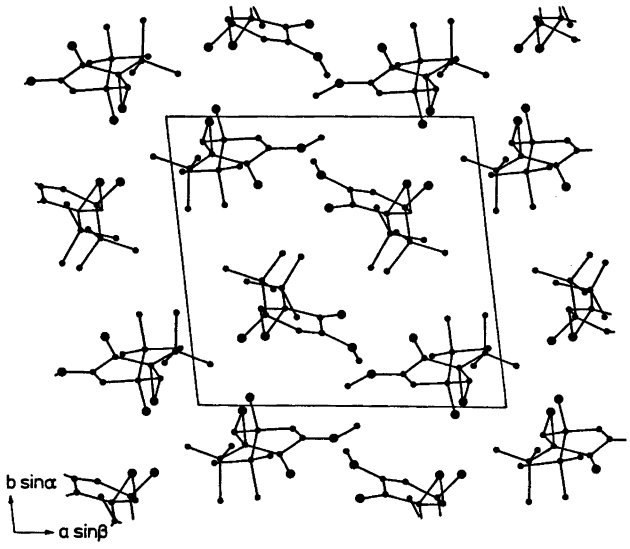


Fig. 4. Projection of the crystal structure along the c direction. ●, carbon; ●, oxygen.

Table 7. Intermolecular distances shorter than 3. 8 Å.

Superscript	Coordinates	Superscript	Coordinates
none	$x;\ y;\ z$	iii	$1-x;\ 2-y;\ -z$
i	$x;\ -1+y;\ z$	iv	$1-x;\ 2-y;\ 1-z$
ii	$-x;\ 2-y;\ -z$	v	$1-x;\ 3-y;\ 1-z$

C(13) – O(15^{ii})	3.632 (4)	C(11) – C′(17^{i})	3.788 (5)
O(14) – C(17^{ii})	3.645 (5)	C(12) – O′(8^{iii})	3.720 (4)
C(3) – O′(15)	3.703 (4)	C(13) – C′(5^{ii})	3.728 (4)
C(4) – O′(15)	3.632 (3)	C(13) – O′(14^{ii})	3.541 (4)
O(7) – C′(9^{iv})	3.285 (5)	C(13) – O′(15^{ii})	3.569 (4)
O(7) – C′(19^{i})	3.629 (5)	O(14) – O′(15)	2.797 (3)
O(8) – O′(8)	3.608 (4)	O(15) – O′(14)	2.967 (3)
O(8) – C′(9)	3.579 (5)	O(15) – O′(15)	3.233 (3)
O(8) – C′(9^{iv})	3.666 (5)	C′(2) – C′(11^{v})	3.740 (5)
C(9) – O′(8)	3.328 (5)	O′(8) – C′(11^{v})	3.413 (4)
C(9) – C′(9)	3.624 (6)	C′(9) – C′(11^{v})	3.724 (6)
C(9) – C′(18^{iii})	3.713 (6)		

Table 8. Least-squares planes and deviations (Å). The planes are described in terms of normalized equations in the orthogonal coordinate system (m, n, p) having $p \parallel c$, n in the bc plane and m in the a,b,c-octant.

Plane A	$0.1693m + 0.9350n + 0.3154p = 9.1427$
Plane B	$0.3487m + 0.9027n + 0.2522p = 9.1738$
Plane C	$-0.0403m + 0.9264n + 0.3745p = 8.1605$
Plane A′	$0.3964m + 0.8868n + 0.2375p = 13.9480$
Plane B′	$0.3145m + 0.9375n + 0.1489p = 14.5499$
Plane C′	$0.4577m + 0.8169n + 0.3263p = 13.4030$

Plane A		Plane B		Plane C	
Atom	Deviation	Atom	Deviation	Atom	Deviation
C(1)[a]	−0.267	C(1)	0.004	C(1)	0.004
C(2)	0.009	C(2)[a]	0.513	C(2)	0.009
C(3)	−0.009	C(3)[a]	0.487	C(3)	−0.009
C(4)[a]	−0.258	C(4)	−0.005	C(4)	0.004
C(5)	0.008	C(5)	0.009	C(5)[a]	0.531
C(6)	−0.008	C(6)	−0.009	C(6)[a]	0.525

Plane A′		Plane B′		Plane C′	
Atom	Deviation	Atom	Deviation	Atom	Deviation
C′(1)[a]	0.169	C′(1)	−0.009	C′(1)	0.007
C′(2)	−0.022	C′(2)[a]	−0.355	C′(2)	−0.017
C′(3)	0.022	C′(3)[a]	−0.303	C′(3)	0.017
C′(4)[a]	0.172	C′(4)	0.010	C′(4)	−0.008
C′(5)	−0.020	C′(5)	−0.020	C′(5)[a]	−0.367
C′(6)	0.019	C′(6)	0.019	C′(6)[a]	−0.328

[a] These atoms were omitted from the calculations of the least-squares planes.

The crystallographic asymmetric unit contains two molecules joined together by two hydrogen-bonds (Fig. 2). The hydroxyl oxygen O(14) in one molecule is hydrogen-bonded to the epoxide oxygen O′(15) of the other. The distances O′(15)···H(O14) and O(15)···H(O′14) are 1.83 and 1.97 Å, respectively, and the angles O′(15)···H(O14)–O(14) and O(15)···H(O′14)–O′(14) are 171 and 167°, respectively.

Fig. 3 indicates the numbering system used in

all tables and shows the bond lengths within the two molecules. Bond distances and angles for the structure are listed in Tables 5 and 6. No correction for thermal vibrations have been made. The estimated standard deviations, based solely on least-squares parameters, are about 0.004 Å for the bonds and 0.3° for the angles. No distance between bonded non-hydrogen atoms in one molecule of the asymmetric unit differs by more than 0.019 Å from the corresponding distance in the other molecule.

The mean $C(sp^3)-C(sp^3)$ bond distance, excluding C(5)–C(6), is 1.532 Å. The longer bonds are between the highly substituted carbon atoms C(4),C(10) and C(6),C(16). The shortness of the C(5)–C(6) bond length reflects the strain caused by the epoxide ring. The $C(sp^2)-C(sp^3)$ bonds have an average length of 1.504 Å in good agreement with usually observed values. The $C(sp^2)-C(sp^2)$ single bond is slightly shorter, 1.492 Å. The $C(sp^3)-O$ bonds and the $C(sp^2)-O$ bond have mean lengths of 1.430 and 1.357 Å, respectively.

The molecular packing is shown in Fig. 4. Intermolecular contacts less than 3.8 Å are listed in Table 7. The hydrogen-bonded dimers are held in position in the unit cell by van der Waals forces. Some of the van der Waals distances are shorter than normal, for example the $O(7)-C'(9^{iv})$ distance of 3.285 Å.

The best least-square planes through the ring are given for each of the dimer molecules in Table 8. In the non-primed molecule, the sets of atoms C(1), C(2), C(3), C(4); C(1), C(4), C(5), C(6); and C(2), C(3), C(5), C(6) are each coplanar within ±0.009 Å. In the primed molecule the deviations from the best planes for the same sets of atoms are ±0.017, ±0.020, and ±0.022 Å, respectively. All hydrogen atoms lie in chemically reasonable positions with apparent bond distances ranging from 0.76 to 1.12 Å, mean value 0.96 Å.

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Crystal Structures of Synthetic Analgetics. IV. Dextropropoxyphene

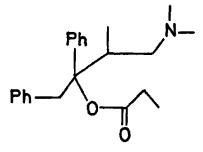
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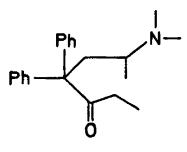
The molecular and crystal structure of dextropropoxyphene has been determined by X-ray methods. The crystals are monoclinic, space group $P2_1$, with unit cell dimensions $a = 9.257(2)$ Å; $b = 9.048(3)$ Å; $c = 12.074(7)$ Å; $\beta = 93.01(4)°$. The phase problem was solved by direct methods and the model refined to an R-value of 0.038 for 1799 observed reflections. E.s.d's are, in average, 0.004 Å and 0.3° in interatomic distances and angles, respectively.

The propylamine chain is nearly fully extended, the dihedral angle C4 – C5 – C7 – N being – 174.2°. The conformation of this side chain is similar to that in the hydrochloride of the title compound. Thus the proposed bioactive conformation is not preferred by propoxyphene in the crystalline state, as was the case for the free base of methadone.

Propoxyphene (I) is one of the few morphine-like synthetic analgetics that are not narcotic.[1] It is a widely used medicinal agent against moderate pain, and related to methadone (II). These two and other analogous diphenylpropylamines have a potential conformational flexibility in the side chain. Extensive stereochemical studies have been performed on these acyclic analgetics [2–4] with the objective to elucidate the structure-activity relationships (SAR) of these compounds. Several X-ray crystallographic determinations have been carried out (on morphine agonists) in the last years.[5–8] So far, however, the cyclic conformation as proposed by Beckett and Casy [9–10] has only been observed in the case of methadone base.[7–8] The two different conformations of methadone, found in the hydrobromide [11] and the free base itself, respectively, do not only clearly depict the conformational flexibility of these molecules but also underline the necessity of studying the molecules in different environments.



I



II

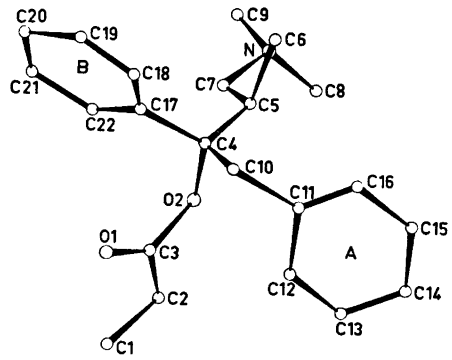


Fig. 1. The propoxyphene molecule with the numbering of the atoms indicated.

In a previous paper the author has reported the structure of the hydrochloride [5] and here the structure of the free base is presented.

Fig. 1 shows the asymmetric unit with the numbering of the atoms.

EXPERIMENTAL

The free base of dextropropoxyphene was prepared from the commercially available hydrochloride, and single crystals were obtained by crystallization from diethyl ether by slow evaporation at room temperature. A crystal of dimensions 0.2 mm × 0.3 mm × 0.4 mm was used for the experiments.

Table 1. Positional and thermal parameters with e.s.d's (10^4).

ATOM	X	Y	Z	B11	B22	B33	B12	B13	B23
O1	572(2)	2500(00)	3036(1)	131(2)	166(3)	89(1)	54(5)	63(3)	-8(4)
O2	2102(2)	3683(3)	1943(1)	106(2)	125(2)	55(1)	15(4)	23(2)	16(3)
N	3720(2)	7153(4)	216(2)	127(3)	296(6)	81(2)	66(7)	28(4)	-97(5)
C1	281(4)	85(4)	1553(3)	198(5)	126(4)	151(3)	16(8)	9(6)	28(6)
C2	1166(3)	1411(4)	1312(2)	155(4)	154(4)	110(2)	64(7)	58(5)	76(6)
C3	1221(2)	2562(4)	2208(2)	96(3)	124(3)	74(2)	-4(6)	20(4)	-3(4)
C4	2336(2)	4951(3)	2691(2)	94(3)	121(3)	52(1)	16(5)	11(3)	8(4)
C5	3367(2)	5242(3)	2036(2)	85(2)	133(4)	64(2)	8(5)	8(3)	-12(4)
C6	3822(3)	7346(4)	2654(2)	153(4)	158(4)	87(2)	72(7)	-14(4)	-18(5)
C7	2726(2)	6358(4)	882(2)	118(3)	149(4)	63(2)	17(6)	8(3)	-26(4)
C8	4739(6)	6187(10)	-234(5)	334(10)	732(22)	227(6)	-597(27)	376(14)	-522(22)
C9	2971(4)	7949(5)	-675(3)	238(6)	218(6)	91(2)	53(9)	32(6)	-103(6)
C10	3084(2)	4486(4)	3797(2)	104(3)	153(4)	53(2)	18(5)	19(3)	2(4)
C11	4403(3)	3448(4)	3709(2)	121(3)	145(4)	47(2)	9(6)	-2(3)	-25(4)
C12	4299(3)	1926(4)	3647(2)	151(4)	146(4)	90(2)	16(7)	-50(5)	-39(5)
C13	5523(4)	1053(4)	3578(3)	232(6)	148(5)	98(3)	-77(8)	-81(6)	-11(5)
C14	6874(3)	1679(4)	3583(2)	163(4)	205(6)	78(2)	-128(8)	-18(5)	-23(5)
C15	7005(3)	3179(5)	3673(2)	121(4)	227(6)	84(2)	-29(7)	26(4)	-63(6)
C16	5781(3)	4046(4)	3736(2)	128(4)	153(4)	88(2)	7(6)	7(4)	-27(5)
C17	902(2)	5727(3)	2872(2)	99(3)	138(3)	68(2)	15(5)	29(3)	18(4)
C18	726(3)	6539(4)	3822(2)	138(4)	222(5)	75(2)	-64(7)	27(4)	53(6)
C19	-564(4)	7296(5)	3970(3)	180(5)	234(6)	112(3)	-96(9)	78(6)	92(7)
C20	-1659(3)	7259(4)	3178(3)	121(3)	185(5)	152(3)	-57(7)	78(6)	14(7)
C21	-1490(3)	6460(4)	2225(3)	101(3)	171(4)	123(3)	2(6)	-3(5)	-9(6)
C22	-228(2)	5705(4)	2075(2)	96(3)	138(3)	81(2)	15(5)	17(3)	9(4)

ATOM	X	Y	Z	B	ATOM	X	Y	Z	B
H1C1	241(37)	-645(43)	921(31)	8,5(,5)	H1C9	3669(44)	8519(51)	-1114(33)	9,7(,6)
H2C1	604(38)	-382(43)	2233(29)	8,5(,6)	H2C9	2390(42)	7188(49)	-1203(33)	9,7(,9)
H3C1	-718(37)	358(45)	1746(30)	8,5(,9)	H3C9	2241(43)	8611(50)	-382(34)	9,7(1,0)
H1C2	2043(36)	1225(43)	1113(27)	7,9(,6)	H1C10	3350(24)	5279(27)	4203(19)	4,1(,3)
H2C2	843(36)	1949(41)	550(28)	7,9(,8)	H2C10	2412(23)	3869(29)	4187(18)	4,1(,5)
HC5	4236(20)	5302(23)	1973(15)	2,7(,3)	HC12	3373(26)	1483(32)	3646(20)	5,2(,5)
H3C6	2971(31)	7962(35)	2721(23)	6,4(,7)	HC13	5425(30)	-27(38)	3518(23)	6,4(,7)
H1C6	4241(32)	7124(37)	3367(25)	6,9(,7)	HC14	7734(28)	1076(32)	3558(21)	5,4(,6)
H2C6	4461(34)	7933(38)	2216(26)	6,9(,7)	HC15	7979(33)	3613(36)	3678(24)	6,5(,7)
H2C7	1914(26)	7043(30)	965(21)	5,0(,4)	HC16	5895(24)	5042(28)	3811(18)	4,1(,5)
H1C7	2346(26)	5491(30)	498(20)	5,0(,6)	HC18	1506(30)	6596(36)	4392(22)	6,0(,6)
H1C8	5309(49)	6672(60)	-628(39)	11,4(,8)	HC19	-647(35)	7877(40)	4689(27)	7,7(,8)
H2C8	5249(50)	5591(61)	262(39)	11,4(1,3)	HC20	-2593(31)	7681(38)	3242(23)	6,5(,6)
H3C8	4155(50)	5252(59)	-553(39)	11,4(,6)	HC21	-2197(27)	6538(32)	1670(20)	5,1(,5)
					HC22	-94(23)	5137(26)	1417(17)	3,6(,4)

The crystals are monoclinic and systematically absent reflections $0k0$ for odd indices is compatible with space group $P2_1$ for an optically active compound. Unit cell dimensions were determined on a Syntex P$\bar{1}$ diffractometer with graphite crystal monochromated Mo$K\alpha$-radiation ($\lambda = 0.71069$ Å).

Three-dimensional intensity data were collected applying the $2\theta - \theta$ autocollection program with variable scan rate ($2 - 8°$ min^{-1}) and a cut off for low intensities above the limit of 0.60 for sin θ/λ. The scan range was from 1.0° below $2\theta(\alpha_1)$ to 1.0° above $2\theta(\alpha_2)$ and the background were counted 0.7 times the scan time. The intensities of three standard reflections were measured periodically during the collection of data. They showed no systematic variation. E.s.d.'s in the intensities were taken as the square root of the total counts with a 2 % addition for instrumental instability.

A total of 2633 independent reflections were recorded within the limit of 0.71 for sin θ/λ; 1799 had a net count larger than 2.5 σ_I.

The data were corrected for Lorentz and polarization effects.

All calculations were performed on a CDC 6600 computer utilizing the programs described in Ref. 12, except for the phase determination.[13] Atomic form factors were those of Hanson *et al.*[14] for O, N, and C and of Stewart *et al.*[15] for H.

A complete list of the structure factors may be obtained from the author on request.

CRYSTAL DATA

Dextropropoxyphene, $C_{22}H_{29}NO_2$, monoclinic. $a = 9.257(2)$ Å, $b = 9.048(3)$ Å, $c = 12.074(7)$ Å, $\beta = 93.01(4)°$, $V = 1010.1$ Å^3, M = 339.49, $Z = 2$. $D_{obs} = 1.09$ g cm^{-3} (flotation), $D_{calc} = 1.11$ g cm^{-3}. Systematic absences = $0k0$ when k is odd; space group $P2_1$.

STRUCTURE DETERMINATION

380 of the highest E-values (≥ 1.20) were used as input for MULTAN,[13] and one of the resulting E-maps gave the positions of all the 25 non-hydrogen atoms. Successive Fourier synthesis, isotropic and anisotropic least-squares refine-

Table 2. Interatomic distances (Å), bond angles (°) and torsional angles (°).

DISTANCE	(Å)	DISTANCE	(Å)	DISTANCE	(Å)
C1 - C2	1.498(5)	C2 - C3	1.501(4)	C3 - O1	1.193(3)
C3 - O2	1.351(3)	O2 - C4	1.469(3)	C4 - C5	1.556(3)
C5 - C6	1.521(4)	C5 - C7	1.533(3)	C7 - N	1.446(3)
C8 - N	1.415(7)	C9 - N	1.442(4)	C4 - C10	1.552(3)
C4 - C17	1.527(3)	C10 - C11	1.396(3)	C11 - C12	1.382(4)
C12 - C13	1.388(4)	C13 - C14	1.373(5)	C14 - C15	1.366(5)
C15 - C16	1.383(4)	C16 - C11	1.384(4)	C17 - C18	1.383(4)
C18 - C19	1.380(4)	C19 - C20	1.357(5)	C20 - C21	1.374(5)
C21 - C22	1.373(4)	C22 - C17	1.386(3)		

ANGLE	(°)	ANGLE	(°)
C1 - C2 - C3	114.6(2)	C2 - C3 - O1	124.9(2)
O1 - C3 - O2	124.8(2)	C2 - C3 - O2	110.4(2)
C3 - O2 - C4	120.8(2)	O2 - C4 - C5	102.3(2)
O2 - C4 - C10	109.8(2)	O2 - C4 - C17	110.2(2)
C5 - C4 - C10	111.2(2)	C5 - C4 - C17	111.7(2)
C10 - C4 - C17	112.8(2)	C4 - C5 - C6	113.2(2)
C4 - C5 - C7	112.6(2)	C6 - C5 - C7	108.9(2)
C5 - C7 - N	113.6(2)	C7 - N - C8	111.3(4)
C7 - N - C9	111.6(2)	C8 - N - C9	109.0(3)
C4 - C10 - C11	116.7(2)	C10 - C11 - C12	121.5(2)
C10 - C11 - C16	121.5(2)	C11 - C12 - C13	121.0(3)
C12 - C13 - C14	120.8(3)	C13 - C14 - C15	119.1(3)
C14 - C15 - C16	119.9(3)	C15 - C16 - C11	122.2(3)
C16 - C11 - C12	116.9(3)	C4 - C17 - C18	121.1(2)
C4 - C17 - C22	121.7(2)	C17 - C18 - C19	121.4(3)
C18 - C19 - C20	120.5(3)	C19 - C20 - C21	119.3(3)
C20 - C21 - C22	120.5(3)	C21 - C22 - C17	121.2(2)
C22 - C17 - C18	117.2(2)		

DIHEDRAL ANGLE	( )	DIHEDRAL ANGLE	( )
C1 - C2 - C3 - O1	-3.5(4)	C1 - C2 - C3 - O2	176.9(2)
O1 - C3 - O2 - C4	.4(3)	C2 - C3 - O2 - C4	180.0(2)
C3 - O2 - C4 - C5	-178.6(2)	C3 - O2 - C4 - C10	63.6(2)
C3 - O2 - C4 - C17	-59.7(2)	C12 - C11 - C10 - C4	-98.6(3)
C11 - C10 - C4 - O2	49.8(3)	C11 - C10 - C4 - C17	172.1(2)
C11 - C10 - C4 - C5	-62.2(3)	C18 - C17 - C4 - C5	-92.6(3)
C18 - C17 - C4 - O2	154.5(2)	C18 - C17 - C4 - C10	32.9(3)
C16 - C11 - C10 - C4	92.3(3)	C22 - C17 - C4 - O2	-28.5(3)
O2 - C4 - C5 - C6	-178.5(2)	O2 - C4 - C5 - C7	57.4(2)
C4 - C5 - C7 - N	-174.2(2)	C6 - C5 - C7 - N	59.4(3)
C5 - C7 - N - C8	77.7(4)	C5 - C7 - N - C9	-160.3(2)

ments gave an R-factor of 0.08. Approximate positional parameters of all the 29 hydrogen atoms were calculated from stereochemical considerations. Hydrogen atoms positioned at the same carbon atom were given common B-values, and all the light atoms were refined isotropically.

Inclusion of the three hydrogen atoms at C8 in the refinement tends to move C8 towards the nitrogen atom, decreasing the C – N distance by about 0.03 Å. This indicates a correlation between the carbon and the hydrogen atom parameters. But due to an improvement of the R-factor of 0.5 % and in spite of a large B-value, these three hydrogen atoms were included in the refinement, which converged at $R = 0.038$ ($R_w = 0.039$). The final parameters are listed in Table 1, where the anisotropic temperature factor is given by

$$\exp -(B11h^2 + B22k^2 + B33l^2 + B12hk + B13hl + B23kl)$$

The mean e.s.d.'s in the positional parameters of the heavy atoms are 0.004, 0.006, and 0.003 in the x-, y-, and z-coordinates.

DISCUSSION

Interatomic distances, bond angles and dihedral angles are given in Table 2. The listed e.s.d.'s are calculated from the correlation matrix.

The bond lengths and angles found in this molecule are normal and do not deviate from standard values,[16] except for the short C8 – N distance (discussed above) and the molecular dimensions at C4. The lengthening of the C4 – C5, C4 – C10, C4 – C17 and C4 – O2 single

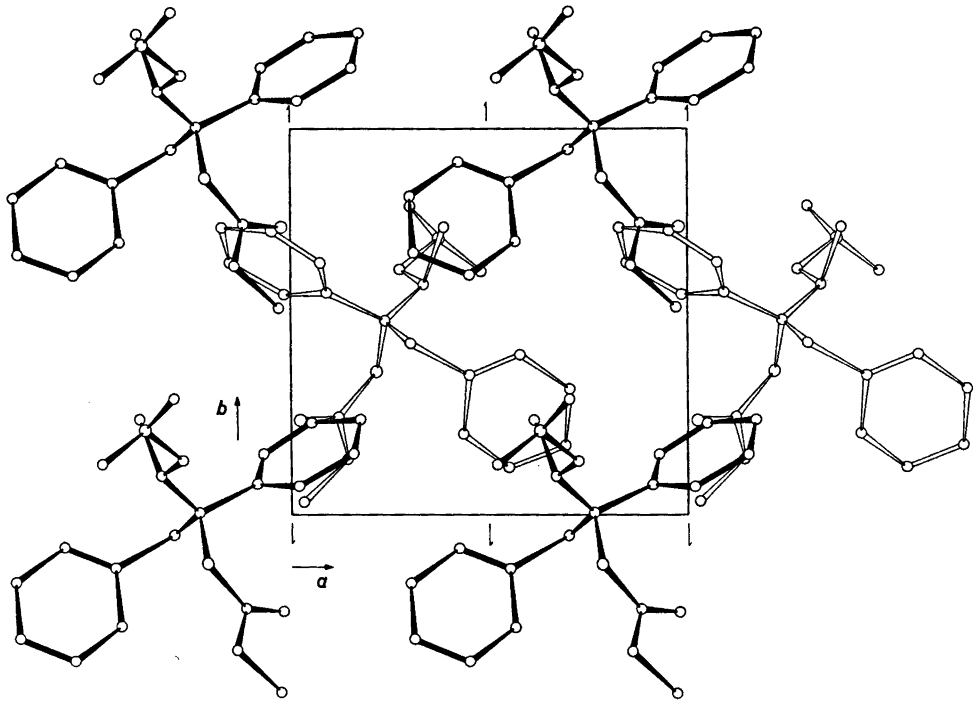


Fig. 2. The crystal packing of dextropropoxyphene as seen along the *c*-axis.

bonds clearly demonstrate the crowded situation around C4. This, together with the corresponding angular distortions, is mainly attributed to nonbonded interactions. This somewhat distorted molecular geometry is in agreement with results reported for the hydrochloride [5] and other related compounds.[6–8,17]

The two phenyl rings A and B (Fig. 1) are strictly planar. C10 is essentially coplanar with plane A (0.011 Å out of the plane), whereas C4 is elevated as much as 0.065 Å out of B. As reported for dextropropoxyphene hydrochloride the angle C4 – C10 – C11 is somewhat opened as compared to the accepted value for $C(sp^3) - C(sp^3) - C(sp^3)$ bond angles. The two planes A and B form an angle of 61.3°. The plane through C4, C10, C11 is nearly perpendicular to plane A whereas the dihedral angle C18 – C17 – C4 – C10 is as small as 32.9°.

The torsional angle C4 – C5 – C7 – N is – 174.2° and the propylamine chain is thus almost fully extended. Hence the free base of propoxyphene prefers a side chain conformation in the crystalline state resembling that of the hydrochloride.[5] This may indicate a less degree of conformational flexibility as compared to the methadone molecule.[6,11] In this manner propoxyphene seems to be similar to isomethadone. Recent spectroscopic investigations on methadone and isomethadone in solution [4] suggested a predominant conformational homogeneity for isomethadone.

The structure reports on analgetics so far seem to confirm that intermolecular forces, for example hydrogen bonds, play an important part in the selection of the preferred conformation in compounds having a quaternary ammonium group. Compounds with an uncharged nitrogen atom and a methyl group on the β carbon atom in the side chain may be more rigid than those having the *s*-methyl group at the α carbon atom.

The crystal structure as seen along the *c*-axis is shown in Fig. 2. There are no short intermolecular distances in the crystal.

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The Reactions of Lignin during Neutral Sulfite Pulping. Part V.* The Reactions of α-(4-Hydroxy-3-methoxyphenyl)-glycerol-β-guaiacyl Ether with Sulfite and their Dependence on pH**

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Dedicated to Professor Dr. Karl Kratzl on the occasion of his 60th birthday.

The sulfonation of the phenolic arylglycerol-β-aryl ether units in lignin at different pH values has been studied using a model compound (I, Scheme 1).

The primary product, the quinonemethide II, undergoes three competing reactions:

(1) nucleophilic addition of sulfite; (2) elimination of the terminal hydroxymethyl group as formaldehyde; (3) elimination of water.

Sulfonic acid groups may be introduced in all positions of the (substituted) glycerol side chain since the conjugated products formed in reactions 2 and 3 add hydrogen sulfite (and/or sulfite) ions giving rise to α- and γ-sulfonic acids and the β-aryl ether bond may be sulfitolytically cleaved giving β-sulfonic acids.

The α-sulfonic acid group of the α,β-disulfonic acids may be eliminated as bisulfite with formation of styrene-β-sulfonic acids. When the pH of the sulfite reaction is increased, this elimination and reactions 2 and 3 become more important.

Guaiacol formed in the sulfite treatment of I condenses with the hydroxymethanesulfonic acid (sulfomethylation, Scheme 2) that is formed by addition of sulfite to the formaldehyde produced in reaction 2. Guaiacol also adds to styrene-β-sulfonic acids affording 1,1-diarylethane- and 1,1-diarylpropane sulfonic acids.

The formation of the different sulfonic acids can be accounted for by the reaction sequences outlined in Schemes 1 and 2. The significance of these reactions for lignin sulfonation is discussed briefly.

Previous communications in this series have described the reactions of the phenolic β-aryl ether units of lignin with sulfite under acidic and neutral conditions.

Model compound I, when treated with sulfite solution at pH 1.5 gives a mixture of *erythro*- and *threo*-forms of the corresponding α-sulfonic acid (III).[1] When the simpler model compound XVIII is treated with neutral sulfite solution and the pH is allowed to rise to 10 during the reaction, the main product is the styrene-β-sulfonic acid IX.[2] This reaction apparently proceeds *via* α-sulfonation, sulfitolytic cleavage of the β-guaiacyl ether bond of the α-sulfonic acid (VI) giving the α,β-disulfonic acid VII, and then elimination of bisulfite to form the corresponding styrene-β-sulfonic acid.[3]

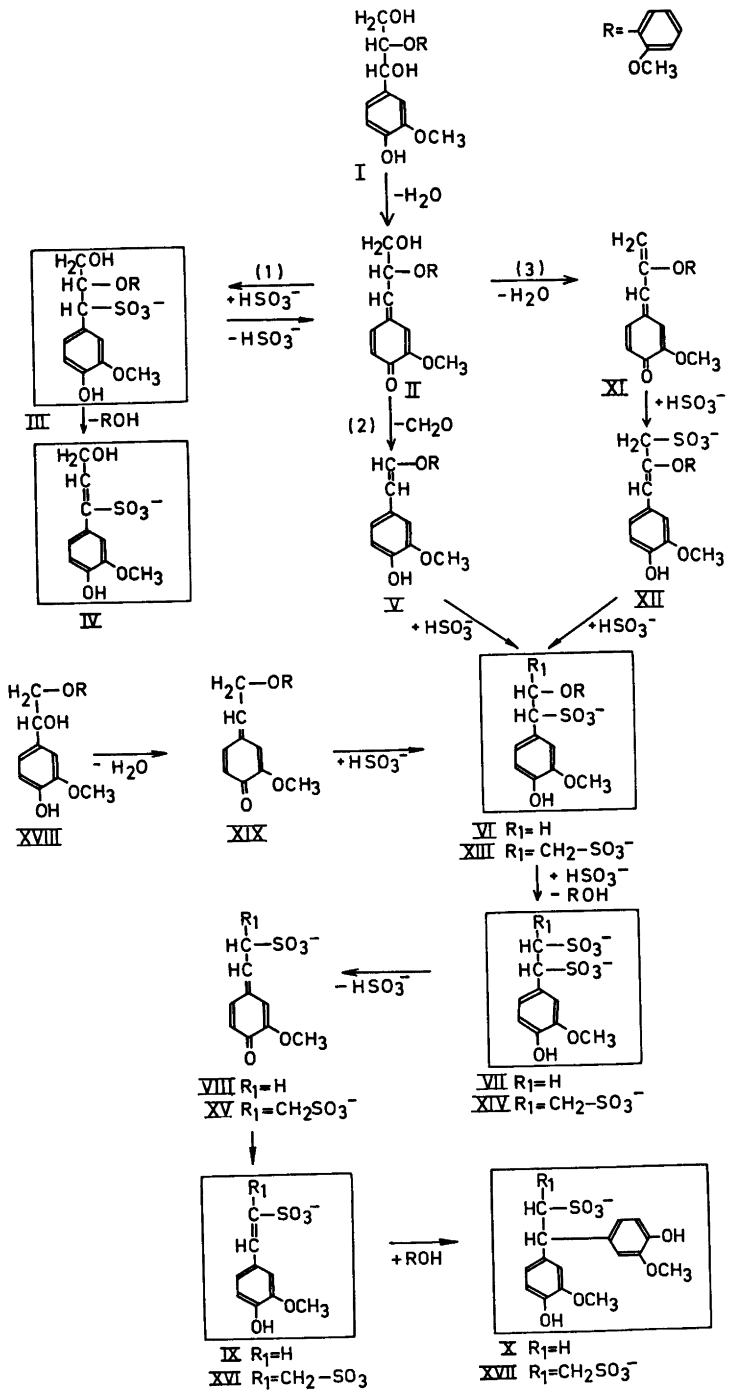
In the present work the reaction of β-aryl ether type structural elements with sulfite at different pH values were reinvestigated using I as a model substance.

RESULTS AND DISCUSSION

Compound I was treated with sulfite solution at different pH values (and at 180 °C unless otherwise indicated). Due to the liberation of hydroxyl and alkoxyl ions in the sulfonation process, the pH of the solution increases unless a sufficiently large excess of sulfite is used. The reaction mixtures, on working up as described previously,[1] give a chloroform-ether soluble fraction containing varying amounts of guaiacol and catechol but no starting material, and a water-

* Part IV, see Ref. 14.

** Preliminary reports on the results of this work have been given (by J. G.) at the International Sulfite Pulping and Recovery Conference in Boston, Mass., October 30—November 3, 1972. Abstr. of Papers, p. 323 and at the Annual Meeting of Zellcheming in Baden-Baden, West Germany, June 25—30, 1973.



Scheme 1. Sulfonic acids are given as anions. Compounds isolated (in the form of acetylated sulfonic acid methyl esters) are shown in frames.

Table 1. Treatment of compound I with sulfite solutions of different pH values.

pH	4.0	7.0	7.0	7.0–8.5	9.5–11.0
Temperature, °C	155	180	180	180	180
Time, h	4	3	6	3	3
Amount of I, g	1.0	3.0	2.0	3.0	4.0
Amount of sulfite solution, ml	80	240	160	160	160
Solvent system	A	A	A	B	A→C, gradient elution
Phenols, mg	traces	375	480	510	853
Compound [a]					
III (*erythro* and *threo*)	*[b,c]	*	+	*	
IV		+[d]	(+)	+	+
VII		+	*	+	+
IX			(+)		+
X				(+)	+
XIII				(+)	
XIV					+
XVI			+		
XVII					+
XXII		+	+	+	+
XXIII		+	+	+	+
XXIV			+		+
XXV			+		
MS		+	+	+	+
Total yield, g	1.75	3.25	2.25	4.0	

[a] Acetylated methyl ester of the corresponding sulfonic acid. [b] +, present; (+), small amount or traces; *, main product. [c] *erythro-threo* 2:1. [d] M.p. 96–98 °C.

soluble fraction containing sulfonic acids. The latter were converted into acetylated methyl esters,[1] and after separation by column chromatography these were identified by NMR and mass spectrometry.

Table 1 shows the sulfonic acids obtained in the treatments at different pH values. Tentative routes for their formation are outlined in Schemes 1 and 2.

Treatment at pH 4, 155 °C (4 h). The simplest reaction mixture was obtained by treatment with a weakly acidic sulfite solution of pH 4. As in the reaction at pH 1.5,[1] the *erythro* and *threo* forms of the α-sulfonic acid III* were formed in a ratio of approximately 2:1. This was independent of the diastereoisomeric form of the starting β-aryl ether (I), supporting the view that α-sulfonation proceeds by a S_N1 mechanism *via* a common intermediate, the quinonemethide II (pathway 1), and/or the benzylium ion derived from I.[1] To give further support for this interpretation, a solution of the quinonemethide II was prepared from compound I and treated with neutral sulfite solution at room temperature. Both stereoisomeric forms of the α-sulfonic acid III were isolated, the *erythro* form being the major component.

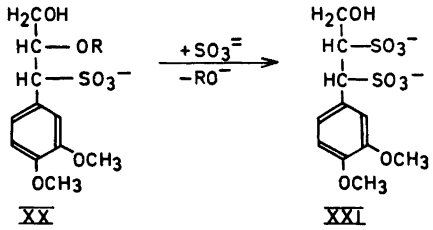
Treatment at pH 7 (3 h). When the pH of the sulfite treatment was raised from 4.0 to 7.0, the *erythro*- and *threo*-forms of the α-sulfonic acid III were still the main products but the sulfonation mixture also contained the sulfonic acids IV, VII, XXII, and XXIII, as well as methanesulfonic acid.

The styrene-β-sulfonic acid derivative IV which was obtained in a small amount apparently arises by elimination of guaiacol from III (β-elimination). It has been previously [4,5] suggested as an intermediate in the alkali-promoted formation of acetoguaiacone and formaldehyde from III.

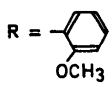
The α,β-disulfonic acid VII can obviously be formed by elimination of formaldehyde from the intermediate quinonemethide II (reverse aldol reaction [6]), followed by addition of bisulfite to the resulting β-guaiacoxystyrene derivative V (formation of the α-sulfonic acid VI) and then

* For the sake of uniformity the side chain carbon atoms are denoted by α, β, and γ (starting from the carbon atom adjacent to the aromatic nucleus) throughout the general part of this work. For systematic nomenclature, see experimental section.

by sulfitolytic cleavage of the β-guaiacyl ether bond in VI [route (2), *cf.* also Ref. 2]. Support for this suggested route was obtained by isolation of the α-sulfonic acid VI and the α,β-disulfonic acid VII in yields of 20 and 70 %, respectively, after treatment of the β-guaiacyl ether XVIII with sulfite under the same conditions. The sulfonic acids VI and VII could arise by addition of bisulfite either to the intermediate quinonemethide XIX [3] or to the intermediate β-guaiacoxystyrene derivative V (see above). The sulfitolytic cleavage of a β-guaiacyl ether bond was demonstrated using the non-phenolic α-sulfonic acid XX. The corresponding α,β-disulfonic acid (XXI) was isolated in the diastereoisomeric forms with a ratio of *threo*- to *erythro*-forms of approximately 2:1.



XX XXI



The benzylsulfonic acids XXII and XXIII are formed by condensation of guaiacol either with hydroxymethanesulfonic acid (sulfomethylation, Scheme 2) or with formaldehyde followed by sulfonation of the resulting *ortho*- and *para*-hydroxybenzyl alcohols. Guaiacol is liberated by sulfitolytic cleavage of the β-arylether linkage and hydroxymethanesulfonic acid is generated by reaction of bisulfite with formaldehyde, eliminated from the quinonemethide II (Scheme 1, route 2). In agreement with this interpretation, the benzylsulfonic acids XXII and XXIII (and the disulfonic acid XXIV) were obtained when guaiacol was treated with neutral sulfite in the presence of formaldehyde under similar conditions. (For the condensation of hydroxymethanesulfonic acid with phenol, see Ref. 7.)

The sulfomethylation of guaiacol is reminiscent of the condensation reactions taking place in alkali andsulfate pulping in which formaldehyde reacts with phenolic structural units to give benzyl alcohol- and then diarylmethane structures.[8–11] In the present instance, the formation of diarylmethane derivatives is prevented by the reaction of formaldehyde or the benzyl alcohol intermediates with bisulfite.

The aliphatic degradation product methanesulfonic acid arises by sulfitolytic cleavage of methyl aryl ether bonds as described previously [2,3,12] (*cf.* also formation of catechol and XXV).

Treatment at pH 7 (6 h). Prolongation of the sulfite treatment at pH 7 from 3 to 6 h resulted in the formation of 4 additional sulfonic acids.

A small amount of the styrene-β-sulfonic acid derivative IX was formed by elimination of bisulfite from the α,β-disulfonic acid VII. The sulfonic acid IX was also obtained, together with the disulfonic acid VII, when compounds XVIII [2] or V were treated in the same way (Scheme 1).

An analogous elimination of bisulfite from the α,β,γ-trisulfonic acid XIV (see below) gave rise to the formation of the styrene-β-sulfonic acid derivative XVI.

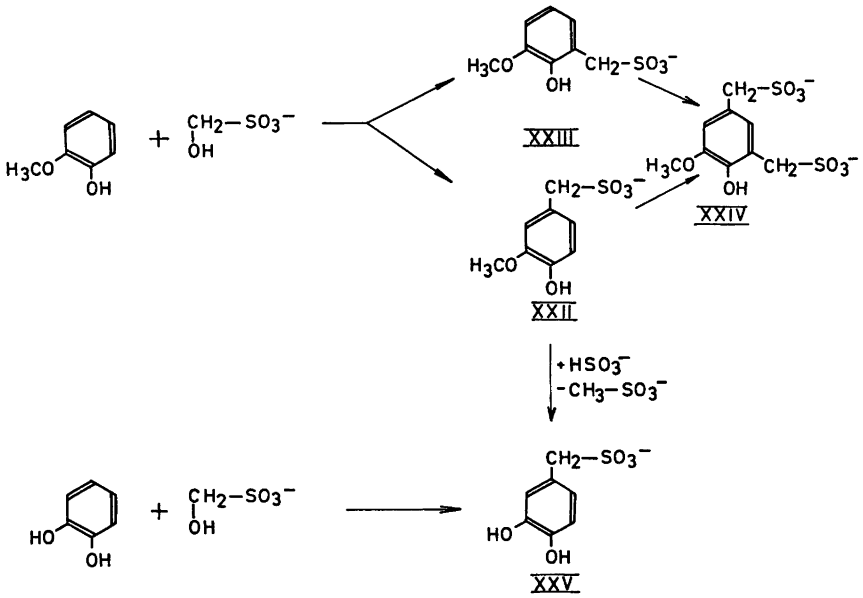
The benzylsulfonic acids XXIV and XXV were produced by sulfomethylation of XXII, XXIII, and catechol, respectively. Compound XXV could also arise from XXII by sulfitolytic demethylation.

Treatment at pH 7.0–8.5 (3h). When compound I was treated with a sulfite solution containing reduced amounts of sodium sulfite, the pH increased during the reaction from 7.0 to 8.5. In addition to the sulfonic acids obtained on sulfonation at a constant pH of 7.0 (3 h), the reaction mixture contained small amounts of two sulfonic acids:

The β-sulfonic acid X arises by the reaction of the α,β-disulfonic acid VII with guaiacol, as was shown in a separate experiment. The quinonemethide-β-sulfonic acid VIII and the styrene-β-sulfonic acid derivative IX are possible intermediates.

The α,γ-disulfonic acid XIII is formed on a route which is described in connection with the formation of the sulfonic acids XIV, XVI, and XVII (see below).

Treatment at pH 9.5–11.0 (3 h). The resulting reaction mixture contained disulfonic acid VII, the styrene-β-sulfonic acid derivative IX, β-sulfonic acid X, and benzylsulfonic acids



Scheme 2.

XXII–XXIV as major components. Small amounts of the styrene-α-sulfonic acid derivative IV, the α,β,γ-trisulfonic acid XIV, the styrene-β-sulfonic acid derivative XVI, and the β,γ-disulfonic acid XVII were also isolated.

The formation of the α,γ- and β,γ-disulfonic acids [XIII (see above) and XVI, XVII, respectively], and of the α,β,γ-trisulfonic acid XIV, can by accounted for as follows:

The intermediate quinonemethide II, on elimination of water, will give the extended quinonemethide XI, route 3, which will afford the α,γ-disulfonic acid XIII by 1,8- and then 1,2-addition of bisulfite. This reaction sequence is analogous to the sulfonations of coniferyl alcohol [13,14] and of pinoresinol [14] in neutral and alkaline solutions. It has been suggested recently [14] that these reactions also proceed *via* extended quinonemethides. Sulfitolytic cleavage of the β-aryl ether bond in XIII can afford the α,β,γ-trisulfonic acid XIV and this, probably *via* the quinonemethide XV, can yield the styrene-β-sulfonic acid derivative XVI. Addition of guaiacol to XVI will then give the β,γ-disulfonic acid XVII.

The α-sulfonic acid III, the main sulfonation product formed at lower pH-values, was absent. This finding may be explained by the competition of the elimination reactions 2 and 3 with the sulfite addition reaction 1 (Scheme 1) and by the reversibility of the latter. Treatment of the α-sulfonic acid III with a sulfite solution of pH 9.5 under similar conditions yielded small amounts of the disulfonic acid VII together with other sulfonic acids. The α-sulfonic acid III which might be formed at higher pH is likely to be consumed by the elimination reaction affording the styrene-α-sulfonic acid derivative IV.

SUMMARY AND CONCLUSIONS

Formation of the sulfonic acids shown in Scheme 1 proceeds *via* a common intermediate, the quinonemethide II, which may undergo three alternative conversions:

(1) Addition of bisulfite to give the α-sulfonic acid III. Elimination of guaiacol can then yield the styrene-α-sulfonic acid derivative IV.

(2) Elimination of formaldehyde to form the β-guaiacoxystyrene derivative V. Subsequent addition of bisulfite, sulfitolytic cleavage of the

β-guaiacyl ether bond, elimination of bisulfite and addition of eliminated guaiacol will convert the intermediate V into the sulfonic acids VI, VII, IX, and X.

(3) Elimination of water to form the extended quinonemethide XI and, by 1,8-addition of bisulfite, the β-guaiacoxystyrene derivative XII. The subsequent reactions of this intermediate, including 1,2-addition of bisulfite, sulfitolytic cleavage of the β-guaiacyl ether linkage, elimination of bisulfite and addition of guaiacol are analogous to those of the β-guaiacoxystyrene derivative V and will give the sulfonic acids XIII, XIV, XVI and XVII.

The formaldehyde eliminated in reaction (2) and bisulfite, or the formaldehyde-bisulfite addition product (hydroxymethanesulfonic acid), will condense with guaiacol or with catechol to afford the benzylsulfonic acids XXII–XXV (Scheme 2).

The relative importance of the three pathways outlined in Scheme 1 and the sulfomethylation reactions outlined in Scheme 2 will be dependent on the reaction conditions used. The pH during the sulfite treatment appears to have a decisive effect on the course of the sulfonation and, thus, on the qualitative and quantitative composition of the final mixture of sulfonic acids. Another important factor seems to be the length of the sulfite treatment.

Although some of the sulfonic acids expected were not isolated as the methyl esters because of separation difficulties in the column chromatography, some general features of the reactions of compound I in sulfite solutions of different pH values can be discerned.

In the pH-range 4 to 8.5 the nucleophilic addition of sulfite to II (pathway 1) is the main reaction. At about pH 7 the elimination pathway 2 and, at a somewhat higher pH, pathway 3 become more and more competitive and predominate at pH values higher than about 9.5. This is reflected by the increased formation of guaiacol and catechol and by the larger amounts of condensation products formed from these phenols and hydroxymethanesulfonic acid or conjugated intermediates. These changes in the reaction patterns of compound I with sulfite solutions are gradual and no distinct pH-limits for the various routes and reaction steps can be drawn.

The finding that all three types of carbon atoms of the side chains (α, β, and γ) in phenolic β-aryl ether structures may be involved in the sulfonation of lignin is in agreement with previous analytical studies.[15] Reactions at these sites and sulfomethylation reactions have to be taken into account, if the reactive groupings in lignin are to be correctly classified into categories according to their ease of sulfonation (*cf.* Ref. 16).

EXPERIMENTAL

Melting points are corrected. Evaporations were carried out under reduced pressure.

Thin-layer and column chromatography. Phenols and sulfonic acid esters were separated on an analytical scale by TLC using silica gel HF_{254}, the solvent systems used unless otherwise stated were: phenols: chloroform-acetone 4:1; sulfonic acid esters: cyclohexane-ethyl acetate 1:1 (A), 3:2 (B), and 7:3 (C). Preparative separations were carried out with a liquid chromatograph (Chromatronix Inc., Berkeley, California) using silicic acid (Bio-Sil A, 20–44 μ, Bio-Rad Laboratories, Richmond, California) as the stationary phase and the same solvent systems as in the analytical separations. The flow rate in a 12.7 × 1000 mm column was 1 ml/min and the pressure approximately 170 kPa.

NMR spectrometry. NMR spectra ($CDCl_3$) were recorded with a Perkin-Elmer R-12 spectrometer. The NMR-data are summarized in Table 2. Chemical shifts (δ-values) are given in ppm downfield from TMS.

Mass spectrometry. Mass spectra were recorded on a Perkin-Elmer 270 instrument at 20 eV using the direct inlet system. The temperature of the probe heater was 50 °C. Mass spectral data are summarised in Table 3.

Model compounds. The model compounds used were prepared as previously described (references are given below).

Sulfite solutions (a) pH 4, $Na_2S_2O_5$ (0.25 mol) in distilled water (1 l); (b) pH 7, $Na_2S_2O_5$ (0.25 mol) in distilled water (1 l) was adjusted to pH 7 with 2 N NaOH; (c) pH 9, Na_2SO_3 (0.5 mol) in distilled water (1 l).

Sulfite treatment. The model compound, suspended in the sulfite solution, was heated under pressure at 180 °C for 3 h unless otherwise stated.

Work-up procedure. The work-up procedure has been previously described.[1] The mixtures of chloroform-ether soluble compounds consisted of guaiacol and catechol only. The sulfonic acids in the aqueous solutions were converted to acetylated sulfonic acid methyl esters. These mixtures were investigated by TLC and then separated by repeated preparative column chromatography into several fractions. Yields of the chro-

Table 2. Proton chemical shifts (δ-values) of acetylated sulfonic acid methyl esters.

Compound acetylated methyl ester of	Aromatic, olefinic H	Aliphatic H	OCH_3 (ester, ether)	Acetyl H	
				Aromatic	Aliphatic
IV	6.8–7.1 (m, 4 H)	4.62 (d, 2 H_γ)[a]	3.80 (s, 3 H) 3.71 (s, 3 H)	2.28 (s, 3 H)	2.00 (s, 3 H)
X	6.7–7.0 (m, 6 H)	3.78 (d, 2 H_β) 4.53 (t, 1 H_α)[b]	3.49 (s, 3 H) 3.74 (s, 6 H)	2.23 (s, 6 H)	
XIV	6.8–7.2 (m, 3 H)	3.4–4.1 (4 H)[c]	3.71 (s, 3 H) 3.77 (s, 3 H) 3.80 (s, 3 H) 3.82 (s, 3 H) 3.89 (s, 3 H)	2.30 (s, 3 H)	
XVII	6.7–7.1 (m, 6 H)	3.4–4.1 (4 H)[c]	3.51 (s, 3 H) 3.64 (s, 3 H) 3.74 (s, 6 H)	2.24 (s, 3 H) 2.27 (s, 3 H)	
XXI (*erythro*)	6.8–7.0 (m, 3 H)	4.00–4.30 (m, H_β) 4.75–5.00 (H_α+2 H_γ)	3.48 (s, 3 H) 3.61 (s, 3 H) 3.86 (s, 3 H)		2.12 (s, 3 H)
XXI (*threo*)	6.85–7.10 (m, 3 H)	loc. uncertain	3.61 (s, 3 H) 3.74 (s, 3 H) 3.87 (s, 3 H)		1.96 (s, 3 H)
XXII	6.98 (s, 3 H)	4.28 (s, 2 H)	3.74 (s, 3 H) 3.80 (s, 3 H)	2.28 (s, 3 H)	
XXIII	6.9–7.3 (m, 3 H)	4.32 (s, 2 H)	3.72 (s, 3 H) 3.80 (s, 3 H)	2.30 (s, 3 H)	
XXIV	7.0–7.2 (broad s, 3 H)	4.31 (s, 4 H)	3.73 (s, 3 H) 3.80 (s, 3 H) 3.82 (s, 3 H)	2.32 (s, 3 H)	

[a] $J_{\beta\gamma}$ 6.0 Hz. [b] $J_{\alpha\beta}$ 6.7 Hz. [c] Location of aliphatic H uncertain due to overlapping of OCH_3.

Table 3. Mass spectral fragmentation of acetylated sulfonic acid methyl esters (all fragments having m/e >130 and intensities >5 % of the base peak are accounted for).

Compound, acetylated methyl ester of	M (m/e)	M−n×42 [a] (n=0, 1, 2)	M−n×42 −95/96 [b]	M−n×42 −2×95/96	M−n×42 −3×95/96	Misc.
IV	358 (11)	316 (100)	221 (21) 220 (64)			205 (37) 192 (16) 179 (83)
VII	382 (1)	340 (5)	245 (41) 244 (12)	150 (100) 149 (10) 148 (21)		
IX	286 (4)	244 (100)	149 (20) 148 (85)			
X	452 (4)	410 (32) 368 (60)	273 (21) 272 (100)			259 (35)
XIII	518	476				352 257 161 } [c]
XIV	490 (3)	448 (17)	353 (72) 352 (16)	258 (24) 257 (36)	163 (19) 162 (6) 161 (19)	
XVI	394 (6)	352 (100)	257 (34)	161 (49)		255 (50) 193 (22) 178 (22)
XVII	560 (3)	518 (28) 476 (61)	381 (16) 380 (43)	285 (7) 284 (9)		353 (8) 259 (11) 257 (10) 163 (22) 149 (100)
XXI *(erythro)* [d]	426 (20)		331 (13) 330 (6)	236 (16)		271 (100) 193 (15) 176 (8) 175 (25)
XXII	274 (3)	232 (18)	137 (100) 136 (14)			
XXIII	274 (5)	232 (82)	137 (100) 136 (80)			
XXIV	382 (8)	340 (45)	245 (100)	149 (18)		
XXV	302 (4)	260 (32) 218 (100)	123 (38) 122 (55)			

[a] m/e=42 corresponds to CH_2CO arising from aromatic acetyl groups. [b] m/e=95 and 96 corresponds to $\cdot SO_2OCH_3$ and HSO_3OCH_3, respectively. [c] M−42−124−n×95/96 (n=0, 1, 2) [d] The *threo*-form afforded the same fragments but with different intensities.

matographic fractions were not determined since this purification procedure involved considerable losses of material. Unless otherwise stated, the acetylated sulfonic acid methyl esters were obtained as colourless amorphous substances. Many of them decomposed rapidly, even when kept at low temperature (about 2 °C).

Treatment of compound I with sulfite solutions

Table 1 summarises the results from the treatments of compound I [17] with sulfite solutions under different conditions.

The following acetylated sulfonic acid methyl esters were isolated (Table 1) and identified by NMR and mass spectrometry (Tables 2 and 3, respectively) and/or by comparison of the spectra with those of authentic samples:

erythro- and threo-*1-(4-Acetoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-3-acetoxypropane-1-sulfonic acid methyl ester* (methyl esters of *erythro* and *threo*-III-diacetate).

α-Methoxysulfonyl-β-acetoxymethyl-4-acetoxy-3-methoxystyrene (methyl ester of IV-diacetate).

1-(4-Acetoxy-3-methoxyphenyl)-ethane-1,2-disulfonic acid dimethyl ester (dimethyl ester of VII-acetate).

trans-*4-Acetoxy-3-methoxystyrene-β-sulfonic acid methyl ester* (methyl ester of *trans*-IX-acetate)

1,1-Bis(4-acetoxy-3-methoxyphenyl)ethane-2-sulfonic acid methyl ester (methyl ester of X-diacetate).

1-(4-Acetoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-disulfonic acid dimethyl ester (dimethyl ester of XIII-acetate).

1-(4-Acetoxy-3-methoxyphenyl)propane-1,2,3-trisulfonic acid trimethyl ester (trimethyl ester of XIV-acetate).

β-Methoxysulfonylmethyl-β-methoxysulfonyl-4-acetoxy-3-methoxystyrene (dimethyl ester of XVI-acetate).

1,1-Bis(4-acetoxy-3-methoxyphenyl)propane-2,3-disulfonic acid dimethyl ester (dimethyl ester of XVII-diacetate).

4-Acetoxy-3-methoxybenzylsulfonic acid methyl ester (methyl ester of XXII-acetate).

2-Acetoxy-3-methoxybenzylsulfonic acid methyl ester (methyl ester of XXIII-acetate).

4-Acetoxy-3-methoxy-1,5-di(methoxysulfonylmethyl)benzene (dimethyl ester of XXIV-acetate).

3,4-Diacetoxybenzylsulfonic acid methyl ester (methyl ester of XXV-diacetate).

Methanesulfonic acid methyl ester.

Treatment of quinonemethide II with neutral sulfite at room temperature

Quinonemethide II, dissolved in chloroform, was prepared from compound I and treated with neutral sulfite solution as described previously [3] for compound XIX. The remaining chloroform solution contained small amounts of starting material and of three other components, which were not characterised. The mixture of acetylated sulfonic acid methyl esters obtained from the aqueous solution following the usual working-up procedure contained, according to TLC (Solvent system B) the *erythro* and *threo*-forms of the methyl ester of III-diacetate, with the *erythro*-form predominant.

Treatment of the α-sulfonic acid III with sulfite at pH 9.5 (3 h)

The methyl ester of III-diacetate (*erythro*-form)[1] (500 mg) was treated with sodium sulfite solution (18 ml). The reaction product contained the *erythro* and *threo*-forms of the starting compound (approximate ratio 2:1), together with small amounts of the dimethyl ester of VII-acetate (TLC and mass spectrometry). Traces of other components were detected by TLC but not identified.

Treatment of the α-sulfonic acid XX with neutral sulfite

The sodium salt of compound XX [18] (800 mg, mixture of *erythro*- and *threo*-forms) was treated with neutral sulfite (80 ml); pH 7 before and after treatment. Preparative separation of the sulfonic acid esters using benzene-acetone (19:1) as the solvent system gave:

threo-*1-(3,4-Dimethoxyphenyl)-3-acetoxypropane-1,2-disulfonic acid dimethyl ester* (dimethyl ester of *threo*-XXI-acetate) (70 % of the isolated material); rapidly decomposing oil.

erythro-*1-(3,4-Dimethoxyphenyl)-3-acetoxypropane 1,2-disulfonic acid dimethyl ester* (dimethyl ester of *erythro* XXI-acetate) (30 % of the isolated material); recrystallized from ethanol; m.p. 119.5 – 120.0 °C.

Treatment of XVIII with neutral sulfite

Compound XVIII [6] (1 g) was treated with neutral sulfite solution (80 ml); pH 7 before and after treatment. The chloroform-ether soluble fraction (190 mg) contained guaiacol and catechol. Preparative separation of the sulfonic acid esters using solvent system A gave: methyl ester of *trans*-IX-acetate (small amount), methanesulfonic acid methyl ester, 1-(4-acetoxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)ethane-1-sulfonic acid methyl ester (methyl ester of VI-acetate) [2] (20 % of the isolated material), and dimethyl ester of VII-acetate (70 % of the isolated material). The last-mentioned compound was identical with the product obtained from compound I.

Treatment of XVIII with sulfite at pH 9

Compound XVIII (1 g) was treated with the sulfite solution (80 ml) (pH 9 before and after treatment). The chloroform-ether soluble fraction (240 mg) contained guaiacol and catechol. Preparative separation of the sulfonic acid esters using solvent system A gave: methyl ester of *trans*-IX-acetate (43 % of the isolated material), identical with the corresponding product obtained from I, methanesulfonic acid methyl ester, dimethyl ester of VII-acetate (43 % of the isolated material) and methyl ester of X-diacetate (14 % of the isolated material), identical with the corresponding product obtained from I.

Treatment of the β-guaiacoxystyrene derivative V with neutral sulfite

The sodium salt of compound V [6] (330 mg) was treated with neutral sulfite solution (18 ml). Catechol, guaiacol, and starting material were found in the chloroform-ether soluble fraction [TLC, chloroform-acetone (9:1)]. Paper chromatography of the aqueous solution [Whatman No. 3 paper; ethanol-conc. ammonia-water (12:1:3)] showed the presence of methanesulfonic acid and of the sulfonic acids VII and IX (co-chromatography of authentic samples).

Treatment of the disulfonic acid VII with guaiacol at pH 10

The disulphonic acid (VII) [3] (sodium salt, 50 mg) was treated with an excess of guaiacol in borate buffer (pH 10). Working-up of the aqueous solution gave the methyl ester of X-diacetate, identical with the product obtained from I and from XVIII (see Scheme 1).

Treatment of guaiacol with neutral sulfite (pH 8) in the presence of formaldehyde

Guaiacol (1.0 g, 8.07 mmol) was treated with a solution of neutral sulfite (80 ml, 40 mmol) and a 30 % formalin solution (3 ml) (pH 8 before and after treatment). The chloroform-ether extract (250 mg) contained starting material and catechol. Chromatography of the sulfonic acid esters (1.8 g) (solvent system A) gave three major components: 2-acetoxy-3-methoxybenzylsulfonic acid methyl ester (methyl ester of XXIII-acetate) (200 mg), recrystallized from ethyl acetate-petroleum ether (60 – 70 °C), m.p. 93 – 94 °C, 4-acetoxy-3-methoxy-benzylsulfonic acid methyl ester (methyl ester of XXII-acetate) (700 mg), recrystallized from ethyl acetate-petroleum ether (60 – 70 °C), m.p. 98 – 99 °C, 4-acetoxy-3-methoxy-1,5-bis (methoxysulfonylmethyl)-benzene (dimethyl ester of XXIV-acetate) (300 mg), recrystallized from ethyl acetate-petroleum ether (60 – 70 °C), m.p. 147.5 – 148.0 °C.

The tree acetoxy-methoxybenzylsulfonic acid methyl esters were identical with the corresponding compounds obtained from I.

Two further components, were present in smaller amounts, but could not be isolated in pure form. Mass spectra indicated that they are methyl esters of diacetoxybenzylsulfonic acids, formed by sulfitolytic demethylation of the appropriate methoxy compounds (XXII and XXIII), or by condensation of catechol with hydroxymethanesulfonic acid.

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Steroid Oxidoreductase Activity of Alcohol Dehydrogenases from Horse, Rat, and Human Liver

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Alcohol dehydrogenase from horse (isoenzyme SS and ES, but not EE), rat and human liver were found to catalyze the NAD-dependent oxidation of 3β-hydroxy groups in 5α- and 5β-steroids of the C_{19}, C_{21}, and C_{24} series. The enzymes from horse and rat liver were more active on 5β- than on 5α-steroids. This difference was most marked with the enzyme from rat liver, especially with 3β-hydroxyandrostan-17-ones and 3β-hydroxypregnan-20-ones as substrates. The K_m of isoenzyme ES from horse liver was lower for 3β-hydroxy-5α-cholanoic acid (0.4 μM) than for 3β-hydroxy-5β-cholanoic acid (0.9 μM). 3α-Hydroxysteroids were not substrates for the enzymes from horse and rat liver. Human liver alcohol dehydrogenase had low affinity for 3β-hydroxy-5α (and 5β)-cholanoic acids, but oxidation could be clearly demonstrated by gas chromatographic analysis of the products.

Alcohol dehydrogenase from horse,[1,2] rat,[3,4] and human [5] liver are known to catalyze the NAD-dependent oxidation of 3β-hydroxy-5β-steroids. In the original paper by Ungar,[1] the enzyme from horse liver was reported to be active also on 3β-hydroxy-5α-steroids, but in a more recent study [3] no activity was observed with 3β-hydroxy-5α-androstan-17-one as substrate. The latter study was carried out using an enzyme preparation mainly consisting of isoenzymes EE and ES [6] (at the time called $LADH_E$ and $LADH_S$), and earlier studies on steroid activity of horse liver alcohol dehydrogenase were made without separation of isoenzymes. Since the different isoenzymes (ES and SS) might differ with respect to specificity and kinetic constants, it was considered of interest to extend the previous studies to pure isoenzymes.

The enzymes from rat and human liver have only been tested with 5β-steroids: 3β-hydroxy-5β-cholanoate and 17β-hydroxy-5β-androstan-3-one. The steroid metabolism in human is markedly different from that in rats (see Ref. 7). The effects of ethanol consumption and alcohol dehydrogenase inhibitors on steroid metabolism are incompletely known. For an evaluation of the *in vivo* significance of the steroid activity of liver alcohol dehydrogenase, it should therefore be of value to have a more complete knowledge of species differences in specificity and kinetic constants.

EXPERIMENTAL PROCEDURES

Materials

Steroids. 3β-Hydroxy-5α (and 5β)-androstan-17-one and 17β-hydroxy-5β-androstan-3-one were from Sigma Chemical Company (St. Louis, Mo.). 3α-Hydroxy-5α (and 5β)-androstan-17-one, 3β-hydroxy-5β-pregnan-20-one and 3β-hydroxy-5α-cholanoic acid were obtained from Ikapharm (Ramat-Gan, Israel). 3α-Hydroxy-5β-pregnan-20-one was from Mann Research Laboratories (New York, N.Y.) and purified 3β-hydroxy-5α-pregnan-20-one was a gift from Dr. H. Eriksson. 3α-Hydroxy-5β-cholanoic acid was obtained from Calbiochem (Los Angeles, Calif.) and was recrystallized and served as starting material for synthesis of 3β-hydroxy-5β-cholanoic acid.[8] The purity of the steroids was checked by gas chromatography-mass spectrometry. The 3β-hydroxysteroids were purified for use as substrates in experiments with human LADH by chromatography of bile acid methyl esters on alumina columns, [8] and of neutral steroids on hydroxyalkoxypropyl Sephadex [9] (Lipidex®-5000, Packard, Downers Grove, Ill.)

Table 1. Rate of formation of NADH in incubations of horse liver alcohol dehydrogenase, isoenzyme SS [6] with 3β-hydroxy-5β-cholanoic acid as substrate together with various steroid solubilizers and 0.5 mM NAD^+.

Concentrations in incubation mixture					Rate of
Enzyme μN	Substrate μM	Tween 80 μg/ml	Na glyco-deoxycholate mM	Acetone mM	NADH formation μM/s
0.72	0	0	0	0	17
0.72	0	0.5	0	0	67
0.72	0	0	0.44	0	50
0.72	0	0	0	450	317
0	0	0	0	450	317
0.72	87	0	0	0	1620
0.72	87	0.5	0	0	1820
0.72	87	5	0	0	2640
0.72	87	0	0.44	0	1690
0.72	87	0	0.88	0	1590
0.72	87	0	0	90	1950
0.72	87	0	0	450	2400
0.01	0	0	0	0	2.0[a]
0.01	0	0.5	0	0	3.8[a]
0.01[b]	0	0	0	0	2.3[a]
0.01[b]	0	0.5	0	0	6.5[a]

[a] Measurements carried out in the fluorimeter, otherwise in the spectrophotometer. [b] Isoenzyme ES.[6]

in the solvent system hexane/chloroform, 9:1 (v/v). Several other steroids were obtained from the reference collection of the laboratory.

Sodium glycodeoxycholate was a gift from Dr. A. Norman. Tween 80 (polyoxyethylenesorbitane monooleate) was purchased from Kebo (Stockholm, Sweden). NAD^+, NADH, and $NADP^+$ were obtained from Sigma Chemical Company.

Enzymes. Alcohol dehydrogenase was isolated from livers of horse (Åkeson, Å. and Lundqvist, G., to be published), rat,[4] and human [5] as described elsewhere. The enzyme preparation from human liver contained all isoenzymes.

Methods

Incubations and reactions. The reactions were carried out in 1 cm cuvettes at 23.5 °C in a total volume of 3.0 ml using NAD^+ or $NADP^+$, 0.5 mM in glycine buffer, pH 10.0 and ionic strength 0.1. In some cases the incubations were carried out using NADH, 0.15 mM in phosphate buffer, pH 7.0 and ionic strength 0.1. Ethanol was used in a final concentration of 8.6 mM. Bile acid solutions, 200 – 400 μM in 0.1 M NaOH, were added to give the desired final concentration. All neutral steroids were dissolved in acetone, 1 mg/ml. Appropriate volumes of this stock solution were mixed with a solution of Tween 80 in acetone, and evaporated to give an oily residue. This was dissolved in buffer and added to the incubation mixture to give a final concentration of Tween 80 of 0.5 μg/ml. Enzyme was added in 5 – 20 μl of 0.05 M Tris-HCl buffer, pH 8.6. Measurements were made on a Beckman DK2 recording spectrophotometer at 340 nm, and a recording fluorimeter [10] modified to yield enhanced stability and sensitivity.[11] Rates of reaction were calculated as mol of NADH formed per mol of active sites per second. K_m and $\bar{V}$ values were determined from Lineweaver-Burk plots.

Steroid analysis by gas chromatography and gas chromatography-mass spectrometry. The reactions were stopped 5 – 20 min after the addition of enzyme by addition of 0.2 ml 3 M H_2SO_4, and the steroids were extracted with 3 × 5 ml of ethyl acetate (methylene chloride in experiments with human LADH). The organic phases were pooled and washed until neutral with 1 ml portions of water. The solvents were evaporated, and bile acids were methylated by diazomethane in ether/methanol, 9:1 (v/v). The steroids were converted into trimethylsilyl ethers [12] or *O*-methyloxime trimethylsilyl derivatives [13] which were analyzed by gas chromatography on a 3 % QF-1 column.

Gas chromatography-mass spectrometry was carried out using an LKB 9000 gas chromatography-mass spectrometry instrument (LKB-Produkter AB, Bromma, Sweden), equipped with a 1.5 % SE-30 or a 3 % QF-1 column. The electron energy was 22.5 eV and the ion source temperature was 290 °C.

RESULTS

Horse liver alcohol dehydrogenase

Studies on steroid solubilizers for use in the enzymatic experiments. The reaction rate was constant at least during the first 5 min after addition of enzyme, and initial velocities were measured in the spectrophotometer (Table 1). Without solubilizers or substrate, the rate of formation of NADH was insignificant, and addition of Tween 80 or sodium glycodeoxycholate resulted in very slow rates of NADH formation. Addition of acetone resulted in much higher rates of NADH formation, and this reaction was not dependent on enzyme. None of the solubilizers tested inhibited the oxidation of 3β-hydroxy-5β-cholanoic acid. Acetone and sodium glycodeoxycholate were not sufficiently effective as solubilizers for the neutral steroids. The reaction of NAD^+ and acetone disturbed the measurements in the fluorimeter, as did production of NADH at concentrations of Tween 80 above 0.5 μg/ml. Thus, Tween 80 at a concentration of 0.5 μg/ml was chosen as solubilizer for the neutral steroids.

Activity of horse liver alcohol dehydrogenase isoenzymes with steroids hydroxylated in various positions. Neutral steroids were solubilized in Tween 80, and bile acids were dissolved in sodium hydroxide. Using the spectrophotometer, the steroids were tested at a concentration of 30 μM. Oxidation of the following steroids was not catalyzed by the SS isoenzyme (1.2 μN): 3α,6α-dihydroxy-5β-cholanoate, 3α,7β-dihydroxy-5β-cholanoate, 20β-hydroxy-4-pregnen-3-one, 6β-hydroxy-4-pregnene-3,20-dione, 11β-hydroxy-4-pregnene-3,20-dione, 6β,11α-dihydroxy-4-pregnene-3,20-dione, 20α-hydroxy-4-pregnen-3-one, and 15α-hydroxy-4-androstene-3,17-dione. Under the same conditions, oxidation of the following steroids was catalyzed by the SS isoenzyme (but not by the EE isoenzyme, 0.8 μN): 3β,12β-dihydroxy-5α-androstan-17-one and 5α-androstane-3β,17β-diol. The products were analyzed by gas chromatography-mass spectrometry of trimethylsilyl ethers and *O*-methyloxime-trimethylsilyl ethers on an SE-30 column, and were identified as an androstanoldione and an androstanolone, respectively. Neither the SS isoenzyme (0.1 μN) nor the EE isoenzyme (0.08 μN) catalyzed the oxidation of the following steroids: 17α,21-dihydroxy-4-pregnene-3,11,20-trione, 17α,19-dihydroxy-4,6-preg-

Table 2. Rate of formation of NADH in incubations of horse liver alcohol dehydrogenase isoenzymes (0.01 μN) with 3-hydroxysteroids. The NAD^+ concentration was 0.5 mM.

Isoenzyme	Substrate [a]	Substrate concentration μM	Rate of NADH formation per active site s^{-1}
EE	5βA-3β-ol-17-one	5.75	0
EE	5αA-3β-ol-17-one	5.75	0
EE	5βP-3β-ol-20-one	5.25	0
EE	5αB-3β-ol	1.77	0
ES	5βA-3β-ol-17-one	5.75	0.88
ES	5αA-3β-ol-17-one	5.75	0.49
ES	5βA-3α-ol-17-one	5.75	0.05[b]
ES	5βP-3β-ol-20-one	5.25	0.53
ES	5αP-3β-ol-20-one	5.25	0.34
ES	5βB-3β-ol	8.80	0.22
ES	5αB-3β-ol	8.85	0.15
ES	5βB-3α-ol	8.80	0.01[b]
SS	5βA-3β-ol-17-one	5.75	1.40
SS	5αA-3β-ol-17-one	5.75	0.74
SS	5βP-3β-ol-20-one	5.25	0.98
SS	5αP-3β-ol-20-one	5.25	0.42
SS	5βB-3β-ol	8.79	0.37
SS	5αB-3β-ol	8.85	0.22

[a] Abbreviations: A=androstane, P=pregnane, B=cholan-24-oic acid; greek letters denote configuration of hydroxyl groups. [b] Values not higher than corresponding blank values (Table 1).

nadiene-3,20-dione 17-acetate, and 19-hydroxy-18-nor-4-androstene-3,17-dione. Reduction of 3α,7α-dihydroxy-12-oxo-5β-cholanoate and 3β-hydroxy-5-androsten-17-one was not catalyzed by the SS isoenzyme (1.2 μN).

Stereospecificity of horse liver alcohol dehydrogenase isoenzymes for the configuration at C-3 and C-5 in 3-hydroxysteroids. The following experiments were carried out using the fluorimeter. The fluorescence increase in the absence of substrate was usually small (Table 1) and was not substracted from the values obtained (Table 2). Isoenzyme EE was not active on the steroids studied, which had a 3β,5β or a 3β,5α configuration. Isoenzymes ES and SS both catalyzed the oxidation of steroids having a 3β,5β or a 3β,5α configuration, but not of steroids having a 3α,5β configuration. The ratio between the activities on steroids with these configurations were approximately the same (3β,5β/3β,5α: 1.8 ± 0.3) for both isoenzymes and with the 3β-hydroxyandrostanes, 3β-hydroxypregnanes, and 3β-hydroxycholanoates as substrates. The ratio between the activities of the SS and ES isoenzymes was about the same for the steroids tested (1.6 ± 0.2). The activities were highest with 3β-hydroxyandrostanes and lowest with 3β-hydroxycholanoates.

3α-Hydroxy-5β-cholanoate (44 or 88 μM) inhibited the oxidation of 3β-hydroxy-5β-cholanoate (3.51 μM) completely.

Determinations of K_m *and* V *of isoenzyme ES and SS for 3β-hydroxy-5α(and 5β)-cholanoate.* The K_m values were at 2 μM or below with the two isoenzymes, and at least with isoenzyme ES it was lower with the 3β,5α than with the 3β,5β epimer (Table 3). The *V* values were higher with the 3β,5β epimer than with the 3β,5α epimer. With the ES isoenzyme, substrate inhibition was observed at concentrations of both epimers above 10 μM.

Table 3. Kinetic parameters from Lineweaver-Burk plots for activities of horse liver alcohol dehydrogenase isoenzymes with 3β-hydroxysteroids.

Isoenzyme [a]	Substrate [b]	K_m μM	*V* s^{-1}
ES	ethanol	1700	2.17
ES	5βB-3β-ol	0.9	0.25
ES	5αB-3β-ol	0.4	0.18
SS	ethanol	8000	3.67
SS	5βB-3β-ol	< 2	0.42
SS	5αB-3β-ol	< 0.4	0.23

[b] Abbreviations see Table 2.

Table 4. Rate of formation of NADH in incubations of rat liver alcohol dehydrogenase (0.01 μN) with 3-hydroxysteroids. The NAD^+ concentration was 0.5 mM.

Substrate [b]	Substrate concentration μM	Rate of NADH formation per active site s^{-1}
5βA-3β-ol-17-one	5.75	0.438
5αA-3β-ol-17-one	5.75	0.060
5βA-3α-ol-17-one	5.75	0.047
5αA-3α-ol-17-one	5.75	0.027
5βP-3β-ol-20-one	5.25	0.480
5αP-3β-ol-20-one	5.25	0.050
5βP-3α-ol-20-one	5.25	0.028
5βB-3β-ol	7.4	0.820
5αB-3β-ol	7.4	0.180
5βB-3α-ol	8.9	0.038
—[c]	0.0	0.042
—[d]	0.0	0.022

[b] Abbreviations see Table 2. [c] Blank with Tween 80. [d] Blank without Tween 80.

Rat liver alcohol dehydrogenase

Studies on the stereospecificity for configuration at C-3 and C-5 were carried out using the fluorimeter. The results are shown in Table 4. The enzyme did not catalyze the oxidation of 3α-hydroxysteroids. The activity on 3β,5β-steroids was higher than on 3β,5α-steroids, the difference being most marked with the neutral steroids. In order to confirm that 3β-hydroxy-5α-androstan-17-one and 3β-hydroxy-5α-pregnan-20-one were oxidized, these steroids were incubated for 2 h at concentrations of 12 μM and 31 μM, respectively, using 0.09 μN enzyme and 0.5 mM NAD^+. Gas-liquid chromatography showed that 70 % of the 3β-hydroxy-5α-androstan-17-one and 30 % of the 3β-hydroxy-5α-pregnane-20-one were oxidized.

The reverse reaction was tested with 17β-hydroxy-5α-androstan-3-one (12.6 μM) and 17β-hydroxy-5β-androstan-3-one (12.3 μM), dissolved in methanol (10 μl). The reaction was

followed in the spectrophotometer, and it was observed that the activity was higher with the 3β,5β epimer (rate of NADH decrease per active site: 0.75 s^{-1}) than with the 3β,5α epimer (rate of NADH decrease per active site: less than 0.05 s^{-1}).

Human liver alcohol dehydrogenase

Since it was found that the steroid activity of human liver alcohol dehydrogenase was much lower than that of horse and rat liver alcohol dehydrogenases, it was difficult to make activity measurements with the fluorimeter. However, formation rates of NADH above blank values with Tween 80 were observed with 3β-hydroxy-5β-androstan-17-one (17.2 μM) and 3β-hydroxy-5β-pregnan-20-one (15.7 μM) with an enzyme concentration of 0.7 μN and 0.5 mM NAD^+. There was no reduction of $NADP^+$ (0.5 mM) when incubations were carried out as with NAD^+ and with the substrates listed in Table 4 at concentrations of about 15 μM. However, this lack of activity may be due to the presence of NAD^+ in the commercial $NADP^+$ preparation used.[14]

In a study of the kinetics of the oxidation of 3β-hydroxycholanoic acids by human liver alcohol dehydrogenase, a gas chromatographic method was used to measure conversions. The reaction was stopped after 10 and 20 min, and the mean conversion rate was calculated. With 3β-hydroxy-5β-cholanoic acid as substrate, K_m was about 40 μM and V was about 0.004 s^{-1}. Negative values for these parameters were obtained with 3β-hydroxy-5α-cholanoic acid as substrate. This is probably due to errors in the measurements, but the results indicate that K_m is at least as high as with 3β-hydroxy-5β-cholanoic acid.

DISCUSSION

In previous investigations,[2,3] horse liver alcohol dehydrogenase was found to catalyze the oxidation of 3β-hydroxysteroids, but hydroxyl groups in the 3α-, 7α-, 7β-, 11α-, 12α-, and 17β-positions were not oxidized. In the reverse reaction, 3-oxosteroids yielded 3β-hydroxysteroids, and no reduction was seen with oxo-groups in the 7-, 11-, and 12-positions. In the present study, purified isoenzyme SS was found to be specific for the 3β-hydroxy group and no oxidation was observed with hydroxyl groups in the 3α-, 6α-, 6β-, 7β-, 11α-, 11β-, 15α-, and 20β-positions. The reduction of oxo-groups in the 12- and 17-positions was not catalyzed by the SS isoenzyme.

Since horse liver alcohol dehydrogenase has been observed to catalyze the oxidation of primary hydroxyl groups, some neutral steroids with hydroxyls in the 19- and 21-positions were tried as substrates, but neither the SS nor the EE isoenzyme was active.

Both isoenzyme ES and SS, but not EE, catalyzed the oxidation of 3β-hydroxygroups in 5α- and 5β-steroids of the C_{19}, C_{21}, and C_{24} series. This result differs from those previously obtained,[3] where only steroids of the 5β-series were found to be substrates. The reason for this discrepancy is not known, but it appears likely that it is due to the use in the previous study of a preparation containing only small amounts of isoenzymes active on steroids. With both isoenzymes, V was higher with 5β-cholanoates. At least with isoenzyme ES, K_m was higher with 5β-cholanoates, and these results indicate that at low concentrations of steroids, such as may be present *in vivo*, this enzyme is more active on the 5α-steroids. The ratio between the activities towards C_{19}, C_{21}, and C_{24} steroids and towards 5α- and 5β-steroids were the same with both isoenzymes, indicating that the E subunit does not change the steroid activity of the S subunit.

Alcohol dehydrogenase from rat liver was also found to catalyze the oxidation of 3β-hydroxy groups in 5α- and 5β-steroids of the C_{19}, C_{21}, and C_{24} series, and the activity was higher with 5β-steroids. The difference in activity towards 5β- and 5α-steroids was more marked with the rat enzyme and this difference was also observed in the reverse reaction.

Alcohol dehydrogenase from human liver had a much lower steroid activity than those from horse and rat liver. This was mainly due to high K_m values, both with 3β-hydroxy-5α-cholanoate and with 3β-hydroxy-5β-cholanoate. Thus, alcohol dehydrogenase may be of minor significance in human steroid metabolism.

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Degradation of Methyl α-D-*gluco*-Hexodialdopyranoside-(1,5) in Aqueous Solution

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The degradation of methyl α-D-*gluco*-hexodialdopyranoside-(1,5) (I) *via* β-elimination to methyl 4-deoxy-β-L-*threo*-hex-4-enodialdopyranoside-(1,5) (II) at various temperatures in aqueous alkali and the further degradation of II in the pH range 1.2 – 12.5 was studied. At low pH values, 2,5-furan-dicarboxaldehyde (III) and 4-*H*-pyran-2-carboxaldehyde-4-one (IV) are formed. The relevance of the result to the yellowing of oxidized cellulose is discussed.

Cellulose products are degraded under the influence of air and light. The degradation, particularly of the hemicellulose polysaccharides, causes yellowing which is accelerated at elevated temperatures. In a previous investigation in this laboratory concerning the formation of aromatic compounds from carbohydrates, reductic acid, furan and benzene derivatives were isolated after treatment of pentoses and uronic acids in slightly acidic solutions.[1] These compounds are precursors of chromophoric products. In another investigation it was shown that the presence of even a small number of 6-aldehydo groups in cellulose resulted in rapid depolymerisation and extensive colour formation during dry or humid aging.[2] Introduction of 6-aldehydo groups into cellulose material is a possible reaction among other oxidative modifications from bleaching and/or through oxidation during storage and use of the material.

Methyl 6-aldehydo-D-glycopyranosides and methyl 6,6′-dialdehydo-β-D-cellobioside have been used as model compounds for celluloses specifically oxidized at the 6-position.[3–5] When these substances are treated at different pH values, elimination at C-4 is the primary reaction from pH 4 and upwards. This leads to 4,5-unsaturated glycosides. The rate of this degradation becomes more important at increasing pH values (*cf.* uronate degradation [6]). At low pH values pure acid hydrolysis is the main reaction.

In our continued efforts at obtaining an understanding of the processes responsible for the yellowing of cellulose we now report on the degradation of methyl α-D-*gluco*-hexodialdopyranoside-(1,5) (I). The degradation of isomers of hexodialdopyranoside-(1,5) at various pH values has recently been studied.[3] Elimination, leading to hex-4-eno-dialdohexopyranosides is the predominant reaction above pH 4.

The rate of degradation of the title compound (I) in 0.1 % aqueous potassium hydroxide (pH 12.5) to produce methyl 4-deoxy-β-L-*threo*-hex-4-enodialdopyranoside-(1,5) (II), at 50, 70, and 90 °C was studied by following the change in intensity of the absorption band at 253 nm for II and also by TLC examination of the reaction mixture. The results are shown in Fig. 1. At the higher temperature a maximum yield of II was obtained after 10 min. Further treatment caused degradation of II. At 50 °C the degradation of I was slow, significant amounts of starting material remaining after 70 min. Treatment of I at 70 °C, however, left no starting material after 40 min, and II was obtained in a 90 % yield. The substance II was stable in aqueous solution at 4 °C for several weeks. The formation of II was found to follow

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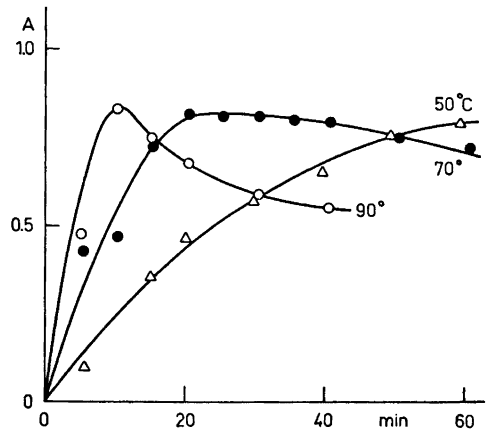


Fig. 1. Variation in UV absorbance at 253 nm during treatment of methyl α-D-*gluco*-hexodialdopyranoside-(1,5) (I) in aqueous alkaline solution (pH 12.5) a various temperatures.

first-order kinetics. The rate constants were found to be 754×10^{-6} s^{-1} at 50 °C and 3270×10^{-6} s^{-1} at 70 °C at pH 12.5. Although compound I was only present in concentrations of 0.3 mM in these experiments, a distinct yellowing was apparent, indicating a high molar extinction for the coloured products. This strongly emphasizes the importance of 6-aldehydoglucose units in the yellowing of oxidized cellulose materials.

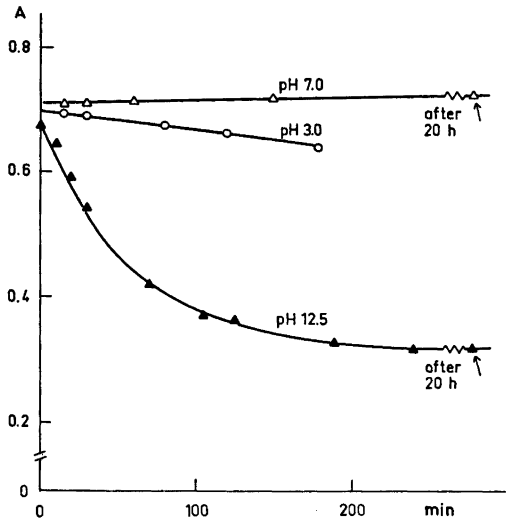


Fig. 2. Decrease in UV absorbance at 253 nm during treatment of methyl 4-deoxy-β-L-*threo*-hex-4-enodialdopyranoside(1,5) (II) at different pH-values at 80 °C.

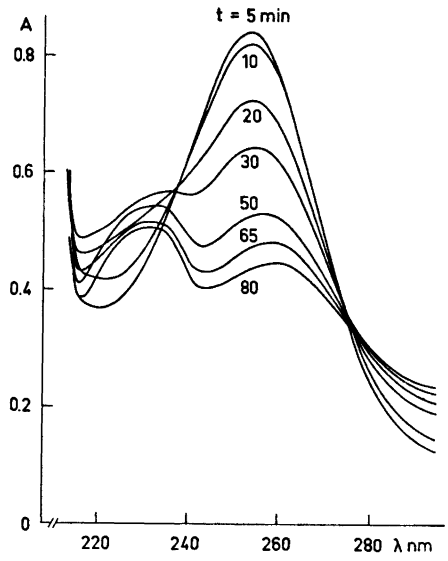


Fig. 3. UV spectra for the degradation of methyl 4-deoxy-β-L-*threo*-hex-4-enodialdopyranoside-(1,5) (II) in aqueous alkaline solution (pH 12.5) at 80 °C.

The rate of degradation of II at pH 3.0, 7.0, and 12.5 and 80 °C was followed by observing the decrease of the absorption band at λ_{max} 253 nm (Fig. 2). The processes followed first-order kinetics and gave rate constants of 13.2×10^{-6}, ~0 and 326×10^{-6} s^{-1}, respectively (compare the rate of I→II at 70 °C 3270×10^{-6} s^{-1}). A yellow colour appeared at an early stage. The UV spectra for the degradation of II at the higher pH value are shown in Fig. 3. These results show that the unsaturated 6-aldehydoglycoside II has a maximum stability at pH 7 or slightly below and is degraded much more slowly at alkaline pH than is the parent substance I.

In previous investigations [3,4] it was found that the elimination reaction of methyl α-D-*gluco*-hexodialdopyranoside-(1,5) (I) and other 6-aldehydo-glycopyranosides operated already at acid pH values forming the corresponding unsaturated glycosides. On the other hand reductic acid, 2-furaldehyde, chromones, and cathecols, all active precursors of chromophoric products, were isolated after acid treatment of pentoses and uronic acids.[1] It was therefore of interest to examine the degradation products from II after acid treatment (at pH 12.5 no low-molecular products were detectable).

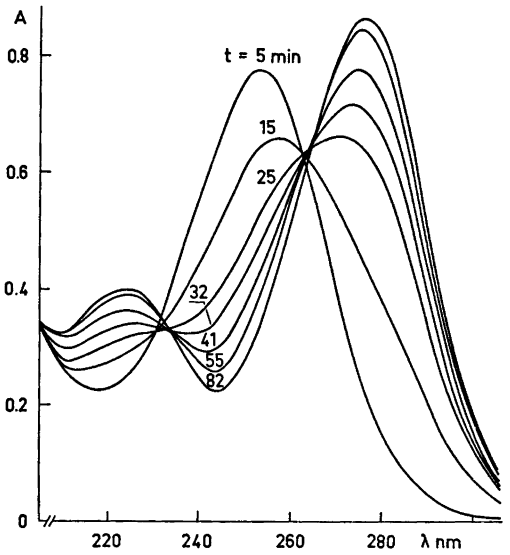
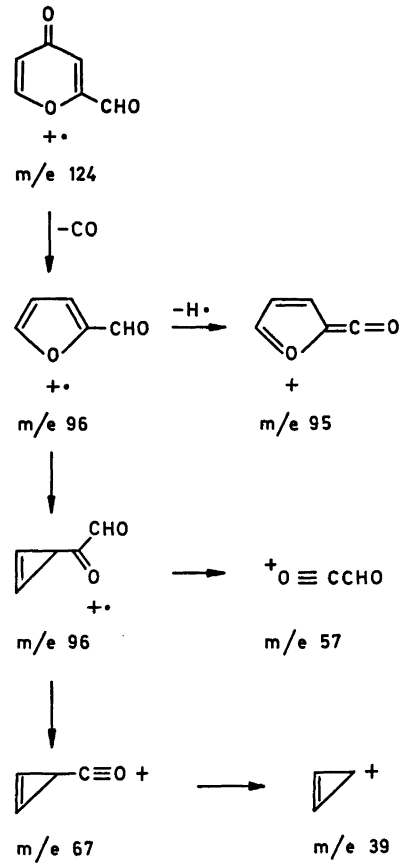


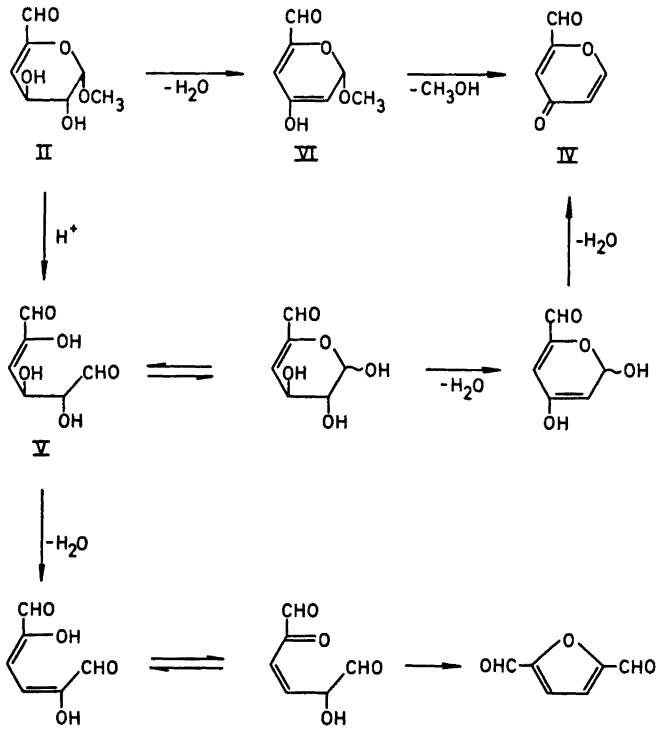
Fig. 4. UV spectra for the degradation of methyl 4-deoxy-β-L-*threo*-hex-4-enodialdopyranoside-(1,5) (II) in aqueous hydrochloric acid (pH 1.2) at 80 °C.



Scheme 1.

For the purpose of studying the formation of chromophoric compounds, II was treated at pH 1.2 and 80 °C. The change with time of the UV spectrum for this process is shown in Fig. 4. The degradation of II obviously is rapid, but kinetic studies using the absorption band λ_{max} 253 nm were not possible. The degradation was followed by TLC which showed the presence of one minor and two more predominant reaction products (III and IV); the two latter were carbonyl compounds, as indicated by strong positive reaction with the spray reagents 2,4-dinitrophenylhydrazine and anisidine hydrochloride. The two compounds were obtained by TLC on the mixture produced by the acidic degradation of II. III was obtained in crystalline form in a yield of 0.4 % and was found to be indistinguishable from an authentic sample of 2,5-furan-dicarboxaldehyde [7] (m.p., mixed m.p., NMR). IV was obtained as an unstable syrup in 1 % yield. By comparison of the spectrum to that of 4-*H*-pyran-4-one-2-carboxylic acid methyl ester [8] (methyl comanate) and by analysis of its mass fragmentation, IV was identified as 4-*H*-pyran-2-carboxaldehyde-4-one which, to the best of our knowledge, is a new substance. A possible rationalization of its fragmentation on MS is outlined in Scheme 1; the MS is analogous to spectra of γ-pyrones reported by Nakata and Tatematsu.[9] Possible pathways for the formation of III and IV from the unsaturated aldehyde II are outlined in Scheme 2.

The formation of III may pass *via* hydrolysis of the glycosidic linkage to the unsaturated dialdose V which is then reacting in a similar way as the formation of 5-(hydroxymethyl)-2-furaldehyde from hexoses [10] (Scheme 2). IV may also be formed from the same acyclic intermediate (V), but a route without ring opening *via* VI is also possible. The latter route is supported by recent results on acid treatment of the 2,3-dimethyl ether of II which yielded III and unchanged dimethyl ether of II.[11,12] It was found that the rate of acid hydrolysis of



Scheme 2.

the glycoside II was of the same order as the rate of degradation of II to III.

The above results indicate that the compounds III and IV are possible precursors of chromophoric products in the acid degradation of celluloses containing aldehydo groups in the 6-position of the anhydroglucose units.

EXPERIMENTAL

Melting points are corrected. Concentrations were performed at reduced pressure below 40 °C unless otherwise stated.

TLC was performed on silica gel HF_{254} (Merck) plates with (a) water-saturated butanone, (b) ethyl acetate-methanol 4:1, and (c) ethyl acetate-cyclohexane 1:1. For preparative TLC 0.5 mm thick layers on 20 × 20 cm plates were used. Zones were detected with UV light. Standard spray reagents were used for sugars, carbonyl compounds and phenols (or enols), namely (a) *p*-anisidine hydrochloride, (b) resorcinol-hydrochloric acid, (c) silver nitrate-sodium hydroxide, (d) 2,4-dinitrophenyl hydrazine, (e) diazotized sulfanilic acid, and (g) ferric chloride. Spots on TLC plates were also studied in UV light (254 nm and 366 nm) before spraying. Products that were unreactive towards the above reagents were detected with 50 % aqueous sulfuric acid (and heating) or with iodine vapour.

UV measurements were carried out on Beckman DK 2 and Zeiss PMQ II spectrometers. IR spectra were obtained with a Perkin-Elmer 337 spectrometer. NMR measurements were carried out with a Perkin-Elmer R-12 60 MHz spectrometer using tetramethylsilane as an internal reference. Mass spectrometry was performed on a Perkin-Elmer 270 spectrometer with ionisation voltage 70 eV and ion current 80 μA.

Kinetic experiments. A sample of I or II (50 mg) was dissolved in water to 100 ml. 1 ml of this solution was added to aqueous buffer solution (50 ml). Experiments were conducted in 0.1 mol/l aqueous buffers: pH 1.2, hydrochloric acid; pH 3.0, potassium formate; pH 7, potassium phosphate, and pH 12.5, potassium hydroxide. The solutions also contained 0.1 mol/l potassium chloride. The solutions were kept in stoppered glass flasks placed in a constant temperature bath of 50, 70, 80 or 90 °C. At time intervals, aliquots (4 ml) were removed, cooled and the absorbance at λ_{max} 253 nm was measured. Plots of absorbance as a function of time are shown in Figs. 1 and 2. The reactions were studied over three half-

lifes in each case. The reaction spectra for degradation of II at 80 °C are shown in Figs. 3 and 4 for pH 12.5 and pH 1.2, respectively. The pseudo-first-order rate constants[13] were calculated from plots of log $[(A_\infty - A_0)/(A_\infty - A_t)]$ *versus* time according to eqn. (1).

$$k = \frac{2.303}{t_2 - t_1}\left[\log\frac{A_\infty - A_0}{A_\infty - A_{t_2}} - \log\frac{A_\infty - A_0}{A_\infty - A_{t_1}}\right] \quad (1)$$

*Methyl 4-deoxy-β-*L*-*threo*-hex-4-enodialdo-pyranoside-(1,5) (II).* Methyl α-D-*gluco*-hexodialdopyranoside-(1,5)[14] (I) (7.0 g) was dissolved in 0.05 M potassium hydroxide (700 ml). The solution was heated at 70 °C for 40 min, cooled in ice-water and neutralized to pH 6 with Dowex 50 (H^+). The solution was concentrated below 40 °C and stored at 4 °C. TLC (solvent a) revealed small amounts of polymeric substance together with II (R_F 0.65), and no other reaction products were detected. The yield obtained by preparative TLC was *ca.* 90 %. The optical rotation and UV data were in accordance with those of an authentic sample.[3] Samples from the aqueous solution of II were used for the kinetic experiments without prior evaporation of the solvent as II was unstable in the solid state.

2,5-Furan-dicarboxaldehyde (III)[7] *and 4-H-pyran-2-carboxaldehyde-4-one (IV) from acid degradation of II.* A sample (210 ml) of the stock solution containing II (3.5 g) was acidified with 1 M sulfuric acid to pH 1.0. The solution was kept at 80 °C for 100 min. At time intervals, aliquots (4 ml) were removed, diluted with water and the UV absorbtion spectra were recorded. The reaction spectrum was similar to that obtained at pH 1.2 (Fig. 4).

TLC analysis showed that almost all starting material, R_F 0.62 (solvent b), had reacted and two new substances, R_F 0.51 (III) and R_F 0.75 (IV), had been formed. In solvent c, however, three new compounds with R_F values 0.30 (III), 0.43 (IV), and 0.62 (minor), respectively, appeared (together with polymeric material).

All of these three compounds were visible in short wave UV light on the TLC plates. The first component with R_F 0.62 gave a faint blue colour with *p*-anisidine spray followed by sulfuric acid spray (after 1 – 2 days) and was not further characterized. III gave a strong red colour whereas IV gave a yellow colour with these spray reagents.

Extraction of the reaction mixture with methylene chloride and evaporation of the organic phase yielded a syrup (200 mg). Compounds III and IV were isolated after separation by preparative TLC with methylene chloride. The plates were developed three times in the solvent. Elution of the preparative plates with methylene chloride gave crystalline III (11 mg, 0.44 %), m.p. 108 – 110 °C. Cooper and Nuttall[7] give m.p. 109.5 – 110 °C. III had λ_{max} (EtOH) 281 nm, ε 11 550. NMR ($CDCl_3$): δ 7.25 (2 H, s, H-3 and H-4), 9.85 (2 H, s, CHO). The chromatographical and NMR data of III were identical with those of an authentic sample of 2,5-furan-dicarboxaldehyde.

IV was obtained as a viscous syrup (30 mg, 1.2 %). By storing at room temperature it was degraded to coloured polymeric material and a small amount of a compound with higher R_F value. This degradation product was visible in short wave UV light but did not react with *p*-anisidine spray. After a week at room temperature IV was completely degraded. No attemps were made to isolate the degradation products. IV showed the following spectra data $\lambda_{max}(CH_2Cl_2)$ 272 nm, ε 11 100; ν_{max} (KBr) 1705 cm^{-1} (CHO) and 1620 cm^{-1} (γ-pyrone C=O); NMR ($CDCl_3$): δ 6.62 (1 H, dd, $J_{3,5}$ 2.7 Hz, $J_{5,6}$ 5.6 Hz, H-5), 7.30 (1 H, broad s, H-3), 7.72 (1 H, broad s, H-6) and 9.68 (1 H, s, CHO). These NMR data are similar to those given by Elvidge and Stevens[8] for 4-*H*-pyran-4-one-2-carboxylic acid methyl ester (methyl comanate). Mass spectrum: *m/e* 39 (66 %), 57(6), 67(14), 95(95), 96(100), 124(4,M^+), as significant fragments of a γ-pyrone structure.[9] These data identified IV as 4-*H*-pyran-2-carboxaldehyde-4-one.

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N-Quaternary Compounds. Part XLI.* Bromination Studies of 2-Pyridylthiovinyl Derivatives

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α-Carbonyl-β-2-pyridylthiovinyl derivatives have been brominated. The preferential reaction path depends on the nature of the pyridine 6-substituent and on the carbonyl group ($CONR^1R^2$, CO_2Et and COMe). Cyclisation to dihydro- and thiazolo[3,2-*a*]pyridinium derivatives constitutes a facile method for the synthesis of the latter; alternative products are the dibromides and the α-bromovinyl thioethers. Stereochemistry and reaction pathways have been rationalised in terms of steric and electronic effects.

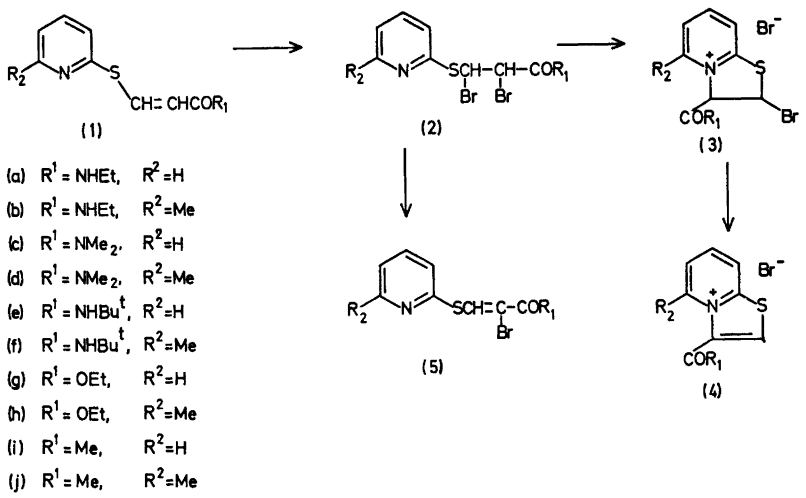
In a recent report we have described a facile method for the synthesis of the thiazolo[3,2-*a*]-pyridinium system.[2] The earlier published methods consist in acid catalysed cyclodehydration of 2-alkylthiopyridines in which the β-carbon of the *S*-side-chain is a carbonyl group.[3–5] In our new synthesis a pyrid-2-thione was added to propiolic or phenylpropiolic acid to form a Michael 1:1-adduct which on treatment with bromine in cold chloroform led to immediate precipitation of the thiazolo[3,2-*a*]pyridinium derivative. The product may be formed by initial bromine addition followed by bromide displacement in the cyclisation, or by direct cyclisation of an intermediate bromonium ion; HBr elimination finally generates the thiazolo system. In this work we report further results on the scope of this reaction by studies of steric and electronic effects. The electronic activation of the double bond was varied through the nature of the attached carbonyl group; the latter was embedded in an ester and a ketone and in amido groups (Scheme 1). Different steric requirements also reside in these groups; the steric influence of the amido group was further changed by the substitution pattern on the amido nitrogen atom. A methyl group was also introduced into the 6-position of the pyridine ring. The available vinyl thioethers had the *cis* configuration with the exception of the ketone 1*i* which was a 1:1 mixture of the *cis* and *trans* isomers and its 6-methyl homologue 1*j* which had the *trans* configuration.[6]

The reactions were run in chloroform at 20 °C; bromine was added as a 10 % solution in chloroform. Addition of bromine to the amides 1*a*, 1*c*, and 1*e*, which carry a hydrogen atom in the 6-position, immediately led to precipitation of a product identified by spectroscopy as the dihydrothiazolo derivative (*3*). A minor product left in solution has been identified as the dibromo adduct (*2*) by comparison of its spectroscopic and chromatographic properties with those of 2*d* and 2*f* discussed below.

The UV spectra of the dihydrothiazolo derivatives (*3*) are significantly different in ethanolic 0.1 N HCl and 0.1 N NaOH; in fact the spectrum in alkaline solution is that of the thiazolo analogue (*4*) which demonstrates HBr elimination from *3*. This is not unexpected as the methine proton on C-3 is activated by both the carbonyl group and the quaternary nitrogen atom; it is readily exchanged in deuterium oxide (NMR) as discussed below. The dihydrothiazolo product has *trans* stereochemistry; this follows from the vicinal coupling constant (TFA) between the C-2 (δ 6.5 – 6.6) and C-3 (δ 6.9 – 7.1) protons which is ≤ 1 Hz.[7,8]

It was pointed out above that in bromination of corresponding acids the product was the

* See Ref. 1.



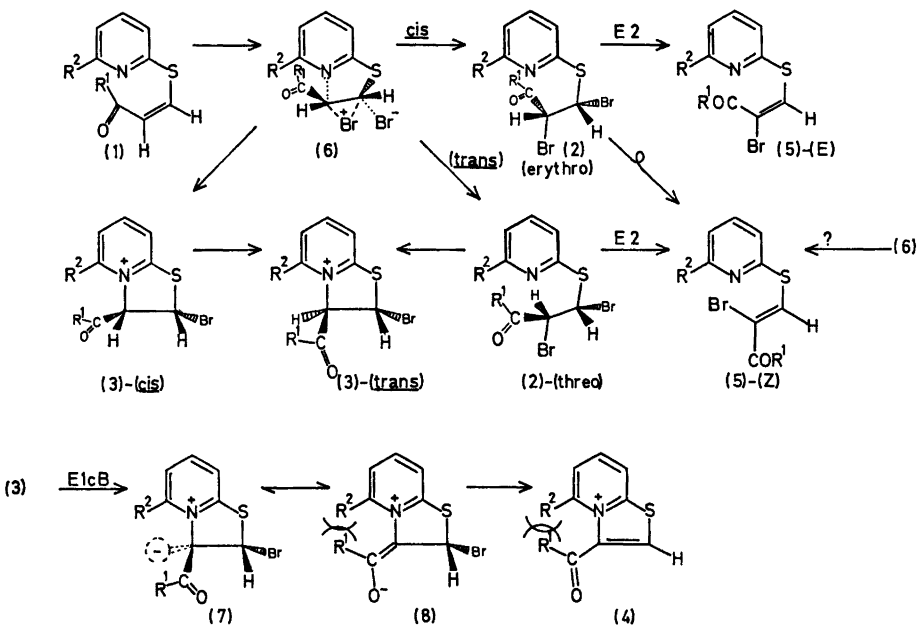
Scheme 1.

thiazole *4*;[3] the differential activation by the carbonyl groups may explain the different product formations. Thus further activation by warming led to HBr elimination; the elimination in recrystallisations of *3* was suppressed by HBr additions. Preparatively, however, the thiazole *4* was generated by warming a solution of *3* in the presence of propylene oxide as HBr scavenger. The NMR spectra (TFA) of the thiazolo[3,2-*a*]pyridinium derivatives are characterised by low field absorption for the C-3 proton (δ 8.7 – 8.9) and the C-5 proton (δ 9.3 – 9.4).

The 6-methyl substituted amides *1b*, *1d*, and *1f* reacted differently after bromine addition. The initial product was the adduct *2*; in the case of the ethylamide *1b*, HBr elimination had partially occurred. HBr was eliminated from all the adducts on warming in solution; no cyclisation, however, occurred. The products are the vinyl derivatives (*5*); the *N*-ethyl derivative has the vinyl proton at δ 8.7 (TFA). The analogues *5d* and *5f* were considerably contaminated by 6-methylpyrid-2-thione; the latter is thus formed in a competitive elimination reaction where the sulfide and not the bromide ion is the leaving group. These results show that a 6-methyl group effectively blocks cyclisation of the amides. The important non-bonded interaction is presumably between the substituents on the amido nitrogen atom and the 6-methyl group. In this respect the amido nitrogen in the examples are perhaps best compared with the bulkiness of a secondary and tertiary carbon. In the esters *1g* and *1h* the non-bonded interaction in the cyclisation step is reduced; the isolated product was the thiazole *4* as in the case of the acids. In the ketones *1i* and *1j*, however, methyl group interference in the cyclisation step was apparent in that the product from the 6-methyl derivative *1j* was the vinyl thioether *5*. The NMR spectrum contained one vinyl proton signal (δ 8.6) suggesting that only one stereoisomer was formed. The desmethyl ketone *1i*, however, yielded both the vinyl thioether *5* and the thiazolo analogue *4*; the vinyl thioether was the major component.

The vinyl thioethers *1* except the ketones *1i* and *1j* have the *cis* configuration as pointed out above. *trans*-Addition of bromine to the *cis*-isomers followed by cyclisation with configurational inversion are consistent with the observed *trans* configuration for the dihydrothiazolo derivatives *3a*, *3c*, and *3e*. NMR showed no isomerisation of the vinyl thioether by bromine or by traces of HBr in experiments using excess of the vinyl thioether reactant.

Interconversions of the *cis* and *trans* isomers of (*3*) cannot be excluded. Reaction paths leading to the *cis* isomer therefore have to be considered. The latter is thought to be thermodynamically the less stable because of steric



Scheme 2.

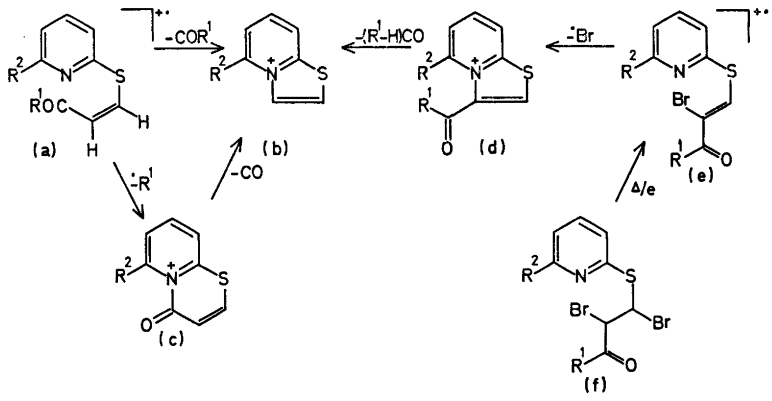
repulsion between the vicinal substituents (Scheme 2). C-3 is highly activated by the quaternary nitrogen atom and the carbonyl group. Epimerisation on C-3 is therefore quite feasible under the experimental conditions used which would result in isolation of the *trans* product irrespective of the original configuration of the product formed. Support for this view was found in a deuteriation experiment with *3a*; dissolution of the latter in a solution of deuterium oxide and acetone-d_6 at 20 °C led to very rapid exchange of the proton on C-3 while no significant exchange of the C-2 proton had occurred after 5 h (NMR). At higher temperatures the C-2 proton was also exchanged.

A cyclic bromonium ion is normally assumed in bromine additions to olefins; an ion pair can initially be assumed. In the case of a solvent of low solvating power and where the onium intermediate carries a strongly stabilising substituent the ion pair may collapse with overall *cis*-addition. With better solvation of the bromonium intermediate the bromide ion preferably adds *trans*. For the vinyl thioethers the charge distribution in the bromonium intermediate is presumably unsymmetrical; the positive charge is better stabilised on the α-carbon to the thioether sulfur atom. The pyridine nitrogen through its lone pair of electrons, however, may be involved in solvation and stabilisation of charge at the other carbon. The solvated onium intermediate *6* may therefore collapse either by *cis/trans* bromide addition or pyridine addition; the latter path leads directly to the *cis* isomer *3* which then is epimerised on C-3 to the *trans* form. The reaction path was further studied by bromination of the ethylamide *1a* with efficient cooling at 0 °C using concentrated bromine and vinylthioether solutions and with rapid addition; the major product was the open-chain dibromide *2a* and the minor product the bicyclic derivative *3a*. The dibromide *2a* was cyclised slowly in dilute chloroform solution in the cold. The slow cyclisation rate compared with the rapid formation of *3a* in the bromination excludes the dibromide *2a* as an intermediate in this reaction and is consistent with direct addition of the pyridine nitrogen to the bromonium intermediate. A 6-methyl group introduces steric repulsion in the approach of the pyridine nitrogen to the bromonium ion *6* for solvation. The activation energy for pyridine addition is thereby increased to the extent

that bromide addition is favoured in the 6-methyl derivatives; the dibromide *2* is thus formed. The esters *1g* and *1h* furnished the bicyclic thiazoles *4* presumably by way of the corresponding dihydrothiazoles *3* as did corresponding acids. The reaction is fast and therefore pyridine attack on the bromonium intermediate is again assumed; this is followed by rapid HBr elimination. The non-bonded interaction between the 6-methyl group and the ester group is thus less than for the amido group. It will be recalled that the 6-methyl ketone yielded the bromovinyl thioether *5j* while the desmethyl derivative *1i* gave a mixture of the bromovinyl product *5i* and the corresponding thiazole *4i*. The observed product formation is explained in terms of steric and electronic effects. The side-chain methyl group is attached directly to the carbonyl carbon which increases the effective non-bonded interaction with the 6-substituent in the pyridine ring. In a bromonium intermediate the proton next to the carbonyl group is highly activated. Intramolecular abstraction of this proton by the basic nitrogen may be a pathway to *5* in the case of the ketones.

The evidence for the formation of only the one stereoisomer of *5* rests on NMR data. Thus the vinyl proton in *5b*, *5i*, and *5j* is in the region δ 8.6–8.7 in TFA solution and at about δ 9.0 in deuteriochloroform solution. These data are used in structural assignment by comparison with data recorded under similar conditions for the parent vinyl thioethers *1*.[6] In deuteriochloroform solution the β-proton of *1* was at δ 8.3–8.5 irrespective of the configuration; in TFA a diamagnetic shift (0.4–1.0 ppm) was observed which depended on the configuration. These observations are rationalised through dominating deshielding effects from the aromatic pyridine ring in chloroform solution which is reduced on protonation of the pyridine nitrogen in TFA solution. Similar shift changes were seen in MeOH-d_4.DBr. The signal from the β-proton in the *trans* isomer is found at the lower field (0.6 ppm) ascribed to the anisotropy of the carbonyl group. The corresponding signal in the bromovinyl thioethers has undergone a diamagnetic shift of 0.4 ppm from deuteriochloroform to TFA which is interpreted in terms of the (*Z*)-configuration for the bromovinyl ethers *5b*, *5i* and *5j*. The stereochemistry of the bromovinyl derivatives *5b* and *5j* is the same although the parent vinyl thioethers *1b* and *1j* have opposite stereochemistry. As the stereochemical course of bromine addition is uncertain and the adduct presumably can be epimerised because of carbonyl activation, discussion of the elimination mechanism is not warranted.

The bromovinyl thioethers *5* contain groups capable of stabilising negative charge. Nucleophilic vinyl substitution of the bromine by the nitrogen atom to yield the thiazole *4* can be visualised. The bromovinyl ketone *5i*, however, did not undergo this reaction in chloroform solution. This further supports thiazole *4* formation from the dihydrothiazole *3* by HBr elimination. The leaving substituents are *cis* in *3*; hence E2 elimination is less favourable. It will be recalled that H-3 in the amide *3a*



Scheme 3.

was very rapidly exchanged in deuterium oxide-acetone-d_6. A carbanion on C-3 is well stabilised by resonance with the carbonyl group 7 and inductively by the quaternary ammonium nitrogen. The best resonance stabilisation is obtained with a keto carbonyl group and the poorest stabilisation by the amido carbonyl group. Only the amides were isolated as dihydrothiazoles *3*; the esters and ketones reacted further to the thiazoles *4*. The results are consistent with an E1cB elimination reaction in which the departure of the leaving group with double bond formation is the rate determining step.[9,10] The E1cB elimination is most often seen in cases with β-proton activation and a poor leaving group. Elimination of the bromide ion is normally encountered in concerted eliminations because of good leaving properties. The unusual behaviour in the present case is attributed to the very good carbanion stabilisation and charge dispersion by the two mentioned groups and to non-bonded interaction as discussed below. On rehybridisation from sp^3 to sp^2 on C-3 the carbonyl group becomes coplanar with the pyridine ring (*8*) which results in differential increased non-bonded interaction between the 6-substituent and the carbonyl substituent. The anionic carbon in the ester and especially in the ketones is closer to sp^2 hybridisation than in the amide because of better resonance stabilisation. The activation energy for bromide expulsion with double bond formation is probably increased for the latter because of the more extensive rehybridisation required on C-3.

The base peak in the mass spectra for the vinyl thioethers is found at *m/e* 136 or at *m/e* 150 depending on whether the molecule contains the 6-methyl group. These mass numbers correspond to the aromatic thiazolo[3,2-*a*]pyridinium cation *b* and correlate well with previous studies of corresponding acids.[11] Secondary fragments are of little importance. The two-step process to *b* with initial R^1 expulsion is also seen, especially in the case of the *t*-butyl derivatives and the esters but hardly any signal at this mass number was present in the spectra of the ketones. In the amides the intensity of the $[M - R^1]$ signal is generally lower in the 6-methyl derivative which suggests non-bonded interaction suppressing a cyclisation reaction such as formation of *c*. Molecular ion intensities for the amides were of the order 0.2 – 2.5 %, for the ketones about 2 % and for the esters about 10 %. The molecular ion intensities in the available bromovinyl thioethers were slightly increased (3 – 6 %). They differ in their fragmentation by initial expulsion of the bromine to give the base peak; the more stable radical is expelled. The other pathway is seen but is of little importance. The [M – Br] species *d* further undergoes rearrangements with transfer of a hydrogen and expulsion of the side-chain to give species with mass number corresponding to the thiazolo[3,2-*a*]pyridinium cation; relative intensity in the range 70 – 90 %. In the dibromides *2* pyrolytic HBr elimination occurred extensively. The dihydrothiazolo derivatives naturally eliminated HBr; the spectra from the thiazolo cations *4* are superimposed on the spectra from pyrolytic products and therefore variable.

EXPERIMENTAL

NMR spectra were recorded with a Varian A-60A or Varian A-100 instrument, the UV spectra with a Cary 14 instrument and the MS spectra with an AEI MS-902 mass spectrometer.

N-t-Butyl-2,3-dibromo-3-(6-methyl-2-pyridylthio)propionamide HBr (2f). Bromine in chloroform (10 % solution) was added dropwise at 20 °C to a solution of *cis-N-t*-butyl-3-(6-methyl-2-pyridylthio)acrylamide (1.25 g, 0.005 mol) in chloroform (100 ml) until rapid bromine decolouration no longer occurred. The excess bromine was destroyed by acetone additions and a little HBr added. The precipitated hydrobromide was recrystallised from ethanol-ether to which had been added a little aqueous HBr; yield 1.92 g (78 %), m.p. 160 °C (decomp.). (Found: C 31.96; H 4.15; N 5.90. Calc. for $C_{13}H_{18}Br_2N_2OS.HBr$; C 31.79; H 3.90; N 6.05); δ(TFA) 3.0 (Me), 1.5 (*N-t*-Bu), 5.1 (H-β, d), 6.3 (H-α), 7.7 – 8.6 (3H-pyr).

N,N-Dimethyl-2,3-dibromo-3-(6-methyl-2-pyridylthio)propionamide HBr (2d) was prepared as above from the corresponding *cis*-ethylene (*1f*); m.p. 124 °C (decomp.) after recrystallisation from ethanol/ether with HBr addition (Found: C 28.78; H 3.33; N 5.85. Calc. for $C_{11}H_{14}Br_2N_2OS \cdot HBr$: C 28.53; H 3.26; N 6.05); δ(TFA) 3.0 (Me), 3.4 (NMe_2) 5.5 (H-β), 6.0 (H-α), 7.6 – 8.7 (3H-pyr.).

trans-*2-Bromo-3-N-ethylcarbamoyldihydrothiazolo[3.2-a]-pyridinium bromide* (3a). A bromine solution in chloroform (10 %) was added dropwise at 20 °C to a solution of *cis-N*-ethyl-3-(2-pyridylthio)acrylamide (0.50 g, 0.0025 mol) in chloroform (50 ml) until a permanent colour of bromine remained. An oily product was precipi-

tated as the addition proceeded. The solvent was removed by evaporation below 20 °C. The residual yellow oily material was then dissolved in ethanol (20 ml) and an equal volume of ether added. The title compound was slowly precipitated from this solution and was recrystallized from ethanol/ether to which had been added a few drops of HBr solution; yield 0.6 – 0.7 g (70 – 80 %), m.p. 140 °C (decomp.). (Found: C 32.44; H 3.43; N 7.40. Calc. for $C_{10}H_{12}Br_2N_2OS$: C 32.63; H 3.28; N 7.61); δ(TFA) 1.3 and 3.5 (NEt), 6.5 (H-2, $J < 1$Hz), 6.9 (H-3), 7.8 – 9.0 (3 H-pyr); λ_{max} (0.1 N HCl EtOH) 242 (log ε 3.90), 310 (3.74), 323 sh. nm (3.70).

trans-*2-Bromo-3*-N,N-*dimethylcarbamoyldihydrothiazolo*[*3,2*-a]*pyridinium bromide* (3c) was prepared as above from the corresponding *cis*-ethylene amide (*1c*); yield 70 – 80 %, m.p. 160 °C (decomp.) (EtOH/Et_2O/HBr). (Found: C 32.50; H 3.58; N 7.45. Calc. for $C_{10}H_{12}Br_2N_2OS$: C 32.63; H 3.28; N 7.61); δ(TFA) 3.2 and 3.5 (NMe_2), 6.6 (H-2, $J < 1$Hz), 7.1 (H-3), 7.8 – 9.1 (3 H-pyr.); λ_{max} (0.1 N HCl EtOH) 240 (log ε 3.81), 310 (3.78), 324 sh.nm. (3.7).

trans-*2-Bromo-3*-N-*t-butylcarbamoyldihydrothiazolo*[*3.2*-a]*pyridinium bromide* (3e) was prepared as above from the corresponding *cis* ethylene amide (*1e*); yield 48 %, m.p. 260 °C (decomp.) (EtOH/Et_2O/HBr). (Found: C 36.07; H 3.93; N 6.92. Calc. for $C_{12}H_{16}Br_2N_2OS$: C 36.38; H 4.07; N 7.07); δ(TFA) 1.5 (*N-t*-Bu), 6.5 (H-2, $J < 1$Hz), 6.9 (H-3), 7.8 – 9.1 (3 H-pyr.); λ_{max} (0.1 N HCl EtOH) 242 (log ε 3.90), 323 nm (3.71).

3-N,N-*Dimethylcarbamoylthiazolo*[*3,2*-a]*pyridinium bromide* (4c). 2-Bromo-3-*N,N*-dimethylcarbamoyldihydrothiazolo[3,2-*a*]pyridinium bromide (0.2 g) was dissolved in ethanol (50 ml) and 1,2-propylene oxide added as HBr acceptor. The resultant solution was heated at 60 °C for 30 min before the solution was evaporated at reduced pressure. The residue was re-dissolved in ethanol before addition of ether and a little pet.ether. The title compound crystallised out in 70 % yield, m.p. 169 °C (decomp.). (Found: C 41.15; H 4.14; N 9.73. Calc. for $C_{10}H_{11}BrN_2OS$: C 41.82; H 3.86; N 9.75); δ(TFA) 3.4 (NMe_2), 8.7 (H-2), 9.4 (H-5), 8.1 – 8.8 (3 H-pyr); λ_{max} (0.1 N HCl EtOH) 225 (log ε 4.21), 299 sh. (4.03), 308 nm (4.13).

3-N-t-*Butylcarbamoylthiazolo*[*3.2*-a]*pyridinium bromide* (4e) was prepared in almost quantitative yield by the above procedure from the corresponding 2-bromodihydrothiazole (*3e*); m.p. 205 °C (decomp.), (EtOH/Et_2O/light petroleum). (Found: C 45.72; H 4.77; N 8.83. Calc. for $C_{12}H_{15}BrN_2OS$: C 45.72; H 4.79, N 8.88); δ(TFA) 1.6 (*N-t*-Bu), 8.9 (H-2), 9.3 (H-5), 7.9 – 8.3 (3 H-pyr); λ_{max} (0.1 N HCl EtOH) 227 (log ε 4.15), 298 sh (3.96), 309 nm (4.05).

3-Ethoxycarbonylthiazolo[*3.2*-a]*pyridinium bromide* (4g). Bromine in chloroform (10 % solution) was added dropwise at 20 °C to a solution of ethyl *cis*-3-(2-pyridylthio)acrylate (1.05 g, 0.005 mol in chloroform (50 ml) until a permanent colour of bromine remained. An oily precipitate was slowly formed. The reaction mixture was evaporated at reduced pressure after about ½ h. The residual brown oily material was triturated with acetone (10 ml) which decolourised the material. The resultant white suspension was dissolved by addition of ethanol. A little ether was then added and the solution left to stand in the cold. The precipitated product was collected after 1 week; yield 80 %, m.p. 158 °C (decomp.). (Found: C 41.65; H 3.67; N 4.84. Calc. for $C_{10}H_{10}BrNO_2S$: C 41.68; H 3.49; N 4.86); δ(TFA) 1.5 and 4.7 (OEt), 9.1 (H-2), 8.0 – 8.9 (4 H-pyr); λ_{max} (0.1 N HCl EtOH) 225 (log ε 4.24), 241 (3.83), 247 (3.73), 296 (4.04), 306 nm (4.14).

3-Ethoxycarbonyl-5-methylthiazolo[*3,2*-a]*pyridinium bromide* (4h) was prepared as described for *4g* by bromination of ethyl *cis*-3-(6-methyl-2-pyridylthio)acrylate (*1h*) yield 80 %, m.p. 140 °C (decomp.) (Acetone/EtOH/Et_2O). (Found: C 43.75; H 3.60; N 4.55. Calc. for $C_{11}H_{12}BrNO_2S$: C 43.72; H 4.00; N 4.63); δ(TFA) 1.5 and 4.7 (OEt), 3.0 (5-Me), 8.9 (H-2), 7.9 – 8.7 (3H-pyr.); λ_{max} (0.1 N HCl EtOH) 228 (log ε 4.16), 247 (3.72), 278 (3.49), 308 (4.04), 317 nm (4.12).

3-Acetylthiazolo[*3.2*-a]*pyridinium bromide* (4i) *and 3-bromo-4-(2-pyridylthio)but-3-en-2-one HBr* (*5i*). Bromine in chloroform (10 %) solution was added dropwise at 0 – 10 °C to a solution of *cis/trans* (1:1) 4-(2-pyridylthio)but-3-en-2-one (0.90 g, 0.005 mol) until the solution assumed the permanent colour of bromine. The product which crystallised out during the bromine addition was triturated with acetone and was recrystallised from ethanol/ether; yield 0.3 g (25 %), m.p. > 260 °C). (Found: C 42.10; H 3.10; N 5.46. Calc. for C_9H_8BrNOS: C 41.88; H 3.12; N 5.43); δ(TFA) 3.0 (Ac), 9.4 (H-2), 10.6 (H-5), 8.9 – 9.0 (3 H-pyr); λ_{max} (0.1 N HCl EtOH) 244 (log ε 3.93), 298 (3.75), 308 (3.88), 326 nm (3.63).

The filtrate from the reaction solution was decolourised by acetone addition. The precipitated HBr salt was a mixture of the title compounds. The salt was dissolved in water and the solution made alkaline by means of sodium bicarbonate. The bromovinyl derivative *5i* was then extracted into chloroform which was dried and evaporated. The residual material was crystallised from ethanol to which had been added a few drops of etheral HBr; yield 0.68 g (40 %), m.p. 150 °C. Runs on larger reaction scales furnished yields up to 70 %. (Found: C 31.92; H 2.74; N 4.32. Calc. for C_9H_8BrNOS. HBr: C 31.88; H 2.67; N 4.13); δ(TFA) 2.7 (Ac), 8.7 (H-β) 8.9 (H-6), 7.7 – 8.7 (3 H-pyr.); λ_{max} (0.1 N HCl EtOH) 243 (log ε 3.67), 276 (3.83), 320 nm (4.23).

N-*Ethyl-2-bromo-3-(6-methyl-2-pyridylthio)-acrylamide HBr* (5b). Bromine in chloroform (10 % solution) was added dropwise at 20 °C to a solution of *cis-N*-ethyl-3-(6-methyl-2-pyridylthio)acrylamide (0.10 g, 0.005 mol) in chloroform until the colour of bromine was no longer

discharged. The solvent was then distilled off and the product recrystallised from ethanol and ethyl acetate; yield 0.14 g (82 %), m.p. 165 °C (decomp.) (Found: C 34.58; H 3.65; N 7.23. Calc. for $C_{11}H_{13}BrN_2OS.HBr$: C 34.57; H 3.69; N 7.33); δ(TFA) 1.4 and 3.6 (NEt), 3.0 (6-Me), 8.7 (H-β), 7.7 – 8.7 (3 H-pyr.); λ_{max} (0.1 N HCl EtOH) 272 (log ε 4.06), 319 nm (4.18).

3-Bromo-4-(6-methyl-2-pyridylthio)but-3-en-2-one HBr (5j). Bromine in chloroform (10 % solution) was added in the usual way at 0 – 10 °C to *trans*-4-(6-methyl-2-pyridylthio)but-3-en-2-one (0.96 g, 0.005 mol) in chloroform (100 ml). Acetone was next added to the reaction solution and the precipitated title compound recrystallised from ethanol-ether-HBr; yield 1.57 g (90 %), m.p. 180 °C (decomp.). (Found: C 34.13; H 3.19; N 3.97. Calc. for $C_{10}H_{10}BrNOS.HBr$: C 34.01; H 3.14; N 3.97); δ(TFA) 2.7 (Ac), 3.0 (6-Me), 8.6 (H-β), 7.7 – 8.5 (3 H-pyr); λ_{max} (0.1 N HCl EtOH) 250 (log ε 3.66), 277 (3.89), 323 nm (4.24).

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Tobacco Chemistry. 27. The Structures of Four Stereoisomeric 8,12ξ-Epoxylabd-14-en-13ξ-ols Isolated from Greek *Nicotiana tabacum* L.

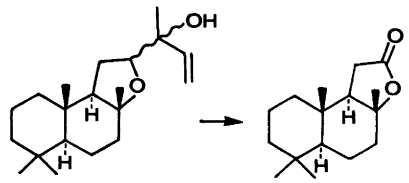
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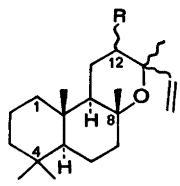
The structures of four stereoisomeric 8,12ξ-epoxylabd-14-en-13ξ-ols** obtained from Greek tobacco were determined by spectroscopic means and by conversion to 12-norambreinolide. Plausible routes for the formation of the new labdanols and related tobacco constituents from a common precursor, abienol, are discussed.

Chemical research on tobacco has revealed the presence of a large number of terpenoids and nor-terpenoids. The vast majority of these either belongs to, or may formally be derived from representatives of three groups of compounds, namely (i) carotenoids – polyisoprenoids, (ii) thunberganes, and (iii) labdanes – drimanes.[1–5] This paper deals with the structural elucidation of four new representatives of the labdane group.

GC-MS examination of a medium-polar, neutral fraction ('B 5') of an extract of Greek tobacco [6] revealed the presence of four compounds which exhibited nearly identical mass spectra suggesting that they were stereoisomers. Two of the isomers (*1a* and *1b*) occurred in quantities permitting isolation and subsequent structure determination. Accurate mass determinations of the M – 15 and M – 18 ions, and the appearance of twenty ^{13}C-resonances in the CMR spectrum of stereoisomer *1b*, established the elemental composition as $C_{20}H_{34}O_2$. Furthermore, the mass spectrum was indicative of a labdane diterpenoid-structure devoid of additional substituents in ring A, [7] *e.g.* ions at m/e 137, 177, and 191. The NMR-spectrum of the stereoisomer *1b* displayed five three-proton singlets in the δ 0.8 – 1.15 region corresponding to three methyl groups on quaternary carbon atoms, and two on oxygenated, tertiary carbons. An ABC-signal pattern in the δ 5.0 – 5.9 region characteristic of a vinyl group bound to a fully substituted carbon was also observed as was a one-proton quartet at δ ~3.80 (*J ca.* 5 and 7 Hz), attributed to a proton on an oxygenated carbon atom. These data made two structures appear likely, namely 8,12ξ-epoxylabd-14-en-13ξ-ol (*1*) and 8,13ξ-epoxylabd-14-en-12ξ-ol (*3*, R = OH). The former of these was favoured since all four stereoisomeric tobacco compounds exhibited a medium intense *m/e* 235 ion ($C_{16}H_{27}O$), only readily rationalized in terms of structure *1* [favoured C(12) – C(13) cleavage], and gave mass spectra differing from that of 8,13β-epoxylabd-14-en-12α-ol. [8]



1 2

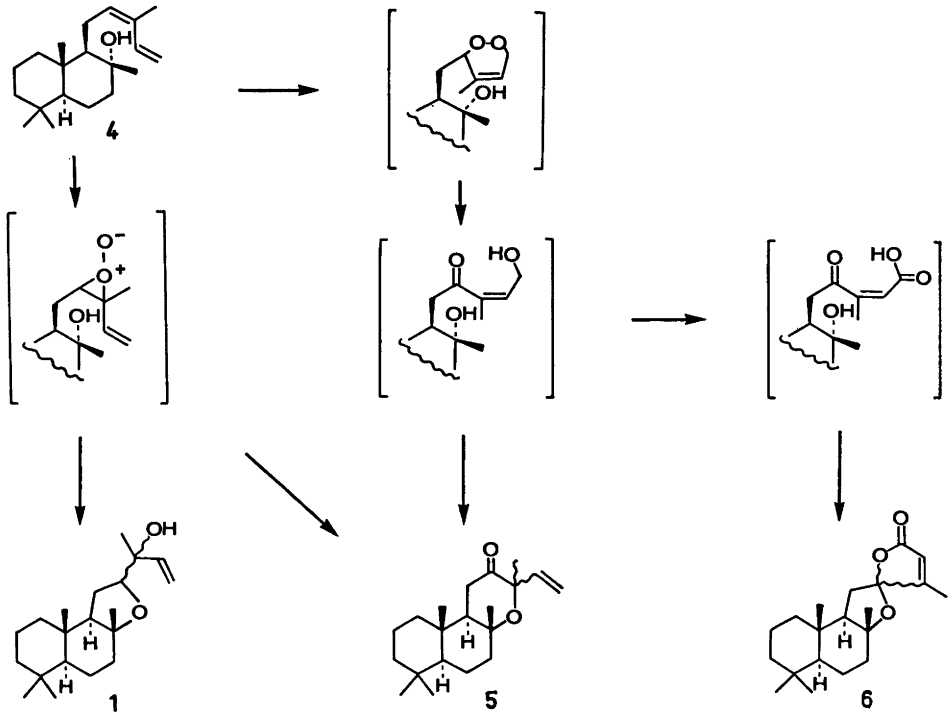


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** Nomenclature according to J. W. Rowe, Oct. 1968; personal communication.

The spectral results were verified chemically since neither of the two main tobacco compounds (*1a*, *1b*) could be acetylated under mild conditions (Ac_2O/py) and oxidation (employing various methods involving CrO_3) did not give ketones, but furnished 12-norambreinolide [9] (*2*) in almost quantitative yield. The latter transformation confirmed the larger part of the carbon skeleton and established the absolute configurations at C(5), C(8), C(9) and C(10); the validity of the 8*R* assignment follows from the facts that the C(8) epimer of the lactone (*2*) has been shown to be the thermodynamically more stable isomer [10] and that authentic 12-norambreinolide (*2*) was virtually unaffected when treated under the same conditions as employed for the oxidation. The above spectral data show how the five carbons beyond C(11) in the side chain are linked and also that C(12) is secondary and carries an ether oxygen function and that C(13) is tertiary and carries a methyl, a vinyl and an oxygen substituent. The only additional structure, 12ξ,13ξ-epoxylabd-14-en-8-ol, consistent with these results, could be excluded on the basis that the proton at C(12) in this compound should resonate at notably higher field (δ 2.5 – 3.2) [11] than actually observed, *i.e.* δ 3.8. Furthermore, the structures of the major stereoisomer (*1b*), and one of the minor ones (*1d*), have recently been confirmed in our laboratory by synthesis, which will be discussed elsewhere.[12] The structure of the fourth isomer (*1c*), however, rests presently on mass spectral evidence only.

The new diterpenoids have the same absolute configuration at C(5), C(8), C(9), and C(10) as the other labdanes previously isolated from tobacco, and are, as the majority of these, oxygenated at C(8) and C(12). The co-occurrence of several sets of labdanoid diastereomers differing only with respect to the side-chain stereochemistry suggests that they are formed from a labdane precursor such as abienol (*4*), recently isolated from tobacco, [13] by non-stereospecific oxidation. Plausible routes for the formation of the new compounds (*1a* – *1d*), and some earlier encountered representatives – the α- and β-levantenolides (*6*) [14] and two 13-



Scheme 1.

epimeric 8,13-epoxylabd-14-en-12-ones (*5*) [15] – involving commonly accepted singlet oxygen intermediates, [16,17] are depicted in Scheme 1. Earlier published views on the biosynthesis of the α- and β-levantenolides differ from ours in assuming that oxidation occurs prior to cyclizations and that the actual precursor is the appropriate acyclic keto-acid. [18,19] It is of interest to note that the new compounds (*1a–1d*) in turn constitute potential precursors for 12-norambreinolide (*2*), which was first identified as a constituent of the aroma of cigar tobacco,[20] and also a series of nor- and seco-terpenoids very recently obtained from Greek tobacco.[5,21]

EXPERIMENTAL

NMR, IR, and mass spectra were recorded on Varian HA 100D and XL 100, Digilab FTS-14 and Perkin-Elmer 257, and LKB 9000 (70 eV) instruments, respectively. Rotations were measured on a Perkin-Elmer 141 instrument. Accurate mass determinations were carried out at the laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm. The mass spectra were obtained by gas chromatography in combination with the mass spectrometer (GC-MS). Analytical gas chromatography was performed on a Varian 1700 instrument using either a steel or a glass capillary column coated with emulphor and Ucon Oil HB 2000, respectively.

Isolation. Fraction B5 [6] was subjected to distillation using CO_2 as carrier gas, furnishing a less volatile fraction which was further subdivided employing column chromatography on silica gel and $AgNO_3$-impregnated silica gel. Thus stereoisomers *1a* (*ca.* 30 mg; numbering *1a* to *1d* according to increasing retention times, glass capillary column coated with HB 2000) and *1b* (*ca.* 200 mg) were obtained in the pure state, while the quantities of stereoisomers *1c* and *1d* only permitted detection by GC-MS. The subfractionation of B5 will be reported elsewhere.

Stereoisomer 1a. δ(CCl_4): 0.81 (3 H, s), 0.82 (3 H, s), 0.87 (3 H, s), 1.10 (3 H, s), 1.18 (3 H, s), 3.80 (1 H, dd, *J* 5 and 7.5 Hz), 5.02 (1 H, dd, *J* 2 and 10 Hz), 5.22 (1 H, dd, *J* 2 and 17.5 Hz), 5.75 (1 H, dd, *J* 10 and 17.5 Hz); ν(film): 3470 (broad), 2974 (s), 2932 (s), 2870 (s), 1461 (m), 1380 (m), 1369 (m), 1152 (m), 1122(s), 1080 (m), 1004 (m), 985 (m), 930 (m), 870 (w), 845 (w); MS: *m/e* 306 (M+, not observed), 191 (100), 69 (38), 137 (37), 109 (32), 95 (26), 81 (26), 41 (24), 43 (23), 55 (22), 192 (19), 217 (18), 235 (17); accurate mass determination: $C_{19}H_{31}O_2$ (M – 15), found 291.2316, calc. 291.2324; $[\alpha]^{20}$ (*c* 0.57; $CHCl_3$), 436 nm: +32.5°, 546 nm: +14.4°, 578 nm: +130°, 589 nm: +12.3°.

Stereoisomer 1b. δ(CCl_4): 0.82 (6 H, s), 0.87 (3 H, s), 1.10 (3 H, s), 1.15 (3 H, s), 2.26 (OH), 3.82 (1 H, dd, *J* 5.5 and 6.5 Hz), 5.00 (1 H, dd, *J* 2 and 10.5 Hz), 5.21 (1 H, dd, *J* 2 and 17.5 Hz), 5.86 (1 H, dd, *J* 10.5 and 17.5 Hz); δ (^{13}C, ppm from internal TMS, in C_6H_6:$CDCl_3$ 1:1, tentative assignments are based on those of manoyl oxide [22]) 39.6 (C-1), 18.4 (C-2), 42.3 (C-3),32.9 (C-4), 57.1 (C-5), 20.1 (C-6), 39.7 (C-7), 73.8 (C-13), 59.7 (C-9), 36.4 (C-10), 23.9 (C-11), 81.1 (C-12), 80.5 (C-8), 142.1 (C-14), 111.5 (C-15), 21.2 (C-17), 23.5 (C-16), 33.3 (C-18), 21.1 (C-19), 14.9 (C-20); ν(film): 3470 (broad), 2929 (s), 2870 (m), 2849 (m), 1461 (m), 1378 (m), 1368 (m), 1325 (w), 1300 (w), 1275 (w), 1255 (w), 1208 (w), 1198 (w), 1181 (w), 1162 (w), 1125 (m), 1080 (m), 1033 (w), 1005 (s), 987 (m), 925 (m), 880 (w), 869 (w), 845 (w), 828 (w); MS: *m/e* 306 (M+, not observed), 191 (100), 69 (38), 137 (36), 109 (31), 81 (26), 95 (25), 41 (23), 43 (22), 55 (22), 192 (18), 217 (17), 235 (15); accurate mass determinations: $C_{19}H_{31}O_2$ (M – 15), found 291.2315, calc. 291.2324; $C_{20}H_{32}O$, found 288.2447, calc. 288.2453; $C_{16}H_{27}O$, found 235.2070, calc. 235.2062; $C_{16}H_{25}$, found 217.1963, calc. 217.1956; $C_{14}H_{23}$, found 191.1802, calc. 191.1800; $[\alpha]^{20}$ (*c* 0.45; $CHCl_3$), 365 nm: −0.2°, 436 nm: −0.3°, 546 nm: −0.3°, 578 nm: −0.3°, 589 nm: −0.2°.

Stereoisomer 1c. MS: *m/e* 306 (M+, not observed), 191 (100), 69 (39), 137 (37), 109 (32), 55 (30), 95 (27), 81 (26), 41 (26), 43 (25), 192 (24), 217 (23), 123 (20), 235 (19).

Stereoisomer 1d. MS: *m/e* 306 (M+, not observed), 191 (100), 69 (54), 43 (50), 137 (47), 41 (45), 109 (43), 95 (37), 81 (36), 55 (33), 192 (24), 217 (22), 235 (21).

Acetylation. Stereoisomer *1b* (5 mg) in dry pyridine (1 ml) was added to a solution of acetic anhydride (0.5 ml) in dry pyridine (1 ml) and the mixture kept at room temperature for 24 h. Excess anhydride was destroyed by the addition of a few drops of methanol. Water was added and the mixture extracted with ether and the extract washed with diluted H_2SO_4, $NaHCO_3$ and water. Concentration of the extract left a colourless oil (4 mg) indistinguishable (TLC) from the starting material.

Oxidation. Treatment of stereoisomers *1a* and *1b*, respectively, with Jones' ($CrO_3 - H_2SO_4 - H_2O$ – acetone),[23] Collins' (dipyridine – CrO_3 (*IV*) – CH_2Cl_2),[24] a two-phase (ether – CrO_3 – H_2O) system,[25] or CrO_3 – 80 % acetic acid furnished a single product which was only slightly less polar (TLC, SiO_2, 30 % ether-pentane) than the starting material (R_F = 0.30 and 0.24, respectively). For instance, stereoisomer *1b* (13 mg) when stirred with CrO_3 (30 mg) in 80 % acetic acid (1 ml) at ambient temperature for 2 h afforded after dilution with aqueous $NaHCO_3$, extraction with ether and distillation of the solvent, a colourless oil (10 mg) which crystallized on standing and was found indistinguishable from authentic 12-

norambreinolide (*2*); m.p. 124 °C (lit.[20] 125–125.5 °C) the IR [10] and mass spectra [26] were found identical to those of authentic 12-norambreinolide (*2*); $[\alpha]^{20}$, *2* prepared from stereoisomer *1a* (*c* 0.1; $CHCl_3$), 365 nm: +166°, 436 nm: +92° 546 nm: +48°, 578 nm: +44° 589 nm: +36°; *2* prepared from stereoisomer *1b* (*c* 0.5; $CHCl_3$), 365 nm: +185°, 436 nm: +107°, 546 nm: +58°, 578 nm: +50°, 589 nm: +48°; authentic 12-norambreinolide (*c* 0.46; $CHCl_3$), 365 nm: +180°, 436 nm: +104°, 546 nm: +57°, 578 nm: +49°, 589 nm: +47°.

Stability-test of 12-norambreinolide (2). The lactone (*2*, 5 mg) in acetic acid (1 ml) was stirred at room temperature for 2 h in the presence of CrO_3 (30 mg). The mixture was worked up as outlined (*vide supra*) and the products (2 mg) examined by GC-MS which revealed the presence of 12-norambreinolide and 8-epi-12-norambreinolide in the ratio 99:1.

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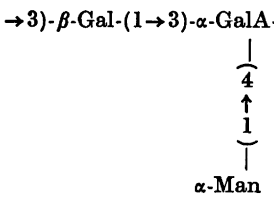
Structural Studies of the *Klebsiella* Type 57 Capsular Polysaccharide

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Dedicated to Professor Fritz Micheel on the occasion of his 75th birthday July 3, 1975.

The structure of the capsular polysaccharide from *Klebsiella* type 57 has been investigated. Methylation analysis, uronic acid degradation, modified Smith degradation and graded acid hydrolysis were the principle methods used. Pure oligomeric fragments were isolated using the three methods of degradation and characterized by chemical and physical methods. These studies show the structure to consist of a tetrasaccharide repeating unit (all sugar residues have the D-configuration and are pyranosidic).



The carbohydrate composition of the capsular polysaccharide from *Klebsiella* type 57 was investigated by Dudman and Wilkinson, who found that it contained mannose, galactose, and hexuronic acid residues in the proportions 44:21:28.[1] Nimmich found the same sugars and demonstrated that the acidic component was galacturonic acid.[2] We now report on structural studies of this polysaccharide.

RESULTS and DISCUSSION

The polysaccharide, isolated as previously described,[2] showed $[\alpha]_{589}$ +104°. As was evident from its IR and ^{13}C NMR spectra, it did not contain *O*-acyl groups. Acid hydrolysates of the original and the carboxyl-reduced [3] polysaccharide contained mannose and galactose in the approximate proportions 1.8:1 and 1:1, respectively. The sugars were isolated from a hydrolysate of carboxyl-reduced polysaccharide and from their optical rotations all belonged to the D-series.

Methylation analyses [4,5] of the original polysaccharide, with and without carboxyl-reduction of the fully methylated product, and of the carboxyl-reduced polysaccharide, are given in Table 1, columns C, A, and B, respectively. In these and other methylation analyses to be described later, the methylated sugars were transferred into the alditol acetates and analysed by GLC-MS.[5,6] Unambiguous identification of the partially methylated alditol acetates was based on retention times on GLC and mass spectra. Carboxyl-reduction of the methylated polysaccharide with lithium aluminium deuteride distinguished D-galactitol derivatives originating from D-galacturonic acid residues from those originating from D-galactose residues. Furthermore, 2,6-di-*O*-methyl- and 2-mono-*O*-methyl-D-galactoses were not present in the original polysaccharide but were found in carboxyl-reduced samples (Table 1, columns B and C). This indicated that these sugars originated from D-galacturonic acid. The results of the analyses reported in Table 1, columns A, B, and C, strongly suggest that the polysaccharide is composed of tetrasaccharide repeating units containing a terminal D-mannopyranose residue,

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Table 1. Methylation analyses of original and modified *Klebsiella* type 57 capsular polysaccharides.

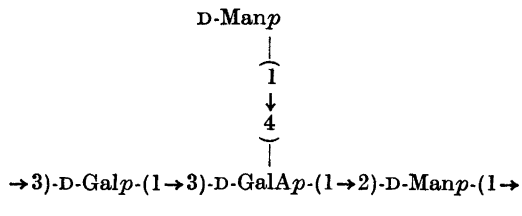
Methylated sugar [a]	T [b]	Mol % [c]						
		A	B	C	D	E	F	G
1,2,4,5,6-Gal [d]	0.42	–	–	–	–	12 [e]	–	–
2,3,4,6-Man	1.00	27	23	25	5 [f]	63 [g]	–	–
2,3,4,6-Gal	1.14	–	–	–	–	–	–	56 [h]
3,4,6-Man	1.67	34	28	29	52	8	–	–
2,4,6-Gal	1.99	39	27	29	43 [i]	17	46	44 [j]
2,6-Gal	2.82	–	22	–	–	–	54	–
2-Gal	5.55	–	–	17 [k]	–	–	–	–

[a] 1,2,4,5,6-Gal = 1,2,4,5,6-penta-*O*-methyl-D-galactitol, 2,3,4,6-Man = 2,3,4,6-tetra-*O*-methyl-D-mannose, *etc.* [b] Retention time of the corresponding alditol acetate relative to that of 2,3,4,6-Glc on a SP-1000 column at 220 °C. [c] A, original polysaccharide; B, polysaccharide, carboxyl-reduced before methylation; C, polysaccharide, carboxyl-reduced after methylation; D, degraded, original polysaccharide (uronic acid degradation); E, degraded, trideuteriomethylated polysaccharide (uronic acid degradation); F, degraded, carboxyl-reduced polysaccharide (Smith degradation); G, degraded, trideuteriomethylated carboxyl-reduced polysaccharide (Smith degradation). [d] Part of this volatile compound and its acetate was probably lost during concentrations. [e] Monodeuterated at C-1 and trideuteriomethylated at O-1 and O-5. [f] Determined from 3 % OV-225 column. [g] ≃90 % triduteriomethylated at O-2. [h] Trideuteriomethylated at O-3. ≃33 % monodeuterated at C-1. [i] ≃80 % trideuteriomethylated at O-4. [k] Dideuterated at C-6.

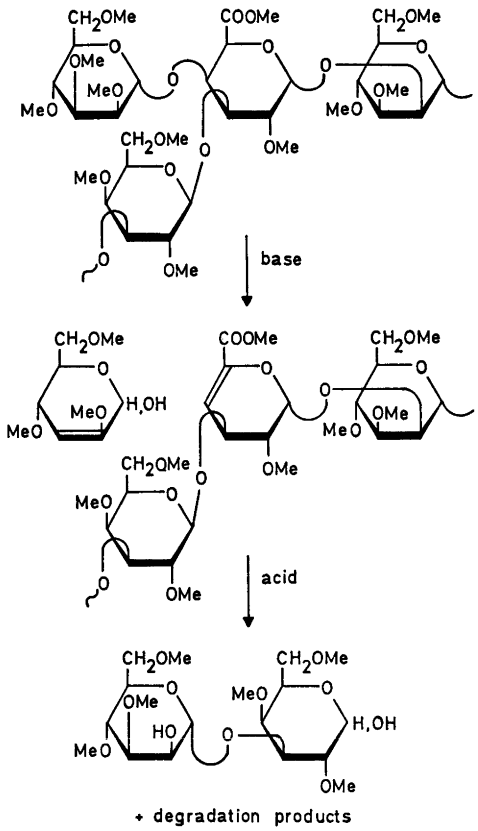
a D-mannopyranose residue linked to O-2, a D-galactopyranose residue linked to O-3, and a branching D-galactopyranosiduronic acid residue, linked to O-3 and O-4. The latter could, according to the methylation analyses, also be furanosidic and linked to O-3 and O-5, but this was disproved by Smith degradation and partial acid hydrolysis studies, as discussed below. The presence of a tetrasaccharide repeating unit was also supported by the ^{13}C NMR spectrum which showed four well resolved signals in the region for anomeric carbons and one signal in the carboxyl region.[7]

The fully methylated polysaccharide was degraded by treatment first with base and then with acid under mild conditions. This uronic acid degradation has recently been described [8] and applied to other *Klebsiella* capsular polysaccharides.[9–12] The product was reduced with sodium borodeuteride and a part was hydrolysed to completion, giving mainly 3,4,6-tri-*O*-methyl-D-mannose and 2,4,6-tri-*O*-methyl-D-galactose, deuterium labelled at C-1 indicating that the D-galactose residue becomes a reducing terminal in the degraded material (Table 1, column D). Another part was re-methylated, using trideuteriomethyl iodide. A hydrolysate of this material (Table 1, column E) contained 2,3,4,6-tetra-*O*-methyl-D-mannose with a trideuteriomethyl group at O-2, demonstrating that the D-galacturonic acid residue was linked to this position which was released during the acid treatment. As expected 1,2,4,5,6-penta-*O*-methyl-D-galactitol, with a deuterium atom at C-1 and trideuteriomethyl groups at O-1 and O-5, was also obtained. As evident from the analytical figures in Table 1, columns D and E, the elimination of the substituent at O-4 in the uronic acid residue was almost complete, as was also the cleavage of the glycosidic linkage in the thus modified residue during the subsequent mild acid hydrolysis. The cleavage of the linkage to O-3 was, however, less complete. Nevertheless, it was sufficient for establishing which sugar residue that was linked to this position.

From the result of this degradation the sequence of the sugar residues in the repeating unit (*1*) could be deduced. The course of the degradation is depicted in Scheme 1 and the correct anomeric configurations, determined as described below, are also given.



1



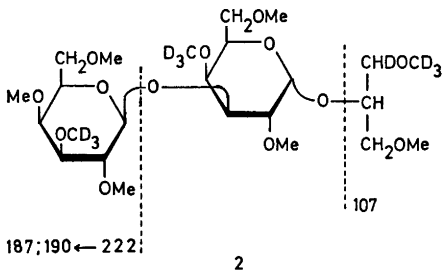
Scheme 1.

In order to determine the anomeric natures of the sugar residues, the polysaccharide was subjected to different degradations and the oligomeric products formed were isolated and characterized. High speed liquid chromatography on silica gel proved to be useful for the isolation of the lipophilic products. These degradations also furnished additional evidence for the proposed sequence (*1*).

The uronic acid degradation was repeated and the oligomeric product isolated after reduction using sodium borodeuteride and remethylation using methyl iodide. MS [6] of the product as well as analysis of its hydrolysate demonstrated that it was the expected nona-*O*-methyl ester of 3-*O*-D-mannopyranosyl-D-galactitol. In the ^{1}H NMR spectrum the anomeric proton appeared at δ 5.09, $J_{1,2}$ 1.5 Hz. The optical rotation, $[\alpha]_{578}$ +15° indicated an α-D-mannopyranosidic linkage. The partial structure α-D-Man*p*-(1→3)-D-Gal is consequently established.

Two acidic components, an aldobiouronic acid and an aldotriouronic acid, were isolated after graded hydrolysis of the polysaccharide. The former was shown to be D-GalA*p*-(1→2)-D-Man by methylation analysis of the carboxyl-reduced disaccharide alditol and by MS of the fully methylated, esterified alditol.[6] The optical rotation of the acid, $[\alpha]_{589}$ +66°, and the ^{1}H NMR spectrum of its alditol with the anomeric proton at δ 5.10, $J_{1,2}$ 3.0 Hz, demonstrate that the D-galacturonic acid residue has the α-configuration. The aldotriouronic acid, investigated by the same methods, proved to be α-D-GalA*p*-(1→2)-α-D-Man*p*-(1→3)-D-Gal. From its optical rotation, $[\alpha]_{589}$ +124°, and the appearance of two anomeric protons in the ^{1}H NMR spectrum of its alditol at δ 5.17, $J_{1,2}$ 3.2 Hz and at δ 5.22, $J_{1,2}$ 1.5 Hz, it was inferred that both the D-galacturonic acid residue and the D-mannose residue have the α-configuration.

The carboxyl-reduced polysaccharide was subjected to a modified Smith degradation procedure, as previously described for other bacterial polysaccharides.[10–13] In this procedure, acetal migration during the mild hydrolysis step is excluded and the positions liberated by this hydrolytic procedure are easily recognized. The product obtained after periodate oxidation–borohydride reduction was fully methylated. Complete acid hydrolysis of this product yielded 2,4,6-tri-*O*-methyl-D-galactose and 2,6-di-*O*-methyl-D-galactose demonstrating that the periodate oxidation had gone to completion (Table 1, column F). Mild hydrolysis with acid followed by reduction using sodium borodeuteride and remethylation using trideuteriomethyl iodide yielded a product which on hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, with a trideuteriomethyl group at O-3, and 2,4,6-tri-*O*-methyl-D-galactose, with a trideuteriomethyl group at O-4 (Table 1, column G). The latter obviously originates from the D-galacturonic acid residue. GLC-MS [6] of the product also demonstrated the presence of a main component with the MS expected for *2*. Some pertinent fragments are depicted in the formula. The glycerol moiety, with a deuterium atom at C-1 and a trideuteriomethyl group at O-1, obviously derives from the D-mannose residue substituted

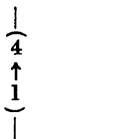


2

at O-2. The experiment was repeated on a larger scale and the nondeuterated analogue of *2* isolated. It showed $[\alpha]_{578}$ +41° and two anomeric protons in the ^{1}H NMR spectrum at δ 5.25, $J_{1,2}$ 3.5 Hz and δ 4.65, $J_{1,2}$ 7.0 Hz, respectively. It consequently contains one α- and one β-D-galactosidic linkage. As it was already demonstrated that the D-galacturonic acid residue is α-linked, the D-galactose residue is consequently β-linked. The partial structure β-D-Gal*p*-(1→3)-α-D-GalA*p*-(1→2)-D-Man is thereby established.

The terminal D-mannose residue in the repeating unit does not appear in any of the degradation products discussed above. The calculated values for the optical rotation of the polysaccharide, using Hudson's rules of isorotation and assuming this D-mannose residue to be α- or β-linked are $[\alpha]_{589}$ +93° and $[\alpha]_{589}$ +50°, respectively. The actual value (+104°) agrees much better with the former, which strongly indicates that this residue is α-linked. From the combined evidence presented above, it is therefore inferred that the *Klebsiella* type 57 capsular polysaccharide is composed of tetrasaccharide repeating units with the structure *3*.

→3)-β-D-Gal*p*-(1→3)-α-D-GalA*p*-(1→2)-α-D-Man*p*-(1→



3

EXPERIMENTAL

General methods. Concentrations were carried out under reduced pressure at bath temperatures which did not exceed 40 °C. For GLC, a Perkin-Elmer 900 instrument fitted with flame-ionisation detector was used. Separations were performed on glass-columns (180 × 0.15 cm) containing (a). 3 % OV-225 on Gas Chrom Q (100/120 mesh) at 170 °C (for partially methylated alditol acetates) or 190 °C (for alditol acetates); (b) 3 % OV-1 on the same support (for permethylated oligosaccharide derivatives). Partially methylated alditol acetates were also separated at 220 °C on a wall-coated glass-capillary column (25 m × 0.25 mm) containing SP-1000 as the stationary phase (LKB-products, Bromma, Sweden). For quantitative evaluation of the GLC a Hewlett-Packard 3370 B integrator was used. For mass spectrometry a Perkin-Elmer 270 instrument (for alditol acetates and derivatives) or a Varian MAT 311-SS 111 MS MS-computer system (for oligosaccharide derivatives) were used. Spectra were recorded at 70 eV, at an ionisation current of 80 μA (Perkin-Elmer) and 1000 μA (Varian MAT) and ion source temperatures of 80 and 250 °C, respectively. For analytical and preparative paper chromatography Whatman No. 1 or 3 MM paper eluted with the solvent system ethyl acetate-acetic acid-water, 3:1:1, was used. The compounds were detected with 3 % *p*-anisidine hydrochloride in ethanol at 120 °C. High speed liquid chromatography was performed with Waters Solvent delivery system 6000 constant flow pump and for monitoring the columns effluent a Waters R401 differential refractometer was used. Separations were performed on two Merck silica gel 60 size A columns connected in series with the solvent system hexane: acetone (2:3). NMR spectra were recorded on Varian XL-100 or HA-100 instruments. The proton-decoupled ^{13}C NMR spectrum was measured at 25.2 MHz on a solution containing ≃100 mg native polysaccharide/ml D_2O. External TMS was used as standard and the spectrum was recorded at 85 °C. An FID acquisition time of 0.8 s was used. The Fourier-transform was computed from 290 000 transients. ^{1}H NMR spectra were recorded by the c.w.- or PFT-mode on $CDCl_3$ solutions using TMS and on D_2O solutions using sodium 3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionate as internal standard, respectively. IR was recorded on a Perkin-Elmer 257 instrument and optical rotations on a Perkin-Elmer 141 instrument using 100 mm semi-microcells.

Isolation of the polysaccharide from *Klebsiella* K-type 57 (strain 4425/51) was performed as earlier described.[2] The polysaccharide showed $[\alpha]_{589}^{20}$ +104° (*c* 0.3, water). In the IR spectrum (KBr), no significant absorptions around 1735 cm^{-1} (*O*-acyl region) were observed. The percentages of nitrogen (0.78 %) and phosphorus (0.67 %) in the material were insignificant. In the ^{13}C NMR spectrum the signal at δ_{TMS} 176.1 was attributed to the carboxyl carbon and the four signals δ_{TMS} 106.1, 102.5, 101.6, and 96.8 to the four anomeric carbons. Skeleton carbons appeared in the region δ_{TMS} 62.0 – 81.0.

Sugar and methylation analyses and carboxyl-reduction of the methylated polymer were performed essentially as described before.[4,5,8,14] The partially methylated and trideuteriomethylated alditol acetates gave mass spectra in which fragments containing a trideuteriomethoxy group were recognized by the shift of three mass units. Mannose and galactose were isolated by paper chromatography from a hydrolysate of carboxyl-reduced polysaccharide and showed $[\alpha]_{578}^{27}$ +11° (*c* 0.4, water) and $[\alpha]_{578}^{27}$ +43° (*c* 0.4, water), respectively.

Carboxyl-reduction of the native polysaccharide was performed by the procedure of Taylor and Conrad.[3] Three consecutive treatments were needed to obtain complete reduction.

Uronic acid degradation of the methylated polysaccharide. A solution of carefully dried, methylated polysaccharide (23 mg) and toluene-*p*-sulfonic acid (≃0.5 mg) in a mixture (4 ml) of methyl sulfoxide and 2,2-dimethoxypropane (19:1) was prepared in a serum vial which was sealed with a rubber cap. The vial was flushed with nitrogen and kept in an ultrasonic bath for 30 min. Methylsulfinyl anion in methyl sulfoxide (2 M, 2 ml) was added and the solution sonicated for another 30 min. The vial was kept at room temperature overnight. The reaction mixture was added dropwise to ice-water (10 ml) and 50 % aqueous acetic acid (10 ml) was added. The aqueous solution was extracted with chloroform (4 × 15 ml) and the combined chloroform extracts were washed with water (5 × 15 ml) and concentrated to dryness. The product was treated with 10 % aqueous acetic acid (20 ml) for 1 h at 100 °C and then concentrated to dryness and dissolved in a mixture (10 ml) of *p*-dioxane and ethanol (8:3). Sodium borodeuteride (65 mg) was added and the mixture was stirred overnight. The mixture was then treated with Dowex 50 (H^+, prewashed with *p*-dioxane-ethanol), and boric acid was removed by repeated distillations with methanol (4 × 5 ml). Part (1/2) of this material was hydrolysed, and the resulting sugars were analysed, as their alditol acetates, by GLC-MS [5,6] (Table 1, column D). Another part (1/2) of the material was trideuteriomethylated, hydrolysed and the sugars analysed (Table 1, column E).

In order to isolate an oligomeric product a larger amount (200 mg) of methylated polysaccharide was degraded as described above using appropriate amounts of reagents. The degraded material was reduced with sodium borodeuteride in *p*-dioxane-ethanol and remethylated using methyl iodide. The remethylated product was recovered by partition between chloroform and water and purified by two successive injections on the liquid chromatography columns yielding permethylated 3-*O*-α-D-mannopyranosyl-D-galactitol (8 mg). On TLC (silica gel, hexane-acetone, 2:3) the compound showed R_F 0.71 and on GLC (OV-1 column at 200 °C) T_{mel} 0.73 (retention time relative to permethylated melibiitol). The MS [6] showed, *inter alia*, the following peaks (relative intensities in brackets): 88(100), 89(32), 101(84), 133(7), 155(7), 187(34), 219(13), 236(8). The compound showed $[\alpha]_{578}^{24}$ +15° (*c* 0.4, chloroform). In the ^{1}H NMR spectrum the signal from the anomeric proton was obtained at δ 5.09, $J_{1,2}$ 1.5 Hz. Part of the material was hydrolysed, and the sugars analysed, as their alditol acetates, by GLC-MS.[5,6] 2,3,4,6-Tetra-*O*-methyl-D-mannose and 1,2,4,5,6-penta-*O*-methyl-D-galactitol (monodeuterated at C-1) were obtained in comparable amounts in this analysis.

Partial acid hydrolysis of the polysaccharide, isolation and characterization of oligosaccharides. Native polysaccharide (150 mg) was hydrolysed with 0.25 M sulfuric acid (35 ml) for 8 h at 100 °C. After neutralisation with barium carbonate the hydrolysate was fractionated on a Sephadex G-15 column (90 × 2.5 cm) eluted with water. The fractionation was monitored by differential refractometry. The fractions eluted with the volumes expected for mono-, di-, and trisaccharides were further investigated. They were purified by paper chromatography yielding mannose (30 mg), galactose (15 mg), galacturonic acid (5 mg), the aldobiouronic acid (10 mg), and the aldotriouronic acid (13 mg). On paper chromatography the latter two compounds showed R_{man} 0.60 and 0.33, respectively. The aldobiouronic acid showed $[\alpha]_{589}^{20}$ +66° (*c* 0.3, water) and the aldotriouronic acid showed $[\alpha]_{589}^{20}$ +124° (*c* 0.4, water). The aldobio- and triouronic acids were reduced to their alditols with sodium borodeuteride. In the ^{1}H NMR spectrum (D_2O, 25 °C) of the aldobiouronic acid alditol the signal from the anomeric proton was obtained at δ 5.10, $J_{1,2}$ 3.0 Hz. The corresponding figures for the aldotriouronic acid alditol were δ 5.17, $J_{1,2}$ 3.2 Hz and δ 5.22, $J_{1,2}$ 1.5 Hz. Part of the alditol preparations was methylated using methylsulfinyl anion-methyl iodide and recovered by partition between chloroform and water. On GLC (OV-1 column at 200 °C) the permethylated aldobiouronic acid alditol showed T_{mel} 1.2 and the MS [6] contained, *inter alia*, the following fragments: 45(100), 46(26), 88(34), 89(41), 101(68), 201(24), 233(9), 236(16). The corresponding figures for the permethylated aldotriouronic acid alditol were T_{mel} 6.4 (OV-1 column at 230 °C) and 45(96), 46(18), 88(52), 89(27), 90(12), 101(100), 201(60), 233(44), 236(20), 405(5). The permethylated samples were carboxyl-reduced with sodium borodeuteride in *p*-dioxane-ethanol (8:3), hydrolysed, transformed into alditol acetates and analysed by GLC-MS.[5,6] The aldobiouronic acid derivative yielded 1,3,4,5,6-penta-*O*-methyl-D-mannitol (monodeuterated at C-1) and 2,3,4-tri-*O*-methyl-D-galactose (dideuterated at C-6). The aldotriouronic acid derivative yielded 1,2,4,5,6-penta-*O*-methyl-D-galactitol (mono-deuterated at C-1), 3,4,6-tri-*O*-methyl-D-mannose, and 2,3,4-tri-*O*-methyl-D-galactose (dideuterated at C-6).

Smith degradation of the polysaccharide. Car-

boxyl-reduced polysaccharide (29 mg) was dissolved in 0.1 M sodium acetate buffer pH 3.9 (20 ml) and sodium metaperiodate (0.2 M, 5 ml) was added. The reaction mixture was kept in the dark at 4 °C for 120 h. Excess of periodate was destroyed by adding ethylene glycol (1 ml) and the mixture was dialysed overnight. The solution was concentrated to 25 ml and sodium borohydride (300 mg) added. After 9 h at room temperature the excess of borohydride was destroyed by adding 50 % aqueous acetic acid (50 ml) under external cooling with ice-water. The solution was then dialysed. Part of the product (1/15) was subjected to sugar analysis; the only sugar detected in the hydrolysate was galactose. The main part of the periodate oxidized-reduced polysaccharide was methylated by two successive treatments with methylsulfinyl anion-methyl iodide. The methylated material was recovered by dialysis and freeze-drying. Part (1/3) of the material was hydrolysed and the sugars were analysed, as their alditol acetates, by GLC-MS [5,6] (Table 1, column F). Another part (2/3) was treated with 90 % formic acid (10 ml) for 1 h at 40 °C. The solution was evaporated to dryness and the residue was dissolved in a mixture (10 ml) of *p*-dioxane-ethanol (8:3). Sodium borodeuteride (60 mg) was added and the reaction mixture was stirred overnight. The reaction was worked up by treatment with Dowex 50-methanol and subjected to trideuteriomethylation. The methylated material was recovered by partition between chloroform and water. Part of this product was investigated by GLC-MS [6] using an OV-1 column at 210 °C. A peak showing T_{mel} 2.6 was obtained. The MS showed, *inter alia*, the following peaks: 45(100), 49(32), 91(66), 101(40), 104(87), 107(25), 187(47), 190(25), 222(29), 377(3). Another part of the trideuteriomethylated material was hydrolysed and the sugars were analysed, as their alditol acetates, by GLC-MS [5,6] (Table 1, column G).

In order to isolate an oligomeric product a larger amount (120 mg) of carboxyl-reduced polysaccharide was periodate oxidized and reduced with borohydride as described above, using appropriate amounts of reagents. The degraded material was methylated twice, hydrolysed with formic acid and reduced with borohydride in *p*-dioxane-ethanol. The product was finally remethylated using methylsulfinyl anion/methyl iodide. The remethylated product was recovered by partition between chloroform and water and purified by two successive injections on the liquid chromatography column yielding permethylated β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→2)-glycerol (3 mg). On TLC (silica gel, hexane-acetone, 2:3), the compound showed R_F 0.65. The retention time on GLC and the MS (allowing for shifts due to deuterium labelling) were the same as for the deuterated analogue described above. The compound showed $[\alpha]_{578}^{25}$ +41° (*c* 0.1, chloroform). In the ^1H NMR spectrum the signals from the anomeric protons were obtained at δ 5.25, $J_{1,2}$ 3.5 Hz and δ 4.65, $J_{1,2}$ 7.0 Hz. Part of the material was hydrolysed and the sugars analysed, as their alditol acetates, by GLC-MS.[5,6] 2,3,4,6-Tetra-*O*-methyl-D-galactose and 2,4,6-tri-*O*-methyl-D-galactose were obtained in equimolecular amounts.

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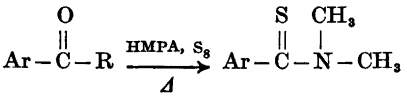
Studies on Organophosphorus Compounds. XII.* About Elemental Sulfur as a Reducing Agent in Hexamethylphosphoric Triamide (HMPA)

J. PERREGAARD, I. THOMSEN and S.-O. LAWESSON

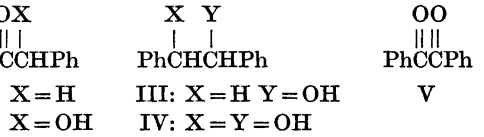
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Deoxybenzoin, benzoin, 1,2-diphenyl-1,2-ethanediol, 1,2-diphenylethanol, and benzil all give *E*-stilbene in fair to good yields, when reacted with S_8 in HMPA at elevated temperatures. Benzoin is first oxidized to benzil and then reduced to stilbene. 1,2-Diphenylethanol undergoes H_2O-elimination to deoxybenzoin and subsequent reduction to stilbene. Isolation of bis(diphenylethyl)polysulfide in the reactions of 1,2-diphenylethanol and 1,2-diphenyl-1,2-ethanediol and further thermal decomposition of the polysulphide to *E*-stilbene and S_8, indicates this compound as a possible intermediate in these reactions. Oxidative degradation of the five substrates produces *N*,*N*-dimethylthiobenzamide, which partly undergoes demethylation to give *N*-methylthiobenzamide. Also the following heterocycles were isolated: 4,5-diphenyl-3*H*-1,2-dithiole-3-thione, 3-methyl-4,5-diphenyl-4-thiazoline-2-thione, 4,5-diphenyl-4-thiazoline-2-thione, and tetraphenylthiophene.

Recently it was shown [1] that elemental sulfur in HMPA smoothly oxidizes different substrates as aldehydes, ketones, alcohols, halides, and amines. The reaction times are short and generally the yields are very acceptable. Contrary to the well-known Willgerodt-Kindler reaction alkyl aryl ketones are oxidized and degraded to aromatic *N*,*N*-dimethylthiocarboxamides.

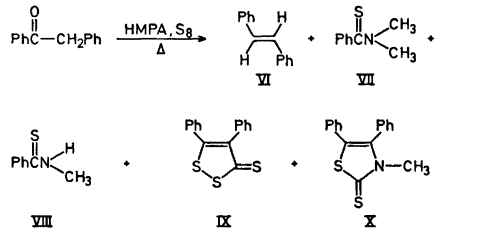


As a logical continuation of that investigation some other types of ketones and alcohols have been studied and this paper deals with the substrates I – V.



RESULTS AND DISCUSSION

The reason for investigating the substrates I – V was, that the ketone I when heated in HMPA at 205 °C with excess of sulfur gave as main product, *E*-stilbene, VI, and also the expected degradation products, *N*,*N*-dimethylthiobenzamide, VII, and *N*-methyl thiobenzamide, VIII, and two heterocycles, 4,5-diphenyl-3*H*-1,2-dithiole-3-thione, IX, and 3-methyl-4,5-diphenyl-4-thiazoline-2-thione, X (Scheme 1).



Scheme 1.

These results were not expected and to our knowledge not observed earlier. Some related substrates, II – V, with two terminal phenyl groups were then studied, and the intention

* Part XI see Ref. 1.

Table 1. Reaction conditions and product distribution from the reaction of elemental sulfur in HMPA with the substrates I – V.

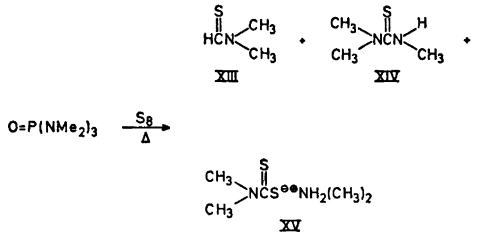
Substrate [a]	Reaction temp. (°C)	Reaction time (min)	Products (%)							
			VI	VII	VIII	IX	X	XI	XII	XVI
I	205	30	73	11	2	1	1			
II	205	120	65	14	4	2	2			
III	205	20	70	3	2	2	1			19
IV	205	25	59	7	3	2	1			10
V	225	45	37	15	4	2	6	4	3	

[a] In all reactions were used 0.3 mol of S_8, 25 ml of HMPA, and 0.05 mol of substrate.

was to find out to what extent elemental sulfur would react as a reducing agent in HMPA. In literature very few reports have been given about sulfur as a reducing agent in the presence of amines. Staneck has reported that benzophenone and sulfur in morpholine give diphenylmethane and bis(diphenylmethyl)disulfide.[2,3] Moreau reinvestigated this reaction [4] and demonstrated that H_2S played a key-role. The same was the case with reduction of chalcone.[5]

In Table 1 are seen the reaction conditions and the product distribution.

Earlier it has been shown that sulfur oxidizes HMPA under similar conditions as used here. The products found resulted from oxidation or oxidative degradation of the dimethylamino groups of HMPA and were the expected products XIII – XV (Scheme 2).

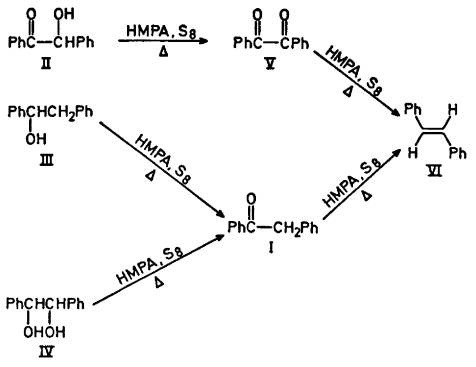


Scheme 2.

E-Stilbene is the main product isolated from all five reactions (Table 1). However, it is not known, if *Z*-stilbene is first formed, as it smoothly and completely is converted into *E*-stilbene by heating with S_8 in HMPA at 200 °C for 1.5 h. Based on the present results it is premature at this stage of the investigation to suggest a mechanism for the formation of stilbene. However, a few features should be noted: The reaction between benzoin, II, and S_8 produces benzil, V, in 86 % yield, when the reaction time is short (a few minutes) (Scheme 3). Benzoin, fused with sulfur, also gives benzil.[6] The reduction of benzoin produces a considerably higher yield of stilbene than benzil does which would indicate that H_2S plays a keyrole in the reaction. It is also observed that H_2S is immediately formed in the reaction of II with S_8, while the detection of H_2S in the reaction of V with S_8 is slow. It is suggested that the formation of H_2S in the last reaction results from oxidation of the dimethylamino groups of HMPA. A search in literature shows that benzoin has not been reacted with H_2S alone, but with HCl present to give 2,3,5,6-tetraphenyl-1,4-dithiacyclohexadiene as the main product and *E*-stilbene in minor amounts (5 %).[7] Also benzil was only reduced to deoxybenzoin or benzoin under basic conditions,[8] so probably the reduction under our conditions goes through other intermediates.

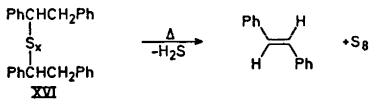
In the reaction of 1,2-diphenylethanol, III, deoxybenzoin, I, could be isolated when the reaction was interrupted before total conversion had occurred. However, as I does react very fast with S_8 to give VI only small amounts of I could be isolated. Also in the reaction of 1,2-diphenyl-1,2-ethanediol, IV, with S_8, I was isolated as an intermediate, formed by H_2O-elimination from IV (Scheme 3).

1,2-Diphenylethanol, III, and 1,2-diphenyl-1,2-ethanediol, IV, both produce bis(1,2-diphenylethyl)polysulfides, XVI, as minor products. According to a microanalysis XVI had an average content of sulfur atoms of 3.2. A disulfide has earlier been reported as intermediate in the reduction of benzophenone with sulfur in



Scheme 3.

boiling morpholine.[4] Thiobenzophenone was suggested to be first formed and then reduced to the disulfide. So probably under our conditions the initially formed deoxybenzoin, I, reacts further, possibly *via* the corresponding thioketone, to XVI, which finally decomposes to *E*-stilbene. In fact, attempts to distill XVI under reduced pressure gave *E*-stilbene and sulfur:



Reduction of XVI with Zn and HCl produces 1,2-diphenylethylmercaptan, XVII. The same reduction of the corresponding disulfide is known.[8] A very small fraction possibly containing the polysulfane, XVIII, was isolated in the reaction of IV.

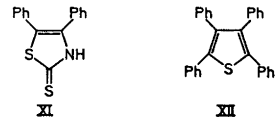


XVII XVIII

TLC (R_F-values) of XVII and XVIII were identical and the NMR-spectra were similar. However, the SH-proton in XVII was found as a doublet at δ 1.9, while the S_xH-proton of XVIII was found as a singlet at δ 2.0. Further, signals in the NMR-spectrum of XVIII at δ 2.4 and 2.5 were probably due to impurities as repeated purification (column chromatography) changed the ratios of these signals to the other signals in the spectrum.

It has recently been shown,[10] that deoxybenzoin when heated in HMPA yields 13 % of *N,N*-dimethylbenzamide. It is also known that carboxamides are easily converted to thiocarboxamides by reaction with S_8 in HMPA and by reaction with dithiophosphoric acids.[11] The formation of *N,N*-dimethylthiobenzamide, VII, (Table 1) can thus be accounted for in several ways.[1] That VII undergoes demethylation at elevated temperatures in HMPA with S_8 present is also since recently a known reaction.[1]

In the reaction of benzil, V, a higher temperature (225 °C) is needed to get fair yields of products. Small yields of tetraphenylthiophene, XII, are also isolated, maybe due to the known reaction of stilbene with S_8 at 240 – 250 °C to give XII.[12] Also in this reaction three other products were isolated, *viz* IX, X, and 4,5-diphenyl-4-thiazoline-2-thione, XI.



XI XII

EXPERIMENTAL

NMR-spectra were recorded at 60 MHz on a Varian A-60 spectrometer. TMS was used as internal reference standard. The chemical shifts are expressed in δ-values (s = singlet, d = doublet, m = multiplet). IR-spectra were recorded on a Beckman IR-18 spectrophotometer. Mass-spectra were recorded on a CEC 21-104 operating at 70 eV, using direct inlet. Direct inlet temperature 180 – 190 °C, ion source temperature 250 °C. Column chromatography was carried out with Silica gel 60 (Merck) R_F-values on TLC are given with Silica gel as supporting material and elution with light petroleum-CH_2Cl_2 (1:1). Commercial HMPA dried over molecular-sieves was used. Elemental analyses were performed by Novo Industry A/S, Copenhagen. M.p's are uncorrected.

General procedure. 0.05 mol of substrates were heated under vigorous stirring with 0.3 mol of S_8 in 25 ml of HMPA until the substrate was consumed (followed on TLC) (in the reaction of benzoin until benzil was used up). After cooling to room temperaure 250 ml of water and 100 ml of ether were added, and non-reacted sulfur was filtered off. After ether extraction (3 × 100 ml) and washing with 2 × 50 ml of H_2O the combined ether phases were dried ($CaSO_4$) and the ether evaporated. The mixture was then dissolved in hot CCl_4. *E*-Stilbene crystallized from the solution on cooling and was filtered off. After evaporation of CCl_4 the remaining mixture was column chromatographed. Mixtures of CH_2Cl_2 and light petroleum were used as eluent, starting with light

petroleum and raising the polarity. Most, but not all of the compounds in the mixtures could be purified and characterized in this way. The yields of the compounds thus isolated are given in Table 1, and the physical properties are as follows:

E-*Stilbene* (VI): M.p. 122 – 123 °C (CCl_4) (lit.[1] m.p. = 122 °C). R_F-value: 0.82. Spectral evidence was in accordance with an authentic sample.[1]

N,N-*Dimethylthiobenzamide* (VII): M.p. 67 °C (EtOH) (lit.[1] m.p. 67 °C). R_F-value: 0.25. Spectral evidence was in accordance with an authentic sample.[1]

N-*Methylthiobenzamide* (VIII): M.p. 77 – 78 °C (EtOH) (lit.[1] m.p. 77 – 78 °C). R_F-value: 0.15. Spectral evidence was in accordance with an authentic sample.[1]

4,5-Diphenyl-3H-1,2-dithiole-3-thione (IX): M.p. 159 °C ($CHCl_3$) (lit.[1] m.p. 159 °C). R_F-value: 0.42. Spectral evidence was in accordance with an authentic sample.[1]

3-Methyl-4,5-diphenyl-4-thiazoline-2-thione (X): M.p. 199 °C (ether – $CHCl_3$) (lit.[13], m.p. 201 – 202 °C). R_F-value: 0.43. (Found: C 67.40; H 4.62; N 5.16; S 22.62. $C_{16}H_{13}NS_2$ requires: C 67.81; H 4.62; N 4.94; S 22.63). NMR ($CDCl_3$): 3.5(s,3H); 7.2(m,10H). IR ($CHCl_3$): 1100, 1435, 1480, 1580 and 2950 cm^{-1}. MS: *m/e* 283 (M^+, 100 %), 267($M-CH_3$, 30 %), 210($M-CH_3NCS$, 19 %), 178(PhCCPh, 33 %), 165($M-PhCNCH_3$, 71 %), 121($PhCS^+$, 23 %), 118($PhCNCH_3^+$, 28 %), 89 (27 %), 77 (52 %).

4,5-Diphenyl-4-thiazoline-2-thione (XI): M.p. 223 – 224 °C (MeOH – $CHCl_3$)(lit.[14] m.p. 224 °C). R_F-value: 0.40. (Found: C 66.37; H 4.08; N 5.18; S 23.76. $C_{15}H_{11}NS_2$ requires: C 66.88; H 4.12; N 5.20; S 23.80). NMR (CF_3COOH): 7.4(m). IR (KBr): 1110, 1480, 1590, 3050 and 3400 cm^{-1}. MS: *m/e* 269 (M^+, 100 %), 210 ($M-CSNH$, 40 %), 178 (PhCCPh, 21 %), 165 ($M-PhCNH$, 68 %), 121 ($PhCS^+$, 40 %), 89 (20 %), 77 (25 %).

Tetraphenylthiophene (XII): M.p. 184 °C (ether – $CHCl_3$)(lit.[1] m.p. 181 – 183 °C). R_F-value: 0.81. Spectral evidence was in accordance with an authentic sample.[1]

Bis(1,2-diphenylethyl)polysulfide (XVI): A viscous liquid with R_F-value (on silica gel eluted with 10 % CH_2Cl_2 in light petroleum): 0.26. (Found: C 72.44; H 5.68; S 22.21, which gives $C_{28}H_{25.6}S_{3.2}$). NMR ($CDCl_3$): 3.3(m, 4 H), 4.4(m, 2 H), 7.2(m, 20 H). IR (CCl_4): 1070, 1440, 1485, 2900 and 3050 cm^{-1}.

Reduction of XVI. To 200 mg of XVI and 2 g of Zn-powder in 10 ml of EtOH were dropwise added 10 ml of 4 N HCl, during 30 min under stirring. Addition of H_2O (50 ml) followed by ether extraction (2 × 25 ml), drying ($CaSO_4$), and evaporation of the ether gave a mixture which was column chromatographed to yield 120 mg of *1,2-diphenylethylmercaptan*, m.p. 37 – 38 °C (lit.[9] m.p. 41 °C). R_F-value (on silica gel eluted with 10 % CH_2Cl_2 in light petroleum): 0.34. NMR ($CDCl_3$): 1.9 (d, 1 H), 3.2 (m, 2 H), 4.3 (m, 1 H), 7.2 (m, 10 H).

1,2-Diphenylethylpolysulfane (XVIII): R_F-value (on silica gel eluted with 10 % CH_2Cl_2 in light petroleum): 0.34. NMR ($CDCl_3$): 2.0 (s, 1 H), 3.4 (m, 2 H), 4.3 (m, 1 H), 7.2 (m, 10 H).

Isolation of intermediates

Benzoin (10.6 g) was heated for 5 min at 190 °C with 9.6 g of S_8 in 25 ml of HMPA. The above described working-up procedure was used. Recrystallization from MeOH of the obtained product yielded 9.0 g (86 %) of *benzil*, m.p. 95 °C (lit.[15] m.p. 95 °C). IR (CCl_4): 865, 1170, 1200, 1440, 1590, 1670 and 3050 cm^{-1} which was identical with an IR-spectrum of a commercially available compound.

I. 1,2-Diphenyl-1,2-ethanediol (5.4 g) was heated for 40 min at 185 °C with 4.8 g of S_8 and 12.5 ml of HMPA. The same working-up procedure as above and column chromatography with CH_2Cl_2 – light petroleum (1:1) yielded first a mixture of products and then 290 mg of *deoxybenzoin*, m.p. 58 °C (lit.[16] m.p. 60 °C). NMR ($CDCl_3$): 4.3 (s, 2 H); 7.4 (m, 8 H); 8.0 (m, 2 H). IR (CCl_4): 990, 1270, 1450, 1500, 1590, 1600, 1690, 3040 cm^{-1}. These spectra were identical with spectra of a commercially available compound. Finally 3.8 g of starting material was eluted with CH_2Cl_2 – ether (1:1).

II. 1,2-Diphenylethanol (9.9 g) was heated for 12 min at 204 °C with 9.6 g of S_8 in 25 ml of HMPA. The same working-up procedure as for 1,2-diphenyl-1,2-ethanediol yielded 180 mg of *deoxybenzoin*, characterized as above, and 4.5 g of starting material.

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Studies on Organophosphorus Compounds. XIII.* About Conversion of Carboxylic Esters into *N,N*-Dimethylthiocarboxamides. Synthesis of 2-Phenylbenzothiazole

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In a one pot reaction esters of carboxylic acids, RCOOR′, are converted directly into the corresponding *N,N*-dimethylthiocarboxamides, $RCSNMe_2$, by heating with sulfur in HMPA. The benzylic part of esters $RCOOCH_2Ph$ is oxidized to *N,N*-dimethylthiobenzamide. Methyl and allyl benzoates produce *N,N*-dimethylbenzamide. The methyl group of methyl benzoate is oxidized to carbon disulfide with dimethylpolysulfides as probable intermediates. 2-Phenylbenzothiazole is formed in the reaction of benzyl benzoate with sulfur and aniline in HMPA at 185 °C. Also benzaldehyde, benzyl alcohol, and thiobenzanilide produce 2-phenylbenzothiazole in high yields under similar reaction conditions.

Recently it was shown that sulfur in hexamethylphosphoric triamide (HMPA) is a very convenient thiation agent at elevated temperatures.[1] Thus different benzylic derivatives, $PhCH_2X$, alkyl aryl ketones, benzaldehydes, and α- and γ-picolines were smoothly converted into the corresponding aromatic *N,N*-dimethylthiocarboxamides. Reducing properties of S_8 in HMPA have also been established.[2] In a reaction of ethyl phenylacetate [1] both *N,N*-dimethylthiobenzamide, I, and phenyl-*N,N*-dimethylthioacetamide, II, were isolated:

$$PhCH_2COOEt \xrightarrow[\Delta]{S_8,\ HMPA} PhCSNMe_2 + PhCH_2CSNMe_2$$

I II

This means that a carboxylic ester directly had been converted into the corresponding *N,N*-dimethylthiocarboxamide. This prompted us to investigate the scope of the reaction, and a series of benzoates, methyl nicotinate and some aliphatic esters were reacted with sulfur in HMPA at elevated temperatures.

RESULTS AND DISCUSSION

Many functional groups have been converted into thiocarboxamides by heating with sulfur and amines,[3] but as far as we know direct conversion of an ester into a thiocarboxamide has not been reported. In fact many reactions with sulfur at high temperatures have been performed, where the ester group has remained unchanged.[4,5] In most of our reactions the esters were smoothly converted into the corresponding *N,N*-dimethylthiocarboxamides as indicated in Table 1. In the reactions of benzyl benzoate and benzyl butyrate the benzylic part of the esters was oxidized to *N,N*-dimethylthiobenzamide, I.

$$RCOOCH_2Ph \xrightarrow[\Delta]{S_8,\ HMPA} RCSNMe_2 + PhCSNMe_2$$

IV I

It appears that esters containing ethyl or longer primary or secondary alkyl groups as the alcoholic parts are quite difficult to react. In these reactions starting material was inevitably

* Part XII, see Ref. 2.

Table 1.[a]

$$\underset{\text{III}}{\text{RCOOR}'} \xrightarrow[\Delta]{S_8,\ \text{HMPA}} \underset{\text{IV}}{\text{RCSNMe}_2}$$

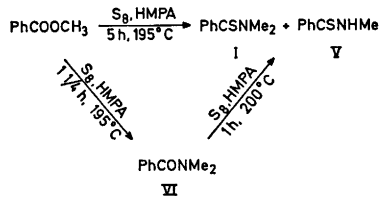
R	R′	React.time (h)	React.temp. (°C)	Yield of IV (%)	Yield of PhCSNHMe (%)	Other products
Ph	Ph	1	207	89	<5	
Ph	$PhCH_2$	1.5	205	91	<5	
Ph	Me	5	195	86	6	[b] CS_2, $CH_3S_xCH_3$, CH_3SH, $PhCONMe_2$
Ph	Et	2	210	53	10	23 % of III recovered
Ph	i-Pr	3.5	205	27	11	58 % of III recovered
Ph	cyclohexyl	2	210	18	9	67 % of III recovered
Ph	*t*-Bu	1.75	200	80	19	
Ph	allyl	0.1	180 – 230	52	5	$PhCONMe_2$ (12 %)
C_5H_4N	Me	4	185	87		
Me	Ph	1.25	185	59		
i-Pr	Ph	1.75	205	71		
Pr	$PhCH_2$	1	210	40		30 % of III recovered, $PhCSNMe_2$ (40 %)
hexyl	Et	5	reflux			III recovered

[a] 0.1 mol of substrate, 0.6 mol of S_8 and 50 ml of HMPA were used in all reactions. [b] Isolated in separate reactions.

recovered (Table 1). In the reactions of the benzoates oxidative demethylation of *N,N*-dimethylthiobenzamide, I, produced *N*-methylthiobenzamide, V, in minor amounts. From NMR-spectra of the reaction mixtures the yields of V were estimated (Table 1). This demethylation has also been observed earlier where *N,N*-dimethylthiobenzamide has been produced at high temperatures in HMPA in the presence of S_8.[1,2]

Detailed mechanistic considerations have to await further investigations of HMPA + S_8 as a thiation agent, but a few observations should be noted. *N,N*-Dimethylbenzamide, VI, was found in the reactions of methyl and allyl benzoates with S_8 in HMPA. Methyl benzoate yielded 61 % of VI after a reaction time of about one hour, but further reaction produced I and V exclusively. Reaction of VI with sulfur in HMPA also gave I and V.[6]

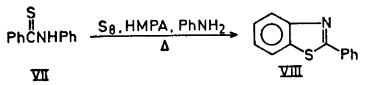
In the reaction of methyl benzoate a low-boiling fraction distilled from the reaction vessel and addition of dimethylamine to it produced,



dimethylammonium dimethyldithiocarbamate which shows the formation of CS_2. Further proof was obtained by a mass spectrum. Small amounts of methylmercaptan were also detected by spectroscopic methods (NMR, MS). In a reaction, run under less severe reaction conditions, dimethyltrisulfide was isolated in 24 % yield, and only very small amounts of CS_2 were isolated. Also other dimethylpolysulfides were probably present, as the fraction from which the trisulfide was isolated gave a distillation residue with the approximate composition of $C_2H_6S_5$. Three singlet peaks in an NMR-spectrum at δ 2.6 further indicate the

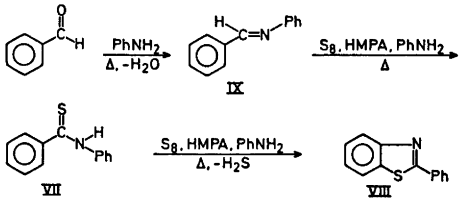
presence of the tetra-, penta-, and hexasulfides. For the formation of these polysulfides it is tentatively suggested that *S*-methyl groups are cleaved from an intermediate in the reaction, and CS_2 is formed by further oxidation of these.

In an attempt to prepare thiobenzanilide, VII, the reaction of benzyl benzoate with S_8 in HMPA was run with aniline present. However, VII was only isolated in minor amounts and the main products were 2-phenylbenzothiazole, VIII, and benzanilide. Apparently VII has reacted further to give VIII. In a separate experiment this was proved, as VII was oxidized to VIII with sulfur in HMPA with aniline present:



VII VIII

These findings made us investigate reactions with other substrates than esters. From benzyl alcohol and benzaldehyde high yields of VIII were obtained. Benzaldehyde first reacts with aniline to give *N*-benzylidene aniline, IX, which was isolated in 85 % yield when the reaction was run at 130 – 140 °C. IX reacts further with sulfur in HMPA in the presence of aniline to give thiobenzanilide, VII. This was confirmed in a separate experiment, when IX was heated with sulfur in HMPA in the presence of aniline at 170 °C for 40 min to give 42 % of VII. Finally oxidative ring-closure of VII produces VIII in an over-all yield of 80 %.



VII VIII

VIII has earlier been prepared in 64 % yield by fusing *N*-benzylidene aniline with S_8 at 250 – 60 °C.[7] Also other reactions are known, when S_8 and aniline are heated with different substrates to give benzothiazoles. Recently it has been reported that formaldehyde, when heated with S_8 and aniline in a sealed tube above 200 °C, produces 2-mercaptobenzothiazole.[8] It has also been shown that α- and γ-picolines produce the corresponding benzothiazoles when reacted with S_8 in the presence of aniline.[9,10]

EXPERIMENTAL

NMR-spectra were recorded at 60 Mc/s on a Varian A-60 spectrometer. TMS was used as internal reference standard. The chemical shifts are expressed in δ-values (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). IR-spectra were recorded on a Beckmann IR-18 spectrophotometer. Mass-spectra were recorded on a CEC 21-104 operating at 70 eV, using direct inlet. Ion source temperature 250 °C. Column chromatography was carried out with Silica gel 60 (Merck). Commercial HMPA dried over molecular sieves (3A) was used. Elemental analysis were performed by Novo Industry A/S, Copenhagen. M.p's and b.p's are uncorrected.

General procedure for the reactions of the esters

0.1 mol of ester was heated under stirring with 0.6 mol of S_8 in 50 ml of HMPA until the ester or all S_8 was consumed, (for reaction conditions, see Table 1.) After cooling to room temperature 300 ml of H_2O and 100 ml of ether were added, and non-reacted S_8 was filtered off. After ether extraction (3 × 100 ml) and washing with 2 × 50 ml of H_2O the combined ether phases where dried ($MgSO_4$), and the ether evaporated. In the reactions of the benzoates the yields of *N*-methylthiobenzamide were estimated from NMR-spectra of these worked-up mixtures. Distillation twice afforded the *N*,*N*-dimethylthiocarboxamides (Table 1). Exceptions to this general procedure and psysical properties of the isolated thiocarboxamides are given below.

All the benzoates were reacted and worked up according to the procedure above to give N,N-*dimethylthiobenzamide*, m.p. 67 °C (EtOH) (lit.[1] m.p. 67 °C), except for *allyl benzoate*, which reacted exothermically. Column chromatography (elution with ether) of the worked-up reaction mixture yielded 8.6 g (52 %) of N,N-*dimethylthiobenzamide* and 1.8 g (12 %) of N,N-*dimethylbenzamide*, which had physical properties in accordance with an authentic sample.[1]

The reaction of methyl benzoate was repeated, and interruption after 1.25 h at 195 °C gave a reaction mixture, which after the working-up procedure above, followed by column chromatography (elution with mixtures of ether/light petroleum) yielded: I. 3.2 g of a mixture of products. Distillation (52 – 58 °C/14 mmHg) gave 1.5 g (24 %) of *dimethyltrisulfide*. (Found: C 19.03; H 4.71; S 76.41. $C_2H_6S_3$ requires: C 19.05; H 4.76; S 76.19).

NMR ($CDCl_3$): 2.5(s). An NMR-spectrum ($CDCl_3$) of the distillation residue showed three singlets at $\delta \sim 2.6$. (Found: C 12.15; H 2.90; S 84.75 which gives $C_6H_{5.8}S_{5.2}$). II. 2.3 g (14 %) of N,N-*dimethylthiobenzamide* and III. 9.1 g (61 %) of N,N-*dimethylbenzamide*. In a third reaction of methyl benzoate at 205 °C for 1.5 h, 6.9 g of a low-boiling liquid were trapped in a cool-bath, b.p. 43–47 °C. NMR (without solvent): 1.2 (q, 1 H), 2.1 (d, 3 H), which was identical with an NMR-spectrum of authentic CH_3SH in CS_2. MS: *m/e* 76(CS_2^+, 100 %), 48(CH_3SH^+, 10 %). 2 g of the liquid were dissolved in 25 ml of CH_2Cl_2 and $NHMe_2$ was slowly bubbled through for 15 min. Evaporation of CH_2Cl_2 and washing with ether yielded 3.1 g of *dimethylammonium dimethyldithiocarbamate*, m.p. 129–130 °C (lit.[11] m.p. 130–132 °C). Methyl nicotinate (0.1 mol) was heated with S_8 (0.6 mol) in HMPA (50 ml) for 4 h at 185 °C. After cooling to room temperature the reaction mixture was poured into 300 ml of H_2O and 100 ml of benzene. Excess S_8 was filtered off. After benzene extraction (4 × 100 ml), drying ($MgSO_4$) and evaporation of benzene the remaining mixture was distilled twice to give 14.5 g (87 %) of N,N-*dimethylthionicotinamide*, b.p. 151–154 °C/0.7 mmHg, $n_D^{20} = 1.6347$. (Found: C 57.59; H 6.16; N 16.98; S 19.51. $C_8H_{10}N_2S$ requires: C 57.83; H 6.02; N 16.87; S 19.28). NMR ($CDCl_3$): 3.2 (s, 3 H); 3.6 (s, 3 H); 7.5 (m, 2 H); 8.6 (m, 2 H).

The reaction mixture from the reaction of phenyl acetate was directly filtered through a column (elution with ether). Column chromatography (elution with ether/light petroleum) yielded 6.1 g of N,N-*dimethylthioacetamide*. Physical properties were in accordance with an authentic sample.[1]

Column chromatography (elution with ether/light petroleum) of the worked-up reaction mixture from the reaction of phenyl isobutyrate yielded 9.3 g (71 %) of *2-methyl*-N,N-*dimethylthiopropionamide*, b.p. 69–71 °C/0.3 mmHg, $n_D^{20} = 1.5393$. NMR($CDCl_3$): 1.2 (d, 6 H); 3.3 (m, 1 H); 3.4 (s, 3 H); 3.5 (s, 3 H).

Column chromatography (elution with ether/light petroleum) of the worked-up reaction mixture from the reaction of benzyl butyrate yielded 5.3 g (30 %) of starting material and 11.8 g of an approximately 1:1 mixture of N,N-*dimethylthiobutyramide* and N,N-*dimethylthiobenzamide*. Distillation twice yielded pure *N,N*-dimethylthiobutyramide, b.p. 119–120 °C/10 mmHg, $n_D^{20} = 1.5412$. (Found: C 55.11; H 9.92; N 10.62; S 24.34. $C_6H_{13}NS$ requires: C 54.96; H 9.92; N 10.69; S 24.43). NMR($CDCl_3$): 1.0 (t, 3 H); 1.8 (m, 2 H); 2.8 (t, 2 H); 3.3 (s, 3 H); 3.5 (s, 3 H).

Preparation of 2-phenylbenzothiazole

Benzyl benzoate (0.1 mol) was heated with S_8 (0.6 mol) in HMPA (15 ml) and aniline (30 ml) at 185 °C for 6 h. After cooling to room temperature the reaction mixture was poured into 300 ml of 2 M HCl and extracted with ether (4 × 100 ml). The combined ether phases were washed with 50 ml of 2 M HCl and further extracted with 1 M NaOH (4 × 50 ml). After drying ($MgSO_4$) and evaporation of the ether column chromatography (elution with ether/light petroleum) yielded 20.2 g (48 %) of *2-phenylbenzothiazole*, m.p. 113 °C (lit.[7] m.p. 114 °C). NMR ($CDCl_3$): 7.2–8.2 (m). IR (CCl_4): 960, 1120, 1315, 1485, 1515, 3030 and 3070 cm^{-1}. Finally 7.1 g (36 %) of *benzanilide* was eluted, m.p. 162 °C (lit.[12] m.p. 160–161 °C). IR (KBr): 1500, 1540, 1590, 1610 and 1670 cm^{-1}. The NaOH-fraction was acidified with 150 ml of 2 M HCl. After ether extraction (3 × 100 ml), drying ($MgSO_4$) and evaporation of the ether, column chromatography (elution with ether/light petroleum) yielded 1.1 g (3 %) of thiobenzanilide, m.p. 99–100 °C (lit.[13] m.p. 99 °C) NMR (CS_2): 7.0–7.8 (m, 10 H); 9.2 (m, 1 H). IR (KBr): 980, 1360, 1430, 1480, 1520, 1580 and 3100 cm^{-1}.

Thiobenzanilide (6.3 g) was heated with S_8 (0.3 mol) in HMPA (7.5 ml) and aniline (15 ml) at 170 °C for 10 h. The same working-up procedure as in the reaction of benzyl benzoate yielded 5.1 g (82 %) of *2-phenylbenzothiazole*.

Benzyl alcohol. (0.1 mol) was heated with S_8 (0.6 mol) in HMPA (15 ml) and aniline (30 ml) at 165 °C for 10 h. The worked-up reaction mixture (as in the reaction of benzyl benzoate) was recrystallized from EtOH to give 15.4 g (73 %) of *2-phenylbenzothiazole*. Column chromatography (elution with ether/light petroleum) of the worked-up NaOH-phase yielded 1.4 g (7 %) of *thiobenzanilide*.

Benzaldehyde. (0.1 mol) was heated with S_8 (0.6 mol in HMPA (15 ml) and aniline (30 ml) at 170 °C for 12 h. The worked-up reaction mixture (as in the reaction of benzyl benzoate) was recrystallized from EtOH to give 16.9 g (80 %) of *2-phenylbenzothiazole*.

Isolation of *N*-benzylidene aniline. Benzaldehyde (0.2 mol) was heated with S_8 (0.3 mol) in HMPA (15 ml) and aniline (20 ml) at 130–140 °C for 1 3/4 h. After cooling to room temperature the reaction mixture was poured into 300 ml of H_2O and 100 ml of ether. S_8 was filtered off. After ether extraction (4 × 100 ml), washing with H_2O (2 × 50 ml), drying ($MgSO_4$) and evaporation of the ether, distillation yielded 30.6 g (85 %) of *N-benzylidene aniline*, m.p. 54 °C (lit.[14] m.p. 53.5 °C). NMR ($CDCl_3$): 7.4 (m, 8 H); 7.9 (m, 2 H); 8.4 (s, 1 H). Benzylidene aniline (9.1 g) was heated with S_8 (0.3 mol) in HMPA (7.5 mol) and aniline (15 ml) at 170 °C for 40 min. The reaction mixture was worked up as in the reaction of benzyl benzoate.

The NaOH-phase yielded after acidification (1 M HCl) and recrystallization from EtOH 4.5 g (42 %) of *thiobenzanilide*.

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A Convenient Method for Conversion of Acetylenic Derivatives into Conjugated Dienes *via* α-Allenic Alcohols*

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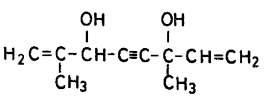
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4-Alkoxy- and 4-(tetrahydro-2-pyranyloxy)-2-butyn-1-ols are in most cases quantitatively converted into conjugated dienes when treated with lithium aluminium hydride in refluxing THF or similar solvents. The reactions proceed *via* α-allenic alcohols. The dienes are free from positional isomers and the formation of alkenynes, which occurs in some cases, can be completely suppressed by a combination of lithium aluminium hydride and aluminium chloride. Most of the conjugated dienes seem to be formed with low stereoselectivity. The *E,E* form is the predominant isomer of 2,4-hexadiene while of 4,6-decadiene the *E,Z* isomer predominates. These latter results may indicate that the attack by hydride on the central carbon atom of the propadienyl group is sterically hindered when the double bond remaining in the same position is formed with *E* configuration.

A few years ago we reported that the acetylenic tetrahydro-2-pyranyloxy (Thp-oxy) compounds *1* and *2* (Table 1) are quantitatively converted into the conjugated dienes *15* and *16* upon treatment with lithium aluminium hydride (LAH) in refluxing tetrahydrofuran (THF).[1] The reactions proceed *via* α-allenic alcohols (strictly alcoholates), which themselves can be prepared according to the scheme in Table 1.[2,3]

As part of a current project covering different aspects of the chemistry of allenes and acetylenes [1,3,10,24,30] we are trying to develop new synthetic methods for other functional groups in which these types of compounds are essential. Consequently, it was of interest to evaluate this apparently simple route to certain conjugated dienes and also to make some mechanistic studies. These continued studies have revealed that (*i*) most primary and secondary α-allenic alcohols are also quantitatively converted into conjugated dienes when treated with LAH in refluxing THF and hence these dienes are directly accessible from the corresponding acetylenes *A* in Table 1, (*ii*) the formation of alkenynes which occurs in some cases [1] can be completely suppressed by a combination of LAH and aluminium chloride in the step $B \rightarrow C$, (*iii*) the attack by hydride on the central carbon atom of the allenic group seems to be sensitive to steric hindrance, giving dienes of different stereochemical forms.

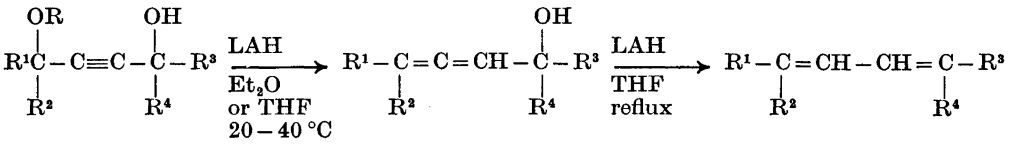
The conversion of α-allenic alcohols into conjugated dienes upon treatment with LAH has been reported by other authors,[4–6,33] most of them starting with substituted 2-butyn-1,4-diols.[4,5] Already in 1954 Whiting and Nayler reported that cosmene (2,6-dimethyl-1,3,5,7-octatetraene) could be easily obtained by LAH reduction of the acetylenic diol *26*, a reaction they presumed to proceed *via* an α-allenic alcohol.[4] To our knowledge three other substituted 2-butyn-1,4-diols have been reported to undergo the same reaction with LAH.[5] Furthermore, skilful carotenoid chemists have reduced the carotenoids, fucoxanthin and neoxanthin, both of which are α-allenic *t*-alcohols, to conjugated



26

* Allenes and Acetylenes X. Part IX: Claesson, A., Olsson, L.-I., Sullivan, G. R. and Mosher, H. S. *J. Amer. Chem. Soc. 97* (1975) 2919.

Table 1. Reactions of acetylenes *A* with lithium aluminium hydride to the conjugated dienes *C*.



A *B* *C*

Acetylenes (*A*)	R	R^1	R^2	R^3	R^4	Dienes (*C*)
1 [1]	Thp	H	H	$-(CH_2)_5-$		$H_2C=CH-CH=$  *15*
2 [1]	Thp	CH_3	CH_3	C_2H_5	C_2H_5	$(CH_3)_2C=CH-CH=C(C_2H_5)_2$ *16*
3 [29]	Thp	H	H	C_4H_9	H	1,3-Octadiene *17* [14]
4 [2]	Thp	C_3H_7	H	H	H	1,3-Heptadiene *18* [15]
5 [24]	*t*-C_4H_9	C_3H_7	H	H	H	1,3-Heptadiene *18* [15]
6	CH_3	C_5H_{11}	H	H	H	1,3-Nonadiene *19* [15,28]
7	Thp	CH_3	H	CH_3	H	2,4-Hexadiene *20*
7-d$_3$	Thp	CH_3	H	CD_3	H	1,1,1-Trideuterio-2,4-hexadiene *20-d*$_3$
8	*t*-C_4H_9	C_3H_7	H	C_3H_7	H	4,6-Decadiene *21*
9 [24]	Thp	H	H	C_3H_7	CH_3	4-Methyl-1,3,-heptadiene *22* [27]
10	CH_3	C_3H_7	H	H	H	4-Methyl-1,3-heptadiene *22* [27]
11	Thp	C_3H_7	H	CH_3	CH_3	2-Methyl-2,4-octadiene *23* [14]
12	Thp	CH_3	CH_3	C_3H_7	H	2-Methyl-2,4-octadiene *23* [14]
13 [3]	CH_3	$(CH_3O)_2CH-CH_2$	CH_3	H	H	$(CH_3O)_2CH-CH_2-C(CH_3)=CH-CH=CH$ *24*
14	CH_3	$HCOCH_2$	CH_3	H	H	$HO-CH_2-CH_2-C(CH_3)=CH-CH=CH_2$ *25*

polyenes with LAH.[6] Grignard reagents also attack α-allenic alcoholates to give conjugated dienes.[7,8]

Finally, the tendency of α-allenic alcohols to be converted into conjugated dienes is also reflected in their smooth rearrangements to halogenated dienes,[9] dienolacetates,[10] and α,β-unsaturated ketones,[10] which reactions probably proceed *via* the mesomeric 2,3-butadienyl↔1,3-butadien-2-yl carbonium ion.[10,11]

The formation of conjugated dienes from acetylenic diols [4,5] has not been fully evaluated as a synthetic method, *i.e.* no diene having a terminal diene group has been prepared, and in all the starting diols at least one of the hydroxy groups has been tertiary as well as

activated by allylic or benzylic position. In contrast, the synthetic method presented here is now rather well understood as to limitations of the structure of the starting acetylenes and in part to the stereochemistry of the products. Owing to the facile preparation of the acetylenic derivatives *A*,[1-3,12] many conjugated dienes of the type *C* are thus easily accessible in three steps from commercial chemicals.

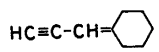
According to our opinion the present synthetic method compares favourably with existing ones [13] owing to the good yields, the absence of positional isomers, and the rather great variation of substituents R^1-R^4.

SYNTHETIC PROCEDURES

The preparations of the starting acetylenes *1–14* follow standard procedures,[1-3,12] and many of them have been described earlier (Table 1).

These acetylenic derivatives were converted into the corresponding dienes by reaction with an excess of LAH in THF or diglyme. The reactions were usually complete (> 90 %) within 4 h as judged by the disappearance of the α-allenic alcohols (GLC). The dienol *25*, however, was formed slowly; only 36 % of impure material was isolated after 8 h.

The dienes were isolated by ordinary work-up followed by simple distillation or, often more conveniently, by preparative GLC on SE-30. Volatile dienes could also be distilled directly from the reaction mixture, a procedure used in the isolation of very pure (> 99 %, GLC) 1,3-heptadiene (*18*) prepared from *5* in diglyme.



27

$HC{\equiv}C-CH=C(CH_3)-C_3H_7$

28

$HC{\equiv}C-CH=CH-C_4H_9$

29

The purity of the distilled dienes is good, better than 90 % in most cases. Furthermore, the dienes are free from positional isomers, and in general further reduction to olefins does not seem to be a problem (< 3 %) as long as a great excess of LAH is avoided. In one preparation of the dienacetal *24* 2.5 mol of LAH were used which, however, led to the formation of 2–3 by-products (20 %). The isolated by-products had a methyl group at a saturated carbon (NMR), clearly indicating that a reduction of the diene had occurred. The dienol *25* is reduced further even if the normal amount of LAH is used.

As already pointed out in our first paper on this subject alkenynes are sometimes formed as by-products.[1] This occurs for acetylenic derivatives having $R^1=R^2=H$ (*i.e. 1*, *3*, and *9*, which give the enynes *27*, *28*, and *29*), or, in other words, when the intermediary α-allenic alcohol *B* has a terminal propadienyl group. The alkenynes are consistently formed in an approximate yield of 10 %.

In attempts to suppress the formation of these alkenynes we soon discovered that the combination of LAH with aluminium chloride had a favourable influence in the step (*B*)→diene (*C*). A molar ratio LAH:$AlCl_3$ of 90–100:1 was found to give the diene *15* as a single product (> 97 % pure, < 0.5 % enyne *27*) from 1-propadienylcyclohexanol (the intermediate allene in *1*→*15*). A molar ratio of 50:1 of the same reagents in a reduction of 1,2-octadien-4-ol (intermediate in *3*→*17*) led to 1,3-octadiene, contaminated with 8 % of monoolefins (GLC-MS), while a molar ratio of 100:1 gave an almost pure product (96 % by GLC, < 0.5 % enyne *29*). However, with this modified reagent it is not necessary to isolate the allenic alcohols, because they can be preformed at a lower temperature (≈40 °C) using LAH alone. The appropriate amount of aluminium chloride is then added and the mixture is refluxed until the allene is completely converted into the diene.

STEREOCHEMISTRY

GLC-data on the stereoisomers of the 1,3-dienes *17–19* do not seem to have been recorded in the literature though some authors have claimed determination of *E* and *Z* isomers of 1,3-octadiene and 2-methyl-2,4-octadiene based on GLC [14] without giving experimental conditions. Hauser *et al.* have observed the inability of some Carbowax columns to resolve 1,3-hepta- and 1,3-nonadiene into *E* and *Z* isomers,[15] and we have encountered similar difficulties though we used longer columns. To obtain a rough estimate of the stereoselectivity in the formation

of 1,3-dienes, we reacted 1,3-heptadiene (prepared from *5* in diglyme), and 1,3-nonadiene with maleic anhydride, these reactions producing adducts in yields of 74 and 71 %, respectively (NMR). Predominant *E* configuration of these dienes is thus indicated,[13] and is further verified by the absorption of medium strength around 950 cm^{-1} in the IR spectra [15,16] of all the mono-substituted 1,3-dienes.

4-Methyl-1,3-heptadiene (*22*) contained comparable amounts of *E* and *Z* isomers (55:45), irrespective of the starting material used (*9* or *10*).

The dienacetal *24* is obtained as a 1:1 mixture of *E* and *Z* isomers, whereas GLC on Carbowax of the dienol *25* was not conclusive (*cf.* Experimental Part).

The 1,4-disubstituted butadienes *20* and *21* exhibit remarkable differences in their content of stereoisomers. While the *E,E* isomer of 2,4-hexadiene (*20*) is the major component (*E,E*:*E,Z*:*Z,Z* = 61:36:3) the same isomer of 4,6-decadiene (*21*) only constitutes 35 – 45 % (*E,E*:*E,Z* approximates 3:5; *Z,Z* ~4 % of the isomers), when both dienes were formed in diglyme. The poor GLC resolution did not permit more exact quantitation of the decadienes. These assignments of the stereoisomers are based on IR spectra of the isolated (*E,E*)-2,4-hexadiene,[17] of a mixture of the (*E,Z*)- and (*Z,Z*)-2,4-hexadienes,[17] and of the partly purified *E,E* and *E,Z* isomers of 4,6-decadiene. The latter isomers had IR spectra closely resembling those of the corresponding 2,4-hexadienes. IR data on other pure isomers of 1,4-disubstituted butadienes also support the assignments of the 4,6-decadienes.[18]

MECHANISM AND DISCUSSION

During formation of a conjugated diene from an α-allenic alcohol, one of the two double-bonds remains in the same position (*a*→*a'* in Fig. 1), while the other (*b*) is shifted to become *c* in the conjugated diene. It is possible and even likely that the two double-bond forming processes will exhibit different stereoselectivity. It can be seen in Fig. 1 that to give an (*E*)-*a'* double-bond, the hydride has to be delivered to the allenic system from the most hindered side, and therefore this process might be sensitive to the size of the group R. The present results (*cf.*

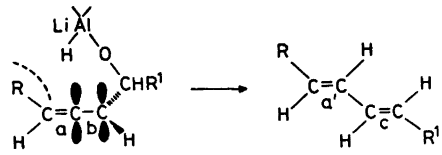
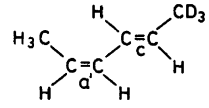


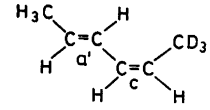
Fig. 1.

Stereochemistry) may corroborate these considerations, as 4,6-decadiene (R = R¹ = C_3H_7 in Fig. 1) contains more *E,Z* isomer than 2,4-hexadiene (R = R¹ = CH_3). This hypothesis is even more likely, since the other process *b*→*c*, which is an allylic rearrangement, can be expected to give more product of *E* configuration the larger R¹ (Fig. 1) is.[19]

We found it of interest to study these processes in greater detail and therefore made the double-bonds of 2,4-hexadiene recognizable by labelling the starting acetylene *7* with deuterium at C-1 to give *7*-d_3. From *7*-d_3 the labelled 2,4-hexadiene *20*-d_3 was prepared in diglyme as for *20* and the isomers *30* and *31* were isolated together by preparative GLC. The deuterium content was 88 % by NMR and 86.3 % by MS. The 2,4-hexadiene system was chosen because all the isomers have been thoroughly investigated by NMR,[20] showing the following methyl resonances relative to TMS (neat): *E,E*, δ 1.663; the *E,Z* isomer has the *E* methyl at δ 1.710 and the *Z* methyl at δ 1.662; *Z,Z*, δ 1.658.



30



31

The *E,Z* isomer exhibits the two methyl signals separated by 0.05 ppm, and consequently the deuterium labelled *E,Z* isomer can be analyzed for its content of (*E,Z*)- and (*Z,E*)-1,1,1-tri-deuterio-2,4-hexadiene (*30* and *31*, respectively). The methyl region of this labelled isomer at 100 MHz is shown in Fig. 2. When corrected for the presence of methyl signals from the *E,E* and *Z,Z* isomers (Fig. 2), both of which are found at 1.66 ppm, signals remain at 1.66 and 1.71 ppm with the approximate ratio

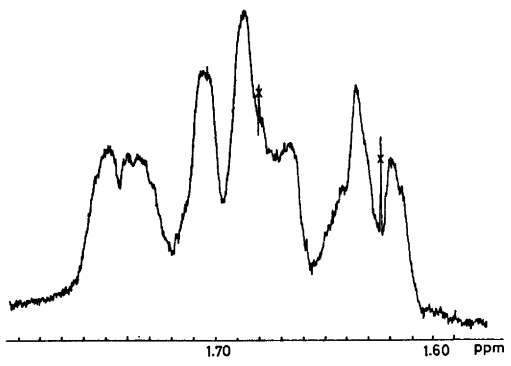


Fig. 2. 100 MHz NMR spectrum of the methyl signals of the deuterium labelled (*E,Z*)- and (*Z,E*)-2,4-hexadienes *30* and *31* containing 15 and 5 % of the *Z,Z* and *E,E* isomers, respectively.

of areas 1:1.6, thus indicating the presence of *30* and *31* in the same ratio.

Assuming the chemical shifts of the *E* and *Z* methyl groups of (*E,Z*)-2,4-hexadiene to be correctly assigned by Albriktsen *et al.*[20] this experiment indicates that for the formation of 2,4-hexadiene there seems to be, very surprisingly, greater stereoselectivity for *E* configuration in the rearrangement $a \rightarrow a'$ than in the step $b \rightarrow c$ (Fig. 1). A major amount of the *E,E* isomer is still formed and the total formation of *E* configuration in the step $a \rightarrow a'$ can be estimated to be around 84 %, while it is approximately 73 % in the allylic rearrangement $b \rightarrow c$. The result is exactly reverse to what one should have expected from the foregoing reasoning (Fig. 1). The formation of (*E,Z*)-4,6-decadiene as the predominant isomer in the other experiment makes the situation still more complex since in this case the allylic rearrangement $b \rightarrow c$ would be expected to be still more *E* stereoselective than in the formation of 2,4-hexadiene. However, it should be noted that the deuterium content of the 2,4-hexadiene, 20-d_3, is only 86.3 % and the found difference between the stereoselectivities (84 and 73 %) is probably greater than the actual figure. Notwithstanding the ambiguous result with 2,4-hexadiene it still seems reasonable to assume that the bulk of the *Z* double-bond of 4,6-decadiene is formed in the $a \rightarrow a'$ mode though the situation might not be as straight-forward as pictured in Fig. 1.

Finally, it is interesting to note that very small amounts of aluminium chloride in combination with the LAH reagent suffice to completely suppress the aforementioned formation of alkenynes. Other authors have also reported selective reactions with this reagent, notably the exclusive attack at C-3 on 3-alkyl substituted propargylic alcohols giving rise to organometallic intermediates [21] or allenic hydrocarbons [22] depending on the ratio of aluminium chloride:LAH. Without added aluminium chloride LAH attack occurs at C-2 as well as C-3.[21] Allylic alcohols can only undergo the above $S_N2'i$ reaction with LAH in exceptional cases.[31,32] The presence of aluminium chloride is sometimes necessary [32] but in other cases inhibitory.[31]

EXPERIMENTAL

General. IR spectra were run on a Perkin-Elmer Infracord 157 G apparatus using liquid films between NaCl discs. NMR spectra were obtained in $CDCl_3$ with TMS as internal standard using a Perkin-Elmer R-12 B spectrometer operating at 60 MHz. These spectra were routinely recorded and are in full agreement with the proposed structures. The 100 MHz spectrum was obtained with a Varian HA-100 D. Mass spectra were obtained at 70 eV using an AEI MS-30 connected to a Pye 104 gas chromatograph. Correct mass spectra (M^+ and fragmentation) were obtained for all conjugated dienes prepared in the present work with the exceptions of the dienes *19* and *24*, which were not run.

For preparative GLC 300×0.96 cm and 600×0.96 cm aluminium columns packed with 20 % SE-30 and Carbowax 20 M, respectively, on Chromosorb W (60 – 80 mesh) were used. The Carbowax column was also used for determinations of stereoisomers.

All reactions involving $LiAlH_4$ and Grignard reagents were performed under nitrogen atmosphere.

Propargylic ethers and acetals used in the subsequent preparations were 3-(tetrahydro-2-pyranyloxy)propyne,[23] 3-(tetrahydro-2-pyranyloxy)-1-hexyne,[2] 3-*t*-butoxy-1-hexyne,[24] and 3-methyl-3-(tetrahydro-2-pyranyloxy)-1-butyne.[25]

2,2,2-Trideuterioacetaldehyde was prepared according to Baldwin,[26] but only two exchanges in D_2O were performed at 40 °C. Yield 50 %.

General procedure for the preparation of 7, 7-d_3, 8, 11, *and* 12. To 0.5 mol of ethylmagnesium bromide in 150 ml of THF was added the appropriate propargylic ether (0.5 mol) in 50 ml of THF (0.5 h). Stirring was continued for 0.5 h at room temperature, when the

appropriate keto compound dissolved in 25 ml of THF, was added dropwise (1 h). After stirring for another 3 h at room temperature the reaction mixture was poured on to ice and ether, enough saturated NH_4Cl solution was added to allow separation of the phases, and the water phase was extracted twice with ether. The organic phase was washed twice with a saturated NH_4Cl solution, dried over K_2CO_3-Na_2SO_4 and distilled.

5-(Tetrahydro-2-pyranyloxy)-3-hexyn-2-ol (7). Yield 74 %. B.p. 102 °C/0.3 Torr. (Found: C 66.3; H 9.30. Calc. for $C_{11}H_{18}O_3$: C 66.63; H 9.15).

5-(Tetrahydro-2-pyranyloxy)-1,1,1-trideuterio-3-hexyn-2-ol (7-d_3). Prepared on a 0.05 mol scale. Yield 67 %. B.p. 102 °C/0.3 Torr.

7-t-Butoxy-5-decyn-4-ol (8). Yield 66 %. B.p. 96 °C/0.25 Torr. (Found: C 74.1; H 11.5. Calc. for $C_{14}H_{26}O_2$: C 74.29; H 11.58).

2-Methyl-5-(tetrahydro-2-pyranyloxy)-3-octyn-2-ol (11). Yield 78 %. B.p. 108 °C/0.4 Torr. (Found: C 69.9; H 9.9. Calc. for $C_{14}H_{24}O_3$: C 69.96; H 10.07).

7-Methyl-7-(tetrahydro-2-pyranyloxy)-5-octyn-4-ol (12). Yield 76 %. B.p. 109 °C/0.1 Torr. (Found: C 69.9; H 9.8. Calc. for $C_{14}H_{24}O_3$: C 69.96; H 10.07).

The acetylenes 6 *and* 10 *were* prepared in analogy to similar compounds.[3,30] The intermediate acetylenic Thp-protected derivatives were methanolyzed without prior distillation.

4-Methoxy-2-nonyn-1-ol (6). Yield 74 %. B.p. 106 °C/0.6 Torr. (Found: C 70.2; H 10.3. Calc. for $C_{10}H_{18}O_2$: C 70.55; H 10.66).

4-Methoxy-4-methyl-2-heptyn-1-ol (10). Yield 80 %. B.p. 66 °C/0.5 Torr. (Found: C 69.0; H 10.2. Calc. for $C_9H_{16}O_2$: C 69.02; H 10.32).

Preparation of the conjugated dienes 18, 19, 21, 22, 23, *and* 24 *from the acetylenes* 4–6, 8 *and* 10–13. To a stirred, ice-cooled suspension of $LiAlH_4$ (1.52 g, 30 mmol) in 20 ml of THF a solution of the starting acetylene (16 mmol of Thp-oxy derivatives and 22 mmol of alkoxy derivatives) in 10 ml of THF was added dropwise. The mixture was refluxed for 4 h, cooled and poured on to a little ice and 75 ml of pentane. Saturated NH_4Cl solution was added to facilitate the separation of the two phases. The water phase was extracted twice with pentane, and the combined organic phases were washed twice with saturated NH_4Cl solution and twice with water, then dried over Na_2SO_4 while protected from air. The dienes were distilled or purified by preparative GLC after cautious evaporation of the pentane. Yields on distillation ranged from 40–70 %. However, it should be noted that GLC yields in most cases exceed 90 % and still higher isolated yields can probably be achieved on a larger preparative scale.

4,6-Decadiene (21). Yield 55 %. B.p. ~75 °C/20 Torr. The *E,E* and *E,Z* isomers were partly separated on a Carbowax 20 M column at 115 °C. (*E,E*)-4,6-Decadiene (containing ~25 % of *E,Z* isomer). IR: 985 cm^{-1} (strong) and 950 cm^{-1} (weak, from the *E,Z* isomer). NMR: δ 6.50–5.20 (m, 4 H), 2.37–1.82 (m, 4 H), 1.40 (sextet, 4 H) and 0.90 (t, 6 H). (*E,Z*)-4,6-Decadiene (containing ~15 % of *E,E* isomer). IR: 982 and 950 cm^{-1} (both medium). NMR: δ 6.60–5.05 (m, 4 H), 2.37–1.83 (m, 4 H), 1.40 (sextet, 4 H) and 0.90 (t, 6 H).

6,6-Dimethoxy-4-methyl-1,3-hexadiene (24). Yield 53 %. B.p. 74 °C/15 Torr. IR: 985 cm^{-1} (strong, $H_2C=$). NMR: δ 7.0–6.3 (m, 1 H), 5.95 (d, 1 H), 5.3–4.9 (m, 2 H), 4.50 (t, 1 H), 3.36 (s, 6 H), 2.50 and 2.38 (two d, 2 H) and 1.83 (s, 3 H). (Found: C 68.8; H 10.2. Calc. for $C_9H_{16}O_2$: C 69.19; H 10.33).

1,3-Heptadiene (18) *from* 5 *in diglyme* A 100 ml one-necked flask containing 30 ml of diglyme was arranged so as to allow continuous distillation while a solution was added from the top. $LiAlH_4$ (2.28 g; 60 mmol) was added in several portions to the ice-cooled, magnetically stirred diglyme. A solution of *5* [24] (7.35 g; 40 mmol) in 20 ml of diglyme was added dropwise to the ice-cooled suspension, while the receiver was cooled to −75 °C. The temperature of the reaction mixture was then slowly raised to 80 °C using an oil-bath. After 2 h the oil-bath temperature was raised to 120 °C, when 1,3-heptadiene started to distill. The reaction system was connected to a water pump *via* a tube containing potassium hydroxide pellets, and the pressure was lowered to ~50 torr. 2.1 g of distilled material containing 10 % of diglyme was obtained. 1,3-Heptadiene, >99 % pure by GLC except for the diglyme, was obtained in 47 % yield.

2,4-Hexadiene (20) *and 1,1,1-trideuterio-2,4-hexadiene* (20-d_3) were prepared in diglyme (30 mmol Thp-derivative and 50 mmol $LiAlH_4$), but the reaction was allowed to proceed in a stoppered 250 ml three-necked flask at 80 °C for 3 h. The reaction mixture was cooled, an efficient condensor was attached and the mixture was cooled to −30 °C. 20 ml of pentane was added, and then 10 ml of water was added dropwise while magnetic stirring was maintained. The mixture was stirred for 0.5 h at 0 °C and then poured into a separatory funnel. It was diluted with saturated NH_4Cl, and the organic phase was separated and the water phase extracted twice with 5 ml portions of pentane. The combined pentane solutions were washed several times with water and dried over Na_2SO_4. The *E,E* and *E,Z* isomers of the 2,4-hexadienes were isolated by injecting 1 ml portions of the pentane solutions on to a 6 m Carbowax column (65 °C) and collecting fractions at 18 and 20 min. IR-spectra agreed with published data.[17]

3-Methyl-3,5-hexadien-1-ol (25). The acetal *13* [3] (2.3 g; 11.4 mmol), was hydrolyzed in 30 ml of dioxane:water (2:1) containing 75 mg of *p*-toluenesulfonic acid (70 °C, 2 h). After cooling, 3 g of $KHCO_3$ was added and then Na_2SO_4 to dry the mixture followed by 50 ml of ether. After filtering the drying process was repeated

once more. Evaporation of solvent *in vacuo* yielded the aldehyde *14* which was ~93 % pure by GLC. IR: 1720 (strong) and 1670 cm^{-1} (medium, H-bonding). NMR: δ 9.85 (t, 1 H), 4.30 (s, 2 H), 3.45 (s, 3 H), 2.65 (d, 2 H), 2.1 (s, 1 H) and 1.50 (s, 3 H). It was not purified, but was allowed to react with $LiAlH_4$ (0.70 g; 18.5 mmol) in refluxing THF for 2 h. Work-up and distillation yielded 100 mg of the title compound (Fraction 1). A residue (810 mg) after the distillation was shown by IR and NMR to be 4-methyl-2,3-hexadien-1,6-diol (*cf.* Scheme in Table 1), better than 90 % pure. This was once more allowed to react with $LiAlH_4$ (0.57 g; 15 mmol) for 6 h. Work-up and distillation yielded 350 mg of *25*. The total yield of *25* was 36 %. The first fraction was ~95 % pure by GLC on OV-25. IR: 900 cm^{-1} (strong, $H_2C=$). NMR δ 6.95–6.30 (m, 1 H), 5.95 (d of two m, 1 H), 5.30–4.90 (m, 2 H), 3.70 (t, 2 H), 3.20 (s, 1 H), 2.47 (q, 2 H; at 100 MHz shown to consist of two overlapping t of almost equal intensity), and 1.80 (s, 3 H). GLC (6 m Carbowax 20 M at 210 °C) showed one main component (mol. wt 112, MS) at 19 min which constituted 80 % of the whole mixture. Except for 5 % of monoolefins (MS) there were two other components at 17 and 20.5 min, representing 8 and 5 %, respectively. The second fraction consisted to approximately 25 % of monoolefins.

Preparations of the conjugated dienes 15, 17, *and* 22 *(from* 9*), $AlCl_3$-procedure.* Molar ratios and amounts of THF were the same as described above for the preparation of the other simple dienes. Instead of refluxing the reaction mixture it was kept stirring at 40 °C for 1 h to allow complete conversion of the starting acetylenes into α-allenic alcohols. The mixture was then cooled to 0 °C and $AlCl_3$ (1 mol % of the $LiAlH_4$ used) was added (in one portion for a 10–15 mmol preparation). The mixture was refluxed for 4 h and worked up as above.

*Reactions of 1,3-heptadiene (*18*) and 1,3-nonadiene (*19*) with maleic anhydride.* 1,3-Heptadiene (375 mg; 3.34 mmol), obtained from *5* as described above, or 1,3-nonadiene (122 mg; 0.97 mmol) was allowed to react with a 10 % excess of maleic anhydride in 1–2 ml of acetone in closed vials at 70 °C for 10 h. The solvent and unreacted dienes were removed *in vacuo*, and the residue was analyzed by NMR for yield. The amounts of olefinic protons in maleic anhydride and the adduct were compared. A 71 % yield of adduct from 1,3-nonadiene and 74 % from 1,3-heptadiene was obtained.

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Large-scale Laboratory Electrolysis in Organic Systems. II.* Construction and Performance of an Undivided 800 cm² Concentric Capillary Gap Cell

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The construction of a concentric capillary gap cell of simple design is described. In principle, it consists of a graphite rod anode (surface area *ca.* 800 cm²) and a steel tube cathode arranged concentrically with a 1 mm gap between them. The electrolyte is circulated through this space *via* a reservoir and a heat exchanger.

This cell has been used for performing acetoxylation and alkoxylation reactions with different organic substrates. Currents generally fall between 20 and 50 A with applied voltages in the range 7 – 60 V. Due to its simplicity, the concentric capillary gap cell should be of value for trying out electroorganic reactions on an extended laboratory scale. A 3000 cm² version, permitting production volumes of the order of 1 kg/h, has been built and successfully tested.

The design of more efficient electrochemical reactors has been actively pursued in recent years.[1–3] The capillary gap cell, in which the distance between the electrodes falls in the region of 0.1 – 1 mm, has proved to be especially useful for large-scale electroorganic synthesis because of the minimized energy consumption and/or the possibility of using very low concentrations of supporting electrolyte. A very ingenious design of this cell type has been developed by Beck for use on a pilot plant or industrial scale.[4–8] It consists principally of a pile of horizontal, circular conducting discs (normally made from graphite) with a centrum hole and separated from each other by thin plastics spacers. The electrolyte solution is pumped into the thus defined "tube" in the center of the pile and forced outward radially between the plates and recycled *via* a heat exchanger (Fig. 1a). The plates are bipolarly connected to the power source.

This design has the advantages of a high space/time yield and of permitting an easy scale-up. However, for laboratories without access to certain specialized techniques for gluing and surface treatment, the construction of such a cell presents some difficult-to-solve problems. Also, it cannot be converted into a divided cell which is a desirable feature in many cases. We have therefore developed a concentric capillary gap cell [9–12] (Fig. 1b) for exploratory work on organic electrode processes on an extended laboratory scale, *i.e.* for currents of the order of 50 A. This cell is greatly simplified as compared to the graphite disc stack cell mentioned above, and has the additional advantage of being convertible to a divided cell. A preliminary report on its construction and use for anodic oxidations of different types was published recently,[9] and a few other applications have appeared elsewhere.[10–12] We now wish to describe the construction of the final version of this cell, a number of auxiliary applications, and our general experience with its operation and performance.

CELL CONSTRUCTION

The first versions of the concentric capillary gap type with anode surface areas of 360 and

* For Part I, see Ref. 9.

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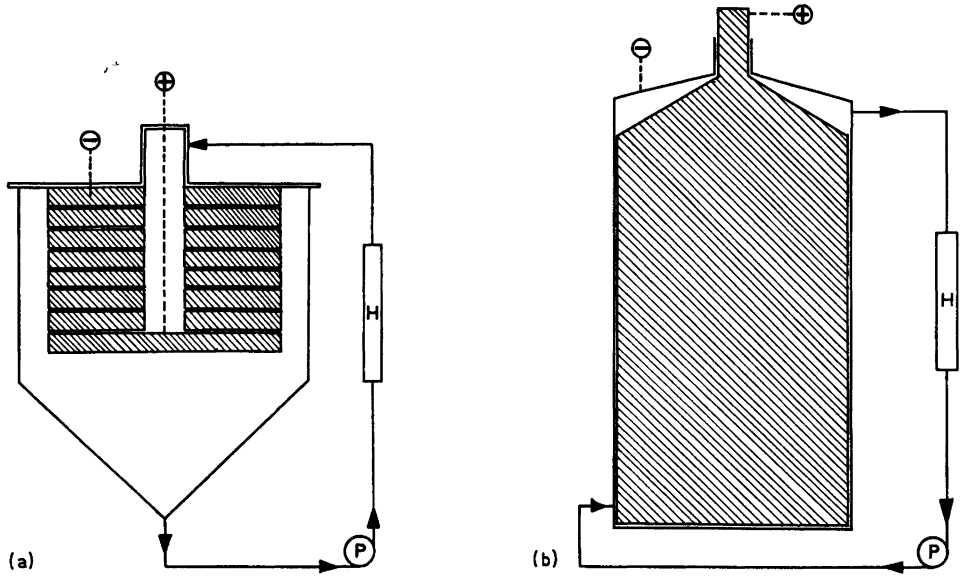


Fig. 1. Schematic representation of the arrangement of electrodes and the flow system for (a) the capillary gap disc stack cell and (b) the concentric capillary gap cell. Cross-sections (through the axis of the cells) have been drawn to illustrate the relative size of the two cell designs when the anode surface areas are identical. P = pump; H = heat exchanger.

580 cm², respectively, had a cylindrical graphite rod anode and a stainless steel tube cathode concentrically arranged and with provision for pumping the electrolyte through the space (1 mm wide) thus defined. The threaded end supports for the graphite anode (AUC quality from Union Carbide) were made from glassfiber-reinforced polytetrafluoroethylene (PTFE) which proved to be an expensive material and unsatisfactory from the leakage point of view. Therefore, the steel tube was provided with flanges at the ends, and the anode supports were constructed from stainless steel (Uddeholm 24L, D17730) with glassfiber-reinforced PTFE for isolation only, as depicted in Fig. 2. For leak-proof sealing, Viton® O-rings were inserted between the flanges and the end supports. These were kept together by six pairs of nuts and bolts at each end. The inlet and outlet tubes for the electrolyte solution were arranged so as to introduce the flow tangentially into the cell.[13]

The size of this version was slightly larger than that of the previous ones, the effective anode area being about 800 cm². With these dimensions the specific anode area (geometric anode area divided by cell volume) is 0.57 cm^{-1}, to be compared with values given in the literature [2] for the graphite disc stack cell, 1 – 5 cm^{-1}. In order to be able to make a realistic comparison between the two cell designs, we have built a graphite disc stack cell which is compatible with ordinary commercial laboratory glassware. It then turned out that the number of graphite discs (size: 9 cm diameter, 1 cm thickness with a centrum hole of 2 cm diameter) could hardly exceed eight per liter of cell volume, provided the volume of the container collecting the flow from the seven capillary gaps in the stack is considered to be the cell volume. With these cell data, the specific surface area of this particular capillary gap disc stack cell is 0.42 cm^{-1}. Refinements in the design (specially designed glassware for the container collecting the flow, thinner graphite discs) might increase the value to about 1 cm^{-1}, but this would be considerably more costly and laborious. Hence the concentric cell type has distinct advantages for use on an extended laboratory scale in its simplicity and cheapness.

The flow system was identical to the one described previously [9] and the power source was a 50A/60V controlled current instrument from Farnell Instruments, Ltd., Wetherby LS22 4DH, England. Photographs of the cell parts and the assembled system in operation have been published (Figs. 2 and 3 from Ref. 12).

CELL OPERATION

The operation of the concentric capillary gap cell is very simple. After assembling the flow system with an adjustable valve and a flask of an appropriate size as storage vessel, the electrolyte solution is introduced into the flask. With the valve almost closed the pump is started and the flow gradually adjusted until a

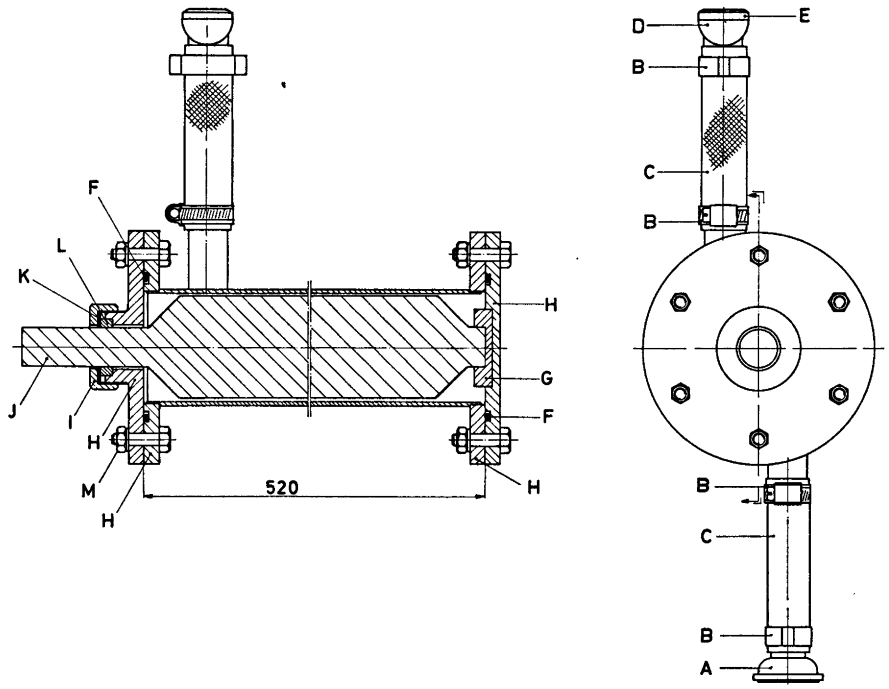


Fig. 2. Drawing of a concentric capillary gap cell with an anode area of *ca.* 800 cm². A, cup of a flexible joint (Rotulex® 4.692 – 15); B, hoseclip; C, PTFE tubing with steel wiring support (Texan® 4201 – 12); D, ball of a flexible joint (Rotulex® 4.691 – 15); E, PTFE sealing ring (Rotulex® 4.690 – 04); F, Viton® O-ring (65.09 × 3.53); G, glass-fiber reinforced PTFE anode support; H, stainless steel (Uddeholm 24L, D17730), flange and end support; I, polypropylene; J, graphite (AUC quality from Union Carbide) anode; K, stainless steel washer; L, sealing ring (Sovirel® 4701 – 26); M, M6 × 25 bolt + nut and washer.

suitable flow rate is attained (generally around 20 l/min). Since there is a tendency for sucking gas into the system if the liquid from the heat exchanger is discharged in a single vertical flow into the storage vessel, a special nozzle for breaking it up into three almost horizontal components was inserted between the heat exchanger and the storage flask. With this arrangement the pump could be run at full capacity without any problems in this respect.

When the flow rate has been adjusted properly the applied potential is gradually increased until the desired current level is reached. The flow of tap water through the condenser is then adjusted so as to maintain the electrolyte solution at a constant temperature. The condenser used as a heat exchanger had a cooling surface area of about 800 cm², which was more than adequate for removing the heat generated at the maximum effect, 3000 W.

REACTIONS

Previously run reactions in the smaller versions of the concentric capillary gap cell include dehydrodimerizations of alkylaromatic hydrocarbons,[9,10] acetoxylations,[9] one case of trifluoroacetoxylation,[10] and one case of additive dimerization.[11] Table 1 shows conditions and yields for additional reactions run in the 800 cm² cell described in this paper. As before, the supporting electrolyte concentration has been kept at a low level in most cases in order to minimize the expenditure of costly tetrabutylammonium salts. This is of course done at the sacrifice of a higher energy consumption. Together with earlier work on this cell type, our experience with the concentric capillary gap cell can be summarized as follows:

(1) In its present size and design, it is preferentially used for large-scale laboratory electrolyses at the anode, 1 – 5 molar runs being feasible within electrolysis periods of 1 – 10 h. For certain anodic reactions and most cathodic ones, a divided cell is desirable and indeed sometimes necessary; we are presently working

Table 1. Results from electrolyses in the concentric capillary gap cell.

Reaction type	Substrate (mol)	Isolated product(s)	Solvent (volume in ml)	Supporting electrolyte (conc. in M)	Current in A	Applied potential, V	Yield Material	Current
Acetoxylation	$HCON(CH_3)_2$ (5)	$HCON(CH_2OAc)(CH_3)$	CH_3COOH (1500)	Bu_4NBF_4 (0.011)	50	40–60	92	92[a]
Acetoxylation	$CH_3CON(CH_3)_2$ (5)	$CH_3CON(CH_2OAc)(CH_3)$	CH_3COOH (1500)	Bu_4NBF_4 (0.011)	50	40–60	78	78[a]
Acetoxylation	$PhCON(CH_3)_2$ (1.5)	$PhCON(CH_2OAc)(CH_3)$	CH_3COOH (1000)	Bu_4NBF_4 (0.008)	50	60	60	60[a]
Acetoxylation	$PhCH_3$ (3)	$PhCH_2OAc$	CH_3COOH (3000)	Bu_4NBF_4 (0.024)	20	53–60	39	26[a]
Acetoxylation	4-*t*-$BuC_6H_4CH_3$ (2)	4-*t*-$BuC_6H_4CH_2OAc$	CH_3COOH (2000)	Bu_4NBF_4 (0.042)	20	60	73	73[a]
Acetoxylation	Mesitylene (1)	$2,4,6\text{-}(CH_3)_3C_6H_2OH$[b]	CH_3COOH (1000)	CH_3COOK (0.90)	20	20	40	40[a]
Acetoxylation	Indene (2)	*cis*- and *trans*-1,2-Indandiol diacetates + additive dimers[d]	CH_3COOH[c] (1200)	CH_3COOK (0.67)	10		39	
Methoxylation	$HCON(CH_3)_2$ (5)	$HCON(CH_2OMe)(CH_3)$	CH_3OH (2500)	Bu_4NBF_4 (0.007)	50	26	75	75
Methoxylation	Furfuryl alcohol (2.4)	2,5-Dimethoxy-2-hydroxymethyl-2,5-dihydrofuran	CH_3OH (1600)	NH_4Br (0.16)	40	7	62	71[e,f]
Ethoxylation	Furan (2.8)	2,5-Diethoxy-2,5-dihydrofuran + maleic aldehyde diacetal	C_2H_5OH	NH_4Br	25	10	73	86[e,f]

[a] Run to 100 % of theoretical conversion. [b] Hydrolyzed before isolation. [c] Acetic anhydride (20 ml) added. [d] Isomers of 1,1′-diacetoxy-2,2′-biindane. [e] Run to 85 % of theoretical conversion. [f] Run at 4–6 °C.

on a divided version of the same size using a tubular Nafion® XR-475 membrane as cell divider and hope to be able to test it soon. The divided cell has a somewhat wider gap (*ca.* 3 mm), though, in order to accommodate both the catholyte and anolyte flow and the cell divider.

(2) Abrasion of the graphite anode has been almost negligible during *ca.* 400 h of intermittent operation using media like acetic acid, methanol, and methylene chloride at current densities of maximally 60 mA/cm^2.

(3) The flow rate through the cell is 330 cm^3/s at a pumping rate of 20 l/min, which is equivalent to a residence time in the cell of about 0.25 s. In a typical run with a substrate concentration between 1 and 2 M, this corresponds to a very low degree of conversion per pass during most of the run, thus ensuring stable and reproducible reaction conditions.

(4) In a medium of low conductivity, such as acetic acid containing *ca.* 0.01 M tetrabutylammonium tetrafluoroborate, the cell resistance is of the order of 1 ohm, whereas with an electrolyte solution of relatively high conductivity, such as methanol/0.16 M ammonium bromide it is about 0.1 ohm. At the current level commonly employed, the power losses are of course relatively high in the low-conductivity media but this is compensated for by savings on supporting electrolyte, a more costly item on the laboratory scale.

(5) The construction of a scaled-up version of the concentric capillary gap cell (anode area about 3000 cm^2; gap width 1 mm), permitting production volumes of the order of kg/h, presented no difficulties in principle. We have run preliminary tests on such a cell, using the acetoxylation of DMF on a 50-molar scale as a model reaction. With 0.02 M tetrabutylammonium tetrafluoroborate as supporting electrolyte, the cell could be operated at 350 A at an applied voltage of 40 V (*i.e.* the ohmic resistance was of the order of 0.1 ohm) at a flow rate of 60 l/min. Again, the heat evolved was easily dissipated by a heat exchanger in the flow loop.

Acknowledgements. We gratefully acknowledge generous financial support from the Swedish Natural Science Research Council and the Swedish Board for Technical Development. We also thank Messrs Braun and Petterson and their skilful coworkers in the mechanic workshop of the Chemical Center for their valuable help with the construction of the cells.

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Mass Spectra of Benzo-2,1,3-thiadiazoles

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The mass spectra of a number of benzo-2,1,3-thiadiazoles are reported and discussed. In most cases they exhibit molecular ions of very great abundance. The fragmentation of substituents is more important than fragmentation of the ring system, except for hydroxy- and alkoxy-substituted compounds.

As a continuation of our studies on the mass spectra of different types of organo-sulfur compounds[1,2] and to examine whether processes analogous to the benzyne formation postulated for benzo-2,1,3-selenodiazoles[3] were important, we have now investigated some benzo-2,1,3-thiadiazoles. Although the mass spectra of the isomeric 1,2,3-thiadiazoles[4,5] and the benzo-1,2,3-thiadiazole[6] have been reported and discussed, the mass spectrum of benzo-2,1,3-thiadiazole (*1*) has not been reported, except for the intensity of the M − 46 (M − NS) peak, which has been compared with the intensities of the analogous M − 30 (M − NO) and M − 28 (M − N_2) peaks from benzo-2,1,3-oxadiazole and benzo-1,2,3-triazole, respectively.[6] The abbreviated mass spectra of the benzo-2,1,3-thiadiazoles listed in Table 1 are given in Table 2.

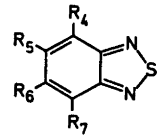
DISCUSSION

The mass spectrum (Fig. 1) of benzo-2,1,3-thiadiazole (*1*) displays the molecular ion as the base peak. The most important fragmentations are losses of H, HCN, and NS.

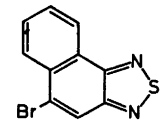
As expected, the halogen-substituted benzo-2,1,3-thiadiazoles (*2* − *8*) underwent stepwise loss of halogen atoms rather than fragmentation of the ring system, although peaks from M − NS

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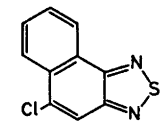
Table 1. Benzo-2,1,3-thiadiazoles (*1* − *35*).



1-33



34



35

No.	R_4	R_5	R_6	R_7
1	H	H	H	H
2	Cl	H	H	H
3	H	Cl	H	H
4	Cl	H	H	Cl
5	Cl	Cl	H	Cl
6	Cl	Cl	Cl	Cl
7	F	Cl	H	Cl
8	Br	H	H	Br
9	CH_2I	H	H	H
10	CH_2Br	Cl	H	Cl
11	CH_3	Cl	H	Cl
12	CH_3	H	Cl	Cl
13	CH_3	Cl	CH_3	Cl
14	OH	H	H	H
15	H	OH	H	H
16	Cl	OH	H	H
17	OH	Cl	H	Cl
18	OCH_3	H	H	H
19	H	OCH_3	H	H
20	OCH_3	H	H	Cl
21	Cl	OCH_3	Cl	Cl
22	OCH_2CH_3	Cl	H	Cl
23	OC_6H_5	NO_2	H	Cl
24	NO_2	H	H	H
25	H	NO_2	H	H
26	NO_2	Cl	H	H
27	NO_2	Cl	H	Cl
28	NO_2	Cl	Cl	NO_2
29	NO_2	Cl	NO_2	Cl
30	NH_2	NO_2	H	Cl
31	NH_2	NH_2	H	H
32	H	SO_2NH_2	H	H
33	CHO	Cl	H	Cl

Table 2. Mass spectra of substituted benzo-2,1,3-thiadiazoles (*1–36*). Values of *m/e* are followed by intensity (%).

1. 138(2) 137(20), 136(100)[M]$^+$, 135(8), 110(6), 109(15), 108(2), 104(2), 103(5), 96(3), 92(7), 90(15), 84(2), 83(5), 82(4), 78(6), 77(9), 76(4), 71(3), 70(8), 69(3), 68(11), 64(10), 63(5), 58(5), 52(10), 51(15), 50(10), 46(19), 45(37).
2. 172(39), 170(100)[M]$^+$, 143(4), 135(22), 126(6), 124(17), 108(50), 103(39), 85(45), 76(6).
3. 172(37), 170(100)[M]$^+$, 143(5), 135(18), 126(6), 124(18), 108(5), 103(4), 85(5), 76(6).
4. 208(16), 207(5), 206(69), 205(9), 204(100)-[M]$^+$, 179(2), 177(2), 171(14), 169(31), 160(7), 158(12), 142(3), 134(5), 110(9), 104(4), 103(5), 98(2), 83(2), 77(2), 76(3).
5. 244(5), 242(35), 241(9), 240(100), 239(8), 238(100)[M]$^+$, 207(5), 205(22), 203(35), 196(3), 194(6), 192(7), 170(5), 168(11), 144(4), 142(5), 120(5), 119(6), 100(3), 98(3), 75(3), 74(5), 59(6).
6. 280(2), 278(14), 276(51), 274(100), 272(72)-[M]$^+$, 241(11), 239(30), 237(31), 230(4), 228(8), 226(6), 207(9), 205(11), 204(18), 202(27), 195(9), 193(8), 181(4), 179(5), 178(5), 176(7), 172(6), 170(7), 167(5), 158(6), 155(10), 146(8), 144(12), 135(9), 134(8), 132(15), 123(8), 121(9), 109(20), 100(24), 86(12), 85(12), 79(9), 74(15), 70(11), 62(11), 47(11), 46(13).
7. 226(12), 225(6), 224(67), 223(8), 222(100)-[M]$^+$, 189(10), 187(24), 178(3), 176(5), 166(3), 164(5), 155(4), 153(10), 143(6), 113(4), 111(4).
8. 297(5), 296(50), 295(8), 294(100), 292(50) [M]$^+$, 250(1), 248(2), 246(1), 220(5), 218(7), 215(28), 213(27), 184(7), 148(5), 134(22), 108(3), 84(5), 51(9).
9. 276(5) [M]$^+$, 151(6), 150(16), 149(100), 128(6), 127(12), 122(17), 105(7), 90(5), 64(8), 63(9), 55(6), 52(6), 51(5), 50(5), 46(7), 45(8).
10. 296(−)[M]$^+$, 221(20), 219(72), 218(14), 217(59), 216(13), 185(8), 184(45), 183(16), 182(100), 180(13), 166(15), 158(8), 156(8), 151(13), 149(13), 148(15), 147(8), 136(33), 135(12), 124(9), 123(12), 110(7), 109(10), 108(8), 88(7), 83(6), 78(7), 77(7), 76(12), 75(9), 70(7), 64(13), 51(12), 50(10), 46(11), 45(17), 44(10).
11. 222(14), 221(8), 220(69), 219(14), 218(100)-[M]$^+$, 217(17), 185(55), 184(14), 183(97), 182(7), 158(3), 156(6), 149(8), 148(8), 147(5), 124(5), 120(2), 109(5), 94(8).
12. 222(14), 221(8), 220(67), 219(17), 218(100)-[M]$^+$, 217(11), 185(30), 184(10), 183(90), 182(5), 151(8), 148(10), 147(4), 124(5), 109(5).
13. 236(13), 235(8), 234(75), 233(15), 232(100)-[M]$^+$, 231(8), 199(32), 197(100), 187(12), 183(8), 162(8), 161(12), 152(8).
14. 154(5), 153(9), 152(100)[M]$^+$, 125(13), 124(17), 97(10), 93(8), 91(3), 65(5), 64(5).
15. 154(6), 153(9), 152(100)[M]$^+$, 125(5), 124(37), 120(4), 106(4), 97(18), 91(6), 81(6), 80(25), 71(9), 70(11), 68(8), 64(10), 53(9), 52(27), 51(12).
16. 190(8), 189(30), 188(43), 187(76), 186(100)-[M]$^+$, 185(18), 184(8), 183(40), 182(8), 181(22), 166(8), 164(14), 160(12), 158(26), 155(12), 152(38), 150(8), 149(18), 145(6), 143(16), 136(12), 134(12), 123(18).
17. 224(12), 223(5), 222(57), 221(7), 220(83)-[M]$^+$, 218(16), 208(6), 206(19), 204(26), 194(8), 192(9), 183(15), 181(7), 168(9), 156(8), 136(10), 135(6), 120(6), 119(6), 110(6), 98(11), 78(56), 77(11), 74(38), 59(67), 52(9), 51(9), 50(7), 45(38), 41(9).
18. 168(4), 167(9), 166(69) [M]$^+$, 165(19), 151(5), 139(5), 138(6), 137(100), 136(19), 135(21), 125(2), 123(20), 109(5), 97(3), 96(4), 93(12), 83(2), 79(2), 77(2), 76(7), 75(2).
19. 168(10), 167(14), 166(100)[M]$^+$, 151(17), 137(10), 136(33), 123(25), 109(4), 80(9).
20. 202(29), 201(11), 200(75)[M]$^+$, 199(11), 197(7), 187(32), 185(29), 183(16), 181(9), 173(34), 172(12), 171(100), 170(12), 159(12), 157(32), 152(11), 143(6), 137(8), 136(7), 135(10), 127(7), 111(6), 100(5), 98(4), 83(5), 76(8), 75(7), 73(6), 70(9), 64(16), 52(5), 51(13), 46(11), 45(10).
21. 274(9), 272(25), 271(6), 270(57), 269(13), 268(57)[M]$^+$, 257(11), 255(33), 253(33), 229(11), 227(30), 225(30), 222(20), 221(12), 220(71), 219(24), 218(71), 217(21), 185(43), 184(17), 183(100), 182(7), 181(26), 166(18), 158(6), 157(7), 151(12), 149(11), 148(13), 137(6), 136(30), 135(13), 132(8), 124(8), 123(11), 110(9), 109(11), 108(7), 79(6), 76(8), 74(71), 70(13), 64(8), 59(100), 52(7), 51(100), 50(7), 46(14), 45(71). 44(14), 43(13), 41(29).
22. 252(3), 250(14), 248(22)[M]$^+$, 224(14), 223(6), 222(72), 221(9), 220(100), 206(7), 204(11), 169(5), 155(5), 98(5).
23. 309(8), 307(20)[M]$^+$, 292(8), 290(10), 262(10), 260(6), 216(7), 214(16), 206(5), 204(8), 186(9), 170(5), 119(9), 118(6), 94(6), 93(100), 86(8), 77(40), 70(5), 65(49), 51(34).
24. 183(7), 182(9), 181(100)[M]$^+$, 151(30), 136(5), 135(50), 123(24), 109(7), 108(23), 104(14), 91(7), 83(5), 82(5), 76(13), 75(5), 70(5), 64(20), 52(6), 51(6), 50(5), 46(5), 45(29).
25. 183(6), 182(6), 181(100) [M]$^+$, 151(11), 136(4), 135(42), 123(22), 109(5), 108(22), 91(5), 83(5), 77(5), 76(8), 64(16), 50(6).
26. 220(6), 218(8), 217(35), 216(9), 215(100) [M]$^+$, 204(5), 187(17), 185(50), 183(5), 181(7), 172(5), 171(7), 170(13), 169(20), 159(17), 142(10), 135(8), 134(25), 133(9), 125(5), 108(12), 107(6), 98(7), 89(5), 83(12), 76(10), 75(10), 70(17), 69(8), 64(9), 52(5), 51(7), 46(7), 45(7).
27. 253(15), 252(11), 251(73), 250(10), 249(100)-[M]$^+$, 233(5), 223(15), 222(13), 221(76),

Table 2. Continued.

220(20), 219(83), 206(16), 205(11), 204(22), 203(19), 195(8), 193(43), 192(5), 171(10), 170(24), 169(12), 168(43), 161(13), 159(17), 158(5), 142(18), 133(12), 117(9), 101(12), 100(12), 98(16), 83(10), 75(15), 74(14), 70(20), 59(19), 46(14), 45(15), 44(12).

28. 298(14), 297(7), 296(63), 295(9), 294(100) $[M]^+$, 248(5), 222(5), 220(8), 219(5), 206(9), 204(13), 202(9), 200(13), 186(5), 184(6), 172(8), 170(13), 169(6), 155(8), 136(7), 135(10), 134(8), 132(27), 123(9), 117(16), 109(10), 100(14), 99(16), 85(12), 83(11), 70(9), 46(11), 44(17).

29. 298(12), 297(5), 296(19), 295(7), 294(80) $[M]^+$, 265(12), 263(17), 250(9), 248(13), 240(7), 238(7), 222(41), 221(7), 220(69), 218(11), 208(12), 206(40), 205(7), 204(51), 202(11), 201(28), 200(9), 199(78), 171(25), 169(21), 167(16), 155(13), 132(31), 117(11), 109(13), 79(20), 52(13), 44(39), 30(100).

30. 232(34), 231(10), 230(100) $[M]^+$, 212(13), 202(14), 200(41), 186(27), 185(10), 184(70), 172(43), 171(9), 170(80), 152(14), 151(24), 150(40), 135(21), 124(16), 108(10), 103(10), 98(13), 94(11), 78(9), 77(19), 76(9), 70(11), 64(13), 63(11), 52(20), 51(14).

31. 166(100) $[M]^+$, 165(16), 151(9), 149(9), 148(7), 139(6), 138(7), 136(27), 135(15), 134(9), 79(13), 64(9), 53(7), 52(11), 51(9), 50(6), 46(7), 45(9), 44(7).

32. 217(5), 216(5), 215(45) $[M]^+$, 199(12), 153(6), 152(20), 151(100), 137(5), 136(7), 135(72), 124(13), 123(6), 109(9), 108(27), 103(15), 91(10), 83(11), 80(7), 77(10), 76(12), 75(11), 70(10), 69(9), 64(35), 52(13), 51(12), 50(17), 46(11), 45(12).

33. 234(18), 233(11), 232(29) $[M]^+$, 231(12), 208(17), 207(8), 206(64), 205(16), 204(100), 203(10), 179(7), 177(11), 171(17), 170(11), 169(33), 168(20), 160(6), 158(7), 142(12), 110(11), 101(17), 100(12), 98(10), 75(21), 70(11).

34. 266(100), 264(100) $[M]^+$, 222(5), 220(16), 218(16), 186(10), 185(67), 183(10), 158(13), 141(10), 133(7), 132(7), 114(13), 77(5), 76(7), 69(7), 51(5), 50(5).

35. 224(5), 223(13), 222(93), 221(34), 220(100) $[M]^+$, 219(5), 193(4), 187(5), 186(9), 185(56), 184(7), 176(11), 174(20), 158(11), 153(11), 141(9), 128(5), 127(10), 114(10), 111(8), 110(20), 100(5), 75(5), 51(5), 50(5).

are present in all of the spectra with M − HCN and M − halogen − HCN often occurring.

The halomethyl compounds (*9,10*) have low molecular ion abundances with significant tropylium type ion peaks at M − halogen.[7] For the iodo compound the only other significant feature is the loss of HCN from this ion while for the bromo derivative Cl loss and Cl + NS loss from this tropylium type ion are significant.

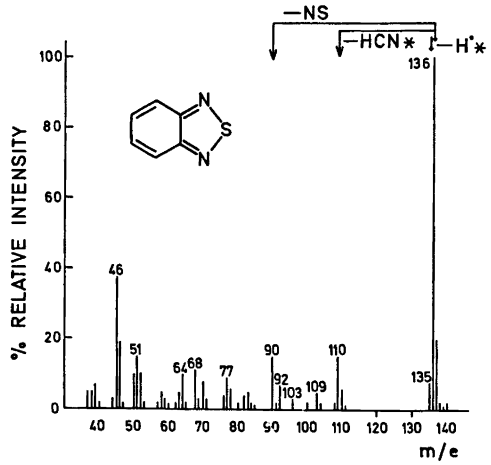


Fig. 1. Mass spectrum of benzo-2,1,3-thiadiazole (*1*).

The isomeric methyl benzo-2,1,3-thiadiazoles (*11,12*) yield virtually identical spectra which have high abundance molecular ions and M − Cl ion with small but significant M − Cl_2 ions. The methyl benzo-2,1,3-thiadiazole (*13*) with one more chlorine has identical characteristics.

The hydroxy isomers (*14,15*) have similar spectra with the loss of HCN, CO, and HCN + CO being significant.[8] The expulsion of carbon monoxide is more important when the hydroxyl is in the 5- than in the 4-position. Expulsion of carbon monoxide is important for the chlorinated hydroxy derivatives (*16,17*).

The difference between the mass spectra of the isomeric methoxy compounds (*18,19*) is interesting (Fig. 2). For both isomers loss of a methyl radical followed by expulsion of carbon monoxide is important as in the mass spectra of anisoles.[9] However, although the 5-methoxy compound expels formaldehyde from the molecular ion to give an intense peak (33 %) with a less significant loss of formyl radical (10 %), the 4-methoxy derivative displays the loss of formyl radical as the base peak with formaldehyde expulsion being less important (19 %). Intense metastables support these transitions in both spectra. Significantly the mass spectrum of the chloro-substituted 4-methoxy derivative (*20*) displays the loss of formyl radical as the base peak. Loss of methyl radical and carbon

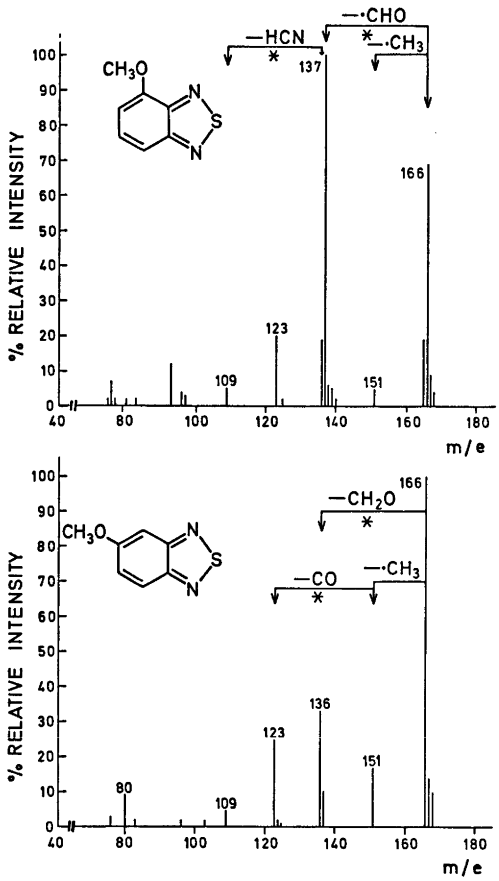


Fig. 2. Mass spectra of 4- and 5-methoxy benzo-2,1,3-thiadiazoles (*18*, *19*).

monoxide is important for both chloro-substituted methoxy derivatives (*20*,*21*).

In the spectrum of 4-ethoxy-5,7-dichloro-benzo-2,1,3-thiadiazol (*22*), the ion giving rise to the base peak is found to be formed in the same way as in many phenetoles.[10] The fragmentation of the phenoxy ion dominates the spectrum of the phenoxy-substituted derivative (*23*).[11]

In the nitro-substituted benzo-2,1,3-thiadiazoles (*24*—*29*) a change in the position of the nitro group does not change the fragmentation pattern significantly. The spectra of the 4- and 5-nitro benzo-2,1,3-thiadiazoles (*24*,*25*) are practically identical. The only important fragmentations are of the nitro group.[12] In the amino-substituted compounds (*30*,*31*) the expected fragmentations of the amino group are found,[13] although where a nitro group is present (*30*) its fragmentation dominates.

The fragmentation of the sulfonamide (*32*) is completely analogues to that of benzene sulfonamide.[14] That is, expulsion of the NH_2 group gives a significant ion (12 %) while further loss of SO affords the base peak. Expulsion of the SO_2NH_2 from the molecular ion produces the second most abundant peak. Loss of carbon monoxide gives the base peak in the aldehyde (*33*) with further loss of chlorine radical producing a significant peak.[15]

The naphthalene derivatives (*34*,*35*) behave similarly. Loss of halogen and NS from the molecular ion base peaks produce the most significantly ions in the spectra.

CONCLUSION

The dominating feature of the mass spectra of the benzo-2,1,3-thiadiazoles is that they almost all exhibit molecular ions of very great abundance (often the base peak as in the benzo-2,1,3-selenodiazoles [3]). The fragmentation of the ring system is far less important than fragmentation of the substituents. Thus it is often easy to deduce from the mass spectra the nature, but not the positions of the substituents except for the hydroxy- and alkoxy-benzo-2,1,3-thiadiazoles. Fragmentations analogous to the benzyne formation postulated for the benzo-2,1,3-selenodiazoles [3] are not found.

EXPERIMENTAL

The benzo-2,1,3-thiadiazoles were synthetized in known ways.[16,17]

The mass spectra were determined on a GEC-AEI MS-902S double focusing mass spectrometer with ionizing potential of 70 eV and an accelerating potential of 8 kV. The compounds were introduced *via* the direct insertion probe at an inlet temperature of 150 °C.

Acknowledgements. Thanks are expressed to Dr. van Daalen for some samples, Statens Videnskabsfond for a grant, and the support of I. J. by the Institute of Petroleum.

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Short Communications

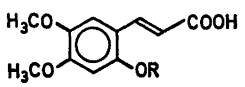
Studies on Orchidaceae Glycosides. 3.* A New Glycoside, 2-(β-D-Glucopyranosyloxy)-4,5-dimethoxy-*trans*-cinnamic Acid (Densifloroside), from *Dendrobium densiflorum* Wall.

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A small number of phenolic glycosides of some *o*-hydroxycinnamic acids have been reported to occur in Nature.[2–5] Results indicating the presence of glycosides of this type in *Orchidaceae* have also been reported. Thus, extracts from some orchids have been shown to give coumarin upon acid hydrolysis or treatment with emulsin.[6] It has further been demonstrated that acid hydrolysis of an extract from *Dendrobium thyrsiflorum* Rchb. f. gives rise to 6,7-dimethoxycoumarin, 6,7-methylenedioxycoumarin, and 7-hydroxy-6-methoxycoumarin.[7] It was suggested that these coumarin derivatives were formed from glycosides of the corresponding *o*-hydroxy-*trans*-cinnamic acids.

In this communication we report the isolation and characterisation of 2-(β-D-glucopyranosyloxy)-4,5-dimethoxy-*trans*-cinnamic acid, named densifloroside (I), and the corresponding *cis* isomer (II).



I

R=β-D-glucopyranosyl

The glycoside I was isolated from a methanolic extract of *D. densiflorum* by gel filtration, chromatography on silica gel and crystallisation. Spectrochemical and elemental analysis indicated that I has the molecular formula $C_{17}H_{22}O_{10}$. Sugar [8,9] and methylation analysis [10] showed I to be a glucopyranoside. Since an aqueous solution of the sugar showed a positive rotation, the sugar moiety in I is D-glucose. Hydrolysis of I gave 6,7-dimethoxycoumarin, and hence the aglycone of I must be either *cis* or *trans* 2-hydroxy-4,5-dimethoxycinnamic acid. The NMR spectrum of densifloroside (I) shows *inter alia* two doublets at δ 6.46 and 7.97 with a coupling constant of 16 Hz, which established that the double bond has the *trans* configuration.[11]

The IR spectrum of I shows a strong absorption at 1705 cm^{-1}, attributed to an α,β-unsaturated carboxylic acid group. After treatment of I with triethylamine, the absorption band at 1705 cm^{-1} was absent but a new band appeared at 1545 cm^{-1}. The absorption bands in the UV spectrum of I showed a hypsochromic shift of 7 nm when the conditions in the solution were changed from acid to alkaline. These results show that I is a phenolic glycoside and not an ester of D-glucose. The β-configuration of the glucopyranose residue is evident from the large negative specific rotation [12] and the chemical shift (δ 4.88) together with the coupling constant (6 Hz) of the anomeric proton.

Another glycoside was found in the methanolic extract. The amount isolated was too small to permit a complete characterisation but, according to its NMR spectrum, it should be the *cis* isomer of densifloroside (I). At present it is not known whether the *cis* or the *trans* isomer prevails in the plant, since the *cis* glycoside was found to isomerize to the corresponding *trans* isomer during the isolation procedure. The proportions of the *cis* and the *trans* isomers may also be influenced by the conditions under which the plants have grown. It has been shown that the ratio of the *cis* and *trans* isomers of 2-(β-D-glucopyranosyloxy)-cinnamic acid in *Melilotus albus* depends on the light during cultivation.[13] If the plants were grown in sunlight, the *cis* isomer was formed, whereas in the shade only the *trans* isomer was formed. On the other hand if the plants were grown first in sunlight and then for a short period in the shade, a mixture of the *cis* and *trans* isomers was found.

Acid hydrolysis of the crude aqueous extract of *D. densiflorum* gave coumarin, 6,7-dimethoxycoumarin, and 6,7-methylenedioxycoumarin. As these compounds do not occur as such in the plant, it seems reasonable to assume that other glycosides of the *o*-hydroxycinnamic acid

* For paper 2 in this series, see Ref. 1.

type are present in the plant in addition to I and II.

Experimental. General conditions were the same as in the previous communication.[1]

Isolation of densifloroside (I). Fresh plants of *Dendrobium densiflorum* Wall. (8.8 kg) were extracted with methanol (20 l). The extract was concentrated to 0.75 l and washed with chloroform (4 × 0.2 l). Part of the crude aqueous extract (200 ml) was evaporated to dryness and the residue was filtered through Sephadex LH-20 (7.5 × 65 cm) using ethanol-water (1:1) as eluent. The progress of the separation was followed by TLC. The fraction containing the glycosides was evaporated to dryness and the residue (4.0 g) was chromatographed on silica gel (5.0 × 35 cm) using ethanol as eluent. The fraction containing crude densifloroside (I) was evaporated to dryness and the residue was recrystallised from water giving white needles, (0.15 g), m.p. 225 – 227 °C; $[\alpha]_D^{22}$ – 119° (*c* 0.23, methanol). (Found: C 49.7; H 6.0; O 44.3. Calc. for $C_{17}H_{22}O_{10}.1\frac{1}{2}H_2O$: C 49.4; H 6.1; O 44.5). UV, nm (ε): λ_{max} (methanol) 332 (8900), 284 (8550), 234 (7650). IR: ν_{max} (KBr) 3550 (m), 3500 – 2450 (m), 1705 (s), 1640 (m), 1612 (m). NMR (DMSO-d_6): δ 3.0 – 3.65 (7 H), 3.76 (s, 3 H), 3.79 (s, 3 H), 4.3 – 5.6 (4 H, exchangeable in D_2O), 4.88 (d, 1 H, J = 6 Hz), 6.87 (s, 1 H), 7.19 (s, 1 H), 6.46 and 7.97 (2 H, AB pattern. J_{AB} = 16 Hz).

Isolation of the cis-*isomer (II).* Part of the concentrated plant extract (50 ml) was fractionated on Sephadex LH-20 as above. The fraction containing II was chromatographed on silica gel (2.8 × 29 cm) using chloroform:methanol:water (65:35:10) as eluent. Preparative TLC in the same system of the eluent fraction containing II from the silica gel column gave a small amount of II (2 mg, R_F 0.19) together with some I (R_F 0.29). NMR (CD_3OD): δ 3.50 – 3.77 (~6 H, partly overlapping with the CD_2HOD signal) 3.81 (s, 3 H), 3.84 (s, 3 H) 4.80 (anomeric proton and HDO signal), 5.96 and 6.76 (2 H, AB pattern, J_{AB} = 13 Hz), 6.91 (s, 1 H) 7.49 (s, 1 H).

Sugar analysis. Densifloroside (I) (11 mg) was hydrolysed in sulfuric acid (0.25 M, 4 ml) at 100 °C for 15 h. The hydrolysate was neutralised with barium carbonate, filtered and washed with chloroform (5 × 1 ml). The solution was concentrated to 1.00 ml and the optical rotation measured (α_D^{22} + 0.133). To a part of this solution (0.400 ml), xylose (0.405 mg) and arabinose (0.500 mg) were added as internal standards and the sugars were converted into the corresponding alditol acetates and analysed by GLC[8] – MS.[9] The amount of D-glucose was determined from the gas chromatogram and found to be 2.63 mg/ml. The specific rotation was found to be + 51°, in good agreement with the published value for D-glucose.

Acid hydrolysis. The crude aqueous extract (100 ml) was refluxed with hydrochloric acid (2 %, 100 ml) overnight. After cooling, the reaction mixture was extracted with chloroform (5 × 50 ml). The combined chloroform extracts were evaporated to an oily syrup which was chromatographed on silica gel (4.5 × 24 cm) using chloroform as eluent. The first fraction yielded coumarin (110 mg, R_F 0.43) which was recrystallised from hexane/ethanol, m.p. 69.5 – 70 °C (Lit.[14] m.p. 67 – 67.5 °C). The second fraction (770 mg) contained a mixture of coumarin, 6,7-dimethoxycoumarin (R_F 0.23), and 6,7-methylenedioxycoumarin (R_F 0.35). Evaporation of the solvent and recrystallisation of the residue from methanol yielded 6,7-dimethoxycoumarin (140 mg), m.p. 143 – 145 °C (Lit.[15] m.p. 144 – 146 °C). From the mother liquor 6,7-methylenedioxycoumarin (3 mg) was isolated by preparative TLC on silica gel using dichloromethane as eluent; m.p. 228 – 229.5 °C (Lit.[16] m.p. 231 – 232 °C) after recrystallisation from ethylacetate. The identities of coumarin, 6,7-dimethoxycoumarin and 6,7-methylenedioxycoumarin were further confirmed by NMR and MS.

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Metal Ion Oxidation. IV.[1] Oxidative Acetoxylation of Arenes by Bis-(2,2′-bipyridine)-silver(II) Peroxydisulfate in Acetic Acid

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Organic complexes of silver(II) are known to be strong oxidants.[2] The stability is dependent on the nature of the ligands as well as the acidity of the medium employed. Some of these complexes have been shown to be effective in the oxidation of carboxylic acids,[3] alkylarenes [4–6] and alcohols.[4–6]

The direct displacement of hydrogen by an acetoxy group in oxidation reactions is of synthetic importance.[7–9] We now wish to report a new route to aryl and benzyl acetates using bis-(2,2′-bipyridine)-silver(II) peroxydisulfate,** *1*, in the oxidative acetoxylation of aromatic compounds. When *1* was stirred with an excess of anisole in glacial acetic acid for 65 h at room temperature the three isomeric acetoxyanisoles were formed in 31 % yield, based on silver(II). (It is assumed that 2 Ag(II) ions are required to form one molecule of aryl acetate.) However, since the brown color of *1* had not disappeared the reaction had not gone to completion.

A remarkable effect was observed when the reaction was repeated in the presence of 0.5 M sodium acetate. After 17 h the reaction was complete as indicated by the change in color from brown to yellow. The yield of acetoxyanisoles was now 157 %. This shows that the peroxydisulfate ion must be involved in the overall reaction. In a control experiment it was shown that no reaction occurred when potassium peroxydisulfate was employed in the absence of silver(II) or silver(I) ion. We therefore assume that the peroxydisulfate ion in *1* is utilized for reoxidizing silver(I) ions and that every peroxydisulfate ion is capable of oxidizing two silver(I) ions.[10] The latter assumption was borne out by the fact that 4-methoxytoluene was oxidized by *1* in the presence of sodium acetate yielding 290 % of a mixture of 4-methoxybenzyl acetate and 4-methoxybenzaldehyde in a ratio of 76:24. Assuming that 2 Ag(I) ions are oxidized by one peroxidisulfate ion the theoretical yield is 300 % based of silver(II). Table 1 contains additional data for several compounds.

Table 1. Product yields in the $Ag(bipy)_2S_2O_8$ oxidation of arenes in 0.5 M NaOAc/HOAc based on Ag(II).

Compound	Reaction time[a] h	Yield of ArOAc %
Anisole	17	157 (*o*:*m*:*p* = 68:1:31)
Biphenyl	22	55 (*o*:*p* = 22:78)[b]
Naphthalene	5	190 (α:β = 95:5)
1,4-Dimethoxybenzene	2	183
Mesitylene	65	42[c]

[a] Complete reaction. [b] 0.1 % *meta* isomer. [c] 3,5-Dimethylbenzaldehyde (7 %) and 3,5-dimethylbenzyl acetate (18 %) were also formed.

The isomer distribution in the silver(II) oxidative acetoxylation of aromatic compounds shown in Table 1 is similar to that obtained in anodic acetoxylation.[11] If the instability of the acetoxy radical [12] is also taken into account, it appears safe to conclude that the initial step in the oxidation of aromatic compounds by silver(II) in acetic acid is the transfer of one electron from the aromatic substrate to the silver(II) ion analogously to the corresponding anodic process.[7] The proposed reaction sequence for the formation of acetoxyarenes compounds in the silver(II) oxidation is shown below. The same mechanism has been suggested

$$Ag(II) + ArH \rightleftharpoons Ag(I) + ArH^{+}$$

$$ArH^{+} + OAc^{-} \rightleftharpoons {}^{\cdot}Ar(H)(OAc)$$

$$Ag(II) + {}^{\cdot}Ar(H)(OAc) \rightleftharpoons Ag(I) + {}^{+}A(H)(OAc)$$

$$+Ar(H)(OAc) \longrightarrow ArOAc + H^{+}$$

$$2Ag(I) + S_2O_8^{2-} \rightleftharpoons 2Ag(II) + 2\,SO_4^{2-}$$

for several reactions involving oxidation by metal ions.[1,13–19] It has been suggested that in aqueous systems the sulfate anion radical, which is an intermediate in the decomposition of peroxydisulfate ions,[20] reacts with aromatic compounds giving cation radicals.[21] Although a similar reaction is possible in the acetic acid

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** Prepared in the same way as the corresponding pyridine complex; see Palmer, W. G., Ed., *Experimental Inorganic Chemistry*, Cambridge University Press, Cambridge 1965, p. 158.

system we consider it unlikely, since no acetoxy-aromatic compounds were formed when potassium peroxydisulfate was stirred in a mixture of acetic acid and an aromatic compound even at elevated temperatures. The effect of sodium acetate on the reaction rate is probably caused by its buffer capacity, since protons are produced in the reaction.

Our assumption that the peroxydisulfate ion reoxidizes silver(I) ions led us to investigate the possibility of running the reaction catalytically in silver(II) by using an excess of peroxydisulfate ion. Thus, when *1* was stirred in 0.5 M sodium acetate/acetic acid containing naphthalene in the presence of an excess of potassium peroxydisulfate * a mixture of α- and β-acetoxynaphthalenes in a ratio of 95:5 was obtained in a 2700 % yield based on silver(II) and a 22 % yield based on peroxydisulfate, respectively. A similar oxidation of 4-methoxytoluene produced a mixture of 4-methoxybenzaldehyde and 4-methoxybenzyl acetate in a 4000 % yield (21 % based on peroxydisulfate).

We are currently investigating other silver(II) complexes than that used in the experiments described above. The tetrapyridine complex of silver(II) peroxydisulfate turned out to be an even stronger oxidant, but its use in acetic acid is limited due to oxidative decarboxylation of the solvent. We have also found that it is possible to acetoxylate aromatic compounds using silver(I) acetate in the presence of 2,2′-bipyridine and potassium peroxydisulfate.

Experimental. Compound *1* was prepared in the same way as the corresponding tetrapyridine complex. Acetic acid was frozen out twice before use. Other compounds were of high commercial purity. Reaction mixtures were analyzed by GLC with a Varian 1400 gas chromatograph on a 2.5 m × 0.3 cm 5 % NPGS on Chromosorb W column or a 3 m × 0.3 cm 10 % APL on Chromosorb P column. The products were identified by comparison with authentic samples. Yields were calculated by integration of the GLC peaks after calibration of the authentic samples against a standard.

A solution of the aromatic substrate (10 mmol) and the complex in 20 ml 0.5 M sodium acetate in acetic acid was stirred at room temperature. Ether was then added and the salts were filtered off. The filtrate was washed with water, dried over anhydrous sodium sulfate and analyzed by GLC. The results are shown in Table 1.

In the catalytic oxidations a suspension of $K_2S_2O_8$ (10 mmol), *1* (0.1 mmol), aromatic substrate (20 mmol), and 40 ml 0.5 M NaOAc/HOAc was stirred at room temperature during 17 h. The reaction mixture was worked up as above.

* The Ag(II) complex and potassium peroxydisulfate are practically insoluble in acetic acid.

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Mutarotational Equilibrium in Water of 3-*O*-Methyl-D-glucose and 3-*O*-Methyl-D-xylose

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In their extensive studies of pyranose-furanose equilibria of aldoses and deoxyaldoses,[1-4] Angyal and Pickles have observed that the proportion of furanose forms in aqueous solution of 3-deoxyglucose is much higher than that for glucose, for which the proportion of furanose forms is insignificant. They rationalize this fact by suggesting the presence of an unfavourable *cis* interaction between the C-3 hydroxyl group and the side-chain at C-4 in glucofuranose. Removal of this interaction by introducing a deoxy grouping at C-3 is thought to be responsible for the higher amount of furanose forms at equilibrium in aqueous solutions of 3-deoxyglucose.[4]

The relative stability of furanose forms of 3-substituted glucose and xylose derivatives as compared to the corresponding pyranose forms has interested us for some time. In previous investigations of the mobility of sugars on paper electrophoresis in germanate buffer [5] at pH about 10 and sulfonated benzeneboronic acid [6] at pH about 7, we found that 3-substituted glucose and xylose have mobilities higher than those observed for the parent sugars. The same effect was produced both on removal of O-3 (3-deoxyaldose) and on substitution at O-3. This effect is noticeable also for the (1→3)-linked disaccharides. Since other results strongly indicated that by far the greatest contribution to complex formation came from *cis*-1,2-hydroxyl groups in the furanose forms, we considered a possible connection between the high mobility on electrophoresis of the 3-substituted sugar and the proportion of α-D-furanose forms present. Some support for this surmise was found in studies of the composition of equilibrium mixtures obtained on methanolyses of aldoses and methylated aldoses subsequently published by Bishop and Cooper.[7]

However, if removal of O-3 and substitution at O-3 both should produce similar increases in furanose content at the mutarotational equilibrium of glucose, we find it difficult to reconcile this with the C-3 substituent/C-4 side-chain interaction suggested by Angyal and Pickles.[4]

Angyal and Pickles studied the mutarotational equilibria by means of NMR spectroscopy on solutions in deuterium oxide.[4] We have now examined the corresponding NMR spectra, in deuterium oxide, of equilibrated solutions of 3-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose. Spectral assignments were made as described by Angyal and Pickles.[2-4] The interfering DOH peak was moved by adding small amounts of acid or suppressed by pulsing techniques. D-Glucose and D-xylose were examined in the same manner. The proportions of furanose forms for these latter sugars were below the limits of detection. The results for the two 3-*O*-methylaldoses are given in Table 1 and in Fig. 1. The small differences in chemical shifts of H-1 in furanose forms and in β-D-pyranosides do not lend themselves to a high degree of accuracy in signal integrations. Clearly, however, alkylation at O-3 produces a significant increase in furanose content.

On the basis of these results we suggest that the observed increase in the amount of furanose forms of 3-deoxy-D-glucose, 3-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose as compared to glucose and xylose is not necessarily caused by an instability factor consisting in OH-3/C-4 side-chain interaction in the parent sugars. We would prefer to describe this phenomenon in terms of destabilization of the pyranose forms relatively to the furanose forms by altering the hydrogen bonding to water on removal or alkylation of O-3. A better understanding of this effect should, however, be desirable.

Table 1. Mutarotational equilibrium and first-order coupling constants of anomeric protons for 3-*O*-methyl-D-glucose, "3-deoxy-D-glucose", 3-*O*-methyl-D-xylose and parent sugars.

Aldose	Molar ratios and H-1 coupling constants ($Y_{1,2}$) α-Pyranose	β-Pyranose	α-Furanose	β-Furanose
D-Glucose [a,b]	0.38[a] (3.5 Hz)	0.62[a] (7.5 Hz)	Nil	Nil
3-*O*-Methyl-D-glucose [b]	0.39 (3.0 Hz)	0.47 (7.7 Hz)	low	0.14 (<1 Hz)
3-Deoxy-D-*ribo*-hexose [a]	0.25 (3.5 Hz)	0.55 (7.8 Hz)	0.05 (3.9 Hz)	0.15 (<1 Hz)
D-Xylose [b]	0.31 (3.2 Hz)	0.69 (7.5 Hz)	Nil	Nil
3-*O*-Methyl-D-xylose [b]	0.38 (3.3 Hz)	0.44 (7.5 Hz)	–	0.18 (2.2 Hz)

[a] Refs. 3 and 4; temperature 31 °C. [b] Present investigation; temperature 30±2 °C. Error in signal integration ±5 %.

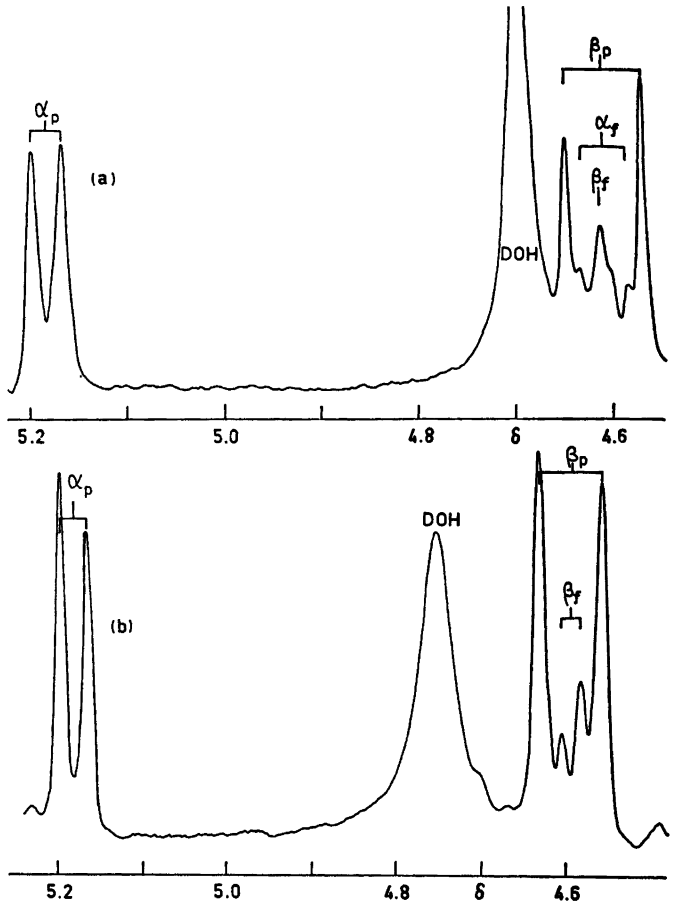


Fig. 1. 100 MHz NMR spectra showing anomeric protons only of 3-*O*-methyl-D-glucose (a) and 3-*O*-methyl-D-xylose (b).

Experimental. The spectra were recorded on a Varian XL-100, spectrometer, at 100 MHz. Deuterium oxide was used as solvent throughout, and the concentrations were 10 – 20 %. The sugars were dissolved in D_2O and their spectra recorded immediately upon dissolution and, after several days, at equilibrium. The DOH signals were suppressed by repeated concentrations and additions of fresh D_2O. Whenever required, the DOH signals were moved by adding small amounts of acid. The DOH signals were also supressed using a two-pulse techniqe (Fourier-transform spectrometry). Spectral assignments were made as described by Angyal and Pickles. Commercial samples of D-glucose and D-xylose were used. Crystalline samples of 3-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose were available in this Department; their physical constants agreed with those in the literature values.

Acknowledgements. We are indebted to the Swedish Natural Science Research Council for financial support.

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An Alternative Synthesis of 3,6-Dideoxy-D-*xylo*-hexose (Abequose)

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In our continued efforts at synthesizing *Salmonella* antigens comprising a 3,6-dideoxyhexosyl containing disaccharide unit covalently linked to an immunogenic protein,[1,2] it became necessary to find a convenient route to abequose (3,6-dideoxy-D-*xylo*-hexose) derivatives benzylated in the 2-position, suitable for use in glycoside synthesis.

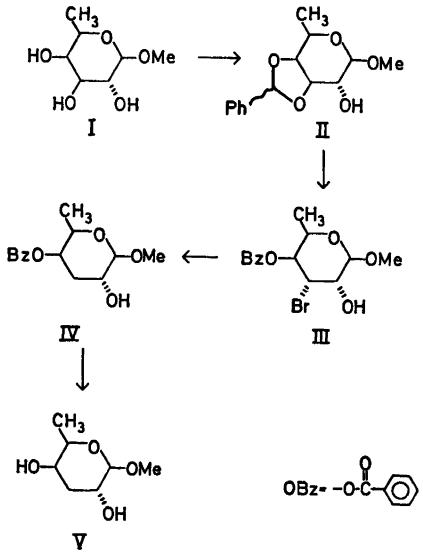
We now report the synthesis of a new abequose derivative IV, useful for this purpose. The key step is the reaction between *N*-bromosuccinimide and a benzylidene acetal devised by Hanessian and Plessas.[3] Siewert and Westphal[4] have previously described a convenient synthesis of abequose from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside.

Methyl β-D-fucopyranoside[5] (I) was prepared from methyl 4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-β-D-galactopyranoside[6] by reaction with *N*-bromosuccinimide, followed by calalytic hydrogenation and debenzoylation.[3] The overall yield of isolated crystalline material was 58 %. Treatment of I with benzylidene bromide and pyridine[7] afforded the 3,4-benzylidene derivative II as a crystalline mixture of stereoisomers. Reaction of II with *N*-bromosuccinimide[3] afforded the bromo compound III which was hydrogenated to give the 3,6-dideoxyhexose IV. Debenzoylation of IV gave methyl β-abequoside (V) in an overall yield of 21 % from I.

The benzylation of IV in the 2-position[8] followed by exchange of the benzoyl group in the 4-position with a *p*-nitrobenzoyl group affords a derivative, useful in synthesis of α-abequosides. This will be described in a forthcoming paper.

Experimental. General methods were essentially the same as those described in a previous paper.[9] GLC-MS was performed using a Varian MAT 311 instrument with a connected Varian 2700 gas chromatograph (ionization potential 70 eV, ionization current 1000 μA and ion source temperature 180 °C). Preparative separations were performed on Merck silica gel 60 prepacked columns for liquid chromatography.

Methyl 3,4-O-benzylidene-6-deoxy-β-D-galacto-hexopyranoside (II). A solution of methyl β-D-fucopyranoside (I) (2.5 g) and benzylidene bromide (3.3 ml) in pyridine (45 ml) was refluxed for 2 h. The reaction mixture was diluted with chloroform and the chloroform solution was washed with water, dried over sodium sulfate, filtered and concentrated to a syrup. TLC (toluene-ethyl acetate 1:1) showed the presence of a major component which was isolated by column chromatography using the same solvent system to yield II (2.45 g) which crystallized from diethyl ether-hexanes, m.p. 97 – 99 °C, $[\alpha]_D$ +2° (*c*, 0.6, chloroform). (Found: C 63.0; H 6.95. $C_{14}H_{18}O_5$ requires: C 63.1; H 6.81). NMR ($CDCl_3$): δ 1.45 and 1.47 (3 H, both d, both $J_{5,6}$ 6.5 Hz, isomeric H-6), 3.54, 3.53 (3 H, both s, OCH_3), 5.93 and 6.17 (1 H, both s, isomeric PhCH), 7.3 – 7.6 (5 H, C_6H_5).



Methyl 4-O-benzoyl-3-bromo-3,6-dideoxy-D-gulo-hexose (III). *N*-Bromosuccinimide (1.1 g) was added to II (1.5 g) and barium carbonate (2.2 g) in carbon tetrachloride (115 ml).[3] The suspension was refluxed with stirring for 1 h, diluted with chloroform and filtered. The filtrate was washed with water and the organic phase dried over sodium sulfate, filtered and concentrated to a syrup. TLC (toluene-ethyl acetate 4:1) showed the presence of one major component which was isolated by column chromatography using the same solvent system yielding pure III (1.23 g) $[\alpha]_D$ +9° (*c*, 0.5, chloroform) NMR ($CDCl_3$): δ 1.28 (3 H, d, $J_{5,6}$ 6.5 Hz, H-6), 2.72 (1 H, broad s, OH), 3.62 (3 H, s, OCH_3), 3.76 (1 H, dd, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 4 Hz, H-2), 4.42 – 4.75 (2 H, m, H-3, H-5), 4,67 (1 H, d, $J_{1,2}$ 7.5 Hz, H-1), 5.35 (1 H, dd, *J* 1.5 Hz, *J* 3.5 Hz, H-4); 7.43 – 7.70 (3 H, C_6H_5); 8.03 – 8.23 (2 H, C_6H_5). The assignments were confirmed by spin decoupling experiments. The axial disposition of the bromine atom is indicated by the coupling constants and is in agreement with the inversion mechanism suggested for this reaction.[3]

Methyl 4-O-benzoyl-3,6-dideoxy-β-D-xylo-hexopyranoside (IV). The 3-bromo compound

III (1.14 g) was hydrogenated at 420 kPa and room temperature with 10 % palladium on charcoal in ethanol (70 ml) containing triethylamine (0.6 ml) overnight.

Filtration and concentration gave a syrup which was dissolved in chloroform. The chloroform solution was washed with aqueous sodium hydrogen carbonate, water, dried over sodium sulfate, filtered and concentrated. The product was purified by column chromatography (toluene-ethyl acetate 1:1) to yield pure IV (0.52 g). $[\alpha]_D$ −105° (*c*, 0.8, chloroform). (Found: C 63.2; H 6.74. $C_{14}H_{18}O_5$ requires: C 63.1; H 6.81). NMR ($CDCl_3$): δ 1.27 (3 H, d, $J_{5,6}$ 6.5 Hz, H-6), 1.7–2.1 and 2.2–2.4 (2 H, m, H-3 and H-3'), 2.65 (1 H, broad s, OH), 3.60 (3 H, s, OCH_3), 3.67–4.05 (2 H, m, H-2 and H-5), 4.23 (1 H, d, $J_{1,2}$ 7.5 Hz, H-1), 5.13–5.33 (1 H, m, H-4), 7.40–7.67 (3 H, C_6H_5), 8.03–8.25 (2 H, C_6H_5).

*Methyl 3,6-dideoxy-β-*D*-xylo-hexopyranoside (V).* The 4-benzoate IV (75 mg) in methanol (10 ml) was treated with barium oxide (excess) at reflux temperature for 30 min. TLC (toluene-ethyl acetate 1:1) showed complete debenzoylation. The reaction mixture was neutralized with solid carbon dioxide, filtered and concentrated to a syrup (38 mg). The syrup was found to be chromatographically pure by TLC (ethyl acetate-methanol-water 85:10:5), having $[\alpha]_D$ −74° (*c*, 0.9, chloroform), $[\alpha]_D$ −66° (*c*, 0.5, methanol) and $[\alpha]_{546}$ −79° (*c*, 0.5, methanol) in agreement with previously recorded values.[10] An NMR spectrum ($CDCl_3$) was identical to that previously recorded for this substance.[10]

A small sample was hydrolyzed with 0.25 M aqueous sulfuric acid for 30 min at 100 °C, reduced with sodium borohydride and then acetylated.[11] The abequitol acetate thus obtained was indistinguishable from an authentic sample by GLC-MS.

Acknowledgement. We are indebted to Professor Bengt Lindberg for his interest and to the Swedish Natural Science Research Council for financial support.

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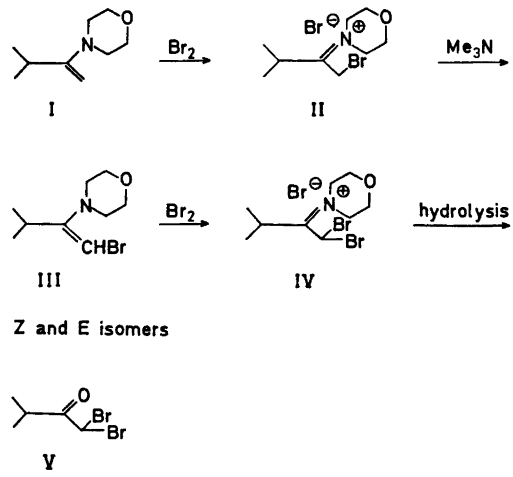
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Bromination of Enamines from Methyl Isopropyl Ketone. II. A Convenient Preparation of Dibromomethyl Isopropyl Ketone

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Dibromomethyl ketones, $CHBr_2COCHR_1R_2$, are rather difficult to prepare; generally they are obtained from the corresponding diazoketone with bromine.[1–3] However, this method is not very attractive for synthesis on a larger scale since it involves the use of excessive amounts of the noxious agent diazomethane. The preparation of bromomethyl isopropyl ketone from the morpholine enamine has recently been reported by us.[4] In our investigation of the scope of this reaction we have developed a convenient synthesis of dibromomethyl isopropyl ketone V; the reaction steps involved are summarized in



Scheme 1.

III (1.14 g) was hydrogenated at 420 kPa and room temperature with 10 % palladium on charcoal in ethanol (70 ml) containing triethylamine (0.6 ml) overnight.

Filtration and concentration gave a syrup which was dissolved in chloroform. The chloroform solution was washed with aqueous sodium hydrogen carbonate, water, dried over sodium sulfate, filtered and concentrated. The product was purified by column chromatography (toluene-ethyl acetate 1:1) to yield pure IV (0.52 g). $[\alpha]_D$ −105° (*c*, 0.8, chloroform). (Found: C 63.2; H 6.74. $C_{14}H_{18}O_5$ requires: C 63.1; H 6.81). NMR ($CDCl_3$): δ 1.27 (3 H, d, $J_{5,6}$ 6.5 Hz, H-6), 1.7–2.1 and 2.2–2.4 (2 H, m, H-3 and H-3'), 2.65 (1 H, broad s, OH), 3.60 (3 H, s, OCH_3), 3.67–4.05 (2 H, m, H-2 and H-5), 4.23 (1 H, d, $J_{1,2}$ 7.5 Hz, H-1), 5.13–5.33 (1 H, m, H-4), 7.40–7.67 (3 H, C_6H_5), 8.03–8.25 (2 H, C_6H_5).

*Methyl 3,6-dideoxy-β-*D*-xylo-hexopyranoside (V).* The 4-benzoate IV (75 mg) in methanol (10 ml) was treated with barium oxide (excess) at reflux temperature for 30 min. TLC (toluene-ethyl acetate 1:1) showed complete debenzoylation. The reaction mixture was neutralized with solid carbon dioxide, filtered and concentrated to a syrup (38 mg). The syrup was found to be chromatographically pure by TLC (ethyl acetate-methanol-water 85:10:5), having $[\alpha]_D$ −74° (*c*, 0.9, chloroform), $[\alpha]_D$ −66° (*c*, 0.5, methanol) and $[\alpha]_{546}$ −79° (*c*, 0.5, methanol) in agreement with previously recorded values.[10] An NMR spectrum ($CDCl_3$) was identical to that previously recorded for this substance.[10]

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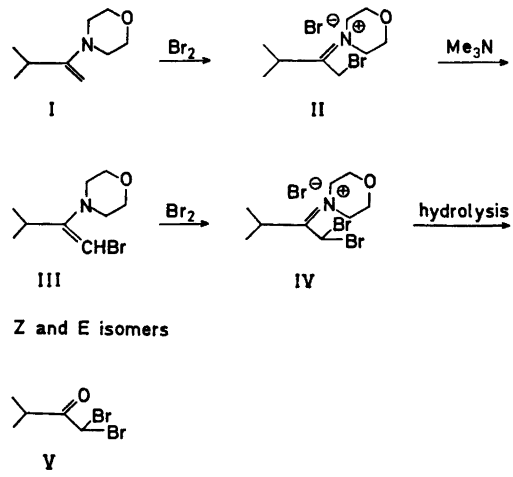
Received April 1, 1975.

Bromination of Enamines from Methyl Isopropyl Ketone. II. A Convenient Preparation of Dibromomethyl Isopropyl Ketone

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Dibromomethyl ketones, $CHBr_2COCHR_1R_2$, are rather difficult to prepare; generally they are obtained from the corresponding diazoketone with bromine.[1–3] However, this method is not very attractive for synthesis on a larger scale since it involves the use of excessive amounts of the noxious agent diazomethane. The preparation of bromomethyl isopropyl ketone from the morpholine enamine has recently been reported by us.[4] In our investigation of the scope of this reaction we have developed a convenient synthesis of dibromomethyl isopropyl ketone V; the reaction steps involved are summarized in



Scheme 1.

Scheme 1. The enamine I is easily prepared from methyl isopropyl ketone [5,6] with an average yield of *ca.* 70 %. The overall yield of V is *ca.* 40 % calculated from the starting ketone.

The bromo immonium salt II is formed by reaction of I with one equivalent of bromine in methylene chloride at −78 °C. II is rapidly deprotonated *in situ* when treated with trimethyl amine or triethyl amine giving the bromo enamine III. The deprotonation of II is complete after 5 min at −20 °C. III is converted to the dibromo immonium salt IV when reacted with a slight excess of bromine at −20 °C. Dibromomethyl isopropyl ketone V is obtained from IV by hydrolysis and the usual work up procedure. The yield of V from I was 60 % calculated from the ^{1}H NMR spectrum of the distilled crude product. The purity was 80 % (NMR). The main impurities was bromomethyl isopropyl ketone (15 %) and methyl α-bromoisopropyl ketone (4 %); other impurities were *ca.* 1 %. However, V is rather unstable and further purification by fractionation has not been successful due to a rapid rearrangement to the more stable 1,3-dibromoketone. The rearrangement is catalyzed by hydrogen bromide; this seems to be a general feature of α,α-dibromo ketones.[2,3]

The intermediates II and III have been investigated by studying their ^{1}H NMR spectra. The spectra were determined from a model experiment using deuteriochloroform as solvent instead of methylene chloride, since the resonance signal from the latter solvent obscured the desired spectra and deuterated methylene chloride was not available. However, conclusions drawn from these experiments are probably valid for explaining reactions in methylene chloride since this solvent and chloroform have very similar dissolving properties towards ion pairs.[7] The ^{1}H NMR spectra were recorded at different temperatures. At −60 °C the absorption signals from II give rise to very broad lines at δ 1.2, 3.9, and 4.4. We explain the line broadening as a result of a massive association of ion pairs to large clusters of ions. This is supported by the fact that II precipitates from the solution if the temperature is only slightly lowered. At 0 °C the lines are sharpened and give the following spectral data: A doublet *J ca.* 7 Hz at δ 1.54 from the geminal methyl groups, a septet from the isopropyl methine proton (overlapping with the morpholine signals) *J ca.* 7 Hz, δ 3.96, and a singlet from the bromomethyl group at δ 4.77. The signals from the morpholine protons appeared as a broad multiplet at δ 4.0−4.7 and not as two distinct bands as in free morpholine or in morpholinium salts. This agrees with results obtained from studies on protonation of the enamine I.[8] The ^{1}H NMR spectrum of III indicates that both the *Z*- and *E*-isomers are formed when II is treated with base (Fig. 1). In the spectrum there are two signals in the region where enaminic vinyl protons are expected to give resonance signals, and we assign the signal at low field to the vinyl proton of the *Z*-isomer. A calculation of the expected chemical shift for the vinyl protons of III (*Z*) and III (*E*) using shielding increments for substituents on ethylene [9] shows that the *Z*-isomer is expected to give resonance at a lower field than the *E*-isomer. Integration over the vinylic signals at −60 °C shows a ratio *Z*:*E* = 4:6. No change in this ratio was found by varying the temperature. This isomeric distribution is probably kinetically determined, but we have not yet studied the equilibrium conditions. No changes in chemical shift were noted in the spectra recorded at different temperatures, −60, 0 and 31 °C. The following data for III (*Z*) and III (*E*) were found: III (*Z*) shows a doublet, *J ca.* 7 Hz, δ 1.11 and a somewhat broadened signal from the vinyl proton at δ 5.52. III (*E*) shows a doublet, *J ca.* 7 Hz, δ 1.19, and a sharp singlet at δ 5.33. The expected septets from the methine protons were not distinguished due to overlap with the morpholine signals. The bromoenamine is not stable at room temperature and it decomposes within an hour at this temperature. Attempts to record ^{1}H NMR spectra from IV has not been successful. IV is not very soluble in deuteriochloroform at low temperature and decomposes rapidly at room temperature.

The ^{1}H NMR samples were hydrolyzed and the organic layer analyzed by means of GLC. The bromoketones found agreed with the results from the ^{1}H NMR analysis of II and III. GLC-analysis of attempted small scale preparations of IV and subsequent hydrolysis did not agree with large scale experiments and are therefore of questionable reliability.

We have examined the optimum conditions for synthesis of V and these are presented in the experimental section. In this context some experimental findings are of importance: The conversion of I to II is best performed by adding the enamine to an equivalent amount of bromine in methylene chloride at −78 °C. The proton abstraction from II is rather fast at −78 °C but the reaction goes still faster if the reaction mixture is warmed to −20 to −10° after the base has been added. Under these conditions proton abstraction is complete within 5 min. Use of excess base did not improve the yield of V. The reaction mixture should be kept below 0 °C during the reactions involving brominations. Brominations performed at room temperature yielded



III (E) III (Z)

Fig. 1.

mostly the 1,3-dibromoketone. Attempts to use the stronger base potassium *tert*-butoxide instead of trimethyl- or triethylamine were unsuccessful, since only traces of V were formed. The main product from these experiments was the bromomethyl isopropyl ketone in *ca.* 80 % yield (GLC). A probable explanation is the low solubility of potassium *tert*-butoxide in methylene chloride and that the proton abstraction from II with base in a two phase system is a very slow reaction. Immediate work up of V is necessary since even traces of acid catalyse the rearrangement to the isomeric 1,3-dibromoketone.

Experimental. ^{1}H NMR spectra were recorded on a Varian A-60 A instrument fitted with a Varian V 60–40 Temperature Programming Unit using liquid nitrogen as cooling agent. Probe temperature was measured with a thermometer inserted in the sample area. The thermometer readings corresponded to the calibrated methanol spectra. The purity of V was checked on a JEOL C-60 HL NMR-spectrometer. TMS was used in all experiments as an internal reference. GLC analysis was performed on a PYE M 64 Gas Chromatograph with flame ionization detector. A 270 cm × 6 mm 12 % QF-1 column at 120 °C was used. Mass spectrum of V was obtained with a LKB 9000 Mass spectrometer *via* the GLC-MS inlet system.

Compound I was prepared by the method of White and Weingarten [5] and the work up procedure was essentially that described by Pocar *et al.*[6] The purity of I prepared by this method was high, > 95 % (^{1}H NMR).

Small scale experiment for obtaining ^{1}H NMR-spectra of II and III. 0.78 g of I (5.0 mmol) was added in one portion to an equivalent amount of bromine in 10 ml of deuteriochloroform at − 50 °C. The resulting mixture was stirred for 1 min and a 2.5 ml aliquot was withdrawn, 0.5 ml of which was taken to ^{1}H NMR analysis the rest being hydrolyzed by shaking with 2 ml of distilled water in a ground glass stoppered test tube. The organic layer was dried ($MgSO_4$) and analysed by GLC. To the reaction mixture containing II 0.6 ml of trimethyl amine (excess) was added and the resulting mixture was warmed to − 20 °C and stirred for 5 min. A 2.5 ml aliquot was taken, filtered and treated as above with the exception that 0.3 ml of concentrated hydrochloric acid was added prior to hydrolysis, to avoid base induced rearrangement of the bromo ketone formed. The ^{1}H NMR samples were kept at − 78 °C between recordings. Details of the attempted preparation of IV have been omitted since we did not succeed in obtaining a ^{1}H NMR spectrum of this compound.

Preparation of dibromomethyl isopropyl ketone V. To a stirred solution of 5.2 ml of bromine in 150 ml of methylene chloride at − 78 °C 15.5 g (0.10 mol) of I dissolved in 50 ml of methylene chloride was rapidly added. The mixture was stirred for 2 min whereafter 11.0 g (0.109 mol) of triethyl amine diluted with 25 ml of methylene chloride was added with vigorous stirring and the resulting mixture was kept at − 78 °C for 2 min. It was then warmed to − 20 °C (crushed ice-acetone) and stirring was continued at this temperature for an additional 5 min. 6 ml of bromine in 25 ml of methylene chloride was then added rapidly with vigorous stirring. After 5 min 100 ml of distilled water were introduced and the cooling bath was replaced by a water bath at room temperature. Stirring with water was continued for 15 min. (It is not advisable to exceed this period since V rearranges rapidly to the 1,3-dibromo ketone in acidic media.) The layers were separated and the aqueous layer was extracted with 2 × 50 ml of methylene chloride. The combined organic layers were shaken with dilute $NaHSO_3$-solution to destroy excess of bromine and washed with 4 × 100 ml of distilled water. The organic layer was dried by shaking with anh. sodium sulfate for 5 min. Filtration and rapid evaporation of the solvent *in vacuo* afforded the crude bromo ketone which was freed from low boiling impurities by an immediate "bulb to bulb" distillation yielding 17.1 g of distilled product, b.p. 63 – 67 °C/0.8 mmHg. ^{1}H NMR analysis showed that it contained 80 % of V, the main impurities being the monobromo ketones $CH_2BrCOCH(CH_3)_2$ (15 %) and $CH_3COCBr(CH_3)_2$ (4 %). The product contained only 1 % of the 1,3-dibromo ketone $CH_2BrCOCBr(CH_3)_2$. The actual yield of V from I was thus 14.7 g (60 %). ^{1}H NMR-spectrum of V (*ca.* 10 % in CCl_4) shows a doublet (6 H) J *ca.* 7 Hz, δ 1.23, a septet (1 H) J *ca.* 7 Hz, δ 3.30, and a singlet (1 H) δ 5.87. Mass spectrum of V shows a typical Br_2-isotopic cluster at $m/e = 242$, 244, and 246 (molecule ion), and a similar cluster at $m/e = 171$, 173, and 175 characteristic for the $CHBr_2$-ion. The base peak appeared at $m/e = 71$ indicating a loss of $CHBr_2$ from the molecule ion.

Acknowledgements. Financial support from the Swedish Natural Research Council is gratefully acknowledged.

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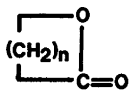
Received February 26, 1975.

12-Methyl-ω-tridecanolide, A New Macrocyclic Lactone from Angelica Root Oil

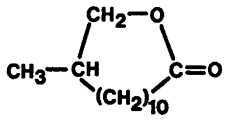
JYRKI TASKINEN

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Three macrocyclic lactones, ω-tridecanolide, ω-pentadecanolide, and ω-heptadecanolide (I, n = 12, 14, and 16, respectively), have been found in the root oil of *Angelica archangelica* L.[1-3] Recently an unknown compound, which also was supposed to be a macrolide, was reported to be present in this oil, of which it constituted less than 0.1 %.[3] On the basis of spectral evidence, structure II, 12-methyl-ω-tridecanolide, is now proposed for this lactone.



I



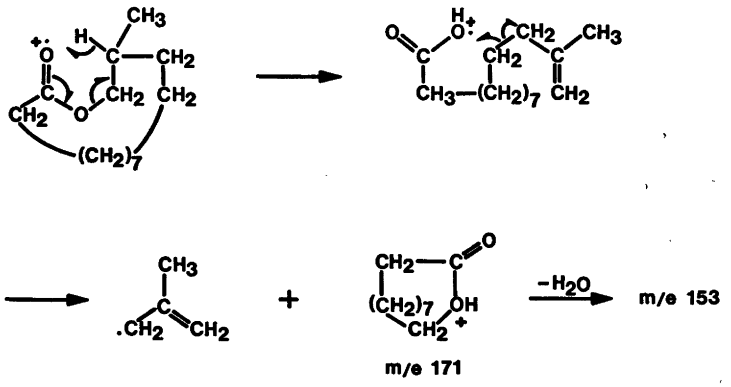
II

The molecular formula $C_{14}H_{26}O_2$ was evident from the mass, IR and ^1H NMR spectra. The mass spectrum was very similar to those of known macrolides, displaying molecular the peak at m/e 226, base peak at m/e 55 and typical M − 18, M − 36, and M − 60 fragments.[4] Some anomalous peaks were present, however, the most abundant of which in the high mass region being at m/e 171 (9 %) and 153 (18 %). A small peak (2 %) for the M − 15 ion was also observed. The IR spectrum had strong absorption bands at 1735 cm^{-1} (C=O) and 1235 cm^{-1} (C−O). The spectrum closely resembled those of the other macrolides, except for a band at 1375 cm^{-1} that implied that a methyl group may be present. The NMR signal at δ 0.92 (3 H, d, J 6.8 Hz) confirmed the presence of a methyl on a carbon bearing one hydrogen. Signals centered at δ 3.68 (1 H, doublet of doublets, J 10.7 and 8.3 Hz) and at δ 4.20 (1 H, doublet of doublets, J 10.7 and 3.4 Hz) were evidently due to two mutually coupled protons, both of which are coupled to another proton, and were assigned to a methylene group carrying an oxygen. Decoupling experiments showed that the methylene and the methyl protons were coupled to the same proton. This establishes that the methyl group is on the carbon β to the ether oxygen. A 2 H multiplet centered at δ 2.38 was assigned to the methylene adjacent to carbonyl.

The lactone was hydrolyzed in alcoholic KOH and the hydroxy acid obtained was methylated with diazomethane. The mass spectrum of the methyl ester had its base peak at m/e 55, and a prominent peak characteristic for methyl esters appeared at m/e 74. All peaks of the series m/e 87, 101... up to 199 were present, and no branching in the middle of the chain was apparent from the spectrum. The molecular peak was not observed. In the high mass range the most abundant peak appeared at m/e 228 (M − 30, 78 %). This peak is probably due to the ion formed by cleavage of the bond between C-12 and C-13 (which carries the hydroxyl group) and rearrangement of one hydrogen atom. Prominent peaks due to ions formed by analogous fragmentation are found in the spectra of some methyl hydroxyoctadecanoates published by Ryhage and Stenhagen.[5]

The corresponding ion (M − 44) produces the base peak in the spectrum of methyl 17-hydroxyoctadecanoate. Because reference spectra of ω-hydroxyesters were not available, methyl 15-hydroxypentadecanoate was prepared from ω-pentadecanolide. The most prominent peak (28 %) in the high mass range in the spectrum of this ester was also produced by the M − 30 rearrangement ion.

From the NMR data and mass spectrum of the methyl ester it is evident that the com-



Scheme 1.

pound has an ω-lactone ring with only one methyl side chain located on C−12, as in II.

The formation of the ions *m/e* 171 and 153, analogues of which are not present in the spectrum of unbranched macrolides, can be rationalized as shown in scheme 1. McLafferty rearrangement of the C-12 hydrogen followed by cleavage of the bond β to the newly formed double bond would form a fragment of *m/e* 171. Loss of water from this ion would produce the *m/e* 153 ion.

Experimental. Gas chromatography was performed on a Hewlett-Packard 7620 A instrument with 6 mm × 3 m stainless steel columns packed with 10 % FFAP or 5 % SE-30 on Chromosorb W AW. IR spectra were recorded on a Perkin-Elmer 521 instrument. Mass spectra were run on a JEOL JMS-D 100 mass spectrometer at the Technical Research Center of Finland. ^{1}H NMR spectra were determined in $CDCl_3$ on a JEOL JMM PFT 100 spectrometer in the Department of Organic Chemistry, University of Helsinki.

The isolation of angelica root oil and separation of the fraction containing the oxygenated compounds have been described before.[3]

12-Methyl-ω-tridecanolide. About 0.2 mg of II was separated gas chromatographically from the oxygenated fraction of angelica root oil.

MS: *m/e* (%) 226 (M^+, 5) 211 (2), 208 (14), 198 (4), 196 (5), 193 (3), 190 (3), 171 (9), 160 (8), 165 (8), 153 (18), 152 (11), 139 (9), 124 (14), 111 (25), 98 (41), 97 (36), 84 (34), 83 (42), 69 (67), 56 (61), 55 (100), 41 (96). IR: ν_{max} 2920, 2875, 1735, 1458, 1375, 1345, 1235, 1170, 1145, 1105, 1060, 1007 cm^{-1}. NMR: δ ($CDCl_3$) 0.92 (3 H, d, *J* 6.8 Hz), 2.38 (2 H, m), 3.68 (1 H, doublet of doublets, *J* 10.7 and 8.3 Hz), 4.20 (1 H, doublet of doublets, *J* 10.7 and 3.4 Hz).

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Synthesis of Triallylmethane

GÖRAN LINDGREN and LARS ADOLFSSON

Department of Chemistry, Div. II, Agricultural College, S-750 07 Uppsala 7, Sweden

The aim of this note is to report the preparation of pure triallylmethane (*4a*), which was needed for the synthesis of 2,4,9-trithiaadamantane (*5a*).

In 1963, Shelden [1] treated the Grignard reagent from diallylmethyl chloride (*1a*) with allyl bromide. He obtained about equal amounts of *4a* and an isomer, believed to be 4-allyl-1,7-octadiene (*3a*), but failed to separate the products. In 1968, Reeve and Bianchi [2] obtained tetraallylmethane (*4b*) and 4-allyl-4-vinyl-1,7-octadiene (*3b*) from triallylmethyl chloride (*1b*) in the same way. They explained the formation of *3b* by the rearrangement shown in Scheme 1.

Following a suggestion by Shelden,[1] we have now prepared *4a* (=*2b*) by hydrolyzing the Grignard reagent from *1b*. 4-Methyl-4-vinyl-1,6-heptadiene (*6b*) was obtained as a by-product but the isomers *4a* and *6b* were cleanly separated by distillation through a spinning band column. Their purity was checked by GLC analysis, and their identity established by ^{1}H NMR spectroscopy (see Experimental). Thus, the integration of either spectrum showed 7 paraffinic and 9 olefinic protons. The latter belong to three vinyl groups, since the elemental analyses and boiling points confirmed the molecular formula $C_{10}H_{16}$. Spin decoupling of the vinyl hydrogens revealed six allylic protons in *4a* and four in *6b*. The coupling of the remaining paraffinic proton in *4a* to all six allylic protons and the absence of further coupling in *6b* exclude alternative structures. As shown in Scheme 1, both *6b* and Shelden's product *3a* are the rearranged hydrocarbons predicted by the mechanism of Reeve and Bianchi.

As might have been expected, the mass spectrum of *4a* showed no molecular peak but was dominated by peaks due to the allyl cation (*m/e* 41, 100 %) and to the loss of an allyl radical (*m/e* 95, 47 %). In the spectrum of *6b*, the former peak (75 %) was weaker than the latter (100 %). This difference may be due to the fact that the fragmentations of *6b* are doubly allylic. Otherwise, the mass spectra as well as the IR spectra of the two isomers were quite similar.

Experimental. Freshly distilled triallylmethyl chloride (*1b*, 40 g, 0.23 mol) was prepared and converted to the Grignard reagent according to Reeve and Bianchi.[2] The resulting solution was poured on to a stirred mixture of ether (100 ml), 2 M hydrochloric acid (200 ml) and crushed ice (200 g). The organic layer was separated, washed with water and dried with magnesium sulfate. The ether was removed by rotary evaporation below 20 °C and the residue

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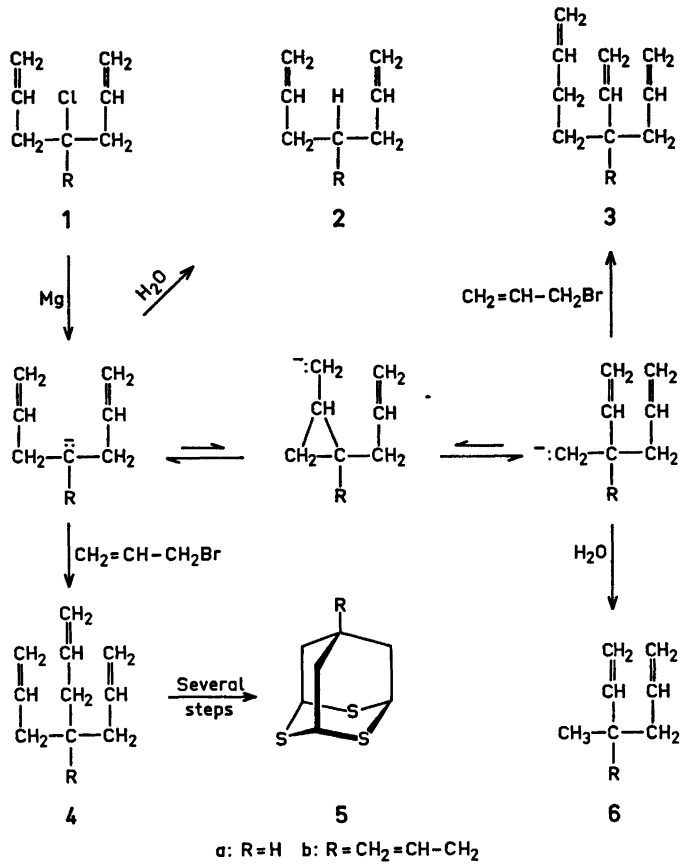
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Experimental. Freshly distilled triallylmethyl chloride (*1b*, 40 g, 0.23 mol) was prepared and converted to the Grignard reagent according to Reeve and Bianchi.[2] The resulting solution was poured on to a stirred mixture of ether (100 ml), 2 M hydrochloric acid (200 ml) and crushed ice (200 g). The organic layer was separated, washed with water and dried with magnesium sulfate. The ether was removed by rotary evaporation below 20 °C and the residue



Scheme 1.

distilled *in vacuo* through a short column. GLC analysis of the distillate collected at 30–45 °C/10 mmHg showed *4a* (8 g, 25 %) and *6b* (3 g, 10 %). Redistillation through a 60 cm tall spinning band column (reflux ratio 20:1) yielded six fractions.

4-Methyl-4-vinyl-1,6-heptadiene (6b). The second fraction consisted of pure *6b* (1.0 g, 3 %), b.p. 38–39 °C/10 mmHg, n_D^{20} 1.4476. IR, ν_{max} (neat): 912vs (broad), 997s, 1375m, 1415m, 1440m, 1458m, 1620s, 2840w, 2920s, 2980s, 3000w, 3080s cm^{-1}. ^{1}H NMR δ (CCl_4): 0.94 (3 H, s), 2.03 (4 H, broad d, J 7 Hz), 4.9 (6 H, m), 5.7 (3 H, m). (Found: C 88.2; H 11.9. Calc. for $C_{10}H_{16}$: C 88.2; H 11.8).

4-Allyl-1,6-heptadiene (triallylmethane, 4a). The last fraction consisted of pure *4a* (6.4 g, 20 %), b.p. 43–44 °C/10 mmHg, n_D^{20} 1.4520. IR, ν_{max} (neat): 912vs, 995s, 1344w, 1412m, 1442s, 1622s, 2840w, 2918s, 2980s, 3000w, 3080s cm^{-1}. ^{1}H NMR, δ (CCl_4): 1.57 (1 H, septet, J 6.2 Hz), 2.04 (6 H, broad t, J 7 Hz), 4.96 (6 H, broad d, J 12 Hz), 5.7 (3 H, m). (Found: C 88.4; H 11.7. Calc. for $C_{10}H_{16}$: C 88.2; H 11.8).

Acknowledgements. We wish to thank Professor Olof Theander for his kind interest in the present work. This was supported by grants from the Knut and Alice Wallenberg Foundation, the Swedish Council for Forestry and Agricultural Research and the Swedish Natural Science Research Council. We are also indebted to Mr. Rolf Andersson for recording the spectra and to Mr. Gunnar Hesselius for performing the elemental analyses.

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Acta Chemica Scandinavica B 29 (1975) 641—646

The Oxidation of Glycosides. XVIII.* Effect of pH on the Degradation of Various Isomers of Methyl Hexodialdopyranoside-(1,5)

HÅKAN F. G. BEVING** and OLOF THEANDER***

Chemistry Department, Swedish Forest Products Research Laboratory, Box 5604, S-114 86 Stockholm, Sweden

The degradation of various isomers of methyl hexodialdopyranoside-(1,5) with the α-D-*galacto* (I), α-D-*gluco* (II), β-D-*gluco* (III). the 4-*O*-methyl ether of III (IV), α-D-*gulo* (V), β-D-*gulo* (VI) and α-L-*manno* (VII) configurations have been investigated at 90 °C and pH 1.5 – 6. A minimum rate in the oxidation was observed at pH 3 – 4. Below this range acid hydrolysis of the glycosidic linkage predominated and above this range β-elimination. The products of β-elimination were detected at pH values, as low as 4. Partial isomerization of the two D-*gulo* derivatives V and VI to the corresponding L-*manno* derivatives *via* C-5 epimerization at pH 4 – 6 was observed.

The rate of alkaline degradation of II has been determined at 23 °C in aqueous sodium and calcium hydroxide.

Methyl β-D-*arabino*-hexopyranosid-2-ulose, methyl β-D-*ribo*-hexopyranosid-3-ulose and methyl β-D-*gluco*-hexodialdopyranoside-(1,5) are formed when methyl β-D-glucopyranoside is treated with chlorine water or other oxidizing agents and these substances have been investigated as models for oxidized cellulose.[1] Carbonyl groups at position 2 or 3 make the glycoside units more labile in acid and alkaline media than the corresponding unoxidized units.[2,3] Methyl β-D-*arabino*-hexopyranosid-2-ulose and methyl β-D-*ribo*-hexopyranosid-3-ulose give dark coloured reaction mixtures when treated with aqueous acid or alkaline solution. In acid solution, reductic acid is the main reaction product whereas in alkaline solution 1,5-anhydro-D-*erythro*-2,3-hexodiulose is formed rapidly by β-elimination and is further degraded to acids. Hydrolysis of methyl β-D-*gluco*-hexodialdopyranoside-(1,5) (III) in aqueous sulfuric acid gives almost exlusively D-*gluco*-hexodialdose.[4,5]

In the present paper we report the results of studies on the degradation of isomers of methyl hexodialdopyranoside-(1,5) with the α-D-*galacto* (I), α-D-*gluco* (II), β-D-*gluco* (III), the 4-*O*-methyl ether of III (IV), α-D-*gulo* (V), β-D-*gulo* (VI) and α-L-*manno* (VII) configuration at 90 °C and pH 1.5 – 6. The rate of degradation of IV was also determined in aqueous sodium and calcium hydroxide at 23 °C and pH 11.5.

The syntheses of I, V, VI, and VII have previously been communicated from this laboratory;[6] Horton *et al.*[7] have reported the synthesis of II. The remaining substances, III and V, were prepared by photolysis of the appropriate 6-azido glycosides by the method devised by Horton *et al.*[7]

The 6-aldehydo glycosides (I – VII) were kept in buffered solutions at 90 °C. L-Rhamnitol was present as internal standard.

Samples were withdrawn at intervals, reduced, acetylated and analyzed by GLC. The rate constants for the degradations were calculated, assuming first-order kinetics. The regression in the lines obtained were in agreement with this assumption except for the two D-*gulo* derivatives V and VI. The rate *versus* pH of com-

* Part XVII. See Ref. 5.

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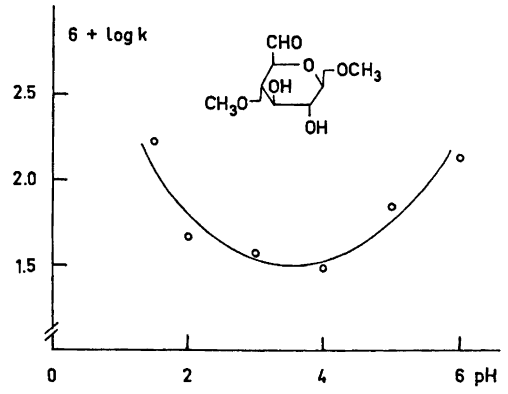


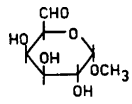
Fig. 1. Degradation of methyl 4-*O*-methyl-β-D-*gluco*-hexodialdopyranoside-(1,5) (IV) at 90 °C in the pH range 1.5 – 6.

pound IV is shown in Fig. 1. The rates of degradation are lowest at pH 3 – 4 (Table 1). At low pH values, the degradation is essentially due to acid hydrolysis of the glycosidic linkage and the corresponding hexitol acetates were observed on GLC of the reduced, acetylated product. At higher pH values, the degradation is essentially due to elimination of the substituent at C-4; the isomers of methyl 4-deoxy-hex-4-enopyranoside-(1,5) thus formed were detected by the strong absorption at 253 nm.[8,9] The formation of these products at pH values as low as 4 was demonstrated. The maximum stability of the methyl 6-aldehydo-glycopyranosides at this pH-range is in accordance with the maximal yield of methyl β-D-*gluco*-hexodialdopyranoside-(1,5) from the chlorine/hypochlorite oxidation of methyl β-D-glucopyranoside at pH 4.[10]

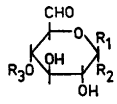
Methyl 4-deoxy-β- and α-L-*threo*-hex-4-enodialdopyranoside-(1,5) (XI and XII, respectively) were also isolated after degradation of II and III at pH 6 and 90 °C for 24 h and transferred into the diacetates, both of which were indistinguishable (NMR, UV) from authentic samples. In addition, the unsaturated aldehyde XI on borohydride reduction afforded methyl 4-deoxy-β-L-*threo*-hex-4-enopyranoside. The influence of 4-*O*-methyl substitution on the rate of acid hydrolysis has been discussed previously.[4] The influence of the size of the group at C-4 on the rate of β-elimination has been noted in earlier studies on monosaccharide derivatives and oligosaccharides.[11] Compound IV was degraded faster than III over the whole

Table 1. Rate constants for the degradation of the isomers of methyl hexodialdopyranoside-(1,5) (I – VII) in buffer solutions at 90 °C.

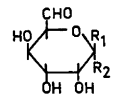
Compound	$k \times 10^6$ s^{-1} pH 1.5 chloride	pH 2 chloride	pH 3 phthalate	pH 4 acetate	pH 5 acetate	pH 6 phosphate
Methyl α-D-*galacto*-hexodialdo-pyranoside-(1,5) (I)	–	127	–	22	94	119
Methyl α-D-*gluco*-hexodialdo-pyranoside-(1,5) (II)	–	13	5	7	7	22
Methyl β-D-*gluco*-hexodialdo-pyranoside-(1,5) (III)	75	17	7	22	34	32
Methyl 4-*O*-methyl-β-D-*gluco*-hexodialdo-pyranoside-(1,5) (IV)	169	47	38	31	71	138
Methyl α-D-*gulo*-hexodialdo-pyranoside-(1,5) (V)	–	15	–	36	52	80
Methyl β-D-*gulo*-hexodialdo-pyranoside-(1,5) (VI)	–	27	23	17	31	28
Methyl α-L-*manno*-hexodialdo-pyranoside-(1,5) (VII)	–	44	–	21	39	45



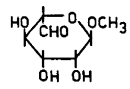
I



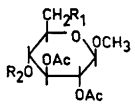
II $R_1 = R_3 = H$; $R_2 = OCH_3$
III $R_1 = OCH_3$; $R_2 = R_3 = H$
IV $R_1 = OCH_3$; $R_2 = H$; $R_3 = CH_3$



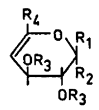
V $R_1 = H$; $R_2 = OCH_3$
VI $R_1 = OCH_3$; $R_2 = H$



VII



VIII $R_1 = N_3$; $R_2 = Ac$
IX $R_1 = Cl$; $R_2 = CH_3$
X $R_1 = N_3$; $R_2 = CH_3$



XI $R_1 = H$; $R_2 = OCH_3$; $R_3 = H$; $R_4 = CHO$
XII $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$; $R_4 = CHO$
XIII $R_1 = H$; $R_2 = OCH_3$; $R_3 = Ac$; $R_4 = CHO$
XIV $R_1 = OCH_3$; $R_2 = H$; $R_3 = Ac$; $R_4 = CHO$
XV $R_1 = H$; $R_2 = OCH_3$; $R_3 = H$; $R_4 = CH_2OH$

pH-range investigated and not only by pure acid hydrolysis as previously found. The L-*manno*-isomer (VII) was degraded at a similar rate as II and III but the D-*galacto* isomer (I) was degraded faster than the corresponding D-*gluco* derivative (II) at all pH-values.

In addition to the above degradation, epimerization of the two aldehydes with the D-*gulo* configuration (V and VI) was observed at pH 4 – 6, being most pronounced at the higher pH value. The epimerized products were first observed by GLC and yielded mannose on borohydride reduction and hydrolysis, thus confirming their structure. Similar epimerizations were not observed for the other 6-aldehydoglycosides studied, probably because they, in contradistinction to the *gulo* compounds, represent the most stable C-5 epimer. In the degradation of the *gulo* derivative (as well as for the other isomers studied), only the decrease of starting materials was determined and the rate constants are, because of the epimerization, therefore not directly comparable with those for the other aldehydoglycosides. Also the degradations of the *gulo* derivatives, contrary to the derivatives of the other isomers, showed some deviation from first-order kinetics.

Table 2. Rate constants for the degradation of methyl 4-*O*-methyl-β-D-*gluco*-hexodialdopyranoside-(1,5) (IV) in aqueous alkaline solution at 23 °C.

Alkali	$k \times 10^6$ s^{-1}
0.02 M NaOH	451
0.01 M $Ca(OH)_2$	363

Methyl 4-*O*-methyl-β-D-*ribo*-hexopyranosid-3-ulose is degraded *via* β-elimination in aqueous solution chiefly at C-4 but also to a certain extent at C-1.[1] To compare the rate of alkaline degradation of methyl 4-*O*-methyl-β-D-*gluco*-hexodialdopyranoside-(1,5) (IV) and methyl 4-*O*-methyl-β-D-*ribo*-hexopyranosid-3-ulose (model compounds for oxidised cellulose), IV was treated in aqueous solution at room temperature. The degradation of IV was very rapid (Table 2) and the rates are of similar order as those obtained for IV in 0.5 M sulfuric acid at 80 °C.[4] Degradation in sodium hydroxide was faster than in calcium hydroxide. Methyl 4-*O*-methyl-β-D-*ribo*-hexopyranosid-3-ulose is degraded approximately 10 times faster than IV in 0.02 M sodium hydroxide at 25 °C.[12]

The data obtained in this investigation support the results from studies on methyl 6,6'-dialdehydo-β-D-cellobioside [13] and 6-aldehydocelluloses.[9] Steric and electronic factors certainly influence the rates at which the various aldehydoglycosides are degraded. Below pH 3 – 4 hydrolysis of the glycosidic bond seems to be the main reaction in which 3,6-hemiacetal formation may increase the rate. Above this pH-range β-elimination at C-4 and formation of methyl 4,5-unsaturated 6-aldehydo-glycopyranoside structures seem to be of increasing importance with increasing pH.

Studies on the further degradation of the methyl 4,5-unsaturated α-D-*gluco*-hexodialdopyranoside-(1,5) (VIII) will be published separately.[14]

EXPERIMENTAL

Melting points are corrected. Concentrations were performed at reduced pressure below 40° C unless otherwise stated.

The irradiation used was unfiltered light from a Hanovia RCR Photochemical Reactor with a medium pressure mercury lamp of 100 W

inserted into a water cooled quartz immersion well.

Paper chromatography was performed on Whatman No. 1 paper. Solvent systems for paper chromatography were (a) ethyl acetate-acetic acid-water 3:1:1 and (b) butanol-ethanol-water 10:3:5.

Thin layer chromatography (TLC) was performed on silica gel GF_{254} (Merck, Darmstadt). Solvent systems for TLC were (a) ethyl acetate-light petroleum (b.p. 60–71 °C) 3:1, (b) ethyl acetate-methanol 4:1, (c) water saturated butanone, (d) propanol-water 85:15. Distilled solvents were used for TLC and paper chromatography.

Paper electrophoresis was run at 40 °C in (a) glycerol-boric acid buffer [15] (0.4 M boric acid, 0.1 M glycerol and 0.096 M NaOH) of pH 6.8 at 1500 V for 2 h and in (b) sodium hydrogen sulfite,[16] pH 4.7, at 1200 V for 1.5 h.

Standard spray reagents were used for sugars, carbonyl compounds, alditols and methyl glycosides namely silver nitrate-sodium hydroxide, *p*-anisidine hydrochloride, resorcinol-hydrochloric acid, periodate-benzidine. The paper chromatograms and TLC plates were studied in UV light (254 nm and 366 nm) before treatment with spray reagents. Acetates and products that were unreactive towards the above reagents were detected on TLC plates with iodine vapour.

GLC was performed on a Perkin-Elmer 801 chromatograph with a single glass column (3 % ECNSS-M on Gas-Chrom Q 100/120 mesh) and flame ionization detector. Peak areas were evaluated with an Infotronics electronic integrator. Optical rotations were measured at room temperature with a Perkin-Elmer 141 polarimeter. UV spectra were recorded on a Zeiss PMQ 2 spectrophotometer. IR spectra were obtained from a Perkin-Elmer 337 spectrometer. NMR measurements were carried out on a Perkin-Elmer R-12 60 MHz spectrometer using tetramethylsilane as internal reference. Mass spectra were recorded on a Perkin Elmer 270 mass spectrometer.

Kinetic experiments

The standard buffer solutions used were prepared as described by Gomori.[17] The degradation experiments were conducted at 90 °C in buffer solution with pH-values ranging from 1.5 to 6 or at 23 °C in aqueous alkaline solution. L-Rhamnitol was used as an internal standard and the extent of degradation was determined *via* GLC on reduced and acetylated aliquots from the reaction mixture. The quotient between the area for the acetylated methyl glycopyranoside (A) and the area for L-rhamnitol acetate (B) was calculated. Log A/B was plotted against time. Assuming first-order kinetics, the rate constant was then calculated from the slope of the line. The molar response factors were assumed to be unity.

The following general procedure was used for the degradation of methyl 6-aldehydo-glycopyranosides (I–VII). To the methyl 6-aldehydoglycopyranoside (10–20 mg) was added a known amount (5–10 mg) of internal standard, L-rhamnitol. Buffer solution (10 ml) of a desired pH was added. The solution was transferred to a glass stoppered test-tube which was heated in a constant water bath at 90 °C. At certain time intervals samples were withdrawn, cooled, neutralized, reduced with sodium borohydride and finally acetylated with acetic anhydride-pyridine 1:1 for 10 min at 90 °C. The obtained mixture was then submitted to GLC.

*Methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-*D*-glucopyranoside (VIII).* A solution of methyl 2,3,4-tri-*O*-acetyl-6-chloro-6-deoxy-β-D-glucopyranoside [18] (20.0 g) in *N*,*N*-dimethylformamide (250 ml) was stirred with sodium azide (20.0 g) for 10.5 h at 100 °C. The reaction mixture was cooled, filtered and evaporated at 50 °C to a syrup which was dissolved in benzene (150 ml) and filtered to remove salts. Evaporation several times from toluene, treatment with charcoal in methanol, and crystallization of the ethanolic solution yielded 5.6 g (27 %) colourless crystals with m.p. 81–83 °C, $[\alpha]_D^{25}$ −35 °(*c* 0.5, chloroform). TLC analysis (solvent a) showed one pure substance, R_F 0.66 (yellow spot with sulfuric acid spray). (Found: C 45.16; H 5.52; N 12.14; O 36.93. Calc. for $C_{13}H_{19}N_3O_8$: C 45.21; H 5.55; N 12.17; O 37.07).

*Methyl β-*D*-gluco-hexodialdopyranoside-(1,5)*[19] *(III):* via *photolysis of methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-*D*-glucopyranoside (VIII).* Methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside (VIII) (0.5 g) was dissolved in benzene (150 ml) and photolysed under a slow stream of nitrogen until TLC examination of the reaction mixture (solvent a) revealed complete conversion (4 h). The pale yellow solution was evaporated to dryness. The residue was deacetylated by dissolving the residue in methanol (15 ml) and treating the solution with a catalytic amount of sodium methoxide solution (0.5 g sodium/100 ml ethanol) (2 ml) at room temperature. The yellow solution was evaporated to a syrup. Water (15 ml) and Dowex 50 W × 8 (H^+) 50–100 mesh (5 ml) was added and the solution was stirred for 2 h at room temperature. Removal of the resin by filtration gave a pale yellow solution which was treated with activated charcoal at 30 °C for 30 min. Filtration through celite 545 and evaporation of the water solution and drying under high vacuum yielded a clear glass (0.21 g) showing chromatographic data in accordance with those for methyl β-D-*gluco*-hexodialdopyranoside-(1,5).[19] Paper electrophoresis in buffer (b) gave M_{vanillin} 1.10. Reduction, hydrolysis, reduction, acetylation, and GLC analysis revealed a pure substance indistinguishable from an authentic sample of D-glucitol hexaacetate.

*Methyl 2,3-di-*O*-acetyl-6-chloro-6-deoxy-4-*O-

methyl-β-D-glucopyranoside (IX). Methyl 4-*O*-methyl-β-D-glucopyranoside[20] (4.0 g) was dissolved in *N,N*-dimethylformamide (40 ml) at 60 °C and methanesulfonyl chloride (15 ml) was added dropwise to the stirred solution, analogously to a method described by Evans *et al.*[18] After 10 h at 60 °C, the mixture was cooled in ice and methanol (40 ml) was added. pH was adjusted to 10 with solid sodium methoxide and the solution was filtered. The precipitated salts were washed several times with small portions of methanol. The solvents were evaporated at 50 °C. After evaporation several times with toluene, the residue was acetylated with acetic anhydride (12 ml) in pyridine (24 ml) over night. The mixture was treated with ice-water and evaporated. Treatment of the syrup with activated charcoal in hot chloroform, evaporation and recrystallization from ethanol gave colourless crystals (3.2 g; 54 %), m.p. 96–98 °C. TLC (solvent a), R_F 0.70; $[\alpha]_D^{25}$ −35° (*c* 0.5, chloroform). (Found: C 46.53; H 6.18; Cl 11.26, O 35.90. Calc. for $C_{12}H_{19}ClO_7$: C 46.38; H 6.16; Cl 11.40; O 36.04).

Methyl 2,3-di-O-acetyl-6-azido-6-deoxy-4-O-methyl-β-D-glucopyranoside (X). Methyl 2,3-di-*O*-acetyl-6-chloro-6-deoxy-4-*O*-methyl-β-D-glucopyranoside (IX) (3.2 g) was dissolved in *N,N*-dimethylformamide (35 ml) at 80 °C. Sodium azide (3.2 g) was added. The mixture was stirred for 16 h at 80 °C and worked up as described for VIII. Recrystallization from ethanol yielded colourless crystals (2.5 g), m.p. 112–114 °C. TLC (solvent a), R_F 0.70 (yellow spot with sulfuric acid spray). $[\alpha]_D^{25}$ −44° (*c* 0.5, chloroform). (Found: C 45.40; H 5.73; N 13.30, O 35.48. Calc. for $C_{12}H_{19}N_3O_7$: C 45.42; H 6.03; N 13.24; O 35.30).

Methyl 4-O-methyl-β-D-gluco-hexadialdopyranoside-(1,5)[21] *(IV)*: via *photolysis of methyl 2,3-di-O-acetyl-6-azido-6-deoxy-4-O-methyl-β-D-glucopyranoside (X).* Methyl 2,3-di-*O*-acetyl-6-azido-6-deoxy-4-*O*-methyl-β-D-glucopyranoside (X) (0.4 g) was dissolved in benzene (150 ml). The photolysis and work up procedures were performed as described for III. The resulting clear glass (0.24 g) had chromatographical data identical with those obtained previously for methyl 4-*O*-methyl-β-D-*gluco*-hexodialdopyranoside-(1,5). Reduction, hydrolysis, reduction acetylation, and GLC analysis revealed a pure substance indistinguishable from an authentic sample of 4-*O*-methyl-D-glucitol pentaacetate.

Degradation of the α- and β-isomers of methyl α-D-gluco-hexodialdopyranoside-(1,5) (II and III) in phosphate buffer (pH 6). Methyl α-D-*gluco*-hexodialdopyranoside-(1,5) (II) (2.0 g) was dissolved in phosphate buffer of pH 6 (100 ml). The solution was heated on a water bath at 90 °C for 24 h. The position of the peak maximum in UV (253 nm) was similar to data obtained by Perlin *et al.*[8] After 24 h, when no starting material was left, the treatment was finished. The pH after the reaction was 6. The solution was extracted with ethyl acetate (3 × 200 ml). The water phase was evaporated to 30–40 ml and extracted with ethyl acetate (3 × 100 ml). The combined extracts were washed with water (100 ml), dried with sodium sulfate and evaporated to dryness. Column chromatography of the residue on silicic acid with dichloromethane-acetonitrile (1:1) as eluent gave a fraction containing 342 mg (19 %). TLC (solvent c) gave R_F 0.55. Paper electrophoresis in buffer b gave M_V 1.16 compared to M_V 1.04 for methyl α-D-*gluco*-hexodialdopyranoside-(1,5) (II): Part of the pure fraction (XI) was acetylated with acetic anhydride-pyridine at 25 °C overnight. After evaporation several times with benzene and purification with activated charcoal, a colourless syrup (XIII), which gave only one spot on TLC (solvent a), was obtained. The optical rotation was $[\alpha]_D^{25}$ +213° (*c* 3.0 methanol). The spectral characteristics for UV, IR, and NMR, of the syrup (XIII) were identical to those of an authentic sample. These data confirmed the structure of XIII as methyl 2,3-di-*O*-acetyl-4-deoxy-β-L-*threo*-hex-4-enodialdopyranoside-(1,5),[8] and obviously XI was methyl 4-deoxy-β-L-*threo*-hex-4-enodialdopyranoside-(1,5).

When part of XI was reduced with sodium borohydride in acetate buffer (pH 3–4) at 0 °C for 20 h, treated with Dowex 50 (H^+) ion-exchange resin and evaporated with methanol, a syrup (XV) was obtained. TLC: (solvent d), R_F 0.35 and optical rotation $[\alpha]_D^{25}$ +184° (*c* 0.5, methanol). Williams[25] reports $[\alpha]_D^{25}$ +192° (methanol) for methyl 4-deoxy-β-L-*threo*-hex-4-enopyranoside (XV), the expected reduction product.

In the same way methyl 4-deoxy-α-L-*threo*-hex-4-enopyranoside-(1,5) (XII) was obtained when methyl β-D-*gluco*-hexodialdopyranoside-(1,5) (III) was treated in buffer solution as above. Paper electrophoresis in buffer b gave M_V 1.23 for XII compared to M_V 1.10 for methyl β-D-*gluco*-hexodialdopyranoside-(1,5) (III). The acetate of XII, methyl 2,3-di-*O*-acetyl-4-deoxy-α-L-*threo*-hex-4-enodialdopyranoside-(1,5) (XIV), was identical to an authentic sample according to UV, IR, and NMR spectral data.[8]

Isomerisation of the α- and β-isomers of methyl D-gulo-*hexodialdopyranoside-(1,5) (V and VI) in phosphate buffer (pH 6).* A sample of V was treated with phosphate buffer (pH 6) and heated at 90 °C for 45 min (corresponding to 50 % conversion). After cooling, sodium borohydride was added and the solution was left at room temperature for 3 h. Treatment with Dowex 50 (H^+) ion exchange resin and evaporation from methanol yielded a syrup. Excess 0.25 M sulfuric acid was added and the solution was left on a water bath (90 °C) overnight. The cooled solution was treated with barium carbonate, filtered, and evaporated to a small volume. A sample of the syrup was examined by paper electrophoresis (buffer a) and by paper chromatography revealing the presence

of equal amounts of gulose and mannose (M_G 0.82 and M_G 0.72, respectively.) Another sample was acetylated with acetic anhydride-pyridine (1:1) for 10 min at 90 °C. The reduced and acetylated product was analyzed by GLC on a 3 % ECNSS-M column at 190 °C. Two peaks were observed which corresponded to glucitol hexaacetate and mannitol hexaacetate. Analogous chromatographic and electrophoretic data were obtained when VI was treated as above.

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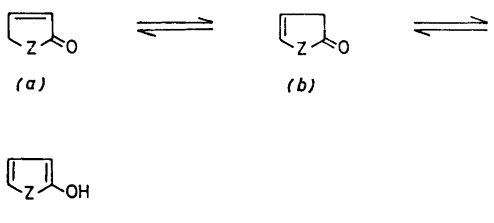
Ionisation Potentials in Tautomeric Analysis of 2-Hydroxy Derivatives of Thiophenes, Selenophenes, and Furans

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Ionisation potentials for tautomeric 2-hydroxy-thiophenes, -selenophenes, and -furans have been analysed by comparison with IP data for compounds derived from either tautomeric form. The enol isomer was not seen. Of the two possible α,β- and β,γ-unsaturated lactones the former was the major isomer; the ratios, however, depend on positional alkyl substitution and on the heteroatom.

The determination of appearance potentials (AP) and ionisation potentials (IP) has recently been found very useful in structure analysis of gaseous molecules in the mass spectrometer;[1–4] such data for the tautomeric hydroxy and amino pyridines have been interpreted in favour of the hydroxy and amino forms.[1–3] Five-membered heterocycles with a potential hydroxy group constitute another important class of tautomeric compounds (Scheme 1). The tautomeric behaviour of 2-hydroxy-thiophenes,[5,6] selenophenes,[7] and furans [8,9] have been elucidated in solution, in solid and in liquid states. In this work we have used IP in structure analysis of the gaseous species from 2-hydroxy derivatives of these five-membered heterocyles.



Scheme 1.

The IP values were obtained by the semilog-plot method as previously described.[10] In most cases the molecular ion signal was the base peak in the spectrum; in the remaining cases the molecular ion signal was strong. For analytical interpretation of the IP data knowledge of the IP's for the possible tautomers is required. The data for the latter are obtained indirectly from the alkylated tautomeric derivatives; the alkylation prevents tautomeric interconversions. Data for such model compounds are presented in the tables. Before discussing the tabulated values it is pointed out that the influence of a substituent on the ease of ionisation depends on the nature of other substituents as well as on the ring system and relative position; generally an electron releasing substituent decreases the IP.[1,11] The aromatic properties of thiophenes resemble closely those of corresponding benzenes; substituent influences in benzenes and thiophenes are therefore assumed to be similar, especially in derivatives derived from the hydroxy tautomer (*c*). Good models for the substituent effects in the lactones (*a*) and (*b*) are not available for comparison. Substituent effects on the ease of ionisation in these structures, however, will be seen in the discussion below to be of the same order as in the "aromatic" systems. The selenophene and the furan systems are treated in the same way. The thiophenes have been studied most extensively because of the availability of a considerable number of model compounds. Findings in the thiophene series are then applied in the structure analyses of the furan and selenophene series assuming similar substituent influences.

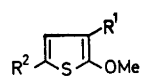
Introduction of a methoxy group into benzene decreases its IP with 0.7 to 8.83 eV (anisol);[11] the IP's for thiophene[12] and for 2-methoxythiophene (*10*) were found to be 8.80 and 8.14 eV, respectively. For methyl, ethyl, isopropyl, and *t*-butyl benzene the IP's have been determined by photoionisation; the successive decreases in IP from the previous member in the series were 0.42, 0.06, 0.07, and 0.01 eV.[13] The IP values determined by photoionisation are generally lower than those obtained by electron impact methods;[11] the differences in a homologue series determined by the two different methods, however, are assumed to be similar. The IP difference between 2-methoxythiophene (*10*) and its 3-*t*-butyl homologue *12* is 0.47 eV (Table 1); the IP differences between *10* and the methyl homologue *11* and *13* are 0.09 and 0.13 eV, respectively. The presence of the methoxy group will by analogy to the benzene system slightly reduce the influences of the alkyl substituents. Non-bonded interaction between *ortho* substituents presumably has little influence on the ease of ionisation in the compounds studied since IP for *o*-methylanisole (8.68 eV) was found to be only 0.25 eV lower than the value reported[11] for *o*-cresol; the difference is in the usual range (0.2 – 0.3 eV) for reduction in IP on *O*-methylation.[1,11] In the five-membered ring the substituents are further apart than in benzene and consequently non-bonded interaction between the vicinal substituents is expected to have less effect on IP than in benzene.

From Table 1 it is seen that IP of 2-methoxythiophene (*10*) is 8.14 eV; the estimated IP value for the enol (*c*) is thus of the order 8.4 eV (Table 2). IP for 3,3-dimethyl-4-thiolen-2-one (*15*) was measured to 8.77 eV; using the effect of the *C*-methyl group in the methoxy systems *11* and *13*, the IP for the β,γ-unsaturated lactone (*b*) can be estimated to ca. 8.9 – 9.0 eV (Table 2); a similar estimation is arrived at from the methyl *t*-butyl derivative (*16*) by application of the alkyl substituent effects in *11* and *12*. The model compound, 5,5-dimethyl-3-thiolen-2-one (*18*), for the α,β-unsaturated lactone (*a*) was not available; estimation of IP by the above technique was thereby prevented. Fortunately the unsubstituted structure *1* has significantly higher IP (9.78 eV) than the estimated IP's for (*b*) and (*c*). Hence IP for isomer (*a*) is at least 9.8 eV which shows that

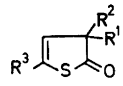
Table 1. Ionisation potentials fo thiophenes.



1 – 9



10 – 14



15 – 17

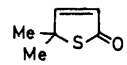
Compound	R^1	R^2	R^3	IP ± 0.05 eV
1	H	H	H	9.78
2	Me	H	H	9.60
3	*t*-Bu	H	H	9.25
4	H	H	Me	9.16
5	H	H	Et	9.08
6	Me	H	Me	9.35
7	Me	Me	H	9.44
8	H	Me	Me	9.13
9	*t*-Bu	H	Me	9.07
10	H	H	–	8.14
11	Me	H	–	8.05
12	*t*-Bu	H	–	7.67
13	H	Me	–	8.01
14	Me	Me	–	7.78
15	Me	Me	H	8.77
16	Me	*t*-Bu	H	8.38
17	Me	Me	Me	8.53

1 exists almost entirely as the α,β-unsaturated lactone (*a*) in the gas phase. This conclusion parallels the results from studies in the non-gaseous states which showed only the presence of the α,β-unsaturated lactone (*a*) within experimental limits.[5] It is also known from previous studies that the IP of a pure compound is rapidly decreased in a non-linear manner on admixture of an isomer with significantly lower IP.[10,12] Therefore the high IP for *1* excludes the presence of lactone (*b*) in significant amounts.

The IP is a measure of the ease of ionisation. The tables operate with IP values which arise as a mathematical result from the semi-empirical evaluation technique of the ionisation efficiency curves for components with the same mass number.[12,14] AP may therefore be a more correct term for these potentials. IP is used in this work, however, because the measured ionisations lead to molecular ions.

The estimated IP's for the three tautomeric forms (*a*), (*b*), and (*c*) of *1* show significant

Table 2. Estimated ionisation potentials.

 (*18*)	 (*a*) 9.8
 (*b*) 8.9	 (*c*) 8.4

differences (Table 2). These values are applied to the interpretation of the figures for the tautomeric compounds *1* – *9*. The figures are all above 9 eV which excludes important contribution from the enol (*c*) (8.4 eV) in the gas phase. Hence further structural discussions are limited to the two lactone isomers (*a*) and (*b*). It is understood, however, that the IP technique does not exclude the presence of minor amounts of the enol (*c*). For the reasons discussed above the parent compound *1* is assigned the α,β-unsaturated lactone structure (*a*). An alkyl group in the 3-position will conjugate or hyperconjugate with an α,β-double bond; this effect adds to the stabilising effect from double bond-carbonyl conjugation in locating the double bond to the α,β-position. Not unexpectedly, therefore, IP for the 3-methyl derivative *2* shows that tautomer (*a*) is the dominant gaseous species, the IP is 0.18 eV lower than for *1* and corresponds to the expected influence from the first methyl group. The same conclusion is reached for the 3-*t*-butyl derivative *3*.

It is seen from Table 1 that the IP for the 3-methyl derivative *2* is 1.5 eV higher than for the isomeric 2-methoxythiophene (*10*). For the other *C*-methyl isomer *4*, however, the IP is 0.6 eV lower than for the parent compound *1*. This difference is too large to be inherent in the methyl group and must reflect another lactone ratio than for the 3-isomer *2* in the gas phase. The IP for *4*, however, is higher than the estimated value for the β,γ-unsaturated tautomer (*b*); it is concluded that the gaseous species are composed of both lactones (*a*) and (*b*). Solution studies have shown that hyperconjugation from a 5-alkyl group stabilises the β,γ-double bond. In fact, both lactone tautomers can be isolated separately. These readily rearrange, however, to a thermodynamic equilibrium mixture under acid or base catalysis. Depending on substituents and medium, the equilibrium mixtures for simple 5-alkyl derivatives contain 10 – 20 % of the β,γ-isomer.[6] The available sample was such an equilibrium mixture which seems in accord with the gas phase data.

For an easily affected equilibrium mixture the question of thermal influence on the equilibrium position becomes important. The tautomeric compounds in this investigation were readily evaporated at 30 – 35 °C using the indirect insertion system. Intramolecular tautomeric changes in the gas phase are probably slow;[15] any changes after evaporation will therefore have to occur on the heated walls of the instrument. The reservoir and its connecting line to the ion source were kept at 130 °C while the ion source temperature was varied from below this temperature to 285 °C; only a very slight increase in the recorded IP's were noticed with increase in temperature. The sample was kept in the reservoir for more than one hour to complete these measurements. The normal time for measurements is of the order 10 – 15 min and hence equilibration towards a gas equilibrium position in the reservoir is probably of little importance; nor is the equilibration rate in the liquid sample exceptionally rapid.[6] The compounds were readily evaporated at 30 – 35 °C and consist essentially of the two lactone isomers which have similar boiling points; the α,β-unsaturated lactone (*a*) has the higher boiling point.[6] Selective evaporation, therefore, probably plays only a minor role and hence the ratios of the lactones (*a*) and (*b*) are not very different in the gas phase and in the liquid sample. Support for this conclusion was demonstrated by using samples with different tautomeric compositions. The isomer ratio can be changed through the polarity of the solvent.[6] Thus nitromethane decreases the amount of the less polar β,γ-isomer from the usual 15 – 20 % in the liquid to 6 – 10 %[6]; in the mass spectrometer the IP for *4* was increased from 9.16 to 9.25 eV after pretreatment of the sample with nitromethane. Further support for direct evaporation is provided by the results in the furan series (Table 3); the available α,β-unsaturated and the β,γ-unsaturated 5-methyl tautomers had significantly different IP's as discussed below.

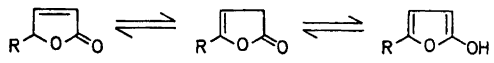
The IP for the 5-ethyl homologue *5* shows a

similar lactone ratio in the gas phase as for *4*. A 4-alkyl substituent will conjugate with the double bond in either form. Not unexpectedly, therefore, the isomer distribution for the 4,5-dimethyl isomer *8* appears to be similar to *4*. The IP for the 3,5-dimethyl isomer *6*, however, is 0.22 eV higher reflecting a relative increase in the α,β-unsaturated lactone; either double bond is conjugated by only one of the methyl groups. A similar conclusion is reached from the IP value of the 3-t-butyl analogue *9*. The highest IP (9.44 eV) for the dimethyl isomers was obtained for the 3,4-dimethyl isomer *7*. The 4-methyl group in this isomer will stabilise either double bond while conjugative stabilisation from the 3-methyl group is limited to the α,β-double bond. The IP difference between *7* and the parent compound *1* is close to the expected substituent effect from two methyl groups. Hence the gas phase molecules consist mainly of isomer (*a*).

In the furan series (Table 3) available model compounds were restricted to 3,3,5-trimethyl-4-oxolen-2-one (*21*). By means of additional data from the thiophene series, however, structure analysis is possible. Thiophene and furan have closely similar IP's, 8.80 and 8.85 eV, respectively.[12] 2,5-Dimethyl-3-methoxythiophene and its furan analogue also have closely similar IP's, 7.89 and 7.86 eV, respectively.[16] By analogy 2-hydroxyfuran is expected to have IP *ca.* 8.4 eV which on methyl substitution is reduced to *ca.* 8.3 eV; the observed values (9.62 and 10.12 eV) for the 5-methyl derivatives *19* clearly exclude the enol form as a major species in the gas phase.

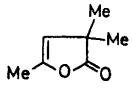
The heteroatom effect on IP is pronounced,

Table 3. Ionisation potentials of furans.



(*a*) (*b*) (*c*)

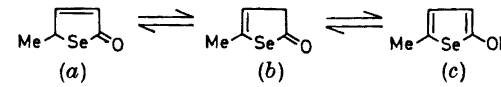
	R	IP ± 0.05 eV
(*19a*)	Me	10.12
(*19b*)	Me	9.62
(*20*)	*t*-Bu	9.03



(*21*)

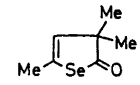
IP 9.00 ± 0.05 eV

Table 4. Ionisation potentials of selenophenes.



(*a*) (*b*) (*c*)

(*22*) IP 8.84 ± 0.05 eV



(*23*)

IP 7.98 ± 0.05 eV

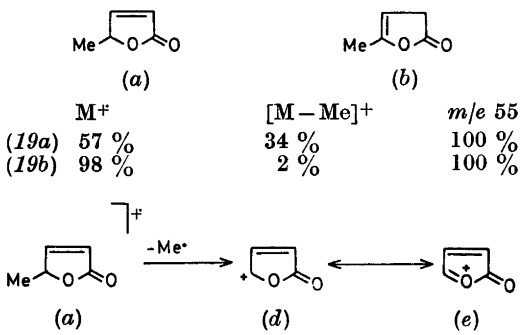
however, when the heteroaromatic system is broken. This is evident from a comparison of the IP data for 3,3,5-trimethyl-4-oxolen-2-one (9.00 eV), and the selen analogue (*23*, 7.98 eV) and the sulfur analogue (*17*, 8.53 eV). The same type of IP differences exist between the isomeric 2,2,5-trimethyl-4-thiolen-3-one and its selen and oxygen analogues.[16] In six-membered oxygen and sulfur heterocyclic systems such differences have also been observed. [17]

The available lactone isomers 5-methyl-3-oxolen-2-one (*19a*) and 5-methyl-4-oxolen-2-one (*19b*) were found to have IP's 10.12 and 9.62 eV, respectively. The high value for *19a* is consistent with the α,β-unsaturated formulation. The IP for the β,γ-unsaturated lactone is estimated to *ca.* 9.2 – 9.3 eV by adding the methyl group substituent effect onto the IP of *21*; the experimental value lies between the estimated value and the experimental value for the α,β-unsaturated lactone *19a*. It follows that the β,γ-unsaturated isomer has been partially tautomerised presumably before evaporation.

The IP (10.12 eV) for *19a* suggests that IP for the unsubstituted α,β-unsaturated lactone (*a*, R = H) is *ca.* 10.2 – 10.3 eV which is in fair agreement with 9.8 eV as estimated for the sulfur analogue *1*; almost the same IP difference is experimentally observed between the 3-methylthiophene *2* and 5-methylfuran *19a* systems which both have been assigned the α,β-unsaturated lactone structure in the gas phase. The same comparison between the 5-methyl analogues *4* and *19a* gives a much larger IP difference; it will be recalled that *4* was found to be a mixture of both lactone isomers in the gas phase.

The *t*-butyl sample *20* was an isomer mixture; the IP suggests that the β,γ-unsaturated lactone (*b*) is the major isomer.

The assignment of different isomer ratios in the gas phase from the two 5-methyl isomers *19* is supported by differences in fragmentation; of special interest is the relative intensities of the molecular ion and the [M−Me] fragment (Scheme 2). *19a* has the lower molecular ion intensity but much larger [M−Me] fragment. This observation can be rationalised in terms of the carbonium ion fragment (*d*) where the positive charge is stabilised by conjugation from the double bond and from the heteroatom. A similar stabilisation by direct methyl group expulsion from the (*b*)-form cannot be visualised. The relative intensity differences also show that extensive tautomerisation does not occur in these molecules on ionisation after electron impact.



Scheme 2.

In the selenophene series (Table 4) the IP (7.98 eV) for the 3,3,5-trimethyl derivative *23* suggests that the β,γ-unsaturated lactone isomer (*b*) of the 5-methyl derivative *22* has IP *ca.* 8.2 − 8.3 eV. In the thiophene and furan series discussed the difference in IP between the lactone forms (*a*) and (*b*) is about 1 eV; if a similar difference is assumed in the selenophene series, the data lead to the conclusion that the α,β-unsaturated lactone (*a*) is the major isomer.

EXPERIMENTAL

The ionisation efficiency curves were recorded on an AEI MS-902 mass spectrometer and interpreted by the semi-log plot method.[10] The ionising current was 20 μA and the repeller was at cage potential. Xenon was the reference gas. The ion source temperature was 230 °C unless specially discussed in the text.

The IP data are the average of three determinations, the deviation being ± 0.05 eV under standardised conditions. The compounds were introduced into the instrument by the indirect inlet system at 30 − 50 °C. The reservoir and the connecting tube to the ion source were at 130 °C.

The compounds used in this investigation were available from other work or were prepared according to the literature; *1*, *4*;[5] *5*;[6] *3*, *9*, *12*, *14*−*16*, *23*;[7] *7*, *8*, *13*, *21*;[8] *19*, *20*;[9] *2*;[18] *6*;[19] *10*;[20] *11*;[21] *22*.[22]

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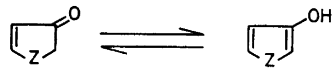
Ionisation Potentials in Tautomeric Analysis of 3-Hydroxy Derivatives of Thiophenes, Selenophenes, and Furans

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Ionisation potentials for tautomeric 3-hydroxy-2,5-dialkyl-thiophenes, -selenophenes and furans have been analysed by comparison with IP data of compounds derived from either tautomeric form. Both the ketone and enol forms are important; the ratios vary from almost pure ketone to considerable amounts of the enol. The isomer ratios depend on the substituents, the nature of the heteroatom and on the relative evaporation rates from rapidly equilibrating samples.

In the preceding paper ionisation potentials (IP) have been applied to the analysis of the tautomeric composition in the gas phase in the mass spectrometer of some 2-hydroxy-thiophenes, -selenophenes and -furans.[1] The gas phase molecules are composed of the two tautomeric lactones with the α,β-lactone as the major species. The ratios, however, depend on the substitution pattern and are similar to what has been found in non-gaseous states. This result stands in contrast to the oxygenated pyridines where the hydroxy structure was the dominating form.[2] In this report the results from the studies of some tautomeric 3-hydroxy-thiophenes, -selenophenes and -furans are reported.



(a) (b)

Scheme 1.

Two tautomeric forms are possible for the 3-isomers (Scheme 1); the ketone (*a*) and the enol or the hydroxy form (*b*). Studies in non-gaseous states have shown that both tautomers are important, but with the ketone (*a*) as the major isomer.

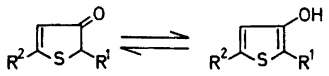
Most of the compounds available for studies belong to the thiophene series (Table 1) and are therefore first discussed. The data for the selenophenes and the furans are collected in Table 2.

3-Hydroxythiophene and presumably its selenophene and furan analogues are chemically unstable; derivatives stabilised by alkyl groups in both α-positions were therefore employed in these studies. The model compounds necessary for comparative interpretation of the IP data for the tautomeric compounds *1* to *6* were in the usual way available by alkylation of either tautomeric form; the ether *7* and the ketone *8* in Table 1 serve such a purpose.

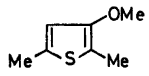
All compounds gave rise to strong molecular ion signals in the spectra; the molecular ion was often the base peak. The strong molecular ion signal facilitated the determination of IP's.

The influence of a substituent on the ease of ionisation depends on the electron releasing properties, the relative position and on the nature of other substituents as discussed in the preceding paper.[1] Methylation of a phenolic oxygen will reduce the IP of the phenol with *ca.* 0.2 – 0.3 eV. Adding this value to the recorded IP value for the ether *7* gives *ca.* 8.1 eV as the estimated IP for the hydroxy tautomer (*b*) of the 2,5-dimethyl derivative *1*. The substituent effect of the third methyl group in the ketone *8* is assumed to be of the order 0.1 – 0.15 eV.[1] The estimated IP for the keto form (*a*) of *1* is thus 8.6 – 8.7 eV; the experimental value is

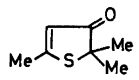
Table 1. Ionisation potentials of thiophenes.



	R^1	R^2	IP ± 0.05 eV
(*1*)	Me	Me	8.55
(*2*)	Me	Et	8.34
(*3*)	Me	i-Pr	8.21
(*4*)	Me	t-Bu	8.10
(*5*)	Et	Me	8.22
(*6*)	t-Bu	Me	8.09



(*7*) IP 7.89 ± 0.05 eV



(*8*) IP 8.49 ± 0.05 eV

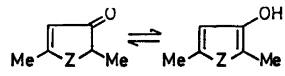
8.55 eV. This value lies between those estimated for the pure tautomers and arise as a mathematical result from the semi-empirical evaluation technique of the ionisation efficiency curves for components with the same mass number.[4,5] The measured quantities are called IP's rather than appearance potentials (AP) because we are measuring the ease of generating molecular ions. As pointed out in the preceding paper the mathematical IP value for a binary mixture of isomeric compounds does not change in a linear manner with the relative amounts of the components; the decrease from the higher component IP value is more rapid and *vice versa*.[6]

On the basis of the above discussion the tabulated data for the tautomeric molecules *1* to *6* clearly show that the keto tautomer (*a*) is the major gaseous component in all cases. Solution studies have shown that equilibrations between the two forms are rapid and neither form was isolated in a pure tautomeric state. For the 2,5-dimethyl compound *1* the ketone-enol isomer ratio varies from about 2 in the neat liquid to almost 10 in chloroform.[3] In the 5-homologue series *1* to *4* the preference for the keto form is slightly reduced with increase in the size of the substituent. The change has been explained by decreasing hyperconjugative stabilization of the double bond in the ketone.[3]

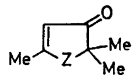
The IP for *1* indicates that only about 10 % of the gaseous species have the hydroxy form (*b*) implying tautomerisation of the liquid sample. The indirect insertion system of the instrument was used, and the samples were evaporated at 30–35 °C into the reservoir which was kept at 130 °C. Intramolecular prototropic shifts in the gas phase are assumed to be slow.[7] Isomerisation of the gaseous molecules on contact with the heated walls is probably not significant as the experimental time is of the order 10 min.[1] This locates the isomerisation to the liquid sample. It is natural to expect the ketone (*a*) and the hydroxy isomer (*b*) to have different volatilities, the ketone being the more volatile. It will be recalled that equilibration in the 3-hydroxy derivatives is rapid.[3] This allows the more volatile component to become enriched in the gas phase.

Inspection of the IP's in the 5-homologue series *1* to *4* reveals that the IP is decreased stepwise. The decrease in each step is slightly larger than expected to be inherent in the additional methyl group substitution on the α-carbon of the side chain. This is interpreted in

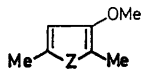
Table 2. Ionisation potentials of furans and selenophenes.



		(a)	(b)
(*9*)	Z = O	IP	9.23 ± 0.05 eV
(*10*)	Z = Se;	IP	8.24 ± 0.05 eV



(*11*) Z = O; IP 9.04 ± 0.05 eV
(*12*) Z = Se; IP 8.21 ± 0.05 eV



(*13*) Z = O; IP 7.86 ± 0.05 eV
(*14*) Z = Se; IP 7.69 ± 0.05 eV

terms of a slight relative increase in the hydroxy isomer (*b*).

The IP difference (0.33 eV) between the 2-ethyl-(*5*) and the 2-methyl-(*1*) isomer indicates that the ketone-enol isomer ratio has been decreased with the increase in the size of the 2-substituent. The *t*-butyl derivative *6*, however, appears to have almost the same isomer ratio as *5*. This observation is not in accord with solution studies in carbon disulfide which show that the ketone-enol ratio depends on the 2-substituent.[3] The gas phase ratios are therefore best understood in terms of relative rates of evaporation of the tautomers combined with rapid equilibrations in the liquid samples evaporated.

In the furan series the IP's for the model compounds *11* and *13* suggest IP *ca.* 9.2 eV for the keto form (*a*) of *9* and *ca.* 8.0 eV for the hydroxy form (*b*). The experimental value is 9.23 eV which shows that the furan in the gas phase is present as the ketone (*a*). The selenophene *10*, however, has IP 8.24 eV which lies between the values which can be estimated for the pure tautomer from the IP's of compounds *12* and *14*. Comparisons of the data indicate 10 – 20 % of the hydroxy isomer. A similar result is arrived at for the thiophenes by comparisons of the corresponding figures. From recent accumulations of data on the physical and chemical properties of selenophenes it is evident that the selenophene system resembles closely the thiophene system.[8] A similar tautomeric behaviour is therefore not unexpected. The aromatic stabilisation of the furan system is less which is probably a major reason why *9* exists preferentially in its keto form (*a*).

Thiophene and furan have IP's 8.80 and 8.85 eV, respectively;[4] the values for the methoxy analogues *7* and 13 are also the same within the experimental error, 7.89 and 7.86 eV, respectively. IP for the selenophene *14* is slightly less (7.69 eV). These observations strongly indicate that the primary ionisation occurs from the aromatic π-electron cloud rather than from the heteroatom. In the keto forms, however, the IP's for the hetero-analogues *8*, *11*, and *12* differ in a manner according to the electron releasing properties of the heteroatom; the same observation has been made in the 2-hydroxy series.[1]

EXPERIMENTAL

The ionisation efficiency curves were recorded with an AEI MS-902 mass spectrometer and the IP's obtained by the semilog-plot method as previously described;[1,6] xenon was the reference gas. The samples were introduced by the indirect method at 30 – 35 °C. The reservoir and the connecting tubes were kept at 130 °C and the ion source at 230 °C. The IP's are the average of three determinations, the deviation being ± 0.05 eV.

The compounds used in this investigation were available from other work or were prepared according to literature: *1 – 6*;[3,9] *7*, *8*, *12 – 14*;[10] *9*;[10,11] *11*.[10,12]

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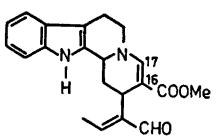
Synthetic Studies in the Alkaloid Field. Part II.* On the Preparation of *N*-(β-Indolylethyl)-dihydro- and *N*-(β-Indolylethyl)-tetrahydropyridines by Catalytic Hydrogenation, and their Acid-induced Cyclization

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Four 1-[β-(3-indolyl)ethyl]-3-carbomethoxy pyridinium salts were converted into the corresponding *N*-alkyldihydro- and/or *N*-alkyltetrahydropyridines by palladium-catalyzed partial hydrogenation. The isolated *N*-alkyldihydropyridines were found to be 1,6-isomers.** The acid-induced cyclization of the obtained compounds into indoloquinolizidine derivatives is described.

Much of the work on indole alkaloid synthesis in recent years has involved utilization of the 1-[β-(3-indolyl)ethyl]tetrahydropyridines in acid-induced transformations into indoloquinolizidine derivatives (for a review article see Ref. 1). One of the common methods of preparing the starting compounds consists of catalytic hydrogenation of the corresponding 1-[β-(3-indolyl)ethyl]-pyridinium salts. Since catalytic hydrogenation of *N*-alkylpyridinium salts customarily yields *N*-alkylpiperidines [2] it was necessary to develop special experimental designs to permit hydrogenation stoppage at the tetrahydropyridine stage. Wenkert *et al.*[3–7] have shown that palladium-catalyzed hydrogenation of pyridines and their *N*-alkyl salts



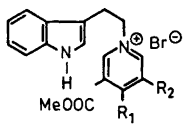
(I)

containing a wide variety of 3-acyl or related substituents yields the corresponding tetrahydropyridines.

In connection with our studies concerning the preparation of indole alkaloids of the vallesiachotamine (I) [8] type we became interested in *N*-alkyldihydro- and *N*-alkyltetrahydropyridines. In the present communication we describe the results obtained by palladium-catalyzed partial hydrogenation of four 1-[β-(3-indolyl)ethyl]-3-carbomethoxypyridinium salts (II–V), followed by the acid-induced transformation of the resulting compounds into indoloquinolizidine derivatives (XVIII–XXIV). Much interest was focused on the possibility of stopping the reduction at the dihydropyridine stage. In particular, it was hoped that the presence of a second carbomethoxy substituent at position 5 of salts (III), (IV), and (V) would permit the isolation of *N*-alkyldihydropyridines, which, especially in the case of 1,4-isomers, could be useful intermediates for the preparation of vallesiachotamine models.[9] Recently Eisner [10] has shown that catalytic hydrogenation of *N*-unsubstituted 3,5-diacyl pyridines can be stopped at the dihydropyridine

* Part I. M. Lounasmaa and C.-J. Johansson, *Tetrahedron Lett.* (1974) 2509.

** In an attempt to make the numbering of the partially hydrogenated pyridine derivatives uniform and thus facilitate their comparison with the tetrahydro compounds prepared earlier, products are numbered in such a way that the 1,2-dihydro compounds described here are referred to as 1,6-isomers.

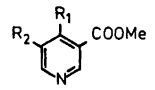


(II) $R_1 = R_2 = H$

(III) $R_1 = H$; $R_2 = COOMe$

(IV) $R_1 = CH_3$; $R_2 = COOMe$

(V) $R_1 = CH_2\text{-}COOMe$; $R_2 = COOMe$

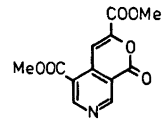


(VI) $R_1 = R_2 = H$

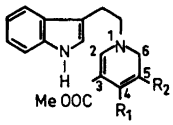
(VII) $R_1 = H$; $R_2 = COOMe$

(VIII) $R_1 = CH_3$; $R_2 = COOMe$

(X) $R_1 = CH_2\text{-}COOMe$; $R_2 = COOMe$



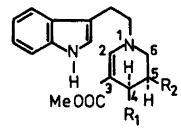
(IX)



(XI) $R_1 = H$; $R_2 = COOMe$

(XII) $R_1 = CH_3$; $R_2 = COOMe$

(XIII) $R_1 = CH_2\text{-}COOMe$; $R_2 = COOMe$



(XIV) $R_1 = R_2 = H$

(XV) $R_1 = H$; $R_2 = COOMe$

(XVI) $R_1 = CH_3$; $R_2 = COOMe$

(XVII) $R_1 = CH_2\text{-}COOMe$; $R_2 = COOMe$

stage, yielding mainly 1,6-isomers, however. Moreover, it is known that *N*-alkyldihydropyridines can be obtained, *e.g.* by complex metal hydride [11] and alkaline dithionite [11,12] reductions of pyridinium salts.

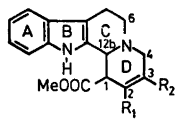
Several pyridines had to be prepared for the present investigation. Methyl nicotinate (VI) and 3,5-dimethyl dinicotinate (VII) were obtained by esterification of the corresponding acids. Dimethyl 4-methylpyridine-3,5-dicarboxylate (VIII), was synthesized as described recently,[13] and transferred by partial alkaline hydrolysis to the corresponding monoacid. Treatment with oxalylchloride and methanol yielded the esterlactone (IX),[14] which by alkaline hydrolysis, alkaline hydrogen peroxide treatment, and esterification was transformed to dimethyl 4-carbomethoxymethylpyridine-3,5-dicarboxylate (X).

Tryptophyl bromide, prepared according to Hoshino and Shimodaira,[15] was treated with appropriate pyridines to give the 1-[β-(3-indolyl)ethyl]-3-carbomethoxy pyridinium salts (II – V).

The next step consisted of palladium-catalyzed hydrogenation of the 1-[β-(3-indolyl)-ethyl]-3-carbomethoxy pyridinium salts in methanol in the presence of triethylamine. In the cases of salts (III – V), which contain a second carbomethoxy substituent in position 5, the reduction took place in two distinct steps, thereby permitting the isolation of dihydro derivatives in reasonable yields. The first mol of hydrogen was taken up within a few hours while the second one required a much longer time. In the case of salt (II), which unlike salts (III – V) does not contain a second carbomethoxy substituent in position 5, the hydrogen uptake was less step-wise, leading mostly to the tetrahydro derivative. Even when hydrogenation was interrupted before 1 mol of hydrogen had been taken up, the reaction mixture contained, besides starting material, mainly the tetrahydro derivative. However, the presence of some 1,6-dihydro derivative was indicated by NMR (multiplet at δ 4.13).

It seems evident to us from these results that clear interruption of the palladium-catalyzed hydrogenation of 1-[β-(3-indolyl)ethyl]-3-carbomethoxy pyridinium salts at the dihydro stage demands a supplementary stabilizing agent, which in the examined cases was the carbomethoxy substituent at position 5.

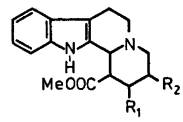
The NMR spectra (*cf.* Experimental part) of the isolated dihydro compounds (XI), (XII), and (XIII) clearly indicate that they are 1,6-isomers, showing characteristic signals due to the α-methylene groups at δ 4.18 – 4.33. Moreover,



(XVIII) $R_1 = H$; $R_2 = COOMe$

(XIX) $R_1 = CH_3$; $R_2 = COOMe$

(XX) $R_1 = CH_2\text{-}COOMe$; $R_2 = COOMe$



(XXI) $R_1 = R_2 = H$

(XXII) $R_1 = H$; $R_2 = COOMe$

(XXIII) $R_1 = CH_3$; $R_2 = COOMe$

(XXIV) $R_1 = CH_2\text{-}COOMe$; $R_2 = COOMe$

the appearence of two distinct methoxy group signals is in agreement with the 1,6-dihydro structure.

The above results, which at least *a priori* seem unfortunate for the preparation of vallesiachotamine models containing a $\Delta^{3(4)}$ ($\Delta^{16(17)}$ in the biogenetic nomenclature of vallesiachotamine) double bond, are in agreement with the results of Eisner.[10] Investigation of the main by-products of the hydrogenation reaction revealed that the 1,4-dihydro compounds (blue fluorescence on thin layer; *cf.* Ref. 16) were present in very low concentration.

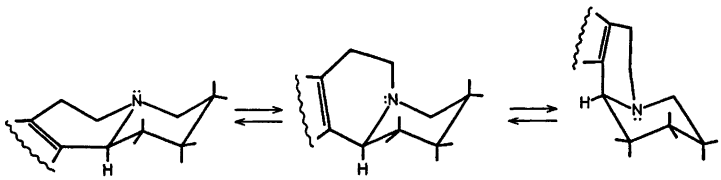
The obtained dihydro (XI–XIII) and tetrahydro (XIV–XVII) derivatives were subjected in anhydrous methanol to acid-induced cyclizations which gave tetracyclic compounds (XVIII–XXIV) in good yields. The mass spectra of the tetracyclic compounds prepared showed, besides the corresponding molecular peaks, *inter al.* typical tetrahydro-β-carboline peaks at *m/e* M–1, 184, 170, 169, and 156.[17–19]

The double bond in the D ring of compounds (XVIII), (XIX), and (XX) can be fixed at the 2,3-position on the basis of several experimental results. Firstly, the intense peak at *m/e* 170 in the mass spectra of compounds (XVIII), (XIX), and (XX) strongly supports the 2,3-position, because only in such a case can the corresponding ion be formed by a retro-Diels-Alder process.[20] Secondly, it would be expected that, due to the relatively strong diamagnetic shielding effect of a double bond either at the 1,2- or at the 3,4-position on the C-12b proton, the NMR signal of the C-12b proton would appear downfield from δ 4.0. The fact that this is not the case in the NMR spectra of compounds (XVIII), (XIX), and (XX) (*cf.* Experimental part and Discussion later) strongly favours the 2,3-position. Thirdly, the C-2 methyl and methylene group signals in the NMR spectra of compounds (XIX) and (XX), respectively, are singlets, which indicates that there are no protons directly attached to C-2 thus eliminating the 3,4-position in these cases. Moreover, if the double bond occupied the 3,4-position there would be strong absorption in the UV spectra of compounds (XVIII), (XIX), and (XX) at about 290 nm, due to the presence of a

$$\backslash \\ N-C=C-COOCH_3 \\ /$$

chromophoric system. The fact that the UV spectra obtained (*cf.* Experimental part) are typical of 2,3-disubstituted indoles,[21] showing only relatively weak absorption in the 280–290 nm region, clearly indicates that the double bond cannot occupy the 3,4-position.

Compounds (XXI–XXIV) can exist in a conformational equilibrium by nitrogen in-



a b c

Scheme 1.

version and *cis*-decalin type ring inversion (Scheme 1; only one enantiomer is illustrated).* Ring C is assumed to be in the half chair conformation and only the chair forms of ring D are considered.[22]

A similar conformational examination can be presented for compounds (XVIII – XX) considering ring D to be in half chair forms.

The presence of the so-called Bohlmann bands**[22–26] in the IR spectra of all tetracyclic compounds prepared (XVIII – XXIV) indicates that conformation *a*, which means *trans*-quinolizidine juncture of C/D rings, dominates the conformational equilibrium between *a*, *b*, and *c* (Scheme 1)***.

In a recent paper Potier and co-workers [20] have indicated that similar compounds having a double bond at 2,3-position (14,15-position in their nomenclature) show Bohlmann bands. From the presence of these bands the French investigators have concluded that introduction of a double bond at 2,3-position has no notable effect on the conformation of the *trans* diaxial hydrogens at C-12b, C-4, and C-6 (C-21, C-3, and C-5 in their nomenclature) in relation to the lone electron pair of the basic nitrogen.

The preponderance of conformation *a* in the conformational equilibrium of compounds (XVIII – XXIV) is also supported by NMR spectroscopy. The absence of any signal downfield from δ 3.8 that could be assigned to the C-12b proton is characteristic of conformation *a* (*trans*-quinolizidine juncture).[20,23,25–28] Owing to the diamagnetic displacement effect of the electron pair of the basic nitrogen, it can be expected that in conformations *b* and *c* (*cis*-quinolizidine junctures) the C-12b proton signal appears at about 0.5 ppm lower field.*

The relative stereochemistry at C-12b and C-1, as well as that at C-2 and C-3 when asymmetric, has not been determined with certainty. The magnitude of the coupling constant (10 Hz) (*cf.* Experimental part) for the interaction between the adjacent C-12b and C-1 protons in the NMR spectrum of compound (XXI) seems to support the *trans* diaxial relationship between these protons. As a consequence, the carbomethoxy group at C-1 would be equatorial in the predominant conformation (conformation *a*). A similar relationship is indicated by the NMR spectrum of compound (XXII), though less clearly, owing to the partial masking of the C-12b – H signal by the peaks of the carbomethoxy groups. Despite these results, confirmation of the relative stereochemistry at C-12b and C-1 must await more rigorous proofs. On the other hand, it can be assumed that the catalytic hydrogenation of the salts proceeds in a *cis*-manner [4] and, as a consequence, that compounds (XXIII) and (XXIV) are 2,3-*cis* products.

According to NMR spectroscopy (*cf.* Experimental part) the cyclization of (XVI) yielded a mixture of two diastereoisomers, which were not separated, whereas in all other cases only one of the possible diastereoisomers was found.

EXPERIMENTAL

The UV spectra were measured on a Perkin-Elmer 137 UV apparatus and the IR spectra on a Perkin-Elmer 237 apparatus. The NMR spectra were taken with a Varian A-60 instrument using TMS as internal standard. The mass spectra were recorded on a Perkin-Elmer 270 mass spectrometer at 70 eV using direct sample insertion into the ion source whose temperature was 80 – 90 °C. The melting points were deter-

* The conformation with a *trans* diaxial C/D ring juncture is not possible.

** The prerequisite for the occurrence of the Bohlmann bands is that the C-12b hydrogen and at least one more adjacent C hydrogen are *trans* diaxial to the lone electron pair of the basic nitrogen.[22]

*** Strictly speaking the IR spectra of compounds (XXI – XXIII) taken in crystalline form (*cf.* Experimental part), where interconversion of various conformations is stopped, only indicate that these compounds crystallize, at least mainly, in conformation *a*. However, it seems evident to us that conformation *a* is predominant even in solution (*cf.* NMR results).

* In mobile systems where several conformations are possible the chemical shift of any given proton will be the weighted time average of its chemical shifts in pure conformations in question, provided that conformational interchange is rapid. The C – 12b – H signal of compound (XXII), which is partly masked by peaks of the carbomethoxy groups, appears at about δ 3.9 (*cf.* Experimental part). Although this value, which reflects *inter alia* the contribution of conformation(s) *b* and/or *c* to the conformational equilibrium, is slightly downfield from δ 3.8, it is considered, nevertheless, to support the predominance of conformation *a*.

mined in a capillary melting point apparatus (Büchi) and are uncorrected.

Esterlactone (IX). 18 g of the diester (VIII) [13] was partially hydrolyzed to the corresponding monoacid (15 g) (MS M+ at *m/e* 195 corresponding to $C_9H_9NO_4$) by the procedure described in the literature.[14] A solution of 25 ml of freshly distilled oxalyl chloride in 25 ml of chloroform was dropped during one hour into an ice-cold mixture of the monoacid and 25 ml of triethylamine in 1000 ml of dry chloroform. The solution was allowed to reach room temperature and stirred for an additional hour. Methanol (125 ml) and chloroform (500 ml) were added and the solution was left to stir overnight. The solution was filtered and the filtrate washed with a sodium bicarbonate solution and dried over anhydrous potassium carbonate. The solvent was evaporated and the residue washed several times with chloroform, giving 10.4 g of esterlactone (IX) (52 %), m.p. 196–197 °C (lit.[14] 196–197 °C). IR (KBr) C=O 1745 (s), 1715 (s), C=C 1635 (s) cm^{-1}. NMR ($CDCl_3$) δ 3.99 (3 H, s, $-COOCH_3$), 4.04 (3 H, s, $-COOCH_3$), 8.52 (1 H, s, vinylic), 9.48 (1 H, s, aromatic), and 9.62 (1 H, s, aromatic). MS M+ at *m/e* 263 corresponding to $C_{12}H_9NO_6$.

Dimethyl 4-carbomethoxymethylpyridine-3,5-dicarboxylate (X). A mixture of 5.0 g of esterlactone (IX) and 5.0 g potassium hydroxide dissolved in 35 ml of water was stirred at room temperature for 0.5 h. The resulting solution was cooled to 5 °C and 6.75 ml of 30 % hydrogen peroxide was added in 0.75 ml portions at 3 h intervals. After a total reaction time of 27 h the solution was concentrated to dryness under vacuum at 40 °C. The residue was dried in a vacuum desiccator overnight, cooled to −70 °C, and treated with 400 ml of methanol saturated with hydrogen chloride and precooled to −20 °C. The mixture was allowed to reach room temperature slowly. After standing for 48 h the mixture was poured slowly onto a suspension of excess of sodium bicarbonate in methylene chloride. The mixture was filtered and the filtrate evaporated under vacuum. The residue was chromatographed on alumina (act. II–III), giving 0.75 g (15 %) of a solid whose crystallization from hexane yielded (X) as colorless crystals. M.p. 61–63 °C. IR (KBr) C=O 1735 (s, broad) cm^{-1}. NMR ($CDCl_3$) δ 3.71 (3 H, s, $-CH_2-COOCH_3$), 3.92 (6 H, s, two $-COOCH_3$), 4.51 (2 H, s, $-CH_2-COOCH_3$) and 9.18 (2 H, s, H-2, H-6). MS M+ at *m/e* 267 corresponding to $C_{12}H_{13}NO_6$.

Preparation of 1-[β-(3-indolyl)-ethyl]-3-carbomethoxy pyridinium salts

General procedure. A mixture of the pyridine derivative and tryptophyl bromide [15] in methanol was stirred under nitrogen atmosphere. After completion of the reaction (TLC) the solution was concentrated to dryness under vacuum and the residue washed with abs. ether.

1-[β-(3-Indolyl)ethyl]-3-carbomethoxy pyridinium bromide (II). Reaction between 425 mg of methyl nicotinate and 770 mg of tryptophyl bromide in 15 ml of methanol gave after 18 h reaction time at room temperature a solid whose crystallization from methanol yielded 1.0 g (84 %) of salt (II). M.p. 213–215 °C (lit.[4] 212–213 °C). IR (KBr) NH 3340 (m) C=O 1740 (s) cm^{-1}.

1-[β-(3-Indolyl)ethyl]-3,5-dicarbomethoxy pyridinium bromide (III). Reaction between 3.5 g of 3,5-dimethyl dinicotinate (VII) and 3.5 g of tryptophyl bromide in 50 ml of methanol gave after 18 h reaction time at room temperature a solid whose crystallization from ether-methanol (1/1) yielded 5.5 g (84 %) of salt (III). M.p. 220–221 °C. IR (KBr) NH 3410 (m) C=O 1740 (s) cm^{-1}.

1-[β-(3-Indolyl)ethyl]-3,5-dicarbomethoxy-4-methyl pyridinium bromide (IV). Reaction between 760 mg of dimethyl 4-methylpyridine-3,5-dicarboxylate (VIII) and 760 mg of tryptophyl bromide in 20 ml of methanol gave after 7 h reaction time at 65 °C a solid whose crystallization from methanol yielded 1.2 g (81 %) of salt (IV). M.p. 193–195 °C. IR (KBr) NH 3440 (m) C=O 1730 (s) cm^{-1}.

1-[β-(3-Indolyl)ethyl]-3,5-dicarbomethoxy-4-carbomethoxymethyl pyridinium bromide (V). Reaction between 193 mg of dimethyl 4-carbomethoxymethylpyridine-3,5-dicarboxylate (X) and 200 mg of tryptophyl bromide in absence of methanol gave after 1 h reaction time at 100 °C 350 mg (71 %) of a solid, which, although essentially pure salt (V), failed to crystallize. IR (KBr) NH 3200 (m) C=O 1740 (s) cm^{-1}.

Reductions

General procedure. A mixture of the 1-[β-(3-indolyl)ethyl]-3-carbomethoxy pyridinium salt, palladium-charcoal (10 %), and triethylamine in abs. methanol was hydrogenated at atmospheric pressure. After the calculated amount of hydrogen had been introduced the hydrogenation was interrupted. The catalyst was filtered, the filtrate evaporated under vacuum, and the residue extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated under vacuum. The residue was purified by column chromatography (Al_2O_3; act. IV).

Dihydropyridines. Hydrogenation of a mixture of 400 mg of the salt (III), 100 mg of palladium-charcoal (10 %), and 0.2 ml of triethylamine in 150 ml of abs. methanol gave after 1 h reaction time 184 mg (56 %) of a solid whose crystallization from methanol yielded (XI) as yellowish crystals. M.p. 175–178 °C. IR (KBr) NH 3345 (s) C=O 1700 (s), 1670 (s) C=C 1645 (s) cm^{-1}. NMR (DMSO-d_6) δ 3.56 (3 H, s, $-COOCH_3$), 3.67 (3 H, s, $-COOCH_3$),

4.34 (2 H, s, C-6-H_2), 7.10 (1 H, d, J = 2 Hz, indolyl α-H), and 7.37 (1 H, s, C-2-H). MS M+ at m/e 340 corresponding to $C_{19}H_{20}N_2O_4$.

Hydrogenation of a mixture of 320 mg of the salt (IV), 100 mg of palladium-charcoal (10 %), and 0.15 ml of triethylamine in 100 ml of abs. methanol gave after 2.5 h reaction time 74 mg (31 %) of (XII) as an oil. NMR ($CDCl_3$) δ 2.53 (3 H, s, $-CH_3$), 3.59 (3 H, s, $-COOCH_3$), 3.70 (3 H, s, $-COOCH_3$), 4.18 (2 H, s, C-6-H_2), 6.92 (1 H, d, J = 2 Hz, indolyl α-H), and 7.38 (1, H s, C-2-H). Owing to the product's instability in air (*e.g.* it rapidly discolors) it was used in acid-induced cyclization (*cf.* later) without further analysis.

Hydrogenation of a mixture of 200 mg of the salt (V), 70 mg of palladium-charcoal (10 %), and 0.1 ml of triethylamine in 100 ml of abs. methanol gave after 3 h reaction time 44 mg (27 %) of (XIII) as an oil. NMR ($CDCl_3$) δ 3.56 (3 H, s, $-CH_2-COOCH_3$), 3.68 (3 H, s, $-COOCH_3$), 3.71 (3 H, s, $-COOCH_3$), 4.24 (2 H, s, C-6-H_2 or $-CH_2-COOCH_3$), 4.32 (2 H, s, $-CH_2-COOCH_3$ or C-6-H_2), 6.98 (1 H, d, J = 2 Hz, indolyl α-H), and 7.37 (1 H, s, C-2-H). Owing to the product's instability in air (*e.g.*, it rapidly discolors) it was used in acid-induced cyclization (*cf.* later) without further analysis.

Tetrahydropyridines. Hydrogenation of a mixture of 500 mg of the salt (II), 150 mg of palladium-charcoal (10 %), and 0.25 ml of triethylamine in 150 ml of abs. methanol gave after 12 hours reaction time 274 mg (70 %) of a solid whose crystallization from benzene-hexane yielded (XIV) as colorless crystals. M.p. 106–107 °C* (lit.[4] 117–118 °C). IR (KBr) NH 3270 (s) C=O 1665 (s) C=C 1590 (s) cm^{-1}. NMR ($CDCl_3$) δ 3.66 (3 H, s, $-COOCH_3$), 6.90 (1 H, d, J = 2 Hz, indolyl α-H), and 7.37 (1 H, s, C-2-H). MS M+ at m/e 284 corresponding to $C_{17}H_{20}N_2O_2$.

Hydrogenation of a mixture of 500 mg of the salt (III), 150 mg of palladium-charcoal (10 %), and 0.2 ml of triethylamine in 150 ml of abs. methanol gave after 24 h reaction time 290 mg (71 %) of (XV) as an oil. IR (film) NH 3350 (m) C=O 1735 (s), 1670 (s) C=C 1615 (s) cm^{-1}. NMR ($CDCl_3$) δ 3.67 (6 H, s, two $-COOCH_3$), 6.91 (1 H, d, J = 2 Hz, indolyl α-H), and 7.36 (1 H, s, C-2-H). MS M+ at m/e 342 corresponding to $C_{19}H_{22}N_2O_4$.

Hydrogenation of a mixture of 270 mg of the salt (IV), 80 mg of palladium-charcoal (10 %), and 0.1 ml of triethylamine in 100 ml of abs. methanol gave after 24 h reaction time 190 mg (85 %) of (XVI) as an oil. IR (film) NH 3320 (s) C=O 1735 (s), 1670 (s) C=C 1610 (s) cm^{-1}. NMR ($CDCl_3$) δ 0.90 (3 H, d, J = 6.5 Hz, $-CH_3$), 3.64 (3 H, s, $-COOCH_3$), 3.68 (3 H, s, $-COOCH_3$), 6.88 (1 H, d, J = 2 Hz, indolyl α-H), and 7.29 (1 H, s, C-2-H). MS M+ at m/e 356 corresponding to $C_{20}H_{24}N_2O_4$.

* Indicated melting point was not altered by repeated crystallizations.

Hydrogenation of a mixture of 175 mg of the salt (V), 120 mg of palladium-charcoal (10 %), and 0.1 ml of triethylamine in 100 ml of abs. methanol gave after 70 h reaction time 47 mg (32 %) of (XVII) as an oil. IR (film) NH 3320 (m) C=O 1735 (s), 1675 (s) C=C 1615 (s) cm^{-1}. NMR ($CDCl_3$) δ 2.29 (2 H, d, J = 6 Hz, $-CH_2-COOCH_3$), 3.62 (6 H, s, two $-COOCH_3$), 3.69 (3 H, s, $-COOCH_3$), 6.98 (1 H, d, J = 2 Hz, indolyl α-H), and 7.31 (1 H, s, C-2-H). MS M+ at m/e 414 corresponding to $C_{22}H_{26}N_2O_6$.

Cyclizations

General procedure. A solution of the reduction product in anhydrous methanol was saturated with dry hydrogen chloride gas during a 2 h period. The solution was left standing at room temperature for 16 h and then was poured slowly into a suspension of sodium bicarbonate in methylene chloride. The inorganic salts were separated and the dried filtrate was evaporated under vacuum. The residue was purified by column chromatography (Al_2O_3; act. IV).

Dihydropyridines. Cyclization of 40 mg of (XI) gave 35 mg (87 %) of (XVIII) as an oil. UV (EtOH 94 %) λ_{max} 208 (ε 27 600), 223 (ε 30 100), 286 (ε 12 700), and 291 (ε 12 500) nm. λ_{min} 213, 255, and 289 nm. IR (film) NH 3430 (m) Bohlmann bands 2815 and 2770, C=O 1720 (s, broad), C=C 1660 (s) cm^{-1}. NMR ($CDCl_3$) δ 3.71 (3 H, s, $-COOCH_3$) and 3.82 (3 H, s, $-COOCH_3$). MS M+ at m/e 340 corresponding to $C_{19}H_{20}N_2O_4$. Other noteworthy peaks at m/e 339, 184, 170, 169, and 156.

Cyclization of 50 mg of (XII) gave 34 mg (68 %) of (XIX) as an oil. UV (EtOH 94 %) λ_{max} 208 (infl.) (ε 24 000), 224 (ε 30 900), 284 (ε 7 670), and 291 (ε 7 080) nm. λ_{max} 211 (infl.), 256, and 289 nm. IR (film) NH 3400 (m) Bohlmann bands 2810 and 2765, C=O 1725 (s), C=C 1655 (m) cm^{-1}. NMR ($CDCl_3$) δ 2.11 (3 H, s, $-CH_3$), 3.81 (3 H, s, $-COOCH_3$), and 3.92 (3 H, s, $-COOCH_3$). MS M+ at m/e 354 corresponding to $C_{20}H_{22}N_2O_4$. Other noteworthy peaks at m/e 353, 184, 170, 169, and 156.

Cyclization of 44 mg of (XIII) gave 40 mg (91 %) of (XX) as an oil. UV (EtOH 94 %) λ_{max} 209 (ε 21 400), 225 (ε 27 850), 284 (ε 7 150), and 292 (ε 6 870) nm. λ_{min} 211, 253, and 290 nm. IR (film) NH 3400 (m) Bohlmann bands 2810 and 2770, C=O 1730 (s, broad) C=C 1655 (m) cm^{-1}. NMR ($CDCl_3$) δ 3.68 (5 H, apparent s, $-CH_2-COOCH_3$ and $-CH_2-COOCH_3$), 3.75 (3 H, s, $-COOCH_3$), and 3.84 (3 H, s, $-COOCH_3$). MS M+ at m/e 412 corresponding to $C_{22}H_{24}N_2O_6$. Other noteworthy peaks at m/e 411, 184, 170, 169, and 156.

Tetrahydropyridines. Cyclization of 170 mg of (XIV) gave 130 mg (76 %) of a solid whose crystallization from ether yielded (XXI) as colorless crystals. M.p. 143–145 °C (lit.[4] 133–134 °C). IR (KBr) NH 3450 (s) Bohlmann bands

2815, and 2760, C=O 1720 (s) cm^{-1}. NMR ($CDCl_3$) δ 3.77 (3 H, s, $-COOCH_3$), and 3.79 (1 H, d, $J=10$ Hz, axial C-12b–H). MS M+ at *m/e* 284 corresponding to $C_{17}H_{20}N_2O_2$. Other noteworthy peaks at *m/e* 283, 184, 170, 169, and 156.

Cyclization of 120 mg of (XV) gave 98 mg (82 %) of a solid whose crystallization from ether yielded (XXII) as colorless crystals. M.p. 142–143 °C. IR (KBr) NH 3440 (s) Bohlmann bands 2810 and 2770, C=O 1730 (s) cm^{-1}. NMR ($CDCl_3$) δ 3.70 (3 H, s, $-COOCH_3$), 3.81 (3 H, s, $-COOCH_3$), and ~3.9 (1 H, d, $J\approx10$ Hz, axial C-12b–H). MS M+ at *m/e* 342 corresponding to $C_{19}H_{22}N_2O_4$. Other noteworthy peaks at *m/e* 341, 184, 170, 169, and 156.

Cyclization of 160 mg of (XVI) gave 134 mg (84 %) of (XXIII) as a mixture of two diastereoisomers A and B (60/40). IR (KBr) NH 3430 (m) Bohlmann bands 2810 and 2780, C=O 1730 (s) cm^{-1}. NMR* ($CDCl_3$) δ 0.92 (3 H, d, $J=7$ Hz, isomer B $-CH_3$), 1.08 (3 H, d, $J=7$ Hz, isomer A, $-CH_3$), 3.66 (3 H, s, isomer B, $-COOCH_3$), 3.68 (3 H, s, isomer A, $-COOCH_3$), 3.77 (3 H, s, isomer B, $-COOCH_3$), and 3.81 (3 H, s, isomer A, $-COOCH_3$). MS M+ at *m/e* 356 corresponding to $C_{20}H_{24}N_2O_4$. Other noteworthy peaks at *m/e* 355, 184, 170, 169, and 156.

Cyclization of 40 mg of (XVII) gave 35 mg (87 %) of (XXIV) as an oil. IR (film) NH 3440 (m) Bohlmann bands 2815 and 2775, C=O 1735 (s, broad) cm^{-1}. NMR ($CDCl_3$) δ 2.76 (2 H, d, $J=7$ Hz, $-CH_2-COOCH_3$), 3.68 (6 H, s, two $-COOCH_3$), and 3.77 (3 H, s, $-COOCH_3$). MS M+ at *m/e* 414 corresponding to $C_{22}H_{26}N_2O_6$. Other noteworthy peaks at *m/e* 413, 184, 170, 169, and 156.

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* The relative intensities given represent the two isomers independently.

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Light Sensitive Chloramphenicol Analogues

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Some chloramphenicol* analogues have been prepared in order to find reagents which could be used in a photoaffinity labelling study of the peptidyl transferase center of the *E. coli* ribosome. The compounds were tested for antibiotic activity *in vitro* in the "fragment reaction" assay, and for their ability to generate very reactive species upon irradiation.

The bacteriostatic action of many antibiotics, *inter alia*, chloramphenicol, is believed to be due to competition with the aminoacylated end of aminoacyl-t-RNA at the A-site on 70 S type ribosomes.[1]

Previously attempts have been made to identify the particular macromolecule(s) involved in the peptidyl transferase center by affinity labelling with derivatives of chloramphenicol (I).[2,3] In these studies, monobromamphenicol (VII) [2] and monoiodamphenicol (IX) [3] were employed. However, in the one report,[2] the antibiotic was irreversibly attached to the L 16 protein of the ribosome, whereas in the other case,[3] the antibiotic was attached to proteins L 2 and L 27 of the ribosome.

In an attempt to clarify this ambiguity we decided to prepare a number of chloramphenicol analogues in order to undertake a photoaffinity labelling study of the peptidyl transferase center. In this paper the preparation of chloramphenicol analogues as well as some of their antibiotic properties is reported.

The principle of photoaffinity labelling consist in letting a traceable, photoactive and biologically active reagent interact with its receptor site in a biological molecule. Upon irradiation with light of a suitable wavelength the reagent should be irreversibly and specifically attached at the active site, and by fractionating and analyzing the products from this, information about the location of the active site can be obtained.[4]

Thus we decided to prepare a series of chloramphenicol analogues, which besides showing biological activity also were photochemically active. In particular we were interested in designing compounds which could be expected to give nitrenes or carbenes upon photolysis.

According to the literature, the biological activity of chloramphenicol analogues is depending on: (*a*) the correct stereochemistry; (*b*) the presence of the two hydroxy groups; (*c*) the acetamido group, substituted with small electronegative substituents; (*d*) an electron withdrawing group in the 4-position of the phenyl group.

On the basis of this, we decided to modify the 4-substituent in the phenyl group or the acyl group in the aliphatic side chain.

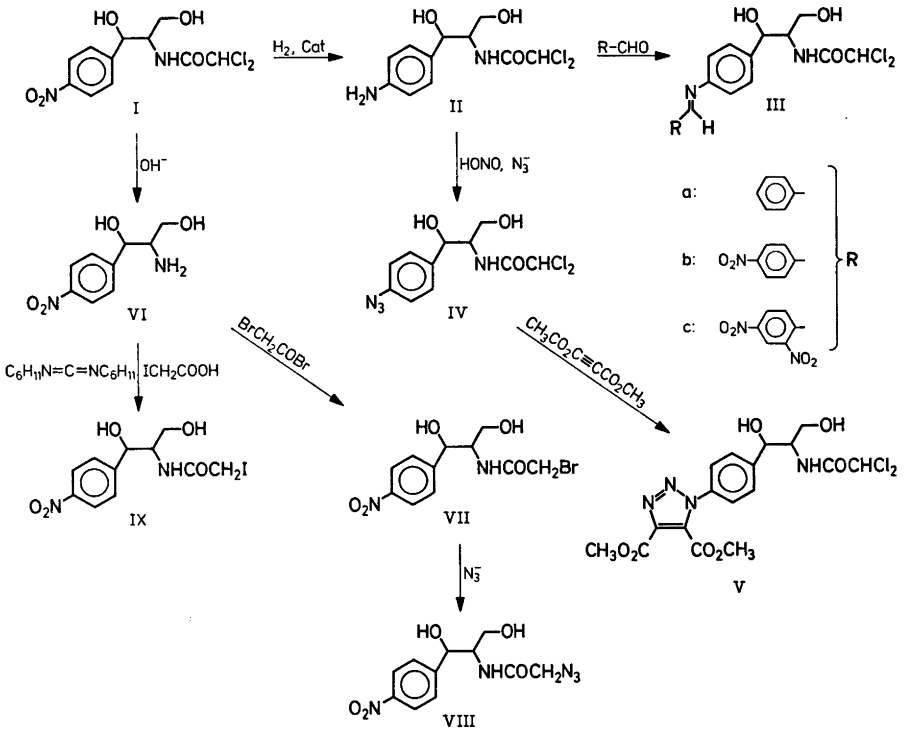
RESULTS AND DISCUSSION

By conventional, and in most cases well known procedures, the compounds listed in Scheme 1 were prepared. The synthetic pathways are also outlined in Scheme 1.

Compounds II,[6] IIIa,[7] IIIb,[6] VI,[8] VII,[9] and VIII [9] were previously known. Compounds IIIc, IV, and V which were not previously known, were identified on the basis of their elemental analysis and their spectroscopic characteristics.

The photochemical activity of the chloramphenicol analogues was examined, and IIIa,

* (1*R*:2*R*)-2-(Dichloroacetamido)-1-(4-nitrophenyl)-1,3-propandiol.



Scheme 1.

IIIb, IIIc, IV, and V were all found to be relatively active (Table 1). In no instances were the photoproducts isolated and characterized. However, it was found for the aromatic azide (IV), that an apparently clean light-induced reaction took place in methanol in $\sim 10^{-4}$ M concentration (Fig. 1), whereas irradiation of more concentrated solutions led to a complex mixture of products.

Table 1. Photoactivity of compounds.

Compound	Quantum yield [a]
IIIa	0.2
IIIb	< 0.1
IIIc	< 0.1
IV	1
V	0.25
VIII	< 0.02

[a] Relative to compound IV, estimated from rate of photolysis.

Interestingly, the Schiff bases (III) all exhibited good photoreactivity.

The aliphatic azide (VIII) showed a surprisingly small photoreactivity, as compared to

Table 2. Antibiotic activity of compounds.

Compound	% activity [a]	% antibacterial activity
Chloramphenicol (I)	100	100
II	2	0 [5]
IIIa	2	1 [12]
IIIb	2	45 [12]
IIIc	2	
IV	10	
V	5	
VI	< 0.5	1.8 [5]
VII	~ 20	15 [5]
VIII	20	
IX	10	

[a] Relative to chloramphenicol in fragment reaction assay.

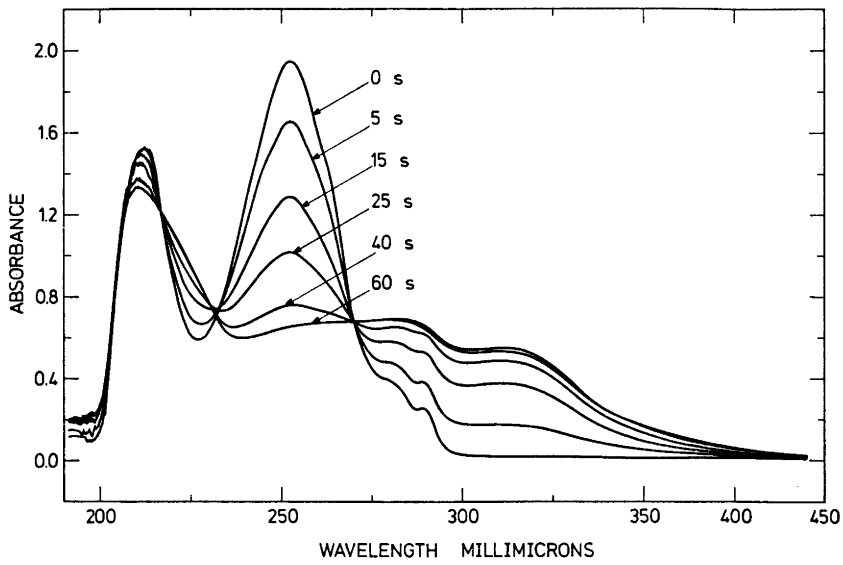


Fig. 1. Photolysis of compound IV.

other less complicated aliphatic azides.[10] This must probably be due to intramolecular energy transfer from the azide chromophore to the aromatic nucleus.

The inhibition of the peptidyl transferase activity of 70 S *E. coli* ribosomes in the fragment reaction [11,13] was determined for the chloramphenicol analogues (Table 2).

From Table 2 it is seen that the inhibition by compounds II, IIIa, VI, and VII are in good agreement with previous antibacterial determinations. However, compound IIIb shows a considerably smaller activity in our assay than that previously recorded.[12]

This difference may be due to the different assay systems.

CONCLUSION

Compound IV and to a smaller extent compound V appeared usable for a photoaffinity labelling study, whereas the other chloramphenicol analogues seem to be unsuitable. Unfortunately the photoreactions of compounds IV and V in methanol solution, in the absence of biological material, required light of such short wavelength that the ribosomes also absorb strongly. Thus it might be expected that eventual excited photoaffinity labels would transform the excitation to the ribosome. However, if the photoreaction was sufficiently fast they would react before energy transfer took place. The photoaffinity labelling experiments will be published in a future paper.

EXPERIMENTAL

Photochemical activity. In order to test the compounds for photochemically activity they were irradiated with UV-light (B & L mercury SP 200 light source) in quartz-cuvettes (MeOH; 0.03 mg/ml) and the reaction followed by UV-spectroscopy.

Test for antibiotic activity. The antibiotic activity of the compounds was determined as their ability to inhibit the peptidyl-transferase activity of 70 S ribosomes from *E. coli*, assayed in the "fragment reaction" as described by Monro.[13] 70 S ribosomes (*E. coli* strain MRE 600) were prepared according to Anderson *et al.*[11] and Ac-^{3}H-leu-pentanucleotide according to Monro.[13] The reaction volume was 200 μl, containing 50 % MeOH, v/v, 5 μmol of Tris.HCl buffer, (pH 7.5), 2 μmol of Mg^{2+}, 0.04 μmol of ethanethiol, 0.1 μmol of puromycin. HCl, 0.2 mg ribosomes, 2000 – 3000 cpm of the "fragment" and $10^{-5} - 10^{-2}$ μmol of the compound to be tested.

Melting points were determined on a Reichardt melting point microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer 337 infrared spectrophotometer and UV spectra on a Unicam SP 1800 UV

spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

(1R:2R)-2-(Dichloroacetamido)-1-(4-aminophenyl)-1,3-propandiol. hydrochloride (II). Chloramphenicol (I) (4 g) was dissolved in methanol (240 ml), containing conc. HCl (3.6 ml), and 10 % Pd/C catalyst (300 mg). The mixture was kept at 0 °C under magnetic stirring and hydrogenated for 2½ h, at which time the absorbtion rate of H_2 had decreased considerably (850 ml H_2 was absorbed). The catalyst was filtered off, the solvent evaporated, and the crystalline residue washed with dry ethanol (2 ml) and diethyl ether (20 ml) to yield 3.8 g (95 %) hydrochloride. M.p. 130 °C decomp. $[\alpha]_D^{20}$ +4.6° (MeOH, *c* 2.05). (Found: C 39.65; H 4.59; N 8.36; Cl 31.28. Calc. for $C_{11}H_{15}O_3N_3Cl_3$ C 40.06; H 4.55; N 8.49; Cl 32.32). IR (KBr): cm^{-1} 2600 (m), 1660 (s).

(1R:2R)-2-(Dichloroacetamido)-1-(4-benzylideniminophenyl)-1,3-propandiol (IIIa). Compound II (500 mg) was dissolved in H_2O (2 ml) and neutralized with an excess of $KHCO_3$. Ethanol (6 ml) was added, followed by benzaldehyde (0.6 ml). After 30 min at room temperature, precipitation of the Shiff base began, and it was completed with addition of water at 0 °C. The crystals were filtered off, and dried in vacuum. Yield: 300 mg (51 %). M.p. 162–163 °C (lit.[7] 153–154 °C). $[\alpha]_D^{20}$ +7.5° (MeOH, *c* 0.82), −45.6° (acetone, *c* 0.49). (Found: C 56.48; H 4.81; N 7.23; Cl 18.71. Calc. for $C_{18}H_{18}O_3N_2Cl_2$: C 56.69; H 4.72; N 7.34; Cl 18.63). UV (MeOH): λ_{max} nm (log ε) 335 (2.14), 272 (2.37). IR (KBr): cm^{-1} 1680 (s).

(1R:2R)-2-(Dichloroacetamido)-1-[4-(4-nitrobenzylidenimino)phenyl]-1,3-propandiol (IIIb). Compound II (400 mg) was dissolved in H_2O (2 ml) and neutralized with an excess of $KHCO_3$. To this mixture was added ethanol (10 ml) containing *p*-nitrobenzaldehyde (200 mg). The reaction vessel was protected from light and incubated for 72 h at room temperature. Thereafter the Shiff base was precipitated with H_2O and filtered off to yield 400 mg (75 %) of the title compound. Recrystallization from 1,2-dichloroethane. M.p. 166–168 °C (lit.[6] 164–166 °C). $[\alpha]_D^{20}$ +8.1° (MeOH, *c* 0.46). (Found: C 50.69; H 4.14; N 9.61. Calc. for $C_{18}H_{17}O_5N_3Cl_2$: C 50.70; H 3.99; N 9.86). UV (MeOH): λ_{max} nm (log ε) 348 (2.30), 262 (2.48). IR (KBr): cm^{-1} 1680 (s), 1510 (s), 1350 (s).

(1R:2R)-2-(Dichloroacetamido)-1-[4-(2,4-dinitrobenzylidenimino)phenyl]-1,3-propandiol (IIIc). This compound was made by condensing compound II with 2,4-dinitrobenzaldehyde analogous to the procedure from compound IIIb. Yield: 350 mg (60 %). Recrystallization from 50 % ethanol. M.p. 144–146 °C. $[\alpha]_D^{20}$ −26.5° (acetone, *c* 0.76). (Found: C 45.80; H 3.87; N 12.13. Calc. for $C_{18}H_{16}O_7N_4Cl_2$: C 45.6; H 3.60; N 11.80). UV (MeOH): λ_{max} nm (log ε) 360 (2.16), 244 (2.72). IR (KBr): cm^{-1} 1675(s), 1520(s), 1340(s).

(1R:2R)-2-(Dichloroacetamido)-1-(4-azidophenyl)-1,3-propandiol (IV). Compound II (1 g) was dissolved in ice cold 2 N H_2SO_4 (12 ml). The solution was stirred, and $NaNO_2$ (400 mg) was added in portions during 60 min, followed by NaN_3 (500 mg) during 45 min. Simultaneously with the NaN_3 addition, ethyl acetate (10 ml) was poured into the mixture to make a two phase system. The phases were then separated, and another extraction with ethyl acetate (10 ml) was undertaken. The combined ethyl acetate fractions were dried over $MgSO_4$ and the solvent evaporated to yield 600 mg (66 %) of the title compound. Recrystallization was made from 1,2-dichloroethane. M.p. 89–92 °C. $[\alpha]_D^{20}$ +5.8° (MeOH, *c* 1.32), −34.6° (acetone, *c* 0.52). (Found: C 41.07; H 3.79; N 17.31; Cl 22.25. Calc. for $C_{11}H_{12}O_3N_4Cl_2$: C 41.37; H 3.76; N 17.55; Cl 22.25). UV (MeOH): λ_{max} nm (log ε) 252 (2.49). IR (KBr): cm^{-1} 2110 (s), 1680 (s).

(1R:2R)-2-(Dichloroacetamido)-1-[4-(4,5-dimethoxycarbonyl-1,2,3-triazolyl)phenyl]-1,3-propandiol (V). Compound IV (200 mg) was dissolved in dry ethanol (6 ml) and refluxed with dimethyl acetylenedicarboxylate (200 mg) for 3 h. The ethanol was removed under reduced pressure, and the residue washed with diethyl ether to yield 170 mg (60 %) of the title compound. Recrystallization from 1,2-dichlorethane. M.p. 174–176 °C. $[\alpha]_D^{20}$ −23.8° (acetone, *c* 0.42). (Found: C 43.83; H 4.01; N 12.13. Calc. for $C_{17}H_{16}O_7N_4Cl_2$: C 44.25; H 3.90; N 12.14). UV (MeOH): λ_{max} nm (log ε) 242 (2.47). IR (KBr): cm^{-1} 1720 (s), 1670 (s).

(1R:2R)-2-(Azidoacetamido)-1-(4-nitrophenyl)-1,3-propandiol (VIII). This compound was made according to Rebstock [9] starting from base hydrolyzed chloramphenicol yielding (1*R*:2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propandiol (VI). M.p. 102–103 °C (lit.[9] 120–121 °C for the DL-form). $[\alpha]_D^{20}$ −15.0° (acetone, *c* 0.99). UV (MeOH): λ_{max} nm (log ε) 272 (2.49). IR (KBr): cm^{-1} 2100 (s), 1660 (s), 1500 (s) 1340 (s).

Hydrolysis. A suspension of chloramphenicol (7 g) in 0.25 N NaOH (170 ml) was stirred for 2 h at room temperature, chilled to 0 °C and the precipitate filtered off. One recrystallization from H_2O yielded the pure chloramphenicol base (VI) (3.5 g, 75 %). M.p. 162–163 °C (lit.[8] 161–162 °C).

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Intramolecular Participation by the Nitro Group. Reactions of Nitrophenylethanols under Acidic Conditions

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The reaction of 2-(*o*-nitrophenyl)-1-phenylethanol, 1-(*p*-nitrophenyl)-2-phenylethanol, and 1-(*o*-nitrophenyl)-2-phenylethanol (*1*) were studied under acidic conditions. The two first alcohols gave *o*-nitrostilbene (95 % *trans*, 5 % *trans*, 5 % *cis*) and *p*-nitrostilbene (91 % *trans*, 9 % *cis*), respectively. 1-(*o*-Nitrophenyl)-2-phenylethanol gave *o*-nitrostilbene, 50 % *cis* and 50 % *trans*, together with 3-benzoylanthranil (*3*) and traces of 2-phenylisatogen (*4*). The results from *1* may be explained by neighbouring group participation of the *o*-nitro group in the reaction.

The interactions between a nitro group and an *ortho* benzyl carbon in aromatic systems have interested us for some time.[1] Recently, the reaction between the nitro group and the hydroxymethyl group in *o*-nitrobenzyl alcohol was reported.[2] The results from that reaction and from several others [3] can be explained by nucleophilic attack of the nitro group oxygens on the *ortho* carbon atom.

We have now studied such interactions further. 1-(*o*-Nitrophenyl)-2-phenylethanol (*1*) was refluxed in toluene with *p*-toluenesulfonic acid, in analogy with the reaction of *o*-nitrobenzyl alcohol.[2] Two substances were isolated from the reaction, *o*-nitrostilbene (7 %) and 3-benzoylanthranil (3-benzoyl-2,1-benzisoxazol) (*3*) (15 %). In addition TLC of the reaction mixture indicated traces of 2-phenylisatogen (2-phenyl-*3H*-indole-3-one-1-oxide) (*4*) to be present.

Analysis of the *o*-nitrostilbene showed it to consist of 50 % *cis* and 50 % *trans*-*o*-nitrostilbene. This is in contrast to the results of dehydrations of the isomeric alcohols, 2-(*o*-nitrophenyl)-1-phenylethanol and 1-(*p*-nitrophenyl)-2-phenylethanol, which gave nitrostilbenes with high *trans*/*cis* ratios, 95/5 and 91/9, respectively.

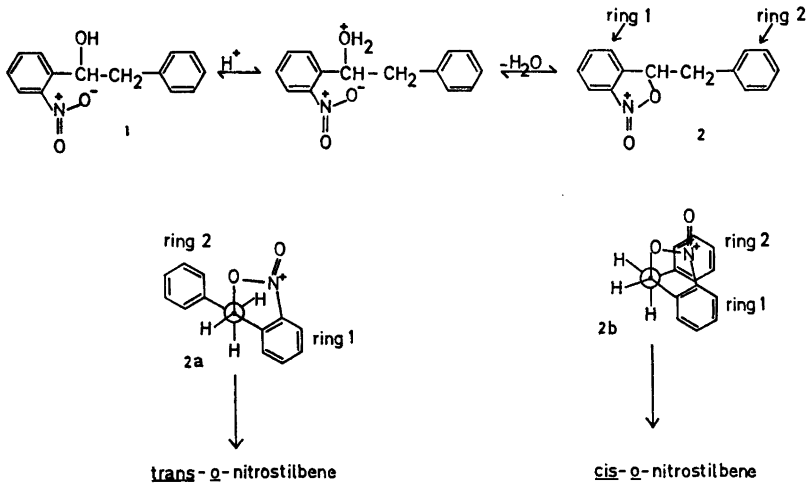
Control experiments showed *trans*-*o*-nitrostilbene to be stable under the reaction conditions, but the *cis* isomer to be slowly transformed to *trans*-*o*-nitrostilbene. Also, *cis*-*p*-nitrostilbene was transformed to the *trans* isomer.

The acidic dehydration of alcohols is generally assumed to proceed by an E1 mechanism. However, the details of the reaction are unknown, and subtle differences in structure may govern the results.[4,5]

The expected high yield of the *trans*-stilbenes in the dehydration of 2-(*o*-nitrophenyl)-1-phenylethanol and 1-(*p*-nitrophenyl)-2-phenylethanol may be explained by a *transoid* orientation of the aromatic rings during the reaction. A different mechanism may be operating in the case of 1-(*o*-nitrophenyl)-2-phenylethanol (*1*) as participation by the nitro group may give rise to the cyclic intermediate *2*. If *o*-nitrostilbene was formed from *2* by an E2-like mechanism, this may explain the high *cis*/*trans* ratio.

The conformer *2b* would be expected to be somewhat more favourable than the ones giving the *cis*-stilbenes from 2-(*o*-nitrophenyl)-1-phenylethanol and 1-(*p*-nitrophenyl)-2-phenylethanol, as ring 1 in *2b* can not rotate freely. Further, this conformer may be favoured by attraction between the positively charged ring system on carbon 1 and the phenyl ring on carbon 2.

The other substance isolated from the reaction of *1* was 3-benzoylanthranil (*3*). TLC of the

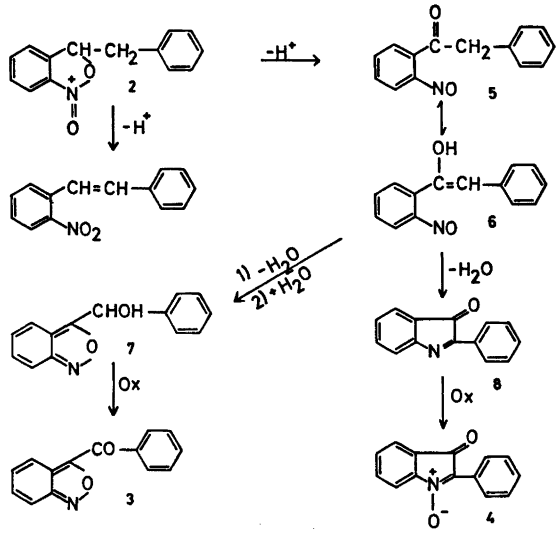


reaction mixture also indicated 2-phenylisatogen (*4*) to be a product. From the results of the investigation on nitrobenzyl alcohol [2] and of other *o*-nitroaryl compounds,[3] an intramolecular reaction was expected between the nitro and hydroxyl groups. However, both benzoylanthranil and phenylisatogen are at a higher oxidation level than 1-(*o*-nitrophenyl)-2-phenylethanol, showing part of the reaction paths of these compounds to be intermolecular.

Control experiments showed both *3* and *4* to be stable under the reaction conditions, and neither of them was therefore a precursor of the other in the studied reaction.

A reasonable way of formation of 3-benzoylanthranil and 2-phenylisatogen could start with the cyclic intermediate *2*, analogous to that proposed in the reaction of *o*-nitrobenzyl alcohol [2] and of several other *o*-nitro aromatic compounds.[3] The five membered ring of *2* could open to give benzyl *o*-nitrosophenyl ketone (*5*). Ring closure reactions of the enol form *6*, might give 3-(hydroxybenzyl)anthranil (*7*) and 3-oxo-2-phenylindolenine (*8*). Both *7* and *8* have to be oxidised if compounds *3* and *4* were formed by these paths.

Oxidation of 3-oxo-2-phenylindolenine (*8*) under slightly acidic conditions did not give



2-phenylisatogen (*4*).[6] However, *8* is reported to be unstable under strong acidic conditions,[6] and oxidation under the present conditions might have yielded *4*.

3-(Hydroxybenzyl)anthranil (*7*) seems not to have been reported earlier. It was readily made by sodium borohydride reduction of 3-benzoylanthranil and was refluxed with *p*-toluenesulfonic acid in toluene in the presence of nitrobenzene as an oxidant. The main product from the reaction was indeed 3-benzoylanthranil, together with several unidentified substances in low yields. Exlusion of oxygen was without effect on the reaction. 3-(Hydroxybenzyl)anthranil may thus be an intermediate in the studied reaction.

Further studies of the reactions of 3-(hydroxybenzyl)-anthranil showed that it was not oxidised by nitrobenzene in the absence of *p*-toluenesulfonic acid.

A rather different reaction path from benzyl *o*-nitrosophenyl ketone (*5*) to *3* and *4* would be by oxidation of the nitroso group to a nitro group. The resulting benzyl *o*-nitrophenyl ketone would be at the same oxidation level as *3* and *4* and the possibility of cyclisations to these compounds could not be excluded. However, an experiment with benzyl *o*-nitrophenyl ketone and *p*-toluenesulfonic acid showed it to be stable under the reaction conditions. Neither *3* nor *4* could be detected in the reaction mixture.

In photochemical reactions, 2-arylisatogens have been obtained from *o*-nitrostilbene.[7] Our own results show that neither *cis*- nor *trans*-*o*-nitrostilbene are transformed to 2-phenylisatogen or 3-benzoylanthranil under the acidic conditions in the present study.

In conclusion, the results from the reaction of 1-(*o*-nitrophenyl)-2-phenylethanol under acidic conditions can be explained by an intramolecular redox reaction occurring *via* the cyclic intermediate *2*. In addition, intermolecular redox reactions take place to give 3-benzoylanthranil and 2-phenylisatogen by oxidation.

EXPERIMENTAL

The general instrumentation has been described.[2] GLC were run on a Perkin-Elmer F-11 gas chromatograph, equipped with a 1.5 m column, 3 % OV-17. GLC-MS were run on the same GLC equipment, followed by a Hitachi-Perkin-Elmer, RMS-4 mass spectrometer. The syntheses of 1-(*o*-nitrophenyl)-2-phenylethanol (*1*)[8] and 2-(*o*-nitrophenyl)-1-phenylethanol[1] have been reported earlier.

Reaction of 1-(o-nitrophenyl)-2-phenylethanol (1). *p*-Toluenesulfonic acid (1 g), *1* (0.5 g), and toluene (50 ml) were refluxed. TLC showed *1* to react slowly and to have disappeared after 5 h. The reaction product consisted of a black powder (0.16 g) with an undistinguished IR spectrum, together with a toluene soluble mixture which was chromatographed on a dry column (silica gel, chloroform). Three products were obtained: *o*-nitrostilbene (32 mg), which GLC-MS showed to consist of 50 % *cis*-*o*-nitrostilbene (16 mg, 3.5 %) and 50 % *trans*-*o*-nitrostilbene (16 mg, 3.5 %), 3-benzoylanthranil (71 mg, 15 %) (IR, NMR, TLC), and 2-phenylisatogen (traces, TLC). This experiment was repeated twice with the same result.

Dehydration of 2-(o-nitrophenyl)-1-phenylethanol. *p*-Toluenesulfonic acid (0.6 g), 2-(*o*-nitrophenyl)-1-phenylethanol (0.3 g) and toluene (30 ml) were refluxed. After 15 min TLC showed the reaction to be complete. The reaction was followed by GC which showed the *trans*/*cis* ratio to be constant from 0.5 min throughout the reaction: 95 % *trans*- and 5 % *cis*-*o*-nitrostilbene. After work up and crystallisation *o*-nitrostilbene (0.19 g, 69 %), m.p. 71–72 °C (99 % *trans*, 1 % *cis*, GC) was obtained.

cis-o-*Nitrostilbene* was obtained by irradiation of a solution of *trans*-*o*-nitrostilbene (1 g) in benzene (170 ml) with a medium pressure Hg lamp. GC showed equilibration (65 % *cis*, 35 % *trans*) after 2.5 h irradiation. The *cis* isomer was isolated by preparative GC followed by crystallisation. The product (97 % *cis*- and 3 % *trans*-*o*-nitrostilbene) had m.p. 62.4–63.2 °C (lit.[9] *cis*-*o*-nitrostilbene 62 °C). IR (KBr): 3090, 3020, 1610, 1570, 1520, 1450, 1345, 865, 795, 780, 760, 710, 695 cm^{-1}. NMR ($CDCl_3$): 7.8–8.2 (1 H, *m*), 6.9–7.5 (8 H, *m*), 6.8 (1.5 H, *d*, *J* 3 Hz).

Stability of cis-o-*nitrostilbene.* *o*-Nitrostilbene (79 % *cis*, 21 % *trans*, 10 mg), *p*-toluenesulfonic acid (400 mg), and toluene (20 ml) were refluxed and the isomerisation followed by GLC. The results are given in Table 1.

Stability of trans-o-*nitrostilbene.* After 5 h reflux of *o*-nitrostilbene (98 % *trans*, 2 % *cis*) in toluene with *p*-toluenesulfonic acid, the composition was 99 % *trans* and 1 % *cis* isomer. TLC of this and of the preceding reaction mixture showed that neither 3-benzoylanthranil nor 2-phenylisatogen were formed in these reactions.

*Dehydration of 1-(*p-*nitrophenyl)-2-phenylethanol*[5] was performed by refluxing 50 mg with *p*-toluenesulfonic acid (100 mg) in toluene (5 ml). After 25 min TLC indicated the reaction to be complete, and *p*-nitrostilbene to be the major product. One more product (*ca.* 5 % from TLC) with R_F slightly smaller than nitrostilbene was

Table 1. Isomerisation of *cis*-*o*-nitrostilbene by reflux with *p*-toluenesulfonic acid.

Time (h)	Composition of *o*-nitrostilbene [a] % *cis*	% *trans*
0	79	21
0.5	44	56
1	30	70
2	36	64
3	25	75
5	28	72

[a] By GLC.

Table 2. Isomerisation of *cis*-*p*-nitrostilbene by reflux with *p*-toluenesulfonic acid.

Time (h)	Composition of *p*-nitrostilbene % *cis*	% *trans*
0	89	11
0.25	82	18
2	62	38
4	40	60
5	32	68

also present. The reaction was followed by GC, which showed the *trans*/*cis* ratio to be constant from 2 min throughout the reaction: 91 % *trans*, 9 % *cis*.

Stability of cis-p-*nitrostilbene*. *p*-Nitrostilbene (89 % *cis* and 11 % *trans*, from irradiation of *trans*-*p*-nitrostilbene) (20 mg) and *p*-toluenesulfonic acid (800 mg) in toluene (40 ml) were refluxed. The results of GC analyses at various times are given in Table 2.

Stability of 2-benzoylanthranil and 2-phenylisatogen. 3-Benzoylanthranil (10 mg), *p*-toluenesulfonic acid (400 mg), and toluene (20 ml) were refluxed for 5 h. TLC, GLC and IR showed no 2-phenylisatogen to be formed and 3-benzoylanthranil to have remained unchanged. An analogous experiment with 2-phenylisatogen showed that this compound too was stable under the reaction conditions.

3-(Hydroxybenzyl)anthranil (7). 3-Benzoylanthranil (40 mg) in methanol (20 ml) was cooled to 10 °C and sodium borohydride (30 mg) added. Water (30 ml) was added after 1 min and the mixture extracted twice with chloroform, the chloroform solution washed with water and evaporated to give a yellow oil (45 mg), showing one spot on TLC. UV ($CHCl_3$), λ_{max}: 303 (ε= 820 m^2 mol^{-1}), 272 (310), 265 (205), 258 (200), 252 (160), 247 (150). IR ($CHCl_3$, 5 %): 3610, 3360, 3080, 3010, 2940, 2860, 1650, 1550, 1500, 1660, 1160, 1145, 1075, 1020, 840 cm^{-1}. NMR ($CDCl_3$): δ 6.7–7.7 (9.5 H, *m*), 6.15 (1 H *s*), 4.1 (1 H, *s*, disappeared on shaking with D_2O). MS: *m/e* 225 (77 %), 208 (14 %), 207 (10 %), 196 (27 %), 180 (30 %), 179 (18 %), 120 (100 %), 118 (16 %), 107 (26 %), 105 (39 %), 92 (25 %), 79 (35 %), 77 (46 %).

Reactions of 3-(hydroxybenzyl)anthranil (7). (a) *With* p-*toluenesulfonic acid and nitrobenzene*. 3-(Hydroxybenzyl)anthranil (15 mg), *p*-toluenesulfonic acid (30 mg), and nitrobenzene (20 mg) were refluxed in toluene (5 ml). After 1 min, TLC showed all the alcohol to have reacted and to have given one product with R_F identical to that of 3-benzoylanthranil together with several other products with smaller R_F values, some of them fluorescing.

(b) *With only nitrobenzene*. The alcohol (8 mg) and nitrobenzene (12 mg) were refluxed in toluene (2.5 ml) for 5 min. TLC showed only starting material.

(c) *With only* p-*toluenesulfonic acid*. The alcohol (8 mg) and *p*-toluenesulfonic acid (15 mg) were refluxed in toluene (2.5 ml). After 1 min, the result, as judged by TLC, was similar to that of experiment a.

(d) *With only* p-*toluenesulfonic acid and exclusion of air*. A mixture of the alcohol (10 mg) and *p*-toluenesulfonic acid (18 mg) in toluene (3.3 ml) was degassed under vacuum by five cold (CO_2/acetone)/hot cycles with addition of N_2 for each cycle. The mixture was then refluxed for 1 min. The result was similar to that of experiments a and c. The reaction mixtures from experiments a, c, and d (from a total of 33 mg hydroxybenzylanthranil) were combined, concentrated and chromatographed. Yield 10 mg of a substance with TLC and IR spectrum identical to that of 3-benzoylanthranil.

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α-*N*-Benzoylarginine-2-naphthylamide Hydrolase (Cathepsin B1?) from Rat Skin. I. Preliminary Experiments with Skin Extract

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The cathepsin B1-like, α-*N*-benzoyl-DL-arginine-2-naphthylamide (BANA) hydrolyzing activity of rat skin extract was studied. The enzyme was extracted into low ionic strength buffers, and was activated by dithiothreitol, EDTA and KCN. Incubation of the extract at acidic pH resulted in a 3.6-fold increase in its BANA hydrolase activity. The presence of inhibitor(s) of BANA hydrolase in the skin extract was indicated by the non-linearity of the activity/enzyme concentration curve and by the increase in total activity of the enzyme by Sephadex G-100 gel chromatography, which also caused separation of several other skin proteases.

Otto [1] observed that the purified cathepsin B preparation of Greenbaum and Fruton [2] was a mixture of two enzymes, cathepsins B1 and B2. Cathepsin B1 is a powerful thiol-activated proteinase that also hydrolyzes synthetic substrates like benzoylarginine amide, naphthylamide, 4-nitroanilide and ethylester.[3–5] Cathepsin B2 hydrolyzes histones, some other basic proteins and benzoylarginine amide, but not the corresponding naphthylamide.[4,6]

Cathepsin B1 has been purified and characterized from beef spleen,[1,3–5,7] calf liver,[8,9] rabbit liver,[10] rat liver,[11] human liver,[12] human placenta,[13] and sheep thyroid gland.[14] The enzyme preparations so far obtained differ markedly in some respects; *e.g.* most of them hydrolyze benzoylarginine 4-nitroanilide, while some of them do not.[11,14] Recently some preparations have been shown to hydrolyze native collagen [7,15] and cartilage protein polysaccharides in co-operation with cathepisn D.[16,17] Some of them also inactivate various glycolytic enzymes [4,18–20] and exhibit kininogenase activity.[21]

The presence of cathepsin B1-like enzymes in various organs of rat [22] as well as in human and rat skin has been shown,[23,24] but a detailed study of the skin enzyme has not been made. The interesting properties of the enzymes that suggest their possible role in inflammatory processes, have initiated our closer investigation of the skin enzyme.

This paper deals with some properties of a crude cathepsin B1-like benzoylarginine-2-naphthylamide hydrolase from rat skin as a starting point for purification and characterization of the enzyme.

MATERIALS AND METHODS

Extract of the skin. Adult Long-Evans rats were used. The rats were rendered unconscious by a sudden replacement of air with nitrogen, decapitated and allowed to bleed. The trunk skin was shaved, removed and cleared of fascia and fat. The skin was cut with scissors into 3 × 3 cm pieces and minced with a meat grinder.

Ten grams of minced rat skin was suspended in 100 ml of ice-cold 10 mM sodium phosphate buffer, pH 6.0, containing 10 g/l (0.134 M)KCl, 0.2 mM dithiothreitol and 1 mM EDTA. The suspension was homogenized for 1 min in an Ultra Turrax T-45 homogenizer with constant cooling in an ice-salt bath (−5 °C). The homogenate was allowed to stand for 2 h at +4 °C, and centrifuged for 1 h at +4 °C, at 25 000 *g*, in a MSE HS-18 centrifuge. The opalescent supernatant was filtered by suction through a Millipore type AP-25 prefilter and AAWP filter (pore size 0.8 μm). The filtered solution is

referred to as the extract. The sediment and flotate after centrifugation at 25 000 *g* and the Millipore filters together with the retained material were rehomogenized to the original volume of the homogenate and their BANA hydrolyzing activities were determined as described for the homogenate.

Substrates, buffers and other reagents. α-*N*-Benzoylarginine-DL-2-naphthylamide hydrochloride (BANA, Sigma Chem. Co.) and L-leucine-2-naphthylamide (Leu-NA, Sigma Chem. Co.) were dissolved in methanol and diluted with water to give final concentrations of 5 mM of substrate and 20 % (v/v) of methanol. α-*N*-Benzoyl-L-arginine-4-nitroanilide hydrochloride (BAPA, Fluka AG) was used as a 1 mM solution in 20 % (v/v) methanol. Methanol (6.7 % in the reaction mixture) that was used to increase solubilities of BANA, Leu-NA, and BAPA had no effect on hydrolysis rates of these substrates. α-*N*-Benzoyl-L-arginine ethylester hydrochloride (BAEE, Merck AG) was dissolved in water (10 mM) just prior to use. α-*N*-Benzoyl-L-arginine amide hydrochloride hydrate (BAA, Merck AG) was dissolved in water (60 mM) and used within two days. Bovine Hemoglobin (Hb, Sigma Chem. Co., type II) was dissolved in water (20 g/l) and used within two days.

The universal buffer of Britton and Robinson,[25] with thiol activators, was modified for the determination of hydrolysis rates of BANA, Leu-NA, BAPA, and BAA. The buffers were prepared by titrating a mixed acid solution containing 80 mM acetic, phosphoric, and boric acids with 0.4 M NaOH to give the desired pH's of 5.8 for BANA, BAPA, and BAA, and 7.0 for Leu-NA, and then adding 0.6 mM dithiothreitol (DTT, Calbiochem), 3 mM EDTA (disodium salt, Merck AG) and 3 mM KCN. The BAEE hydrolyzing enzyme was assayed in Britton-Robinson buffer, pH 6.0, without added activators.

The hemoglobin hydrolyzing activity was assayed in a lactate-acetate buffer, which was made by titrating a solution of 0.4 M sodium lactate and sodium acetate with 5.0 M HCl to the desired pH (4.3 for routine assays).

Enzyme assays. To a test tube 0.1 ml of enzyme solution and 0.1 ml of suitable buffer were pipetted and the mixture was allowed to stand for 10 min at room temperature. The tubes were transferred to a water bath (37 °C), reactions were started by adding 0.1 ml of substrate solution and the mixtures were incubated for 15 min – 4 h depending on enzyme activities. The pH's of reaction mixtures were measured during the incubations. All assays were performed in duplicate.

Naphthylamine, liberated from BANA and Leu-NA, was assayed by stopping the enzyme reaction with 0.4 ml of 4-dimethylaminobenzaldehyde reagent.[26] The reagent was made by dissolving 250 mg of 4-dimethylaminobenzaldehyde in 25 ml of methanol, and adding 25 ml of an acetate-HCl buffer, pH 1.4. The buffer was made by titrating 1 M sodium acetate to pH 1.4 with concentrated HCl. The solutions were allowed to stand for 30 min at room temperature and their absorbance was measured with a spectrophotometer at 450 nm against a blank containing water instead of the enzyme solution. The stopping reagent caused precipitation of some proteins when crude enzyme preparations were used. The turbidity caused by the precipitate was removed by centrifugation (10 min at 1000 *g*), or corrected against a blank, to which 4-dimethylaminobenzaldehyde reagent was added immediately after the enzyme solution. A standard curve was prepared by pipetting 2-naphthylamine solutions (0 – 15 nmol in 20 % methanol) to the reaction mixture instead of the enzyme solution. The BANA hydrolase activity of the skin homogenate was assayed by using 0.5 ml of the homogenate, buffer and substrate, incubating in a shaking water bath (37 °C) and stopping the reaction with 2 ml of 4-dimethylaminobenzaldehyde reagent. The colour solution was filtered through Whatman No. 1 paper and the absorbance of the filtrate was measured at 450 nm.

The hydrolysis of hemoglobin was assayed by estimating the solubilized tyrosine and tryptophan containing peptides by the Folin-Ciocalteau reagent.[27] The enzyme reaction was stopped by adding 0.4 ml of 0.61 M trichloroacetic acid to the reaction mixture and centrifuging (1000 *g*, 10 min), after standing at room temperature for 15 min. To 0.4 ml of the supernatant, 2 ml of 0.1 M NaOH containing 20 g/l Na_2CO_3 was added. Finally, 0.2 ml of Folin-Ciocalteau reagent (Merck AG, diluted 1:1 with water) was added with rapid mixing. The solution was allowed to stand for 30 min and the absorbance was recorded at 750 nm against a blank of water instead of enzyme solution. The absorbance caused by the enzyme solution was subtracted by using a blank containing water instead of the substrate. A standard curve was prepared with 0 – 0.25 μmol tyrosine instead of the enzyme solution, and hemoglobin hydrolyzing enzyme activities were expressed in tyrosine equivalents (nmol) solubilized in one minute.

The hydrolysis of BAEE was determined by measuring unhydrolyzed substrate according to the method of Hestrin.[28] After incubation, the reaction was stopped by adding 0.2 ml of cold, alkaline hydroxylamine solution, which was freshly made by mixing equal amounts of 2 M hydroxylamine hydrochloride and 3.5 M NaOH, to the reaction mixture. After 15 min, 0.1 ml of 4 M HCl was added, followed later by 0.1 ml of 0.7 M $FeCl_3$. The colour developed was measured after 20 min, at 540 nm, against a blank containing water instead of BAEE. These values were subtracted from corresponding blank values containing water instead of the enzyme solution. A standard curve was constructed from increasing concentrations of BAEE.

The hydrolysis of BAA was estimated by measuring the ammonia liberated during the reaction by a modified phenol-hypochlorite reaction.[29] After incubation, the reaction was stopped by adding 1 ml of phenol-nitroprusside (17 g/l phenol and 50 mg/l sodium nitroprusside) and 1 ml of alkaline hypochlorite solution (5 g of NaOH and 5 ml of 10 % sodium hypochlorite solution was dissolved in water and diluted to 1 l) to the reaction mixture. The colour was developed at 55 °C for 10 min, and after cooling, measured at 625 nm against a blank containing water in place of the enzyme solution. In every assay standards were run containing 0.05 and 0.1 mmol ammonium sulfate in place of the enzyme solution.

The hydrolysis of BAPA was assayed by measuring the 4-nitroaniline formed. The reaction was stopped by adding 0.2 ml of zink sulfate solution (100 g/l), the solution was centrifuged for 10 min at 1000 *g* and the absorbance of the supernatant measured at 383 nm. A standard curve was prepared by replacing the enzyme solution with 4-nitroaniline (0 – 10 nmol) dissolved in 20 % methanol.

The protein concentration was determined according to Lowry *et al.*[30] with bovine serum albumin (Sigma Chem. Co.) as a standard. The proteins from chromatographic separations were estimated by absorbance at 280 nm with an Isco UA-2 monitor.

Gel chromatography. A 5 × 70 cm column of Sephadex G-100 was equilibrated with 10 mM sodium phosphate buffer, pH 7.5. The sample, 60 ml of extract, was applied by a peristaltic pump and elution was carried out with the equilibrating buffer at +4 °C. The flow rate was 60 ml/h and the fraction volume 10 ml.

RESULTS

Extraction. The effect of pH on the extraction of the enzyme was studied by extracting minced skin with 10 mM phosphate buffers, at pH 8.0 and 6.0, or with 10 mM citrate buffer, pH 4.0, in the presence of 0.2 mM DTT and 1 mM EDTA. A similar set of extracts was prepared with the same buffers, but containing KCl (10 g/l) in order to study the effects of salt concentration on the extraction. BANA-hydrolase activities of the extracts, when assayed at pH 5.8, are given in Fig. 1. Acidic pH increased both the specific and total activities of the extracts. The addition of salt increased the total activity, but the extraction of inactive proteins was increased to the same proportion. Although acidic extraction at pH 4.0 gave the best activity, 10 mM sodium phosphate buffer, pH 6, with 1 % (w/v) KCl was used in later extractions. The reason for avoiding the acidic

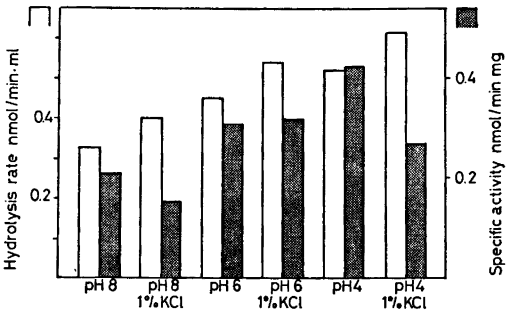


Fig. 1. Effect of pH and KCl (1 %) on extraction of rat skin BANA hydrolase. Phosphate buffers (10 mM) were used at pH 8 and 6, and citrate buffer (10 mM) at pH 4. Buffers contained 0.2 mM DTT and 1 mM EDTA.

pH in extraction was that acidic pH seemed to increase activity of purified BANA hydrolase preparations (details to be described). Avoiding treatment with acidic pH until the enzyme was purified, made it possible to study the activation phenomenon closer. The extraction procedure described in "Materials and Methods" gave a recovery of 72 % for BANA hydrolase, as compared to the total activity of the skin homogenate. About 18 % of BANA hydrolase was retained in the sediment after centrifugation of the homogenate at 25 000 *g* for 1 h and about 0.4 % was found in the floating lipid material. The material retained on Millipore filters contained less than 0.1 % of the total BANA hydrolase activity of the homogenate.

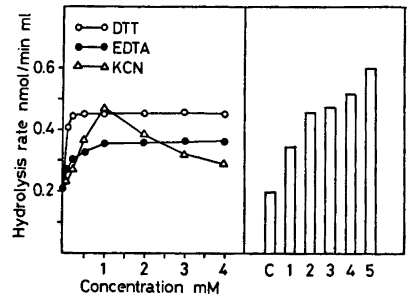


Fig. 2. Activation of BANA hydrolase of rat skin extract with DTT, EDTA, and KCN. On the left: Effect of activator concentration. On the right: Additional effects of activators. C = control with no activators, 1 = 1 mM EDTA, 2 = 0.2 mM DTT, 3 = 1 mM KCN, 4 = 1 mM EDTA + 0.2 mM DTT, 5 = 1 mM EDTA + 0.2 mM DTT + 1 mM KCN.

Enzymatic characteristics of the extract. The BANA hydrolase of the extract behaved like a typical thiol-activated enzyme (Fig. 2). DTT, EDTA, and KCN all increased the hydrolysis rate of BANA. In the case of KCN a distinct optimal concentration (1 mM) was found. At this concentration KCN activated the enzyme even better than did DTT or EDTA. In the cases of DTT and EDTA the activity of the enzyme reached a plateau at 0.2 and 1.0 mM concentration, respectively. Maximal activation of the enzyme was obtained when all activators, DTT, EDTA and KCN, were simultaneously present at the concentrations mentioned above.

The pH-optima of the skin extract in Britton-Robinson buffer were pH 5.8 for BANA, and pH 5.5 for BAA (Fig. 3). An upward bend in the hydrolysis rates of BANA and BAA was noticed between pH 6 and 8. The hydrolysis of both

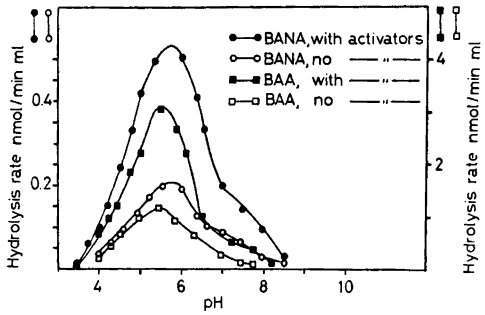


Fig. 3. Effects of pH and thiol activators on BANA and BAA hydrolase activities of rat skin extract in Britton-Robinson buffer. Activators were 1 mM EDTA, 0.2 mM DTT and 1 mM KCN.

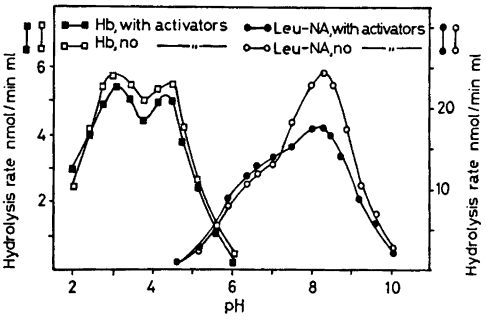


Fig. 4. Effects of pH and thiol activators on hemoglobin and Leu-NA hydrolase activities. The hydrolysis of hemoglobin was assayed in a lactate-acetate buffer. Other conditions were as in Fig. 3.

substrates was increased by DTT, EDTA, and KCN. The pH-optima for the hydrolysis of hemoglobin and Leu-NA by the extract are given in Fig. 4. Hemoglobin hydrolase had two pH-optima, pH 3.0 and 4.3, and thiol activators did not effect the hydrolysis rate. Leu-NA was hydrolyzed maximally at pH 8.0, with a lower optimum at pH 7.0. The thiol activating mixture (DTT, EDTA and KCN) inhibited the enzyme maximally at pH 8.0, while activating it slightly at pH 7.0. BAPA was not hydrolyzed by the extract.

The BANA hydrolase activity of the extract was increased, when the pH of the solution was lowered to between 3.0 and 4.0 by adding 0.5 M HCl, and the temperature of the solution was elevated to 55 °C for 20 min (Fig. 5). At the same time, some protein was precipitated, and after centrifugation the specific activity of the undiluted extract was increased 3.6 times. The effect of elevated temperature was to accelerate the rate of activation. At 0 °C, no activation was evident during 2 h, but some protein was

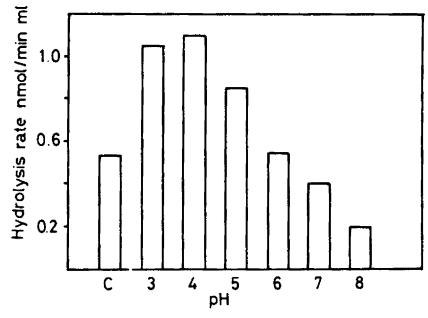


Fig. 5. Effect of preincubation of the extract at 55 °C, for 20 min, at various pH's, on BANA hydrolase activity. C = control, incubated at 0 °C, at pH 6.0.

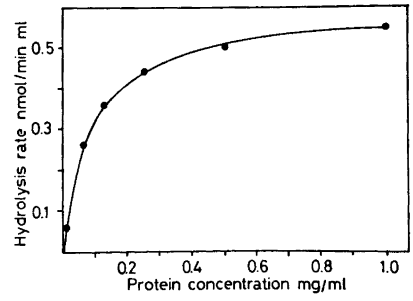


Fig. 6. Effect of enzyme concentration of the assay mixture on BANA hydrolase activity of the extract.

precipitated. At alkaline pH, elevated temperatures destroyed the BANA hydrolase.

The hydrolysis of BANA by the extract increased linearly with time. The effect of enzyme concentration on the hydrolysis rate of BANA was tested by diluting the extract with the homogenization buffer and plotting enzyme activity against protein concentration of the reaction mixture. The activity/enzyme concentration curve was in no way linear (Fig. 6), suggesting the presence of some dissociable inhibitor(s) of BANA hydrolase in the extract. Dialysis of the extract against 10 mM phosphate buffer, pH 7.5, did not abolish the effect of dilution on enzyme activity. This suggests that the possible inhibitor is a high-molecular-weight protease inhibitor. In later experiments undiluted enzyme preparations were used, unless otherwise stated.

Chromatography on Sephadex G-100. Fig. 7. represents a Sephadex G-100 gel chromatography of the skin extract. The BANA hydrolase was eluted as two peaks. The first peak was eluted together with the void volume (V_0, fraction No. 44). The second peak (fraction No. 75) with a lower molecular weight was more active than the first. The main Leu-NA hydrolyzing enzyme and a minor BAA hydrolyzing enzyme were eluted at V_0. The major BAA hydrolase peak (cathepsin B2) was eluted slightly before (fraction No. 68) the low molecular weight

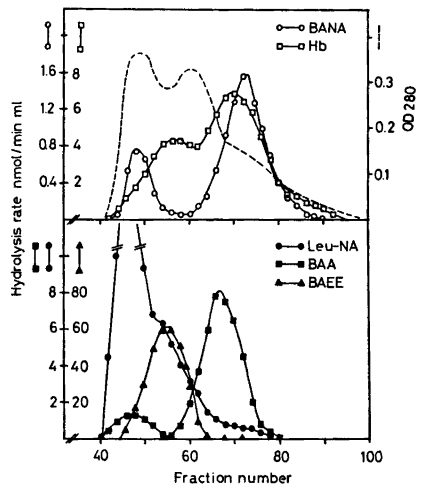


Fig. 7. Sephadex G-100 gel chromatography from rat skin extract. Details of the chromatography are given in "Materials and Methods".

BANA hydrolase, and the main hemoglobin hydrolyzing enzyme was eluted between the BAA and BANA hydrolases. The BANA hydrolase containing fractions from the Sephadex G-100 chromatography were pooled (fraction Nos. 66 – 78). The specific activity of the pooled enzyme was increased 7.4-fold, *i.e.*, from 0.26 nmol/min × mg to 1.92 nmol/min × mg. The total activity of the pooled preparation was increased 2.3-fold from the original activity of the extract. This increase in total activity suggests removal of some inhibitor(s) during the gel chromatography.

The pooled BANA hydrolase from the Sephadex G-100 gel chromatography increased in activity during incubation at pH 4, at 55 °C, as did the activity in the extract (see Fig. 5). The enzyme was fairly stable, and the activity decreased by about 16 – 20 % in a week in 10 mM sodium phosphate buffer, pH 7.5, at +4 °C. EDTA (1 mM) and DTT (0.2 mM) increased the stability of the enzyme. The frozen enzyme (– 18 °C) was stable for at least three months.

DISCUSSION

The acidic rat skin BANA hydrolase can, like the human skin enzyme,[32] be extracted with low ionic strength buffers. The recovery of the enzyme in acidic extraction was higher than in alkaline extraction, and incubation at acidic pH and elevated temperatures increased the activity of the extract. Corresponding phenomena have also been observed with human liver and bovine spleen cathepsin B1 extracts, where the enzyme activity was similarly increased during autolysis at acidic pH.[7,12] In the present investigation an acidic extraction was avoided in order to be able to study the activation phenomenon more thoroughly. The enzyme preparation, when purified by Sephadex G-100 gel chromatography, was still activated by treatment at pH 4. A possible explanation of the acid activation of the BANA hydrolase is irreversible dissociation of some inhibitor from the enzyme at acidic pH, or autolytic modification of the enzyme by some acidic proteinase.

Rat and human skin BANA hydrolases have been shown to be activated by DTT and other thiols as well as EDTA.[23,24,31] In addition, the rat skin BANA hydrolase is also activated by KCN.

The activation with KCN was maximal at a 1 mM concentration. Otto and Bhakdi [3] observed slight activation of beef spleen cathepsin B1 with KCN, while in some other tissues inhibition with KCN has been reported.[11,12] Besides the hydrolysis of BANA, the hydrolysis of BAA was activated by thiol activators, while the hemoglobin hydrolyzing enzyme was not effected, and the main Leu-NA hydrolyzing enzyme was inhibited by the activation mixture (DTT, EDTA and KCN), at pH 8.0. BANA was hydrolyzed optimally at pH 5.8 and BAA at pH 5.5. In addition, an upward bend in pH curves of both substrates was noticed between pH 6 and 8. This bend is probably caused by the alkaline BANA hydrolyzing enzyme demonstrated earlier from rat skin.[24]

The activity/enzyme concentration curve reached a plateau with increasing enzyme concentrations. This phenomenon suggested the presence of inhibitor(s) of BANA hydrolase in the extract. Sephadex G-100 gel chromatography separated some of these inhibitors from the enzyme, as indicated by an increase in total enzyme activity. The presence of an inhibitor of thiol proteases in rabbit skin has earlier been demonstrated.[33,34] A purified preparation is known to inhibit the so called "Arthus protease" of Udaka and Hayashi [33] as well as papain, and according to Lazarus and Dingle [34] rabbit skin extract inhibits human liver cathepsin B1. The inhibitor(s) in the rat skin may be similar to the one shown by Udaka and Hayashi,[33] and Lazarus and Dingle.[34]

The stability of the rat skin BANA hyrolase is fairly good when partially purified by Sephadex G-100 chromatography. Since the enzyme is stable, and the estimation of its activity is not difficult, it is very suitable for extensive purification and characterization; details of which will be presented in our forthcoming reports.

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Tobacco Chemistry. 29. (7*S*)-10-Oxo-4ξ-methyl-7-isopropyl-5*E*-undecen-4-olide, a New Thunbergan-type Nor-isoprenoid Isolated from Greek *Nicotiana tabacum* L.

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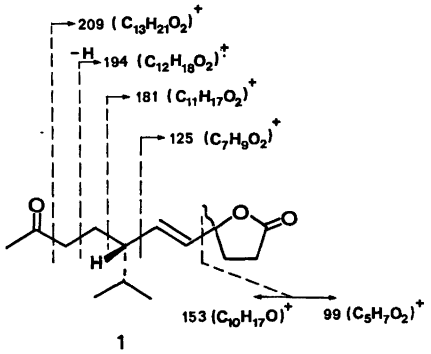
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The structure of a new C_{15}-lactone, (7*S*)-10-oxo-4ξ-methyl-7-isopropyl-5*E*-undecen-4-olide, isolated from Greek tobacco, has been determined mainly by ^{1}H NMR, ^{13}C NMR and high resolution MS, and its absolute configuration established by degradation to (2*S*)-5-oxo-2-isopropylhexanal. The carbon skeleton of the new compound indicates that it is derived from a thunbergane precursor and constitutes the first C_{15} nor-thunberganoid in tobacco.

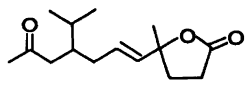
Some 200 nor-isoprenoids have so far been encountered in tobacco leaves and tobacco smoke. Virtually all of these can be viewed as derived from either (i) carotenoids/aliphatic polyisoprenoids, (ii) labdanes/drimanes, or (iii) thunberganes, and are conveniently grouped accordingly.[1] They constitute a substantial amount of the tobacco essential oil and many of them are considered important for the tobacco flavour and aroma.[2] In the present communication** we present evidence for the structure (*1*) of a new lactone isolated from sun-cured Greek *Nicotiana tabacum* L. and evidently derived from a thunbergane precursor.

The presence of fifteen lines in the ^{13}C NMR spectrum and precise mass determination established that the new compound had the elemental composition $C_{15}H_{24}O_3$. The three oxygen atoms, as judged from IR, ^{13}C NMR, and ^{1}H NMR, were accomodated in a methyl ketone grouping (1716 cm^{-1}; δ_C 208.9, s; δ_H 2.1, 3 H, s) and a γ-lactone moiety (1777 cm^{-1}; δ_C 176.9, s). Of the additional three methyl groups revealed by the spectral data, two were present in an isopropyl group (1370 and 1386 cm^{-1}; δ_C 19.1, q, and 20.7, q; δ_H 0.82, 3 H, d, and 0.88, 3 H, d), and the third was linked to the γ-atom of the lactone ring, and, according to the low field chemical shift of its proton resonance (δ_H 1.5, 3 H, s) allylic to the *trans* disubstituted double bond present in the molecule (981 cm^{-1}; δ_C 131.6, d, and 134.1, d; δ_H 5.5, 2 H, m). Examination of the high resolution mass spectrum confirmed the presence of these structural features and implied structure *1* for the new compound. Thus, a doublet at *m/e* 209 ($C_{13}H_{21}O_2$ and $C_{12}H_{17}O_3$) was consistent with the occurrence of the acetyl and isopropyl groups, and peaks at *m/e* 153 ($C_{10}H_{17}O$), 99 ($C_5H_7O_2$), and 125 ($C_7H_9O_2$) were in accord with the presence of the 4-methyl-4-vinylene γ-lactone unit. Moreover, an ion at *m/e* 194 ($C_{12}H_{18}O_2$) demonstrated the loss of the elements of acetone from the molecular ion by a McLafferty reaction and hence, the attachment of the acetyl group to a methylene group. The remaining methylene group could be inserted in three ways giving 10-oxo-4-methyl-7-isopropyl-5*E*-undecen-4-olide (*1*), 10-oxo-4-methyl-8-isopropyl-5*E*-undecen-4-olide (*3*), or 9-oxo-4-methyl-7-isobutyl-5*E*-decen-4-olide (*4*) of which the two last possibilities were disfavoured by MS and ^{13}C NMR evidence. Thus, the presence of an $C_{11}H_{17}O_2^+$ ion is only readily rationalized on the basis of structure *1*, which on electron impact should undergo simple cleavage of the allylic C(7)—C(8) bond extending from the tertiary C(7).

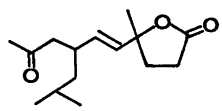
** Presented in part at the EUCHEM-conference, Canary Islands, Sept. 16—20, 1974, and the CORESTA-meeting, Montreux, Sept. 22—27, 1974.



1



3



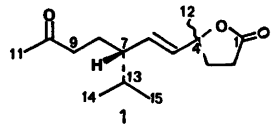
4

Similarly, the ^{13}C NMR data were consistent only with structure *1*, since in the case of structure *3* the resonance for the methylene carbon α to the keto group should occur at a lower field than observed (δ_C 41.9, t) because of the β-substitution, and in the case of structure *4* the signals due to the *geminal* methyl carbons, here incorporated in an isobutyl group, should occur at a lower field (δ_C 22 – 23) than observed (δ_C 19.1, q, and 20.7, q).[3,4] Moreover, the excellent agreement of relevant ^{13}C NMR signals observed for solanone (*2*) and 4-methyl-5-hexen-4-olide (*7*) with those of the tobacco isolate strongly supported the suggested structure: 10-oxo-4-methyl-7-isopropyl-5*E*-undecen-4-olide (*1*, see Table 1).*

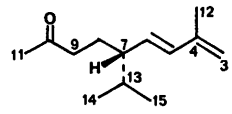
Conclusive evidence for the presence of a 2-oxo-5-isopropylhex-1-ylidene moiety was obtained by the isolation of 5-oxo-2-isopropylhexanal (*5*) on ozonolytic degradation of the lactone. This ketoaldehyde (*5*) was optically active and exhibited the same positive rotatory power as the corresponding material (*5*) similarly prepared from solanone (*2*) thereby establishing the absolute configuration at C(7) as *S*.**

Additional evidence for the chemically unverified part of the molecule and for the *trans* configuration of the double bond was obtained by spectral examination of the triol diacetate

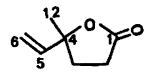
Table 1. ^{13}C chemical shifts (ppm relative to TMS) and assignments recorded for (7*S*)-10-oxo-4-ξ-methyl-7-isopropyl-5*E*-undecen-4-olide (*1*), and model compounds *2* and *7*.



1



2



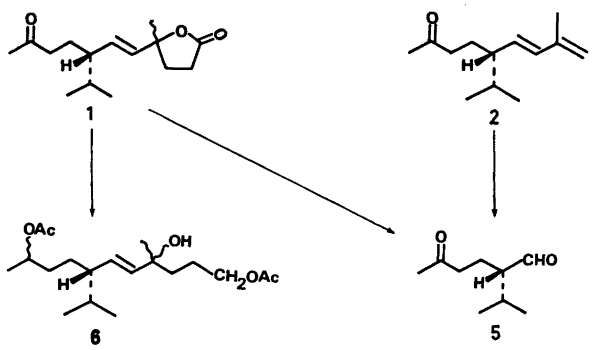
7

^{13}C assignment	Chemical shifts[a] (δ) and No. of lines[b] (n) 1 δ	n	2 δ	n	7 δ	n
C(1)	176.9	s			176.7	s
C(2)	29.1	t			28.7	t
C(3)	34.4	t	114.7	t	33.6	t
C(4)	85.4	s	141.8	s	85.4	s
C(5)	131.6[c]	d	132.2[c]	d	140.4	d
C(6)	134.0[c]	d	134.6[c]	d	113.4	t
C(7)	48.7	d	49.5	d		
C(8)	26.0	t	26.4	t		
C(9)	41.9	t	41.9	t		
C(10)	208.9	s	208.2	s		
C(11)	30.0	q	29.9	q		
C(12)	26.6	q	18.7	q	26.1	q
C(13)	31.9	d	32.5	d		
C(14)	19.1	q	19.3	q		
C(15)	20.7	q	20.8	q		

[a] Measured in proton noise-decoupled spectrum; [b] No. of lines observed in off-resonance proton-decoupled spectrum ('s' = singlet etc.); [c] Tentative.

* Dr. E. Demole, Firmenich SA, Geneve, has independently and simultaneously identified 10-oxo-4-methyl-7-isopropyl-5*E*-undecen-4-olide as a new constituent of Burley tobacco (private communication).

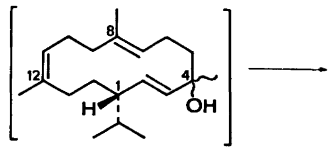
** Originally the L-configuration was erroneously attributed to solanone.[5] The correct *S* (or D)-configuration was later established through correlation with (2*S*)-5-oxo-2-isopropylhexanoic acid by Fukuzumi *et al.*[6] who, however, applied the sequence rules of Cahn, Ingold and Prelog,[7–9] (further exemplified by Weedon[10]) incorrectly by assigning the *R*-configuration to solanone.



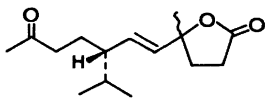
(*6*; 3460 cm^{-1}; δ_H 4.1, 2 H, t, and 4.8, 1 H, m) prepared by $LiAlH_4$-reduction of the tobacco constituent (*1*) and subsequent acetylation under mild conditions. Thus, electron impact generated the structurally required fragments, notably the important M − 101 (74 %) produced by cleavage of the labile allylic C(3) − C(4) bond extending from the hydroxylated tertiary carbon, and its daughter ions M − 101 − 60 (100 %, m* 135.9) and M − 101 − 60 − 18 (42 %, m* 146.8) formed by consecutive losses of acetic acid and water. It exhibited IR absorption (979 cm^{-1}) characteristic of a *trans* disubstituted double bond and displayed, after addition of $Eu(dpm)_3$ to the ^1H NMR sample, a 16 Hz vicinal spin-spin coupling constant for the two olefinic protons constituting the AB-part of an ABX-system.

Shortage of material prevented configurational assignment of the second chiral centre, C(4). However, the CMR and ^1H NMR spectra (including those recorded after addition of shift reagent) indicated that the tobacco isolate was configurationally homogeneous as no additional resonances were observed; this, as previously found for the structurally related methyl 3-hydroxy-3-methyl-6-isopropyl-4*E*-octenoate,[11,12] would have been expected if two C(4)-epimers were present.

The structural relationship of this new lactone with the macrocyclic thunbergane diterpenoids, which are quite abundant in tobacco, places it in the growing family of volatile tobacco constituents assumed to be derived from such diterpenoids. A possible precursor, although as yet not detected in tobacco, is the hydrated thunbergene, thunberga-2,7-11-trien-4-ol (*8*)* which under the influence of oxygen and light could undergo oxidative cleavages of the C(7) − C(8) and C(11) − C(12) bonds furnishing the C_{15}-lactone. Most of the thunbergan-derived nor-isoprenoids previously encountered in tobacco are either C_{12}- or C_{13}-compounds and the new lactone therefore represents the first member of a new subgroup. Nine of the eleven thunbergan-diterpenoids found in tobacco [1] are hydroxylated and unsaturated at positions corresponding to those in the present lactone, and, being potential precursors, they might all possess the same *S*-configuration at the carbon atom carrying the isopropyl group.



8



EXPERIMENTAL

^1H NMR, ^{13}C NMR (accuracy of δ_C: ± 0.05 ppm), IR, UV, and mass spectra were recorded on Varian XL-100, Digilab FTS-14, Beckmann DB-2A, and LKB 9000 instruments, respec-

* Nomenclature according to J. W. Rowe Oct. 1968; private communication.

tively. Rotations were measured on a Perkin-Elmer 141 instrument, and precise mass determinations were carried out at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm.

7(S)-10-Oxo-4-ξ-methyl-7-isopropyl-5E-undecen-4-olide (1, 35 mg) was isolated from a medium-volatile, neutral fraction [13] of an extract,[14] obtained from 295 kg sun-cured Greek *Nicotiana tabacum* L., employing liquid chromatography on silica gel and $AgNO_3$-impregnated silica gel. The fractionation of this medium-volatile material will be described elsewhere.[13] MS (20 eV): *m/e* 252 (M^+, 6), 43 (100), 121 (44), 107 (33), 99 (31), 93 (31), 95 (25), 81 (24), 109 (24), 97 (22), 71 (22), 123 (22), 135 (22), 149 (22), 151 (22), 111 (21), 194 (19), 55 (19), 69 (18), 119 (18), 125 (18), 83 (18), 137 (17), 134 (17), 139 (16), 112 (12), 105 (11), 108 (11), 133 (11), 122 (9), 179 (9), 181 (9), 161 (9), 163 (8), 59 (8), 234 (8); accurate mass determinations: $C_{15}H_{24}O_3$, found 252.1726, calc. 252.1725; $C_{13}H_{21}O_2$, found 209.1538, calc. 209.1541; $C_{12}H_{17}O_3$, found 209.1175, calc. 209.1178; $C_{12}H_{18}O_2$, found 194.1302, calc. 194.1307; $C_{11}H_{17}O_2$, found 181.1219, calc. 181.1228; $C_{10}H_{17}O$, found 153.1277, calc. 153.1279; $C_7H_9O_2$, found 125.0595, calc. 125.0602; $C_5H_7O_2$, found 99.0450, calc. 99.0446; ν_{max} (film): 2965 (s), 2948 (s), 2876 (m), 1777 (s), 1716 (s), 1386 (shoulder), 1370 (m), 1240 (m), 1205 (m), 1169 (m), 1141 (m), 1078 (m), 981 (m), 932 (m); δ_H ($CDCl_3$): 0.82 (3 H, d, *J* 6.5 Hz), 0.88 (3 H, d, *J* 6.5 Hz), 1.50 (3 H, s), *ca.* 2.0–2.8 (*ca.* 4 H, m), 2.12 (3 H, s), 5.5 (2 H, m); addition of Eu(dpm)$_3$: *r* (relative induced shifts) 0.57 and 0.65 [C(7)-CH(C*H*$_3$)$_2$, overlapping doublets], 1.0 [C(4)C*H*$_3$, s], 1.24 [C(5)*H*=C(6)*H*, m], 3.35 [C(11)*H*$_3$, s]; δ_C ($CDCl_3$): see Table 1; $[\alpha]^{20}$ +1.3° (589 nm), +1.9° (578), +2.4° (546), +5.7° (436), (*c* 1.6, $CHCl_3$).

4-Hydroxy-1,10-diacetoxy-4-methyl-7-isopropyl-5E-undecene (6): (7*S*)-10-oxo-4-ξ-methyl-7-isopropyl-5*E*-undecen-4-olide (*1,* 10 mg) dissolved in dry ether (3 ml) was added dropwise to a solution of $LiAlH_4$ (8 mg) in dry ether (20 ml) and the mixture stirred for 1 h at room temperature. Moist ether, followed by water (10 ml) was added to the reaction mixture and the products extracted with ether. Removal of the solvent left a homogeneous (TLC) product (13 mg) which was acetylated without further purification. The residue was dissolved in dry pyridine (1.5 ml) and acetic anhydride (0.3 ml) and kept at ambient temperature for 6 h. Excess anhydride was destroyed with a few drops methanol, water (10 ml) was added and the acetate extracted with ether. The extract was washed with H_2SO_4 (dil.), water, $NaHCO_3$, water, dried and evaporated leaving the diacetate as a colourless oil which was chromatographed on a silica gel column. The diacetate (9 mg) was eluted with 50 % ether/pentane. MS (20 eV, ion source temp. 120 °C): *m/e* 342 (M^+, not visible), 181 (100), 241 (74), 43 (42), 111 (42), 163 (42), 137 (29), 71 (33), 123 (28), 264 (21), 182 (15); metastables were observed at *m/e* 215.1, 185.0, 135.9, 146.8, and 172.6, respectively, corresponding to the transitions 324 (M−18)→264 (M−18−60)→221 (M−18−60−43), 241 (M−101)→181 (M−101−60)→163 (M−101−60−18), and 207 (M−15−2×60)→189 (M−15−2×60−18); ν_{max} (film): 3460 (broad), 2959 (m), 2936 (shoulder), 2871 (m), 1739 (s), 1455 (w), 1383 (shoulder), 1372 (m), 1368 (m), 1247 (s), 1130 (w), 1031 (m), 979 (w), 952 (w); δ_H ($CDCl_3$): 0.91 (3 H, d, *J* 6.5 Hz), 0.96 (3 H, d, *J* 6.5 Hz), 1.19 (3 H, d, *J* 6 Hz), 1.30 (3 H, s), 2.01 (3 H, s), 2.03 (3 H, s), 4.07 (2 H, t with further splittings), *ca.* 4.83 (1 H, m), 5.43 (2 H, m); irradiations at δ 1.19 and 1.58, respectively, simplified the multiplet at δ *ca.* 4.83 to a distorted triplet and the multiplet at δ 4.07 to a broad singlet; irradiation at δ *ca.* 4.83 collapsed the doublet at δ 1.19 to a singlet; addition of Eu(dpm)$_3$: *r* (relative induced shifts) 0.18 and 0.23 [C(7)CH(C*H*$_3$)$_2$, m], 0.42 and 0.46 [C(11)*H*$_3$, 2×d], 0.65 and 0.67 [2×COC*H*$_3$], 1.0 [C(1)*H*$_2$, t], 1.23 [C(10)*H*, m], 1.26 [C(6)*H*, m], 1.81 [C(5)*H*, d, *J* 16 Hz], 1.75 and 1.82 [C(4)C*H*$_3$, 2×s]; decoupling experiments performed on the Eu(dpm)$_3$-treated sample: irradiation at δ 5.80 [C(10)*H*, *r* 1.23] collapsed the two doublets at δ 1.52 (*r* 0.42) and δ 1.55 (*r* 0.46), respectively, to a broad singlet [C(11)H$_2$]; irradiation at δ 1.54 sharpened the multiplet at δ 5.80 [C(10)*H*].

Ozonolysis of (7S)-10-oxo-4ξ-methyl-7-isopropyl-5E-undecen-4-olide (1). The natural lactone (*1,* 15 mg) dissolved in CH_2Cl_2 (15 ml) and pyridine (0.1 ml) was treated with excess ozone at −65 °C. Zinc-powder (500 mg) and acetic acid (2 ml) were added and the mixture stirred 35 min, during which the temperature was slowly raised to 20 °C. The solution was decanted, the reaction vessel with its residue was washed with CH_2Cl_2, and the combined solutions washed four times with water. Removal of the solvent left a slightly yellow oil which was chromatographed on silica gel furnishing pure (2*S*)-5-oxo-2-isopropylhexanal (*5,* 2 mg); MS: *m/e* 156 (M^+, 2), 43 (100), 58 (75), 41 (35), 71 (23), 69 (22), 55 (19), 95 (18), 70 (10), 59 (10), 96 (10), 86 (9), 110 (9), 138 (8), 85 (8), 81 (8), 123 (7); ν_{max} (film): 2967 (m), 2935 (m), 2879 (w), 2720 (w), 1722 (s), 1719 (s), 1372(m), 1170 (m); lit.:[15] ν_{max} (film): 1709 (C=O); δ_H ($CDCl_3$) 0.97 (3 H, d, *J* 7 Hz), 1.00 (3 H, d, *J* 7 Hz), 2.13 (3 H, s), 2.3–2.6 (2 H, m), 9.63 (1 H, d, *J* 2 Hz); lit.:[15] δ 1.0 (isopropyl methyls, *J* 6 Hz), 2.07 (acetyl methyl);$[\alpha]^{20}$ +47° (589 nm), +49° (578), +60° (546), +165° (436), +415° (365), (*c* 0.2, $CHCl_3$). The ketoaldehyde did not separate from the corresponding ketoaldehyde *5* prepared from solanone (*vide infra*) when co-injected on a glass capillary GC-column.

Ozonolysis of solanone. Solanone ((5*S*)-8-methyl-5-isopropyl-6*E*,8-nonadien-2-one, 18mg); $[\alpha]^{20}$ +16.7° (589 nm), +17.1° (578), +20.6° (546), +45.6° (436), +114.3° (365), (*c* 0.62, $CHCl_3$);

lit.: $[\alpha]_D^{23}$ + 13.6° (neat),[5] $[\alpha]_D^{28}$ + 14.2° ($CHCl_3$);[6] previously procured from the same tobacco (fraction B3),[14,16] was ozonolyzed and the ketoaldehyde *5* (6.2 mg) isolated as described above; $[\alpha]^{20}$ +43.7° (589 nm), +45.9° (578), +55.6° (546), +133.3° (436), +364.8 (365), (*c* 0.54, $CHCl_3$); the NMR, IR and mass spectra were indistinguishable from those of the ketoaldehyde obtained from the tobacco constituent.

Model compounds. Solanone (*2*), isolated from Greek tobacco,[14,16] and 4-methyl-5-hexen-4-olide (*7*), obtained in almost quantitative yield by CrO_3/AcOH oxidation of linalool oxide, was used for comparison of ^{13}C NMR data, see Table 1.

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A Study of ^{13}C—^{19}F Coupling Constants in Glycosyl Fluorides

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Proton decoupled ^{13}C NMR spectra of a number of glycosyl fluorides gave information about the $^{13}C-^{19}F$ coupling constants. The $^{1}J\{^{13}C1F\}$ values were found to be negative and to be numerically higher in α-pyranosyl fluorides than in β-pyranosyl fluorides. An axial substituent at C2 in a pyranosyl fluoride increases both $^{2}J\{^{13}C2F\}$ and $^{1}J\{^{13}C1F\}$. $^{3}J\{^{13}C3F\}$ values are higher in β-pyranosyl fluorides than in α-fluorides indicating a Karplus type of relation. In furanosyl fluorides both $^{1}J\{^{13}C1F\}$ and $^{2}J\{^{13}C2F\}$ were found to be smaller when the fluorine atom and the substituent at C2 are *trans* than when they are *cis*-oriented.

Since a rather large number of glycosyl fluorides have been prepared in connection with other work [1] and since relatively few $^{13}C-^{19}F$ coupling constants have been described [2] it was decided to measure ^{13}C NMR spectra of a number of glycosyl fluorides, to see whether $^{13}C-^{19}F$ coupling constants showed a stereochemical dependance. $^{1}H-^{19}F$ coupling constants have been found to give valuable configurational information in carbohydrate derivatives.[3]

Proton decoupled ^{13}C NMR spectra have therefore been measured on a number of glycopyranosyl and furanosyl fluorides (Tables 1 and 2). These spectra give the ^{13}C chemical shifts and the $^{13}C-^{19}F$ coupling constants.

The signals were assigned by the principles generally used for pyranoses [4] or by selective proton decoupling.[5] The chemical shifts of the pyranosyl fluorides are very similar to those of other pyranoses.[6] The furanosyl fluorides mostly have C1 at slightly lower field than the corresponding pyranoses. Most noticeable in the spectra of the furanosyl fluorides is the low field position of the signals of C2 and C4. The assignment of C2 in furanosyl fluorides is easy because of its rather large coupling to fluorine.

The one bond $^{13}C-^{19}F$ coupling constant of the pyranosyl fluorides is in almost all cases 10 Hz higher in α-fluorides than in β-fluorides. This might indicate that the lone pair electrons of the ring-oxygen affect the coupling constant.[6] The α-fluorides, with an axial fluorine atom, have a numerically larger coupling constant than the β-fluorides. We have, however, found by selective decoupling of H1 in (*α-1b*) that the sign of $^{1}J\{^{13}C1F\}$ is opposite to that of $^{2}J_{HF}$, and the latter has been found to be positive.[7] This is in accordance with the results found by Weigert and Roberts.[8] Thus pyranosyl fluorides with an equatorial fluorine have a higher $^{1}J_{C1F}$ value than those with an axial fluorine atom in agreement with the results obtained for $^{1}J\{^{13}CH1\}$ values.[6]

The $^{2}J\{^{13}C2F\}$ value in α- and β-pyranosyl fluorides is found to be *ca.* 25 Hz when the substituent at C2 is equatorial. When, however, this substituent is axial, as in D-mannose derivatives, $^{2}J\{C2F\}$ is 35 – 40 Hz. Selective decoupling of H2 in (α-1b) showed that $^{2}J\{^{13}C2F\}$ is positive. An axial substituent at C2 also increases (numerically decreases) the $^{1}J\{C1F\}$ values as seen, *e.g.*, by comparison of (*α-1b*) and (*α-3b*).

This may explain why the anomeric tetra-*O*-acetyl-D-mannopyranosyl fluorides (α- and *β-3b*) have almost the same $^{1}J\{C1F\}$ values. The xylopyranosyl fluorides (α- and β-7) have different conformations,[9] both with an axial fluorine atom. In (*β-7*), which has an axial substituent at C2, $^{1}J\{^{13}C1F\}$ is only 222.8 Hz whereas it is 228.5 Hz in (α-7).

In β-pyranosyl fluorides, which have the fluorine atom and C3 *trans*-oriented, the $^{3}J\{^{13}C3F\}$ value is 4 – 10 Hz whereas it is only 0 – 6 Hz in α-fluorides, in which the fluorine

Table 1. ^{13}C chemical shifts (ppm) and $^{13}C-^{19}F$ coupling constants (Hz) of glycopyranosyl fluorides.

Compound	C1 ^1J(C1F)	C2 ^2J(C2F)	C3 ^3J(C3F)	C4 ^4J(C4F)	C5 ^3J(C5F)	C6	OCH_3 J(CF)
α-*1a*[10]	108.23 222.2	71.89 24.85	73.25	69.44	75.03 2.9	61.04	[b]
β-*1a*[11]	110.02 211.3	75.97 22.1	73.56[a] 2.2	69.91	76.86[a] 4.30	61.36	[b]
α-*1b*[12]	103.65 228.92	69.90 24.29	69.16	67.13	69.66 4.39	60.99	
β-*1b*[13]	105.97 218.96	70.81 22.25	71.77[a] 3.81	67.26	71.63[a] 2.05	61.53	
α-*1c*[14]	103.93 229.9	78.39 23.4	71.01	67.45	69.54 4.4	61.30	58.84 [b]
β-*1c*[14]	108.43 217.38	80.27 24.93	72.53 10.59	67.67	71.29 4.77	61.48	59.62 1.83
α-*1d*[15]	103.90 227.4	72.01 24.7	77.74	68.42	70.00 4.6	61.43	60.20
β-*1d*[15]	106.06 218.3	71.00 27.8	79.55 5.9	67.90	72.11 3.8	62.21	58.60 [b]
α-*2*[16]	104.25 228.3	67.30 24.1	67.28	66.86	68.79 3.7	61.10	
β-*2*[16]	107.20 217.29	69.15 24.88	70.08 10.54	66.61 1.20	71.33 4.68	61.42	
α-*3b*[16]	104.71 223.1	67.55 39.7	68.08 1.8	64.95	70.82 3.1	61.72	
β-*3b*	104.05 223.6	67.75 25.0	68.37 5.9	66.36	72.71 4.4	62.72	[b]
α-*3c*[14]	104.6 224.1	75.9 35.2	70.1 ~1	64.8	70.0 2.9	61.4	59.5 [b]
α-*3d*[15]	104.96 221.19	65.87 39.55	76.12	66.35	70.94 2.9	62.01	57.74 [b]
α-*4*[16,17]	104.26 226.74	67.68 38.76	66.18 0.91	63.99 0.5	66.39 2.44	62.03	
β-*4*[16,17]	105.03 227.35	67.13 22.89	67.72	64.52 1.12	74.70	63.17 2.13	
5[17]	104.5 224.7	64.6 36.6	65.4	65.1	65.8 2.9	61.8	[b]
6e[15]	103.76 227.7	70.77 24.7	69.52	72.74	67.86 4.6	17.12	
6f[15]	104.68 221.3	67.79 41.3	68.08 2.1	69.76	68.72 3.1	17.1	

Table 1. Continued.

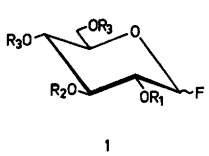
α-*7*[12]	103.8 228.5	70.3 23.4	68.8	68.0	60.1 4.4	*b*
β-*7*[18]	105.27 222.8	67.03 36.0	67.10 2.8	66.82 0.6	60.56 3.4	
8[18]	104.6 227.1	67.5 26.4	68.1	66.4	62.4 2.8	*b*

[a] May be reversed. [b] Digital resolution only ± 1.5 Hz.

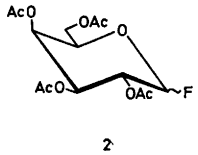
Table 2. ^{13}C chemical shifts (ppm) and coupling constants (Hz) of glycofuranosyl fluorides.

Compound	C1 ^1J(C1F)	C2 ^2J(C2F)	C3 ^3J(C3F)	C4 ^4J(C4F)	C5 ^3J(C5F)	C6	OCH_3 J(CF)
α-*9b*[19]	106.88 234.19	78.21 19.61	73.13	76.65 1.17	67.55 0.7	62.35	
β-*9b*[19]	111.51 228.46	78.96 35.36	71.69	80.39 2.46	68.15	62.51	
α-*9c*[15]	107.73 233.8	86.15 18.9	73.95	76.78 1.2	67.75 0.9	62.55	58.91 0.9
β-*9c*[15]	112.15 226.5	87.00 31.4	71.59	80.14 2.4	68.48	62.79	57.93
α-*9d*[15]	106.68 231.6	77.74 19.5	77.74	81.11 1.5	68.74 1.2	62.46	58.06
β-*9d*[15]	112.26 227.3	77.81 34.5	81.67 0.7	81.94 2.6	69.26	63.11	58.53
α-*10b*[19]	112.62 225.2	75.96 36.0	69.84 0.9	77.96 2.1	67.69 1.5	62.40	
β-*10b*[19]	106.52 236.8	71.57 20.1	68.33	77.88 2.4	67.10 1.2	62.18	
β-*10c*[15]	106.74 234.4	80.72 20.5	66.28[a]	78.06	68.35[a]	62.14	58.90 *b*
11[15]	112.27 228.0	80.23 36.8	73.83	81.63	63.17		*b*
α-*12*[15]	112.27 224.7	75.72 37.4	70.77	78.31 1.8	62.91 1.8		
β-*12*[15]	107.07 236.1	72.68 20.5	68.54 1.2	79.00 2.6	63.11		
α-*13*[20]	107.39 234.4	71.91 20.5	69.59	82.92	63.57		*b*
β-*13*[20]	112.44 225.5	75.00 35.9	71.35 1.5	81.27 2.9	64.29 0.5		
14[20,21]	112.71 225.81	80.94 40.3	76.65	84.42	63.49		

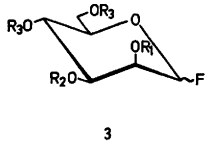
[a] May be reversed. [b] Digital resolution only ± 1.5 Hz.



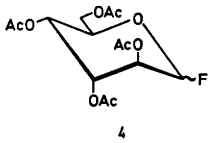
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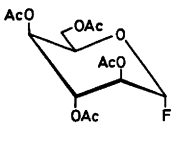
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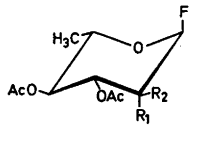
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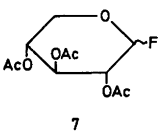
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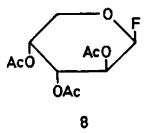
5



6



7



8

(a) $R_1 = R_2 = R_3 = H$

(b) $R_1 = R_2 = R_3 = CH_3CO$

(c) $R_1 = CH_3$, $R_2 = R_3 = CH_3CO$

(d) $R_1 = R_3 = CH_3CO$, $R_2 = CH_3$

(e) $R_1 = H$, $R_2 = CH_3COO$

(f) $R_2 = H$, $R_1 = CH_3COO$

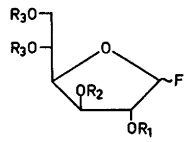
atom and C3 have a *gauche*-orientation. This may indicate a Karplus type of relation. In all pyranosyl fluorides, irrespective of anomeric structure, a $^3J\{^{13}C5F\}$ value of 2 – 5 Hz is found. In a single case (*β-2*) a small coupling was observed between the fluorine atom and C4. A small four bond coupling was also observed to the *O*-methyl group of (*β-1c*).

The $^1J\{C1F\}$ values of the furanosyl fluorides (Table 2) seem to be dependent of the relative configuration of C1 and C2. When the fluorine atom and the substituent at C2 are *cis*-oriented $^1J\{C1F\}$ is numerically larger (*ca.* 6 Hz) than when the two substituents are *trans*-oriented. The $^2J\{C2F\}$ value, on the other hand, is numerically much larger when the substituents at C1 and C2 are *trans* than when they are *cis*. Selective proton decoupling of H1 and H2 has shown that the sign of $^1J\{C1F\}$ in (*α-9b*) is negative whereas $^2J\{C2F\}$ is positive, in agreement with the results found for the pyranosyl fluorides. Thus the absolute values of $^1J\{C1F\}$ and $^2J\{C2F\}$ are smallest in the furanosyl fluorides which have the fluorine atom and the substituent at C2 *cis*-oriented. It may be noted that only few of the furanosyl fluorides investigated show any coupling between fluorine and C3. A small coupling between fluorine and C4 is, on the other hand, found in almost all compounds.

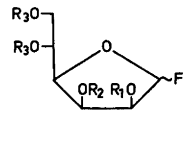
EXPERIMENTAL

The ^{13}C NMR spectra were obtained on a Bruker WH-90 instrument at 22.63 MHz. The two unacylated glucosyl fluorides (α- and *β-1a*) were measured as *ca.* 20 % solutions in water using dioxane (10 %) as internal reference (67.40 ppm). All other compounds were measured as *ca.* 20 % solutions in deuteriochloroform using tetramethylsilane as internal reference. In order to measure the $^{13}C-^{19}F$ coupling constants accurately a sweep width of 1200 Hz was used in most cases with 8K computer memory for spectrum accumulation. This gives a digital resolution of ± 0.3 Hz.

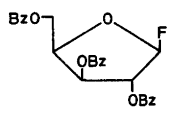
Tetra-O-acetyl-β-D-mannopyranosyl fluoride (*β-3b*). A solution of 1,3,4,6-tetra-*O*-acetyl-*β*-D-



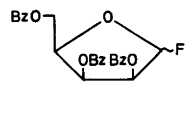
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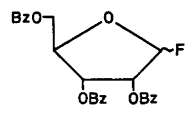
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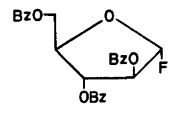
11



12



13



14

(b) $R_1 = R_2 = R_3 = CH_3CO$

(c) $R_1 = CH_3$, $R_2 = R_3 = CH_3CO$

(d) $R_2 = CH_3$, $R_1 = R_3 = CH_3CO$

mannopyranose [22] (500 mg) in anhydrous hydrogen fluoride (2 ml) was kept at −70 °C for 30 min. It was then diluted with dichloromethane and poured on ice. The organic phase was washed with aqueous sodium hydrogencarbonate, dried and evaporated. The residue was acetylated with acetic anhydride (2 ml) in pyridine (2 ml). The product thus obtained was separated into two fractions by preparative TLC using ether-pentane (3:1) as eluent. The fastest moving fraction gave 220 mg (44 %) of tetra-*O*-acetyl-α-D-mannopyranosyl fluoride (*α-3b*), identified through its ^1H and ^{19}F NMR spectra. The next fraction yielded 33 mg (7 %) of (*β-3b*) which was crystallized from ether, m.p. 105.5 − 107 °C, $[\alpha]_D^{20}$ − 1.0 ° (*c* 1.5, $CHCl_3$). (Found: C 47.82; H 5.50. Calc. for $C_{14}H_{19}FO_9$ C 48.00; H 5.43).

A ^1H NMR spectrum in deuteriochloroform gave the following data: H1 δ 5.53; H2 5.42; H3-4 5.1 − 5.3; H5 3.90; H6 4.2 − 4.4. J_{12} 1.8 Hz; J_{23} 3.1; J_{45} 7.0; J_{56} 5.6. A ^{19}F NMR spectrum gave $\Phi = -142.0$ ppm (from $CFCl_3$); J_{1F} 50.3 Hz; J_{2F} 10.7.

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Strained Heterocyclic Compounds. 8. Synthesis of *N*-[Bromo-(*t*-butyloxycarbonyl)phthalimidoacetyl]piperidine and Some Related Compounds and Their Reactions

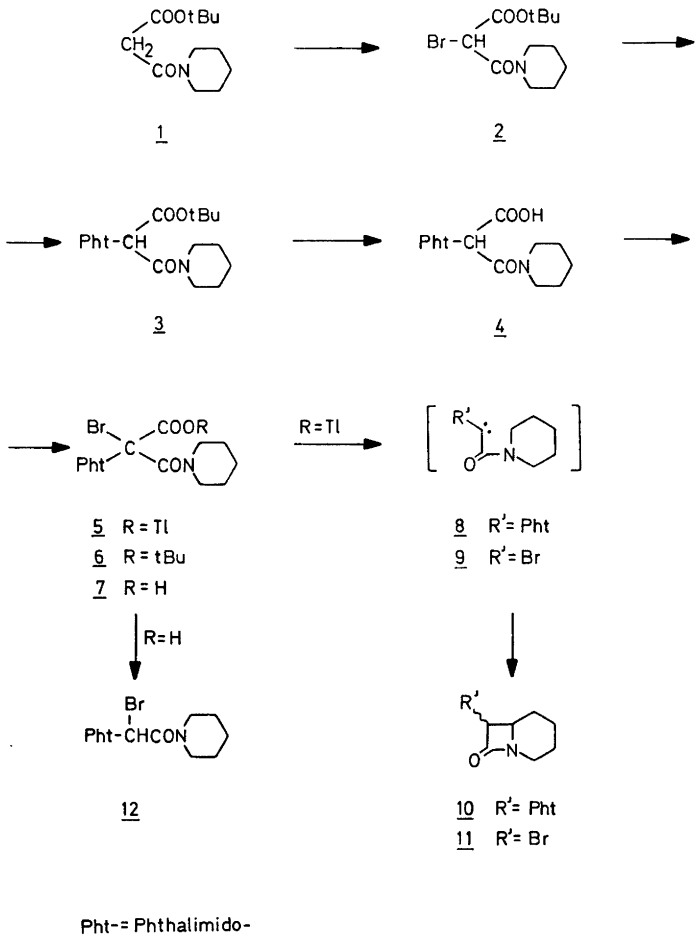
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The thallium(I) salt of *N*-(bromocarboxyphthalimidoacetyl)piperidine (*5*) has been prepared. Decarboxylation in bromobenzene at 150 °C gave thallium bromide and a complex mixture of organic products. Only a trace of the desired phthalimido-β-lactam (*10*) was formed.

We have been interested in developing general methods for the synthesis of penicillin and cephalosporin analogues for some time. The main part of our early work was directed towards the construction of the appropriate β-lactam rings, by carbene insertion and we have synthesized fused α-halo-β-lactams [1] using this method. After considerable effort to replace the halogen in these β-lactams with an amide side chain, leaving the β-lactam intact, recently we were successful [2] in synthesising the phthalimido-β-lactam *10*. We have also investigated systems which would allow the introduction of the appropriate side chain prior to cyclization. The thallium(I) carboxylate *5* appeared to be a suitable intermediate for this purpose since it would be expected to yield a carbene on decarboxylation by analogy with other thallium [3] and mercury [4] salts of α-halocarboxylic acids.

The crude thallium salt *5* was prepared by the following reaction sequence. The carbodiimide promoted condensation of *t*-butyl hydrogen malonate and piperidine in a manner similar to that described by Brunwin *et al.*[5] gave the ester amide *1*. The yield was considerably improved by the use of dichloromethane as the solvent (65 % *vs.* reported yield of 15 %).[5] Bromination of *1* in chloroform solution, using acetamide as the hydrogen bromide acceptor,[6] gave a good yield of the monobromo compound *2*. This method should be of general value since the bromination of β-dioxocompounds frequently yields a mixture of mono- and dibromo derivatives. The compound *2* was converted to the phthalimido compound *3* by reaction with potassium phthalimide in DMSO or DMF solution. Hydrolysis with trifluoroacetic acid at 0 °C gave the acid *4* which was moderately stable in the solid state but decarboxylated in solution (*cf.* Sheehan *et al.*[7]). The acid *4* was added as a solid to a TFH solution containing the amount of thallium ethoxide required to convert it to the dithallium salt. On subsequent addition of one equivalent of bromine per mol of salt, approximately one equivalent of thallium bromide was precipitated, indicating that bromination had occurred. Filtration and evaporation of the solvent from the filtrate at 0 °C gave the crude thallium salt *5*, which tended to decompose on attempts at purification. The crude salt was dissolved in bromobenzene and heated to 150 °C. Rapid evolution of carbon dioxide and the precipitation of thallium(I) bromide indicated carbene formation. However, only traces of the desired phthalimido-β-lactam *10* could be detected in the reaction mixture by means of preparative TLC combined with mass spectrometry. A tarry residue was obtained on evaporation of the solvent and only small amounts of low molecular weight compounds could be isolated. The major isolable products, which were formed in yields of about 5 % each were phthalimidoacetylpiperidine, diphthalimidoacetylpiperidine, and phthalimide. The forma-



Pht-= Phthalimido-

tion of the latter two products indicates that partial elimination of thallium phthalimide takes place during the reaction. This leads to the formation of the bromo-β-lactam *11* which would react further to the phthalimido-β-lactam *10*.[2] Although the carbene *8* is probably formed, the traces of phthalimido-β-lactam observed may well be derived not from this but from the halo-β-lactam.

A different route to the thallium salt *5* was also investigated. The ester *3* was converted [8] to the bromoester *6*, which was hydrolyzed at −15 °C to the acid *7*. However, attempts to isolate this in a reasonably pure state failed due to its rapid decarboxylation to the compound *12* even at low temperature.

EXPERIMENTAL

All melting points were determined on a micro hot stage apparatus and are uncorrected. Elemental analyses were carried out by Centrala Analyslaboratoriet, Uppsala, Sweden and Mikroanalyslaboratoriet, Uppsala, Sweden. IR-spectra were recorded using a Perkin-Elmer Model 421 spectrophotometer, using KBr discs for solids and neat liquid films for oils. The numbers are given in cm^{-1}. NMR-spectra were recorded using a Varian A60 instrument, on $CDCl_3$ solutions except where otherwise stated. The chemical shifts are given as δ-values relative to TMS as internal standard. Mass spectra were recorded using an LKB 9000 instrument. Column chromatography was performed using silica gel (Merck 0.05 – 0.2 mm). Preparative thin layer chromatography was performed using silica gel (Merck Fertigplatten 2 mm). All THF used was

distilled from potassium metal/benzophenone in an N_2-atmosphere.

N-(t-Butoxycarbonylacetyl)piperidine (1). To a solution of crude *t*-butyl hydrogen malonate [5,9] (12 g, 0.075 mol) in dry dichloromethane (75 ml) cooled to 0 °C was added freshly distilled piperidine (6.4 g, 0.075 mol). The mixture was stirred and cooled for 15 min. A solution of dicyclohexylcarbodiimide (15 g, 0.075 mol) in dry dichloromethane (50 ml) was then added dropwise during 1/2 h, the mixture being held at 0 °C. After an additional period of stirring at room temperature for 3 h the dicyclohexylurea formed was filtered off. Unreacted dicyclohexylcarbodiimide was destroyed by adding 2 M HCl dropwise to the cooled filtrate and the mixture was filtered again. The filtrate which was two-phased was separated into organic and aqueous phases. The aqueous phase was extracted with 2 portions of diethyl ether, the combined organic phases were extracted with an aqueous saturated $NaHCO_3$ solution and water, then dried over $CaCl_2$ and the solvent was evaporated. Distillation yielded *N*-(*t*-butoxycarbonylacetyl)piperidine (*1*) (11.0 g, 65 %) b.p. 124 °C/0.5 mmHg.

N-[Bromo(t-butoxycarbonyl)acetyl]piperidine (2). *N*-(*t*-butoxycarbonylacetyl)piperidine (1.6 g, 7 mmol) and acetamide (0.83 g, 14 mmol) were refluxed in dry chloroform (25 ml) and a solution of bromine (1.1 g, 7 mmol) was added dropwise through the condenser. After 1/2 h the acetamide-hydrobromic acid complex[6] was filtered off, the filtrate was washed with one portion of an aqueous $Na_2S_2O_4$ solution and one portion of water, then dried over $CaCl_2$ and the solvent was evaporated. Chromatography using silica gel (110 g SiO_2/2 g crude product) with light petroleum (b.p. 40–60 °C) containing increasing amounts of diethyl ether as the eluent gave 1.5 g (70 %) of *N*-[bromo(*t*-butoxycarbonyl)acetyl]piperidine (*2*) as a colourless oil. An analytical sample was purified by preparative TLC using distilled diethyl ether as eluent. (Found: C 47.2; H 6.6; N 4.3; Br 26.1. Calc. for $C_{12}H_{20}NO_3Br$: C 47.1; H 6.6; N 4.6; Br 26.1). IR: 1750, 1730 C=O; 1650 C=O. NMR: 1.40 [s, 9 H, OC(CH_3)$_3$]; 1.62 (s, 6 H, CH_2); 3.48 (s, 4 H, N-CH_2); 5.03 (s, 1 H, CHBr). MS: *m/e* 249, 251 (M−C_4H_8), 226 (M−Br) 170 (M−C_4H_8Br), 112, 84.

N-(t-Butoxycarbonylphthalimidoacetyl)piperidine (3). *N*-[Bromo(*t*-butoxycarbonyl)acetyl]-piperidine (0.5 g, 1.63 mmol) and potassium phthalimide (0.3 g, 1.63 mmol) were heated in 30 ml freshly distilled DMSO at 50 °C for 1 1/2 h. The solvent was evaporated *in vacuo* and the reaction mixture was dissolved in chloroform, washed with water, then dried over $CaCl_2$ and the solvent was evaporated. Column chromatography using silica gel with diethyl ether as eluent gave 0.48 g (79 %) of (*3*) m.p. 132–134 °C (dec.). (Found: C 64.3; H 6.5; N 7.4. Calc. for $C_{20}H_{24}N_2O_5$: C 64.5; H 6.5; N 7.5). IR: 1770, 1730 >C=O, 1660 >C=O. NMR 1.52 [s, 9 H, −OC(CH_3)$_3$]; 1.66 (s, 6 H, -CH_2-); 3.4–3.9 (s, 4 H, N-CH_2); 5.65 (s, 1 H, Pht-CH); 7.78 (m, 4 H, aromatic protons). MS: *m/e* 372 (M); 316 (M−C_4H_8), 272 (M−$C_5H_4O_2$).

N-[Bromo(t-butyloxycarbonyl)phthalimidoacetyl]piperidine (6). To a suspension of 0.93 g (0.018 mol) of sodium hydride (50 % dispersion in oil) in 250 ml of dry THF was added in portions 6.8 g (0.018 mol) of *3*. The reaction mixture was refluxed for 1 h, then cooled to 0 °C in an icebath. A cooled solution of 2.9 g (0.018 mol) of bromine in 25 ml of dry dichloromethane was added rapidly dropwise. The solution was washed with one portion of an aqueous $Na_2S_2O_4$ solution, one portion of water, then dried over $CaCl_2$ and the solvent was evaporated. The crude product was crystallized from dichloromethane/diethyl ether to give 5.4 g (66 %) of *4*, m.p. 150–152 °C (dec.). A second crop of crystals was obtained, weighing 0.9 g (11 %). (Found: C 53.2; H 5.25; N 6.15; Br 17.9. Calc. for $C_{20}H_{23}N_2O_5Br$: C 53.2; H 5.14; N 6.2; Br 17.7). IR: 1790, 1760, 1735 >C=O; 1650 >C=O. NMR: 1.5 [s, 9 H, OC(CH_3)$_3$]; 1.66 (s, 6 H, -CH_2-); 3.4–3.9 (s, 4 H, N-CH_2); 7.85 (m, 4 H, aromatic protons). MS: *m/e* 394, 396 (M−C_4H_8), 371 (M−Br), 349, 351 (M−$C_5H_9O_2$), 112, 84.

N-(Carboxyphthalimidoacetyl)piperidine (4). *N*-(*t*-Butoxycarbonylphthalimidoacetyl)piperidine (0.37 g, 0.001 mol) was stirred in 5 ml of trifluoroacetic acid at 0 °C for 1/2 h. Evaporation of the TFA *in vacuo* and crystallization of the residue from cold, dry diethyl ether gave crystals of *N*-(carboxyphthalimidoacetyl)piperidine (0.25 g, 79 %) m.p. 88–90 °C (dec.). (Found: C 60.8; H 5.2; N 8.8. Calc. for $C_{16}H_{16}N_2O_5$: C 60.8; H 5.1; N 8.9). IR: 3200–2800 (broad)-OH, 1770, 1740, 1715 >C=O, 1610 >C=O. NMR: (DMSO) 1.55 (s, 6 H, CH_2); 3.40 (s, 4 H, N-CH_2); 5.76 (s, 1 H: Pht-CH); 7.83 (s, 4 H, aromatic protons). MS, *m/e* 316 (M), 272 (M−CO_2), 112, 84.

Hydrolysis of 6 *to* N-*(bromophthalimidoacetyl-piperidine (12).* Compound *6* (0.45 g, 0.001 mol) was stirred in 10 ml of trifluoroacetic acid at room temperature for 10 min. The solvent was evaporated and the product was dissolved in chloroform, washed with water, then dried over $CaCl_2$ and the solvent was evaporated. Crystallization of the residue from dichloromethane/diethyl ether gave 0.29 g (82 %) of *N*-(bromophthalimidoacetyl)piperidine m.p. 105–107 °C. (Found: C 51.4; H 4.33; N 7.92; Br 23.0. Calc. for $C_{15}H_{15}N_2O_3Br$: C 51.4; H 4.32; N 7.98; Br 22.7). IR: 1790, 1770, 1725 >C=O; 1655 >C=O. NMR: 1.57 (s, 6 H, CH_2); 3.5 (s, 4 H, N-CH_2); 6.76 (s, 1 H, -CHBr-); 7.82 (m, 4 H, aromatic protons). MS: *m/e* 350, 352 (M), 271 (M−Br), 112, 84.

Preparation and decomposition of 5. *N*-(Carboxyphthalimidoacetyl)piperidine (*4*) (0.632 g, 2 mmol) was added to a solution of 1.00 g (4 mmol) of thallium(I) ethoxide in THF (30 ml) at room temperature. The mixture was

cooled to 0 °C and an ice-cold solution of 0.32 g (2 mmol) of bromine in dry dichloromethane was added dropwise. The yellowish precipitate of thallium(I) bromide (0.49 g, 86 %) was removed by filtration and the solvent from the filtrate was evaporated *in vacuo*. Dry bromobenzene (25 ml) was added to the residue and the solution was refluxed for 1/2 h. After that period of time the gas evolution accompanying refluxing was observed to cease. The precipitate of thallium(I) bromide (0.505 g, 89 %) formed was removed by filtration and the solvent from the filtrate was evaporated. The residue contained a large number of products the separation of which was accomplished by preparative TLC. Those products identified were phthalimide (~4 %), *N*-(phthalimidoacetyl)piperidine (~5 %), *N*-(diphthalimidoacetyl)piperidine (~5 %), and traces of 7-phthalimido-8-oxo-1-azabicyclo[4.2.0]octane (*10*).

N-(Diphthalimidoacetyl)piperidine. *N*-(Dibromoacetyl)piperidine (0.7 g, 2.5 mmol) and potassium phthalimide (1.1 g, 6 mmol) were refluxed in freshly distilled dimethylformamide (20 ml) for 1 h, after which period the solvent was evaporated *in vacuo*. The residue was dissolved in chloroform, washed with water, then dried over $CaCl_2$ and the solvent was evaporated. Purification of the residue on a column of silica gel eluted with diethyl ether gave *N*-(diphthalimidoacetyl)piperidine (0.371 g, 37 %).[10] An analytical sample was obtained by recrystallization from chloroform/diethyl ether. M.p. 247 – 249 °C. (Found: C 65.9; H 4.7; N 9.7. Calc. for $C_{23}H_{19}N_3O_5$: C 66.2; H 4.58; N 10.1). IR: 1780, 1765, 1720 >C=O; 1655 >C=O. NMR: 1.55 (s, 6 H, $-CH_2-$); 3.21 (s, 2 H, N-CH_A); 3.57 (s, 2 H, N-CH_B); 6.70 (s, 1 H, -*CH*); 7.65 (m, 8 H, aromatic protons). MS: *m/e* 417 (M), 306 (M – C_6H_9NO), 112, 84.

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Properties of the Glycoprotein Laccase Immobilized by Two Methods

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Laccase (*p*-diphenol:oxygen oxidoreductase; EC 1.10.3.2) from *Neurospora crassa* has been immobilized by two different procedures: (1) Covalent attachment to Sepharose 4B activated with cyanogen bromide, and (2) Adsorption to Concanavalin A-Sepharose *via* the carbohydrate moiety. Except for small changes in the Michaelis-Menten constants, no differences were noted in the enzymological properties of the immobilized enzymes when compared to free enzyme.

The carbohydrate moiety of laccase involved in the interaction with Concanavalin A does not appear to be closely associated with the active center since binding to the lectin has no effect on the enzymological parameters investigated.

The immobilization of enzymes has been accomplished by several procedures, including covalent attachment to various matrices, adsorption to insoluble substances, or entrapment within a matrix. Such insoluble preparations have been useful not only for industrial applications but also in the study of multienzyme systems and as tools in biological research.[1–3] We report here the immobilization of laccase (*p*-diphenol:oxygen oxidoreductase, EC 1.10.3.2) from *Neurospora crassa* by two different procedures. The first, covalent attachment to CNBr-activated Sepharose, has been widely used.[4] The second takes advantage of the ability of *Neurospora* laccase to bind to Concanavalin A.[5] This plant lectin, which is commercially available attached to CNBr-activated Sepharose, adsorbs α-D-mannopyranosyl, α-D-glucopyranosyl and sterically similar residues in a manner reminiscent of the antigen-antibody reaction. *Neurospora* laccase is a glycoprotein of the type which binds Concanavalin A and thus can be immobilized by adsorption to Concanavalin A chemically bound to Sepharose.

MATERIALS AND METHODS

Insoluble matrices. CNBr-activated Sepharose 4B and Concanavalin A-Sepharose (Con A-Sepharose) were both purchased from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Preparation of pure laccase. Laccase was prepared from cycloheximide-induced cultures of *Neurospora crassa* [5] as described by Froehner and Eriksson.[6] The enzyme was homogeneous according to polyacrylamide gel electrophoresis and had a specific activity of 28 U/mg (see below for definition of U). $E(1\ \%,\ 1\ \text{cm}) = 14.0$ at 280 nm was used to calculate protein concentrations.

Assay for laccase activity. Oxygen consumption was measured with an oxygen electrode as previously described.[6] One unit (U) of activity is defined as the amount of enzyme required to consume 1.0 μmol/min of oxygen at 25 °C at pH 6.0 with 10 mM guaiacol as the reducing substrate. For the substrate specificity studies, each compound was used at a concentration of 10 mM in 0.10 M phosphate buffer, pH 6.0.

Preparation of immobilized laccase with CNBr-activated Sepharose. The procedure described in the Pharmacia brochure ("CNBr-activated Sepharose 4B for immobilization of biopolymers") was used with minor modifications. 1.5 mg of enzyme in 100 ml of 0.50 M sodium phosphate buffer, pH 7.2, was incubated with CNBr-activated Sepharose (previously washed

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and swelled in 10^{-3} M HCl) with constant stirring at 22 °C. The reaction was monitored by measuring laccase activity in the supernatant at various times. After 4 h, the gel was collected by filtration and washed once with 20 ml of 0.50 M sodium phosphate buffer, pH 7.2. The preparation was then incubated for 45 min at 22 °C with 10 ml of 1.0 M ethanolamine, pH 8, with constant stirring. After filtration, the gel was washed with 25 ml of 0.50 M sodium phosphate buffer, pH 7.2, followed by 25 ml of 1.0 M sodium acetate, pH 4.5, on a sintered glass funnel. This washing procedure was repeated three times. The gel was suspended in 0.10 M sodium phosphate buffer, pH 6.0, containing 0.01 % merthiolate (total volume = 5.0 ml) and stored at 5 °C. 20 µl was used for each assay.

Preparation of immobilized laccase with Concanavalin A-Sepharose. 0.75 mg of enzyme in 10.0 ml of 0.10 M sodium phosphate buffer, pH 6.0, was stirred with 1.8 ml (packed volume) Concanavalin A-Sepharose for 1 h. The volume was reduced to 5.0 ml by removing part of the supernatant, merthiolate added to 0.01 %, and the preparation stored at 5 °C. 20 µl was used for each assay.

RESULTS

The immobilization procedure, utilizing CNBr-activated Sepharose yields about 60 % of the input laccase activity. As shown in Fig. 1, the immobilization reaction is about 75 % complete after 1 h as measured by the amount of activity left in the solution. This, however, usually gives an overestimate of the amount bound to the matrix. By the Concanavalin A-Sepharose procedure, yields of nearly 100 % were obtained as determined by the amount of activity bound. Furthermore, the reaction is complete in less than 1 h. Both immobilized preparations could be stored for at least several months without detectable loss of activity. No activity could be detected in the supernatant

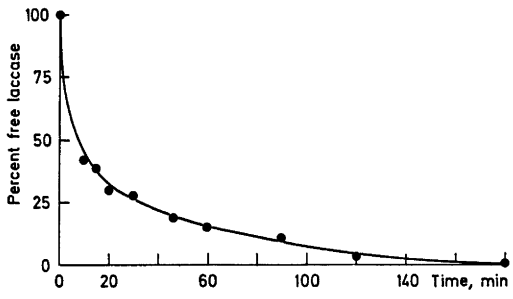


Fig. 1. Binding rate of laccase to CNBr-activated Sepharose.

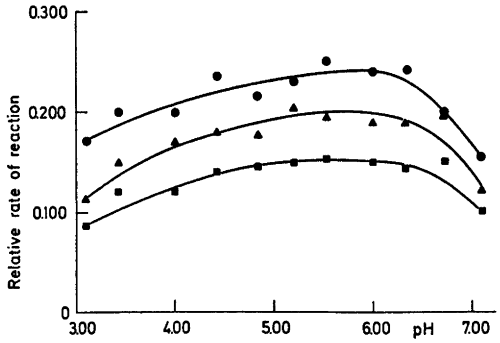


Fig. 2. pH-profiles of rates of reaction with 10 mM guaiacol. Free laccase, ●; laccase-Con A-Sepharose, ■; laccase-CNBr-Sepharose, ▲.

above the gel, indicating that leakage of the enzyme off of the matrix did not occur. Immobilization of an enzyme often changes some of its properties.[2] We have carried out investigations to compare both enzymological and structural properties of free laccase, laccase immobilized with CNBr-activated Sepharose (laccase-CNBr-Sepharose), and laccase immobilized with Concanavalin A-Sepharose (laccase-Con A-Sepharose). First, all three preparations are active over a wide pH range and no major differences in the profiles are apparent (Fig. 2).

A comparison of the Michaelis-Menten constants is shown in Fig. 3. For free laccase, the K_m for guaiacol is 0.36 mM, while this value is larger for the immobilized enzymes. Laccase-CNBr-Sepharose exhibits a K_m of 0.74 mM, while that for laccase-Con A-Sepharose is 0.67 mM. Although significant, the differences in these values are not very large since immobilized enzymes often have a K_m which is 10 – 20 times larger than for the free enzyme. The rate of oxygen consumption with different substrates did not show any significant differences among free laccase, laccase-CNBr-Sepharose, and laccase-Con A-Sepharose (Table 1). As expected from previous results,[6] laccase is most active with *ortho* and *para* diphenols, whether free or immobilized.

A comparison of the rate of oxygen consumption as a function of temperature failed to show any significant differences among free laccase, laccase-CNBr-Sepharose, and laccase-Con A-Sepharose. However, these experiments were complicated by the change in oxygen

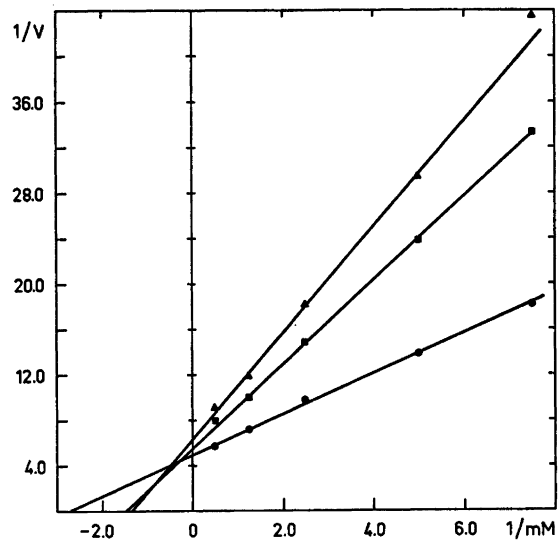


Fig. 3. A comparison of the Michaelis-Menten constants with guaiacol in phosphate buffer, pH 6.0. Free laccase, ●; laccase-Con A-Sepharose, ■; laccase-CNBr-Sepharose, ▲.

concentration in solution as a function of temperature. A plot of the log of activity *versus* the reciprocal of the temperature was not linear.

With the hope of detecting structural changes induced by the immobilization, the stabilities of the enzymes were investigated. Since laccase is released from Con A-Sepharose in the presence of moderate concentrations of urea or at temperatures above about 55 °C, comparisons of heat stability and resistance to urea denaturation could be carried out only on free laccase and laccase-Sepharose. Again, no significant differences were noted. Both have a half-life of about 25 min at 70 °C and are approximately 50 % active in 7 M urea. Similarly, no differences in sensitivity to trypsin were noted among the three preparations.

Table 1. Relative reaction rates with different substrates for free laccase, laccase-Con A-Sepharose, and laccase-CNBr-Sepharose.

Substrates	Free laccase	Laccase bound to Con A-Sepharose	Laccase bound to CNBr-Sepharose
Hydroquinone	1.11	1.03	1.02
Gallic acid	1.35	1.19	1.31
DOPA	0.70	0.48	0.58
Protocatechuic acid	0.70	0.61	0.86
Guaiacol	1.00	1.00	1.00
Pyrogallol	1.80	1.29	0.78
Pyrocatechol	0.76	0.90	1.10
Resorcinol	0.07	0.13	0.10
m-Cresol	0	0.14	0.17
Phloroglucinol	0.48	0.50	0.61
Orcinol	0	0.13	0.14
Tyrosine	0	0.05	0
Potassium ferrocyanide	0	0	0

DISCUSSION

Immobilization of laccase by these two procedures seems to change the enzymatic properties of this enzyme very little. In fact, the only significant difference noted, an increase in the K_m for the two immobilized preparations, is usually attributed to reduced diffusion of the substrate caused by the proximity of the matrix to the enzyme.[2] In view of a recent observation that lactoperoxidase is attached almost exclusively to the surface of Sepharose beads,[11] other factors may be important also. Since the free enzyme is quite stable to heating and to moderate concentrations of urea, it is not surprising that covalent attachment to Sepharose does not increase the stability. Similarly, the free enzyme is active over a wide

pH range, making any changes in pH optimum due to immobilization difficult to detect.

Covalent binding of an enzyme to a matrix can lead to a decrease in enzyme activity due to shielding of the active center,[7,8] or modification of the enzyme structure. Full inactivation of an enzyme is obtained when a covalent bond is formed with the residues in the active center.[9,10] Attachment of laccase to Con A-Sepharose did not reduce the activity nor did it affect the substrate specificity of the enzyme, suggesting that the carbohydrate moiety is not involved in the catalytic center. A similar conclusion was reached in investigations of mammalian dopamine-β-hydroxylase bound to Con A-Sepharose.[12]

Immobilization of glycoenzymes with Concanavalin A-Sepharose is a technique which may be useful for several reasons. First, the procedure is very simple and rapidly performed. Also, the extremely mild conditions required and high yields obtained offer advantages over covalent attachment. Since the carbohydrate moiety is not generally involved in the active site of the enzyme, attachment to Con A-Sepharose should not affect the substrate specificity. The commercial availability of immobilized plant lectins with different specificities should make it possible to attach almost any type of glycoprotein to a matrix by this procedure. Finally, the detection of differences between free and Con A-bound enzymes may clarify the function of the carbohydrate moiety of glycoenzymes.

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Carbon-13 NMR Studies of Labdane Diterpenoids

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Studies of the ^{13}C NMR spectra of ten labdane derivatives using shift reagents, deuterium labelled compounds, and spin-lattice relaxation time measurements have permitted assignment of all signals. Axial, and sterically distorted methyl groups are shown to have longer relaxation times than their equatorial counterparts.

Considerable interest has been focused on carbon-13 nuclear magnetic resonance (^{13}C NMR) spectrometry of complex natural products [1] and its utility for solving structural,[2–12] stereochemical [13–16] and conformational [17] problems in the terpenoid field has been demonstrated.

The labdane group of diterpenoids is of general occurrence in Nature and comprises members regarded as intermediates in the biosynthesis of tri and tetracyclic diterpenoids.[18] In tobacco, which contains a series of labdanes, some of them probably also constitute precursors of many of the nor-terpenoids encountered.[19] A ^{13}C NMR study of the labdanes was therefore deemed of interest and this work * describes the ^{13}C NMR spectra of ten labdane derivatives. The utility of spin-lattice relaxation time (T_1), which has been shown to reveal unique structural information,[20–21] is also briefly explored as a tool for solving assignment problems in this series.

RESULTS AND DISCUSSION

All compounds studied (I–X) possess a labdane or nor-labdane (IX) skeleton and differ with respect to the oxygenation pattern. Their pulsed Fourier transform ^{13}C NMR spectra were obtained at 25.16 MHz in $CDCl_3$ using TMS as internal standard; in most cases both proton noise-decoupled and off-resonance decoupled spectra were recorded. The assignments of the observed resonances, which are given in Table 1 and are discussed in some detail below, were in many cases aided by studies of T_1 and lanthanide induced chemical shifts (LIS), by intercorrelation and by comparison of literature data for structurally related compounds. The chemical shifts of the quaternary carbons for two of the compounds examined here, manool (VI) and sclareol (VII), have been reported previously, but without assignment.[22]

Manoyl oxide (I 8,13-epoxylabd-14-ene).[23] A preliminary assignment of the non-trivial resonances was achieved by comparison with published data [17] and use of additivity rules;[24,25] for the carbons bound to oxygen, C(8) and C(13), the chemical shift values of C(2) in tetrahydropyran (69.7 ppm) was used as the constant term.[26] Some relevant values are included in Table 1 and it follows that there is in most cases an astonishingly good agreement, despite the approximations made, between predicted and observed values in the case of the methylene carbons. However, large deviations are generally encountered for the methyl, methine, and quaternary carbons, some of which therefore are omitted in Table 1.

Although neither the C(5) and C(9) signals, nor the C(8) and C(13) signals could be assigned on a one-to-one basis on the above grounds alone, differentiation could be accomplished with the aid of the shifts encountered on reduction of the C(14)–C(15) double bond (II, IIa) and oxygenation of ring A (III–V) (*vide infra*). Similarly, the C(16) and C(17) resonances could

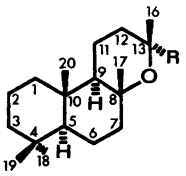
* Part of this work has been presented at Organikerdagarna, Stockholm 1972.

Table 1. Carbon-13 chemical shifts

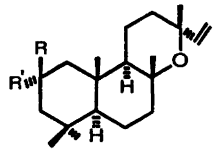
Compound	Chemical shift in ppm from internal TMS									
	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)	C(9)	C(10)
I Manoyl oxide	39.0 37.2	18.6 18.3	42.1 41.6	33.2 28.7	56.4 49.6	19.9 19.5	43.2 42.4	74.8 72.1	55.7 53.2	36.9 33.6
II Dihydromanoyl oxide	39.2	18.7	42.2	33.3	56.5	19.9	43.1	74.5	58.3	36.8
III 2-Oxomanoyl oxide	54.6	211.0	56.4	38.7	55.6	20.1	42.6	74.6	55.1	42.2
IV 2α-Hydroxymanoyl oxide	48.1	64.8	51.1	34.7	54.8	19.5	43.0	74.5	55.8	38.5
V 3-Oxomanoyl oxide	37.8	33.8	217.3	47.3	54.9	20.8	42.5	74.4	54.9	36.5
VI Manool	39.0	19.3	42.2	33.3	55.6	24.3	38.3	148.6	57.2	39.7
	7	2	0	0	5	2	5	9	15	8
VII Sclareol	39.5	18.8 [a]	41.9	33.1	55.9	20.4	43.9 [b]	74.3	61.4	39.0
VIII Labd-13*E*-en-8-ol	39.8	18.5	42.1	33.3	56.2	20.6	43.3	74.1	61.6	39.3
IX 14,15-bisnorlabdan-8-ol-13-one	39.9	18.8	41.9	33.2	56.0	20.4	44.5	73.6	60.6	39.2
X Marrubiin	35.3 [a] 51	18.2 79	28.6 [a] 169	43.9 127	44.9 137	76.6 186	31.6 [a] 53	32.3 81	75.7 94	39.8 108

be assigned as these were the only methyl signals (28.5 and 25.5 ppm) whose positions changed on hydrogenation.

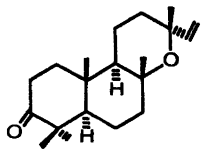
Of the C(18), C(19), and C(20) methyl resonances, that at lowest field was ascribed to the equatorial C(18), while the highest field signal was associated with C(20), which is subjected to three 1,3-diaxial hydrogen interactions.[24] These correlations were confirmed by T_1 measurements (*vide infra*).



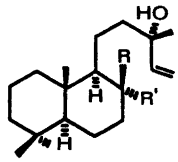
I R= $CH{=}CH_2$
II R= CH_2CH_3



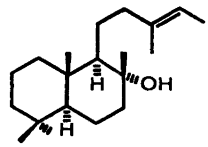
III R,R'= O
IV R'=OH, R'=H



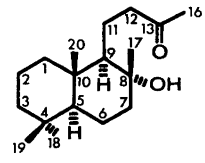
V



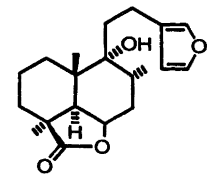
VI R,R'= CH_2
VII R= CH_3, R'= OH



VIII



IX



X

and lanthanide induced shifts.

C(11)	C(12)	C(13)	C(14)	C(15)	C(16)	C(17)	C(18)	C(19)	C(20)	Comments
15.4	35.8	73.0	147.8	110.1	28.5	25.5	33.4	21.3	15.3	
15.7							30.7	21.5	17.4	Predicted
15.4	35.8	72.8	37.9	8.0	27.2	24.8	33.4	21.3	15.7	
15.5	35.5	73.2	147.5	110.4	28.5	25.0	33.4	22.9	16.5	
15.4	35.3	73.0	147.1	109.7	28.6	25.4	33.3	22.1	16.3	
15.6	35.7	73.4	147.9	110.3	28.5	24.8	26.6	20.8	15.0	
17.6	41.4	73.5	145.1	111.4	27.9	106.3	33.6	21.6	14.4	
20.0	42.4				25.9					Predicted [46]
37	51	164	41	44	51	7	0	0	5	$Eu(FOD)_3$ LIS(Hz)
18.4 [a]	44.8 [b]	73.0	146.2	110.3	26.2	24.0	33.3	21.4	15.3	*a,b*: may be interchanged
23.9	44.6	137.0	118.3	13.3	15.9	23.9	33.4	21.5	15.5	
	42.0	135.5	118.4	12.8	15.0					Predicted [25]
18.4	46.2	?	—	—	29.8	24.0	33.4	21.4	15.1	
28.3	21.0	125.5	111.0	143.2	138.8	16.6	23.0	184.3	22.3	*a*: may be interchanged
81	40	22	18	31	31	33	158	666	113	$Eu(FOD)_3$ LIS(Hz)

The assignments made for manoyl oxide (I) are in agreement with results obtained for pimaradienes,[17] and podocarpanes.[27]

Dihydromanoyl oxide (II 8,13-epoxylabdane).[28,29] The only resonances due to the carbons of rings A and B, including substituents, which do not occur at the same frequencies in the spectra of manoyl oxide (I) and dihydromanoyl oxide (II) are those assigned to the carbons also representing part of ring C, *i.e.* C(8), C(9), and C(17). While these alterations were useful in distinguishing between the methyl signals and between the C(5) and C(9) signals, the minor shifts observed for the other ring C carbon signals did not aid the individual assignment of the C(8) and C(13) resonances. However, examination of a labelled derivative, 14,15-d_{2-4}-dihydromanoyl oxide (IIa),[29] prepared by catalytic reduction of manoyl oxide using deuterium gas, resolved this ambiguity, since the intensity of the C(13) signal decreased drastically, while that of the C(8) signal remained the same;[27] the signals due to C(14) and C(15) were hardly visible.

2-Oxomanoyl oxide (III 8,13-epoxylabd-14-en-2-one).[30,31] The spectrum of this compound confirmed most of the assignments made for the ring A carbons of manoyl oxide (I). Thus the resonances for C(1) and C(3) are displaced 15.6 and 14.3 ppm downfield in agreement with what has been found on introducing a keto group into cyclohexanes [32] and steroids.[33,34] A deshielding of C(4) and C(10) by 5.5 and 5.3 ppm was also observed and since this is a noticeably larger effect than that encountered for the cyclohexanes and steroids, it might partially be associated with a conformational change on introduction of the keto group. The resonances due to the proximate methyls, C(18), C(19), and C(20) all move less than 2 ppm downfield.

2α-Hydroxymanoyl oxide (IV; 8,13-epoxylabd-14-en-2α-ol).[31] As for 2-oxomanoyl oxide (III), the changes in chemical shifts caused by introduction of an oxygen function confirmed the initial assignments made for manoyl oxide (1). Thus, the 46.2, 9.0, and 9.1 ppm shifts towards lower field of the C(2), C(3), and C(1) signals and the 1.6 ppm upfield shift of the C(5) signal agree well with values re-

ported for cyclohexanols.[36] In contrast, the C(10) and C(4) resonances move 1.6 and 1.5 ppm to lower field, a result which differs from that reported for cyclohexanols but is in accord with results obtained for steroids.[34]

3-Oxomanoyl oxide (V; 8,13-epoxylabd-14-en-3-one).[37] The chemical shifts of the B- and C-ring carbons remained, as expected, approximately the same as in manoyl oxide (I). The downfield shifts of 15.2 and 14.1 ppm observed for the C(2) and C(4) signals, and the upfield shifts of 1.2 and 1.5 ppm for the C(1) and C(5) signals conform with steroid results.[34] The carbonyl group exerts a marked effect on the vicinal geminal dimethyl groups, C(18), C(19), as already noted for corresponding triterpenes (upfield shifts of 6.8 and 0.5 ppm).[16,38]

Manool (VI; labd-8(17),14-dien-13-ol).[39] Although manoyl oxides functionalized in rings B and C are known,[40–42] none of these were available to us in sufficient quantities. Some bicyclic labdane diterpenoids (VI–X) capable of providing similar information were examined instead. The resonances due to the ring A carbons were readily assigned on the basis of the results obtained for manoyl oxide (I).

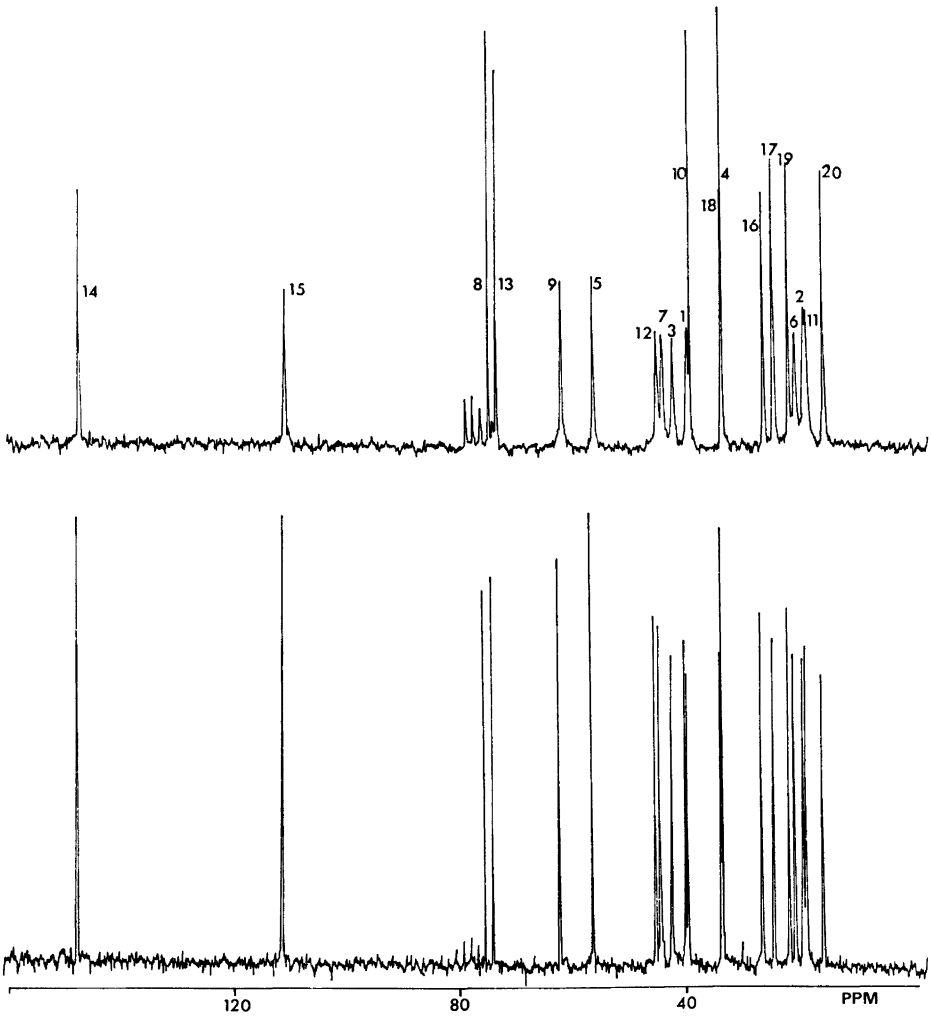


Fig. 1. ^{13}C NMR spectra of sclareol (VII) recorded at 25.18 MHz as 3.2 M (upper) and 1.3 M (lower) solutions in $CDCl_3$.

Addition of $Eu(FOD)_3$ [43,44] allowed the two exo-methylene carbon signals, C(15) and C(17) to be distinquished as the former, due to the carbon closest to the coordination site, suffered the largest shift.

Although the europium shift reagents are known to cause considerable contact shifts in addition to the pseudocontact interaction,[45] they were found useful for the present spectral assignment. The observed LIS, listed in Table 1, along with corresponding estimated chemical shifts,[24,25] allowed unambigous assignment of all signals. The shifts of the side-chain aliphatic carbons were estimated from values for the appropriate alkane (2,5-dimethyl-heptane) [46] allowing for the influence of the double bond [47] and the hydroxyl group [36] and in the case of C(11) accounting for two γ-interactions from C(17) and C(20). The main reason for the 4.4 ppm deshielding of C(6) in manool relative the corresponding carbon in manoyl oxide, is considered to be due to the removal of one of the three methyl 1,3-diaxial interactions with the β-hydrogen on this carbon.

Sclareol (VII; labd-14-ene-8,13-diol).[39] The proton noise-decoupled spectrum of a concentrated solution of sclareol, Fig. 1, showed large differences in the peak heights, the quaternary carbon signals being highest, followed in turn by the methyl, methine, and methylene carbon resonances. Dilution of the sample produced a spectrum with peaks of roughly the same heights. This broadening of the peaks in the spectra of the concentrated solution is ascribed to a more effective spin-spin relaxation because of increased viscosity,[20] the effect being most noticeable for carbons with short T_1's, *i.e.* the methylenes. This can effectively be used for an estimate of T_1's, yielding information otherwise also available from off-resonance decoupling experiments.

Assignment of the resonances was based in the case of the A and B ring carbons and methyls on a comparison with the values for manoyl oxide (I) and in the case of the side chain carbons with those for manool (VI). Unequivocal distinction between C(2) and C(11), between C(7) and C(12), and between C(16) and C(17) could not be made at this stage due to the small shift differences, but confirmatory evidence for the assignments made in Table 1 was obtained from the spectrum of labd-13*E*-en-8-ol (VIII) and from T_1 measurements (*vide infra*).

Labd-13E-en-8-ol (VIII).[48] The ring A and B carbons were again readily identified utilizing the results for sclareol (VII) and manoyl oxide (I) and comparison with values for 3-methyl-2*E*-hexene [25] (relevant values are included in Table 1) allowed unambiguous assignment of the side-chain resonances. The chemical shifts for C(2), C(7), and C(17) were virtually the same as those for the corresponding carbons in sclareol and hence strongly support the assignments made for the latter compound. The only remaining signal not accounted for is due to C(11) and appears at an expected position, *i.e.* 5.5 ppm downfield of the C(11) resonance in the sclareol spectrum.

Bis-14,15-norlabdan-8-ol-13-one (IX).[49] Comparison with the data for sclareol (VII) and labd-13*E*-en-8-ol (VIII) leaves only the C(11), C(12) and C(16) resonances to be accounted for. Of these, the signal at 29.8 ppm is clearly due to C(16), a value consistent with previously published results for long-chain methyl ketones,[50] and hence that at 46.2 ppm due to C(12), since C(12) is expected to resonate at lower field than C(11).*

Marrubiin (X; 15,16-epoxy-9-hydroxy-8α-labda-13(16),14-dien-19,6β-olide).[53] Assignment of the signals due to the carbonyl,[54] furyl,[55] and hydroxylated carbons left 13 resonances to be considered more closely and required the use of $Eu(FOD)_3$ and selective decoupling.[56]

Coordination of the shift reagent is expected to take place mainly at the lactone carbonyl and the tertiary hydroxyl group, and to a much lower extent at the furan oxygen. This was largely confirmed by the strong shifts observed for the unambiguously identified resonances of the carbons in the vicinity of the lactone ring. Thus C(19), C(6), C(5), and C(4) are shifted 26.5, 7.1, 5.5, and 5.1 ppm, respectively, while the hydroxylated C(9) and the vicinal C(8) and C(10) are shifted 3.7, 3.2, and 4.3 ppm respectively. Selective proton irradiation at

* The C(12) and C(16) resonances are weaker than those of the other carbons, either due to longer relaxation times, or because of the presence of small amounts of the ring-closed vinyl ether (IXa), easily formed in acidic media.[51,52] The spectrum of the latter compound is expected to differ from that of the parent ketone (IX) only with respect to the C(12), C(13), and C(16) signals.

2.34 ppm, the expected position for the 5β-H resonance in the proton spectrum, sharpened the two peaks at 44.9 [C(5)] and 32.3 [C(8)] ppm.

The methylene resonances were assigned as follows. The signal at 21.0 ppm, undergoing the smallest LIS, is assigned to C(12) and is sharpened on irradiation of the proton triplet at 2.57 ppm. As introduction of the axial hydroxyl group at C(9) is expected to shift the C(1) signal about 5 ppm towards higher field relative to that for sclareol (VII), the 35.3 ppm resonance can be ascribed to C(1). Of the resonances at 28.6 and 31.6 ppm, the former, being the more strongly shifted on $Eu(FOD)_3$ addition, is assigned to C(3) and the latter to C(7). Only one methylene signal (28.3 ppm) then remains to accomodate C(11).

Of the three methyl signals the least shifted (16.6 ppm) is ascribed to C(17), while the most shifted one, also appearing at the lowest field, is assigned to C(18), thereby leaving only C(20) to account for the 22.3 ppm signal. Selective decoupling of the C(18) methyl proton singlet at 1.34 ppm sharpened the corresponding carbon signal at 23.0 ppm, while irradiation at the C(20) methyl proton singlet at 1.11 ppm and the C(17) methyl proton doublet at 0.99 ppm removed the splittings of the corresponding carbon peaks at 22.3 and 16.6 ppm.

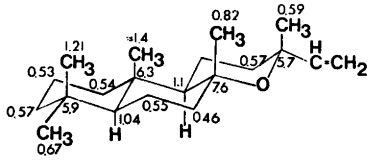
The above assignments are consistent with results obtained for other similar lactones.[12]

SPIN-LATTICE RELAXATION DATA

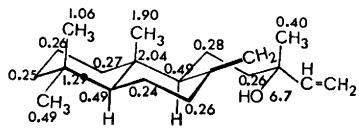
Spin-lattice relaxation times were determined according to the inversion-recovery method.[57] The relaxation times of individual carbons were measured for manool oxide (I), dihydromanool oxide (II), manool (VI), and sclareol (VII) and the data are given in Scheme 1.

Extremely rapid relaxation was found for the carbons of the rigid backbone of the bicyclic molecules (VI, VII). This is due to molecular association which tends to slow down over-all molecular motion. Longer T_1's, however, are observed in the case of the tricyclic compounds (I, II), which is a consequence of more rapid tumbling due to the absence of polar groups. The almost equal T_1 values obtained for the backbone methylene carbons within each compound suggest essentially isotropic over-all motion. Under these circumstances the dipolar relaxation rate is proportional to the number of directly bonded protons [58] in agreement with the experimental results.

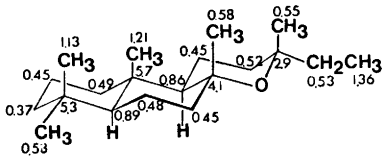
It is known that internal motion is equivalent to a shortening of the effective correlation time τ_{eff} thus resulting in lengthened T_1's.[59] However, no significant increase in the T_1's of the sidechain carbons 11 and 12 was observed for the bicyclic compounds (VI, VII) suggesting low conformational mobility for these carbons, at the concentration used.



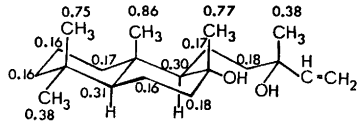
Manoyl oxide (I)



Manool (VI)



Dihydromanoyl oxide (II)



Sclareol (VII)

Scheme 1. Spin lattice relaxation times (T_1) for the various carbons of manoyl oxide (I), dihydromanoyl oxide (II), manool (VI), and sclareol (VII) measured as *ca.* 2.0 and 1.3 M solutions in $CDCl_3$ at 50 °C.

Particularly well investigated is the internal rotation of a group having only one degree for internal motion.[60,61] For a methyl group undergoing internal rotation at a rate which is fast compared to over-all motion, one expects a relaxation time, $T_1(CH_3)$, which is nine times longer than in the absence of internal motion.[59] This behaviour was for example shown to apply for the angular methyl carbon in cholesteryl chloride. In manool (VI), the T_1's of the methyl carbons were found to range from 0.40 to 1.90 s, clearly showing that some methyl groups do not spin freely. It has been demonstrated by several workers [61–64] that steric crowding lowers the barrier to internal rotation. The concomitant increased rotation rates lead to lengthened spin-lattice relaxation times. In the case of manool, the methyl carbon 20, experiencing 1,3 diaxial interactions with the 2β and 6β protons and the 19 methyl group, exhibits the longest T_1 (1.90 s) of the methyl carbons, while C(19) displays a T_1 of 1.06 s. The equatorial C(18) as well as the sidechain C(16) give rise to significantly lower values (0.49 and 0.40 s). Analogous findings were made for the other three compounds (I, II, and VII). Although in sclareol (VII) much shorter T_1's were generally observed, again a significantly increased relaxation time was found for C(20) when compared with that of the remaining methyl relaxation times in this molecule. It should be pointed out in this context, that absolute values of the methyl relaxation times are of no relevance when a comparison of data is made for different molecules. This is only valid on condition that the T_1's are normalized, which can be done by dividing T_1 (CH_3) by T_1 (CH), where the latter implies the relaxation time of a methine carbon which does not undergo internal reorientation.[63] These results suggest that examination of methyl carbon T_1's provides a useful assignment technique in the diterpenoid field.

EXPERIMENTAL

The Fourier transform ^{13}C NMR spectra were obtained on a Varian XL-100-12 WG spectrometer operating at 25.16 MHz and equipped with an S-124 XL FT accessory. The instrument was controlled *via* a Varian 620 L 16 K computer.

The samples were usually examined as 1 – 2 M solutions in $CDCl_3$ using TMS as internal standard. The influence of concentration on chemical shifts, as judged from data obtained for sclareol (VII) at two concentrations (3.2 and 1.3 M), was negligible, except for C(7), C(8) and C(12) – C(16), which ranged from 0.15 to 0.35 ppm.

An RF flip-angle of about 45° was usually used to achieve optimum sensitivity enhancements in survey spectra. 8 K datapoints were used in all experiments and the precision of the peak positions in the proton noise-decoupled (PND) spectra was about ±0.05 ppm. Single frequency off-resonance (SFOR) spectra were obtained by irradiating 400 Hz upfield from TMS in the proton spectrum. Partially relaxed Fourier transform (PRFT) spectra were obtained by a modified inversion-recovery method [57,65] in which a pulse sequence of $(90° - T - 180° - t - 90° - T)$ was employed.

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Note added in proof: We are grateful to Professor E. Wenkert for informing us prior to publication about his ^{13}C NMR results on labdanes, which are consistent with ours except for some assignments of a few closely spaced peaks.

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Intestinal Glycoproteins of Germfree Rats. IV. Oligosaccharides Obtained by Chemical Degradation of a Water-soluble Glycoprotein Fraction*

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Removal of sialic acid from the glycoprotein and subsequent degradation with sodium hydroxide—sodium borohydride resulted in the formation of a number of low-molecular weight carbohydrate compounds of which three were isolated and characterized: 2-deoxy-2-acetamido-D-galactitol; *O*-α-L-fucopyranosyl (1→2)-*O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol (oligosaccharide I); *O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol (oligosaccharide II). From an acetolysate of the desialized glycoprotein a fourth component was obtained and characterized to be *O*-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-D-glucose (oligosaccharide III).

The mucin layer that covers the epithelial surface of the intestinal mucosa in mammals is known to contain glycoproteins as major constituents.[1-3] Detailed chemical studies of these biopolymers have been impeded by the fact that they are rapidly degraded by the intestinal microflora; however, by the use of germfree animals the intestinal mucin glycoproteins may be obtained essentially in the undegraded state. A water-soluble glycoprotein fraction from the intestinal mucus of germfree rats has been characterized recently [4] and the present communication reports studies on the carbohydrate side chains of this glycoprotein.

RESULTS AND DISCUSSION

It has been well established for glycoproteins of the mucin type that the carbohydrate-peptide linkage involves an α-*O*-glycosidic bond between *N*-acetylgalactosamine and the hydroxyl group of serine or threonine in the polypeptide chain. This linkage is labile to alkali, resulting in β-elimination of the carbohydrate moiety. By performing the alkali treatment in the presence of excess borohydride the alkali catalysed peeling reaction with concomitant degradation of the carbohydrate chains is greatly diminished, as first demonstrated by Carlson [5] who succeeded in isolating from pig submaxillary mucin a number of oligosaccharides, all terminating in *N*-acetylgalactosaminitol. The germfree rat intestinal mucin was subjected to alkali treatment under similar conditions, followed by separation of acidic and neutral oligosaccharides on an AG 1 formate ion exchange column. Further fractionation by gel chromatography and preparative paper chromatography (PC) revealed a large number of compounds present in quantities too small for extensive examination. More than half of the carbohydrate content of the mucin was excluded from a Bio-Gel P-2 column indicating the presence of long-chain oligosaccharides or incomplete release of carbohydrate from the polypeptide chain. The failure to detect *N*-acetylgalactosaminitol in this high-molecular weight fraction would favour the latter possibility. Rechromatography of the front peak from the P-2 column on Bio-Gel P-6 gave no further resolution, the peak being excluded also from this gel; evidently a molecular weight of more than 5000 must be assumed for this fraction. Removal of the sialic acid residues from the glycoprotein and sub-

* Part III, see Ref. 4.

sequent treatment with alkali-borohydride furnished an oligosaccharide mixture of a less complex composition, thus facilitating the isolation of individual sugars. After deionization the degraded material was fractionated on a Bio-Gel P-2 column (Fig. 1). Final separation was achieved by preparative PC. However, only two oligosaccharides (I and II) in addition to *N*-acetylgalactosaminitol were isolated in sufficient yield and purity for structural investigation.

The final peak from the P-2 column (fractions 35 – 39) contained *N*-acetylgalactosaminitol and small amounts of fucose and *N*-acetylglucosamine. The *N*-acetylgalactosaminitol was isolated by chromatography on thick paper; it had the same mobility on PC and the derived trimethylsilyl ether had the same retention time on gas liquid chromatography (GLC) as an authentic sample, while it could be easily separated from *N*-acetylglucosaminitol by PC and GLC. Periodate oxidation of the *N*-acetylgalactosaminitol followed by treatment with sodium borohydride gave 2-*N*-acetylamino-1,3-propanediol, identified by cochromatography (PC and GLC) with a reference sample.

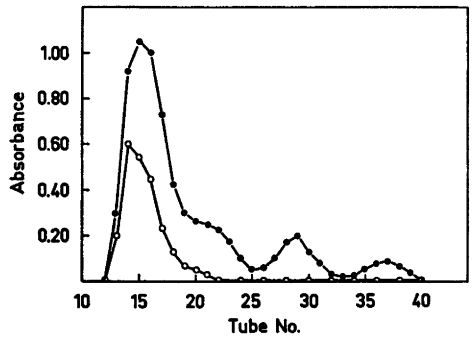


Fig. 1. Chromatography on Bio-Gel P-2 of the products obtained by degradation of desialized glycoprotein with alkali-borohydride. Effluent fractions were analysed for neutral sugar (●) and protein (○). Tubes 26 to 32 and 35 to 39 were pooled, respectively, concentrated and subjected to preparative PC. Experimental details are given in the text.

Oligosaccharide I was a trisaccharide consisting of fucose, galactose, and *N*-acetylgalactosaminitol. Weak acid hydrolysis gave fucose and a non-reducing compound with the chromatographic mobility of oligosaccharide II. By subsequent incubation of this compound with β-galactosidase, galactose and *N*-acetylgalactosaminitol were liberated.

Oligosaccharide I was methylated with methyl iodide-methylsulfinyl carbanion in dimethyl sulfoxide [6] according to the modification of Sandford and Conrad. [7] After hydrolysis and conversion into their alditol acetates the methylated sugars were analysed by GLC [8] – mass spectrometry. [9] The three peaks obtained on the gas chromatogram indicated equimolar proportions of the three component sugars. The first and second peak had the retention time of 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylgalactitol, and the respective mass spectra showed the primary fragments characteristic of the per-

Table 1. Methyl ethers from hydrolysates of methylated oligosaccharides I and III, and the major ions (*m/e*) observed in the mass spectra of their alditol acetates.

Oligosaccharide	Methyl ether	r^a	Major ions (m/e)
I	2,3,4-Tri-*O*-methylfucose	0.60	117,[b] 131,[b] 161,[b] 175[b]
	3,4,6-Tri-*O*-methylgalactose	1.96	45,[b] 101, 129, 145, 161,[b] 189,[b] 205, 234
	1,4,5,6-Tetra-*O*-methyl-2-deoxy-2-*N*-methylacetamidogalactitol	3.25	45,[b] 56, 88, 101, 130,[b], 133,[b] 142, 246,[b] 290[b]
III	2,3,4,6-Tetra-*O*-methyl-galactose	1.15	45,[b] 101, 117,[b] 129, 145, 161,[b] 205[b]
	3,6-Di-*O*-methyl-2-deoxy-2-*N*--methylacetamidoglucitol	2.87	45,[b] 74, 116, 128, 142, 158[b] 202,[b] 233[b]

[a] Retention times of the corresponding alditol acetates relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. [b] Primary fragments.

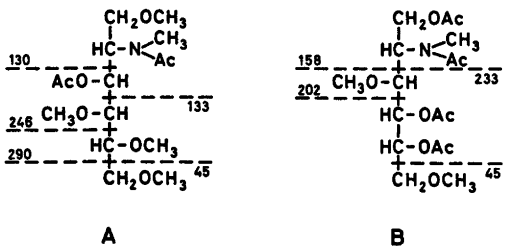


Fig. 2. Primary fragments formed by electron impact on the alditol acetates of the partially methylated amino sugar components of oligosaccharide I (A) and III (B).

acetates of a 2,3,4-tri-*O*-methyl-6-deoxyhexitol and a 3,4,6-tri-*O*-methylhexitol [10] (Table 1). The mass spectrum of the compound in the third peak on the gas chromatogram showed predominant ions at *m/e* 45, 130, 133, 246, and 290 (Table 1). The main diagnostic ion for 2-deoxy-2-*N*-methylacetamidoalditol acetate derivatives is that at *m/e* 158, which is formed by fission between C_2 and C_3 resulting in the ion $[CH_3CO-O-CH_2-CH-N(CH_3)COCH_3]^+$. This ion is absent in the spectrum of the amino sugar derivative from oligosaccharide I. However, methylation of a 2-deoxy-2-acetamidoalditol would introduce a methyl ether group at C_1; thus, in the mass spectrum of such a compound a peak at *m/e* 130 should be expected (Fig. 2 A). The formation of the other primary ions present in the mass spectrum of the amino sugar component is evident from Fig. 2 (A). From the experimental data the following structure is proposed for oligosaccharide I: *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol. The α anomeric configuration is assigned to the fucose since this sugar was released from the rat intestinal mucin [4] on incubation with a specific α-(1→2)-L-fucosidase.[11]

The small quantity isolated of oligosaccharide II did not permit methylation analysis. It was composed of galactose and *N*-acetylgalactosaminitol and was indistinguishable from the compound obtained on the removal of fucose from oligosaccharide I. Incubation of oligosaccharide II with a β-galactosidase furnished galactose and *N*-acetylgalactosaminitol. Periodate oxidation of the disaccharide followed by treatment with sodium borohydride and mild acid hydrolysis led to total destruction of the galactose. PC of the resulting product revealed, in addition to glycerol, the presence of a compound which could be detected with periodate-benzidine and also by a hypochlorite-potassium iodide-starch reagent. When oligosaccharide I was subjected to this Smith degradation procedure, again the same compound was detected; furthermore it was also obtained, together with 2-*N*-acetylamino-1,3-propanediol, by incomplete periodate oxidation of *N*-acetylgalactosaminitol and subsequent borohydride reduction. The formation of this compound, possibly *N*-acetylthreosaminitol, from oligosaccharide I and II strongly indicates that galactose is linked to C_3 of the *N*-acetylgalactosaminitol in both oligosaccharides. Accordingly the structure of oligosaccharide II is proposed to be *O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol.

Baig and Aminoff [12] have also reported the isolation of oligosaccharides I and II after alkali-borohydride treatment of a hog submaxillary glycoprotein.

Besides the alkali-borohydride degradation procedure acetolysis was also attempted to obtain oligosaccharides from the germfree rat intestinal glycoprotein. The desialized glycoprotein was acetylated to give a product which was soluble in the acetolysis medium. After acetolysis the products were deacetylated and fractionated on a Bio-Gel P-2 column (Fig. 3.)[13] The front peak contained high-molecular weight

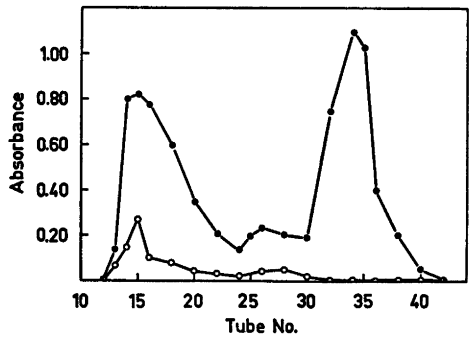


Fig. 3. Chromatography on Bio-Gel P-2 of the products obtained after acetolysis of desialized glycoprotein. The effluent was analysed for neutral sugar (●) and protein (○). Tubes 24 to 30 were pooled, concentrated and subjected to preparative PC. Experimental details are given in the text.

material, the middle one oligosaccharides and glycopeptides, and the last peak mostly monosaccharides. The middle fraction (tubes 24 – 30) was further fractionated by chromatography on thick paper. A large number of compounds was present but only one (oligosaccharide III) was isolated for further examination. It was a disaccharide consisting of galactose and *N*-acetylglucosamine; incubation with β-galactosidase gave the two component sugars. Oligosaccharide III was methylated, hydrolysed, the sugars converted into their alditol acetates and analysed by GLC-mass spectrometry. The first peak on the gas chromatogram had the same retention time as a reference sample of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, and the mass spectrum showed peaks corresponding to the typical fragmentation pattern of a peracetylated 2,3,4,6-tetra-*O*-methylhexitol [10] (Table 1). The mass fragments of the amino sugar derivative (Table 1) are consistent with those obtained by Stellner *et al.*[14] and by Schwarzmann and Jeanloz [15] for 1,4,5-tri-*O*-acetyl-3,6-di-*O*-methyl-2-deoxy-2-*N*-methylacetamidoglucitol; the primary fragments are depicted in Fig. 2 (B). The data presented agree with the following structure for oligosaccharide III: *O*-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-D-glucose.

It is evident from the present work that neither of the two methods employed for degradation appeared ideal for oligosaccharide formation. Both the acidic and the alkaline mode of degrading the glycoprotein resulted in a large number of oligomers of which none could be demonstrated to occur in a comparatively high proportion. This may reflect the presence of microheterogeneity in the polymer as well as a generally intricate molecular structure, both well known features of most glycoproteins. However, the results obtained so far suggest that the molecular constitution of the carbohydrate portion of the germfree rat intestinal mucin does not differ basically from that of other mucin glycoproteins.

EXPERIMENTAL

N-Acetylgalactosaminitol and *N*-acetylglucosaminitol were prepared as described by Crimmin;[16] the two recrystallized products had m.p. 175 – 176 °C and 148 – 150 °C, respectively, and both were chromatographically homogeneous on PC and GLC.

2-*N*-Acetylamino-1,3-propanediol was prepared from DL-serinol (2-amino-1,3-propanediol), Sigma, by acetylation with acetic anhydride in pyridine at 20 °C for 15 min. The product after recrystallization from ether, had m.p. 84 – 85 °C. The ion exchange resins AG 1, AG 3, and AG 50 and the Bio-Gel P-2 for gel chromatography are products of Bio-Rad Laboratories, Richmond, California, U.S.A. The mixed bed ion exchange resin Zerolit DM-F was purchased from BDH Chemicals, Poole, England.

Analytical methods. Gas-liquid chromatography (GLC) was performed with a Varian 1400 gas chromatograph on a stainless steel column (200 × 0.3 cm) packed with 3 % SE-52 on Varaport 30 for the separation of pertrimethylsilyl ethers of free sugars, alditols, and methyl glycosides. Partially methylated alditol acetates were separated on a glass column (200 × 0.3 cm) containing 3.5 % OV-225 on Varaport 30 at 185 °C.

For GLC-mass spectrometry of partially methylated alditol acetates the OV-225 column was mounted in a Varian 1400 gas chromatograph combined with a Varian CH 7 low-resolution mass spectrometer. The instrumental details are published elsewhere.[17]

Paper chromatography (PC) was carried out on Whatman No. 1 and, for preparative purposes, on Whatman No. 3 MM filter paper in solvent systems (v/v): A. Ethyl acetate, pyridine, acetic acid, water, 5:5:1:3. B. Butanol, pyridine, water, 6:4:3. C. Ethyl acetate, pyridine, water, 8:2:1. Sugars were located on chromatograms by a periodate-benzidine reagent (*a*)[18] or by a hypochlorite-potassium iodide-starch reagent (*b*)[19] which reacts with CH_3CONH-groups.

Methanolysis was performed by heating the oligosaccharide (0.25 mg) with 1 M hydrogen chloride in methanol (0.5 ml) at 85 °C for 20 h, and then preparing the product for GLC as described by Reinhold.[20] Incubation with β-D-galactosidase (Grade IV, Sigma, purified from *E. coli*) was performed by adding the enzyme (5 units) to the sample (1 mg) dissolved in 0.10 M phosphate buffer pH 7.0 (0.5 ml), and keeping the digest at 35 °C for 16 h under toluene. The mixture was then diluted with water (2 ml), deionized by treatment with Zerolit DM-F resin, and the product concentrated to dryness and examined by PC and by GLC.

The elution of ion exchange or gel chromatography columns was monitored by the phenol-sulfuric acid test [21] and by the method of Lowry *et al.*[22] for neutral sugars and protein, respectively.

Removal of sialic acid from the intestinal glycoprotein. The purified glycoprotein (1.1 g) with a sialic acid content of 13.5 % was dissolved in 0.05 M hydrochloric acid (200 ml) and the solution kept at 80 °C for 80 min. After cooling

and neutralization with sodium hydroxide the mixture was concentrated to 50 ml and dialysed against distilled water (4 × 1 l) during 36 h at room temperature. Deionization of the dialysate followed by concentration and PC revealed trace amounts of fucose as the only neutral sugar. The non-dialysable fraction was freeze-dried to give a product (0.92 g) with a sialic acid content of 1.3 %, as estimated by the method of Jourdian *et al.*[23]

Isolation of oligosaccharides after alkali-borohydride treatment of the desialized glycoprotein. The desialized material (0.9 g) was dissolved in 2 M sodium borohydride (100 ml); then 0.1 M sodium hydroxide (100 ml) was added to give a final concentration of 0.05 M sodium hydroxide-1 M sodium borohydride and the solution was kept at 45 °C for 15 h. After cooling and neutralization with acetic acid to pH 6 the turbid solution was clarified by filtration and passed through a column (3 × 50 cm) of AG 50 × 2 (H^+) resin, 50 – 100 mesh. The column was eluted with water until the eluate gave a negative reaction for carbohydrate. After concentration and removal of boric acid by repeated evaporations with methanol the eluate was still acidic, and was neutralized by treatment with AG 3 × 4 A (OH^-) resin, 100 – 200 mesh. The resulting neutral solution was concentrated to a syrup (0.61 g), which was subjected to fractionation on a column (2.5 × 80 cm) of Bio-Gel P-2, 200 – 400 mesh. The syrup was dissolved in 10 % ethanol in water (4 ml) and a third of the portion was taken out each time for chromatography on the P-2 column, which was eluted with 10 % aqueous ethanol. Fractions of 5 ml were collected and aliquots analysed for carbohydrate and protein. The three runs gave almost identical elution curves (Fig. 1) and appropriate fractions were pooled; yield, tubes 13 to 25: 393 mg; tubes 26 to 32: 42 mg; tubes 35 to 39: 38 mg.

The low-molecular weight fraction (38 mg) on GLC and PC proved to contain a compound with the chromatographic mobility of *N*-acetylgalactosaminitol together with minor proportions of fucose and *N*-acetylglucosamine. By preparative PC in solvent system C and B, successively, the substance was isolated in a pure state, yield 10 mg. It cochromatographed with authentic *N*-acetylgalactosaminitol as one compound on GLC and PC, but was clearly separated from *N*-acetylglucosaminitol. The compound (2 mg, *ca.* 10 μmol) was dissolved in an unbuffered aqueous solution (2.5 ml) of 0.02 M sodium metaperiodate (50 μmol) and left in the dark at 20 °C for 2 h. After addition of ethylene glycol (1 drop) and sodium borohydride (5 mg) the solution was left overnight at 5 °C. Then excess acetic acid was added and boric acid was removed by codistillation with methanol; the resulting product was deionized with mixed bed resin prior to analysis by GLC and PC.

The trimethylsilyl derivative of the compound when cochromatographed with the di-trimethylsilyl ether of 2-*N*-acetylamino-1,3-propanediol gave one peak on the gas chromatogram. To separate this peak from that of the solvent a temperature programme was used, starting at 70 °C with an increase of 8 °C per min. Under these conditions the di-trimethylsilyl ether of ethylene glycol, also present in the sample, was eluted in the solvent peak. On PC in solvent systems A and B the isolated compound had the same mobility as 2-*N*-acetylamino-1,3-propanediol.

The middle fraction from the P-2 column (42 mg) was further fractionated by preparative PC in solvent system B. Several compounds were revealed by the periodate-benzidine reagent, of which two, oligosaccharides I (11 mg) and II (4 mg) were obtained as chromatographically homogeneous products.

Oligosaccharide I had R_{Lactose} 1.25 and 1.34 in solvent system A and B, respectively. Methanolysis and subsequent GLC showed the presence of fucose, galactose, and *N*-acetylgalactosaminitol. When oligosaccharide I (2 mg) was hydrolysed with 0.1 M hydrochloric acid (0.5 ml) at 100 °C for 1 h fucose was detected in addition to a non-reducing compound with the chromatographic mobility of oligosaccharide II in solvent systems A and B. The deionized hydrolysate was evaporated to dryness, dissolved in 0.1 M phosphate buffer pH 7.0 and incubated with β-galactosidase. Examination of the product by PC and GLC showed the presence of fucose, galactose, and *N*-acetylgalactosaminitol. After methanolysis and trimethylsilylation *N*-acetylgalactosaminitol gave one, and sometimes two, minor peaks on the gas chromatogram besides the main peak obtained as the single one by direct trimethylsilylation of the alditol. The origin of these smaller peaks is unknown.

Methylation.[6] Oligosaccharide I (6 mg) in a 10 ml serum bottle sealed with a rubber cap was dissolved in dry dimethyl sulfoxide (1 ml). Methylsulfinyl sodium in dimethyl sulfoxide (1 ml, 2 M)[7] was added and the reaction mixture stirred for 1 h at room temperature. Methyl iodide (0.5 ml) was added slowly through a syringe under external cooling and the stirring was continued for 1 h. Water (5 ml) was added and excess methyl iodide was evaporated by bubbling nitrogen gas through the solution. After a further addition of water (50 ml) the methylated sugar was extracted into chloroform by shaking with 2 × 15 ml in a separatory funnel. The combined chloroform extracts (30 ml) were then shaken with water (6 × 100 ml) to remove dimethyl sulfoxide, and the organic phase was dried with anhydrous sodium sulfate and concentrated to dryness. The methylated product was hydrolysed with 90 % formic acid (1 ml) at 100 °C for 3 h. After dilution of the mixture with 4 volumes of water the heating was continued for another 2 h followed by evaporation

of the formic acid. The residue was dissolved in water (2 ml), sodium borohydride (5 mg) was added and the solution left overnight at 5 °C. After acidification with acetic acid and concentration, boric acid was removed by distillation with methanol, the product dissolved in a 1:1 (v/v) mixture of acetic anhydride and pyridine (2 ml) and heated at 100 °C for 15 min. After concentration to dryness the residue was dissolved in chloroform and subjected to GLC-mass spectrometry; the results are given in Table 1.

Oligosaccharide II had $R_{Lactose}$ 1.65 and 1.80 in solvent system A and B, respectively. Methanolysis followed by GLC showed the presence of galactose and *N*-acetylgalactosaminitol. When the oligosaccharide (1 mg) was incubated with β-galactosidase, the resulting material after deionization was shown by PC and GLC to contain galactose and *N*-acetylgalactosaminitol as the only free sugars. Oligosaccharide II (2.2 mg, *ca.* 6 μmol) was dissolved in 0.02 M sodium metaperiodate (2.5 ml, 50 μmol) and kept at 20 °C in the dark for 2 h. After addition of ethylene glycol (1 drop) and sodium borohydride (5 mg) the solution was left overnight at 5 °C and then neutralized and freed of boric acid the usual way. The product was dissolved in 0.05 N sulfuric acid and kept at 80 °C for 70 min, followed by neutralization, deionization, and concentration to dryness. PC of the resulting material established the absence of galactose and the presence of glycerol and another non-reducing compound which could be detected with both reagents *a* and *b*. This latter compound had a $R_{N\text{-acetylglucosamine}}$ of 1.18 and 1.37 in solvent system A and B, respectively, while the corresponding values for 2-*N*-acetylamino-1,3-propanediol were 1.47 and 1.80.

When oligosaccharide I (1 mg, *ca.* 2 μmol) was subjected to the same procedure (oxidation with 50 μmol) of periodate, followed by reduction and acid hydrolysis) a non-reducing compound with the same chromatographic mobility on paper as the one obtained from oligosaccharide II was detected with reagent *a;* however, no spot was revealed with the less sensitive reagent *b*. By oxidation of *N*-acetylgalactosaminitol (2 mg, *ca.* 10 μmol) in 0.02 M periodate (1 ml, 20 μmol) at 20 °C in the dark for 2 h and subsequent treatment with borohydride the same non-reducing compound was detected together with a small proportion of 2-acetylamino-1,3-propanediol.

Isolation of oligosaccharide III after acetolysis of the desialized glycoprotein. The desialized glycoprotein (0.89 g) was dissolved in formamide (20 ml) by stirring 1 h at room temperature. A 2:1 (v/v) mixture of acetic anhydride and pyridine (66 ml) was added and the stirring continued for another 48 h. The brown liquid was diluted with water and dialysed against distilled water for 3 days. The acetylated polymer which precipitated gradually, was filtered off and dried, yield 0.85 g. The acetylated product (0.85 g) was stirred with a 10:10:1 (v/v) mixture of acetic anhydride, acetic acid, and sulfuric acid (120 ml) at 40 °C for 5 h.[18] After cooling pyridine (30 ml) was added to terminate the reaction and the mixture was poured into ice water and left overnight at 5 °C. The acetolysis products were recovered by extraction with chloroform (3 × 200 ml) and the combined chloroform phases were extracted with water to remove acetic acid. After drying with anhydrous sodium sulfate the chloroform was evaporated off and the residual syrup dissolved in methanol (60 ml). A 0.5 % solution of sodium methoxide in methanol (20 ml) was added and the deacylation was allowed to proceed for 30 min at room temperature. The reaction was terminated by the addition of ethyl acetate (15 ml) followed by concentration to *ca.* 20 ml, dilution with water and treatment with Dowex 50 (H^+). The resulting solution was concentrated to a syrup (0.57 g) which was dissolved in 10 % ethanol in water (4 ml). This solution was divided into three parts, each being successively subjected to gel chromatography on a Bio-Gel P-2 column; fractions of 5 ml were collected. The fractions (tubes 24 to 30) corresponding to the middle peak of the elution curve (Fig. 3) were pooled and concentrated to a syrup (59 mg) which was further fractionated by preparative paper chromatography. One sugar, oligosaccharide III, (8 mg) was isolated in the pure state; it has $R_{Lactose}$ 1.25 and 1.45 in solvent system A and B, respectively. GLC after methanolysis showed the presence of galactose and *N*-acetylglucosamine, and incubation of the oligosaccharide (1 mg) with β-galactosidase gave rise to the same sugars, as demonstrated by GLC and PC. Oligosaccharide III (5 mg) was methylated, hydrolysed and the products converted into alditol acetates as described for oligosaccharide I, followed by examination by GLC-mass spectrometry; the results are summarized in Table 1.

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Studies on the Degradation of Pterine and Pterine-6-carboxylic Acid by *Pseudomonas fluorescens* UK-1

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Pseudomonas fluorescens UK-1 has been incubated in basal mineral medium which was 0.5 mM in pterine or pterine-6-carboxylic acid. During incubation the test organism splits the pteridine ring by liberating carbon dioxide from position 2. Glucose added to the medium greatly enhances both the growth of the organism and the carbon dioxide formation. Despite the structural similarities between pterine and pterine-6-carboxylic acid, only the degradation products derived from pterine are fluorescent in UV-light. Among the degradation products lumazine, pyrazine-2-carboxylic acid, and pyrazine-2-carboxamide have been identified. Also the activities of pterine deaminase and a carbon dioxide-liberating enzyme have been determined.

In a previous report we have shown that during incubation of *Pseudomonas fluorescens* UK-1 in a medium containing pterine-6-carboxylic acid, compounds as yet unidentified accumulate in the growth medium.[1] In preliminary experiments we have found that pterine-6-carboxylic acid and pterine (2-amino-4-hydroxypteridine) give rise to slightly different degradation products in the growth medium, despite their structural similarity. In this report we describe the nature and amounts of compounds accumulating in the growth medium when pterine--6-carboxylic acid and pterine are used as carbon and nitrogen sources. The effect of glucose on the degradation of these compounds and appearance of the degradation products has also been studied.

In addition to studying the degradation, we also determined the activities of two enzymes that are operative during the first stages of the degradation of the pteridines, namely a carbon dioxide-liberating enzyme and pterine deaminase. The studies of the last-mentioned enzyme are based on the findings of Levenberg and Hayaishi.[2] They showed that *Alcaligenes metalcaligenes*, which uses pterine-6-carboxylic acid as a source of carbon and nitrogen, possesses a pterine deaminase activity.

METHODS

Reagents. All chemicals used in experiments were guaranteed reagents from Sigma Chemical Co. or AnalaR reagents from The British Drug Houses Ltd., except folic acid-2-^{14}C which was a product of Radiochemical Centre, Amersham, England.

Culture conditions. Ps. fluorescens UK-1 was preserved and cultivated in 5 mM pantothenate medium as described earlier.[3] The main cultivation in degradation studies was carried out in a medium which was 0.5 mM in pterine-6-carboxylic acid or pterine, these providing the only source of carbon and nitrogen. The pH of the medium was 8.0 to facilitate the dissolving of pterine-6-carboxylic acid.

In the studies of enzyme activities we used, for purposes of comparison, a medium which was 10 mM in sodium glutamate.

Determination of degradation products. During the growth of the test organism, 5 ml samples were withdrawn and the cells centrifuged off. A 200 μl sample of clear supernatant was pipetted onto Whatman No. 3 MM paper and paper chromatograms were run in a 1:2 1% NH_3-propanol mixture as solvent.[4] After drying, the chromatograms were photographed in ultraviolet light with a Desaga MinUVIS lamp at a wave length of 254 nm. Spots visible in UV-light were cut off and eluted with 4 ml of 0.1 M potassium phosphate buffer, pH 8.0. The absorbances of the eluates were measured with a Unicam SP-800 spectrophotometer over the range 200 – 400 nm.

In preliminary experiments, we used glucose in concentrations of 0.1, 0.2, and 0.5 % to

determine its effect on the uptake and degradation of pteridines. In other experiments a concentration of 0.2 % was used.

For the study of CO_2 liberation from pterine and pterine-6-carboxylic acid by *Ps. fluorescens* UK-1, folic acid labelled with ^{14}C at position 2 (total activity 250 000 dpm) was added to 100 ml of culture medium. Inlet air was conducted to the culture vessel through three washbottles containing successively 5 N KOH, 1 N H_2SO_4, and distilled water. The liberated CO_2 was collected in 0.1 % $Ca(OH)_2$ solution, which was changed at intervals of 6 to 12 h. The precipitated calcium carbonate was filtered through a Millipore membrane filter and dried. The filter with precipitate was weighed and then counted in a Wallac NTL-314 liquid scintillation counter. Permablend III (Packard Instrument Company Inc.) was used as the scintillator in a concentration of 5.5 g to 1000 ml of toluene.

To study the uptake velocities of the pteridines used, the cells were grown for 25 – 30 h in basal medium supplemented with either pterine-6-carboxylic acid or pterine in a concentration of 0.5 mM. The washed cells (*ca.* 20 mg dry weight) were suspended in 10 ml of a reaction mixture which was similar to the growth medium but supplemented with folic acid-2-^{14}C, 2×10^5 dpm/ml. Specimens (1 ml) taken at intervals from 30 s to 40 min were cooled rapidly, filtered through a Millipore membrane filter, and washed twice with cold 2 % Na_2CO_3 solution. The filter with cells was dried and counted.

Determination of enzyme activities

(a) *Carbon dioxide-liberating enzyme. Ps. fluorescens* UK-1 was grown to the middle of the logarithmic phase in 100 ml of the pterine-glucose medium and the glutamate medium. The cells were centrifuged off, washed twice with cold 0.9 % NaCl solution, and ruptured in 2 ml of 0.05 M potassium phosphate buffer pH 8.0 using a MSE ultrasonic disintegrator 60 W, 18 000 kc, 1.5 A. The rupturing time was 7 min. The disintegrating vessel was kept in an ice bath during handling. After the disintegration the suspension was centrifuged and the protein content of the clear supernatant was determined by the sulfosalicylic acid method.[5] The enzymatic reaction was carried out in a Warburg apparatus at 30 °C. Two millilitres of reaction mixture was pipetted into the main cup. The reaction mixture was the same as the growth medium but supplemented with folic

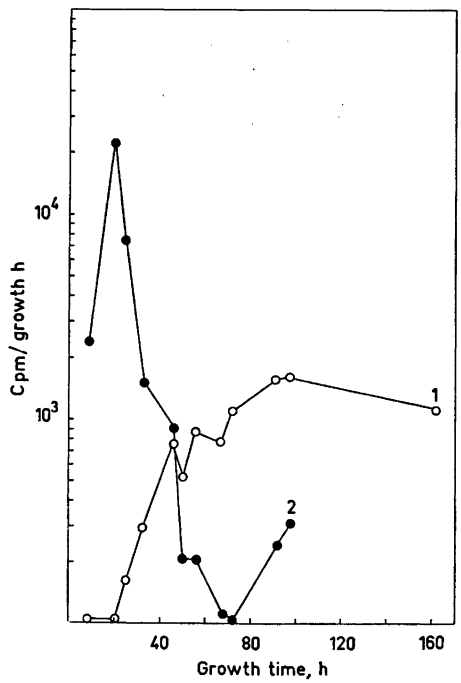


Fig. 1. CO_2 liberation during cultivation of *Ps. fluorescens* UK-1 in pterine medium. Curves: (1) without glucose; (2) with glucose.

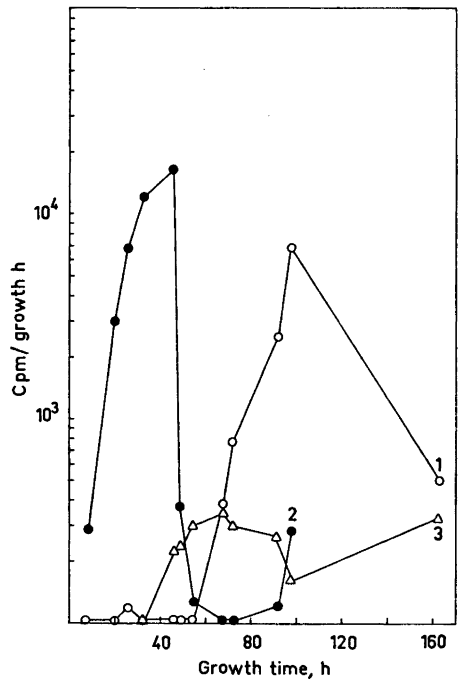


Fig. 2. CO_2 liberation during cultivation of *Ps. fluorescens* UK-1 in pterine-6-carboxylic acid medium. Curves: (1) without glucose; (2) with glucose; (3) CO_2 liberation due to spontaneous degradation.

acid-2-^{14}C, 2×10^5 dpm/ml. The side arm contained 0.5 ml of cell extract, and the middle well a filter paper strip which had been moistened with 0.5 M KOH. The reaction was started by pouring the cell extract into the main cup. The reaction time was 60 min. Thereafter the strip was removed, dried, and its radioactivity counted.

(b) *Pterine deaminase.* The cell extract for pterine deaminase activity determination was made as in the case of the carbon dioxide-liberating enzyme. The amount of ammonia which is liberated in the course of deaminase reaction at 30 °C was measured according to Conway's micro-diffusion technique.[6] Several attempts were made to purify the enzyme and to determine its activity spectrophotometrically according to Levenberg and Hayaishi,[2] but they were unsuccessful perhaps due to a very rapid inactivation of the enzyme.

RESULTS AND DISCUSSION

Carbon dioxide liberation from pterine and pterine-6-carboxylic acid during the growth of *Ps. fluorescens* UK-1 is shown in Figs. 1 and 2. The carbon dioxide originated from carbon atom 2 in the pteridine ring, which was labelled with ^{14}C in that position. Instead of labelled pterine or pterine-6-carboxylic acid we used folic acid-2-^{14}C, for it has earlier been shown that *Ps. fluorescens* UK-1 splits the bond between C-9 and N-10 in the folic acid molecule.[3] In addition, the amount of radioactive folic acid is so small (0.85 μg) that it could not serve as an effective carbon and nitrogen source. When pterine was used as the carbon and nitrogen source the $^{14}CO_2$ formation started after 20 h of incubation and reached its maximum at 95 h (Fig. 1). In the case of pterine-6-carboxylic acid the $^{14}CO_2$ formation started only after 55 h of incubation but reached its maximum about the same time as with pterine (Fig. 2). In both experiments only 50 % of the added activity was recovered as $^{14}CO_2$. About 10 % of the pteridine was degraded spontaneously, as can be seen in Fig. 2, curve 3.

When the culture medium contained 0.2 % glucose the carbon dioxide formation from pterine and pterine-6-carboxylic acid was greatly increased. With pterine in the growth medium, maximal carbon dioxide formation was noted after 20 h of growth, whereas with pterine-6-carboxylic acid the formation was at maximum at 45 h. In both cases almost the entire ^{14}C activity was liberated in the form of $^{14}CO_2$ during 70 h of growth.

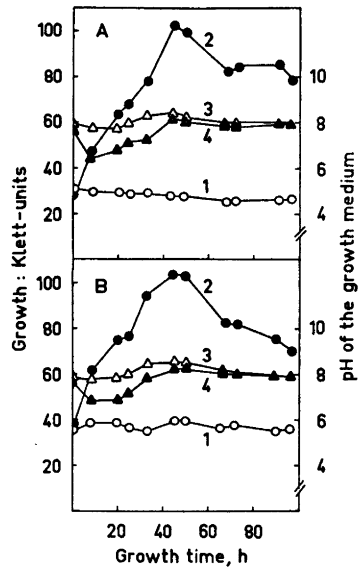


Fig. 3. Effect of glucose on growth rate and pH during cultivation of *Ps. fluorescens* UK-1. Part A: conditions in pterine medium. Part B: conditions in pterine-6-carboxylic acid medium. Curves: (1) growth without glucose; (2) growth with glucose; (3) changes in pH when the growth medium was lacking glucose; (4) changes in pH when the growth medium contained glucose.

When testing the uptake of pterine and pterine-6-carboxylic acid into cells we found that the maximal uptake velocities were reached after 30 sec and 2 min with pterine and pterine-6-carboxylic acid, respectively. The amounts of these compounds transported were about 50 % higher when glucose was present in the reaction mixture.

In the experiments described here glucose plays a remarkable role. As mentioned above, the degradation of the pteridine ring at position 2 is a quick and complete process when glucose is present in the growth medium. In the absence of glucose only one-half of the added pteridine is degraded. Obviously the transport of pterine or pterine-6-carboxylic acid into the cell is enhanced by the energy supplied by glucose. Glucose has a growth promoting effect, too, as can be seen in Fig. 3. When pterine or pterine-6-carboxylic acid was the only carbon and nitrogen source, no growth occurred. However, the cells survived, as is indicated by carbon dioxide formation (Figs. 1 and 2). In

Table 1. Some properties of compounds which can be detected chromatographically in the culture medium of *Ps. fluorescens* UK-1 during incubation. Pterine was the source of carbon and nitrogen. The medium did not contain glucose.

Compound	Appearance time, h	R_F-value	Colour	Absorption maxima, nm
(Pterine)		0.37	light blue	274, 340
I	20	0.30	yellow-green	255, 360
II	20	0.50	dark red-violet	269
III	20	0.78	dark red-violet	269
IV	44	0.27	blue-green	274, 328
V	15	0.17	blue-green	230, 255, 280, 335

Table 2. Activity of the carbon dioxide-liberating enzyme in cells grown in different media to the middle of the logarithmic phase.

Medium	Specific activity mU/mg of protein
Glutamate	1.2
Pterine-glucose	1.25×10^3

Table 3. Pterine deaminase activity of cells grown in glutamate medium to the late logarithmic phase. Specificity of pterine deaminase towards different substrates.

Substrate	Specific activity mU/mg of protein
Pterine-6-carboxylic acid	0.7
Pterine	2.9

the presence of glucose, the growth reaches its maximum within 50 h. From this we can conclude that *Ps. fluorescens* UK-1 easily utilizes the nitrogen of the pteridines, but the actual carbon source in these circumstances is glucose.

The pteridine ring is also degraded by *Ps. fluorescens* UK-1 without glucose and the degradation products accumulate in the medium. Five degradation products could be detected in chromatograms illuminated with UV-light when pterine was used as the carbon and nitrogen source. The appearance time from the beginning of the growth, the R_F-value, absorption maxima, and colour of different spots are presented in Table 1. The presence of glucose accelerates the disappearance of pterine from the culture medium. Also the number of spots of the degradation products is smaller since the degradation products are metabolized more rapidly in the presence than in the absence of glucose.

By using several standards and identification tables for substituted pteridines, and by comparing R_F-values and colours of the spots, we

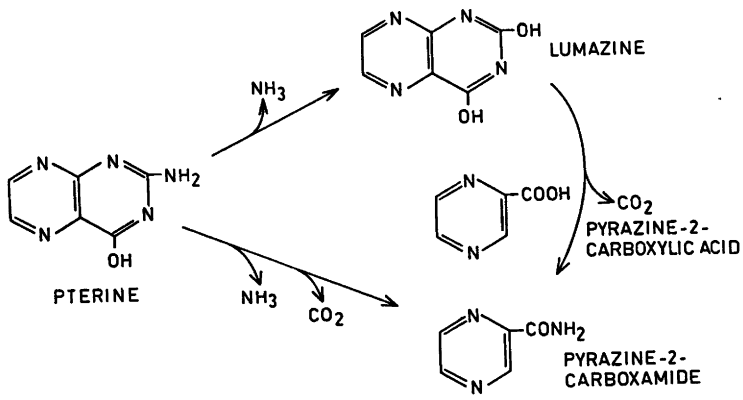


Fig. 4. Hypothetical model of the first stages during the degradation of pterine by *Ps. fluorescens* UK-1.

have identified three compounds which originate from pterine during the incubation of *Ps. fluorescens* UK-1. Compounds I, II, and III are lumazine, pyrazine-2-carboxylic acid, and pyrazine-2-carboxamide, respectively. Compounds IV and V are still unidentified, but there is strong evidence that they are hydroxylated lumazines.

Lumazine, pyrazine-2-carboxylic acid, and pyrazine-2-carboxamide appear in the growth medium after 20 h from the beginning of the incubation. The formation of pyrazine-2-carboxylic acid and pyrazine-2-carboxamide is in good agreement with the liberation of carbon dioxide, the formation of which also starts after 20 h from the beginning of the incubation (Fig. 1). The formation of lumazine, which is the deamination product of pterine, indicates the presence of pterine deaminase.

On the basis of the results described above it can be concluded that the first stages in the catabolism of pterine in *Ps. fluorescens* UK-1 occur as shown in Fig. 4. By the action of pterine deaminase, lumazine is formed which, catalyzed by the carbon dioxide-liberating enzyme, forms pyrazine-2-carboxylic acid and pyrazine-2-carboxamide. The last-mentioned compounds can also be formed directly from pterine. It must be pointed out that the mechanism is presented here only as a hypothesis. In further studies we will attempt to explain the mechanism in more detail.

To determine the activity of the carbon dioxide-liberating enzyme we cultivated *Ps. fluorescens* UK-1 in pterine-glucose medium and, for comparison, in glutamate medium. The cells were harvested in the middle of the logarithmic phase (after 20 h of growth), treated as described above, and their activity was measured. The results are presented in Table 2. The enzyme activity in cells grown in pterine-glucose medium was about 1000 times greater than in cells grown in glutamate medium, which possibly indicates that this enzyme is inducible.

For the determination of pterine deaminase activity we cultivated *Ps. fluorescens* UK-1 in glutamate medium to the late logarithmic phase (cells grew 40 h) and treated it as described above. In activity determinations we used both pterine-6-carboxylic acid and pterine as substrates. The pterine deaminase activity in the presence of these substrates is presented in Table 3. With pterine, the activity was about four times as great as with pterine-6-carboxylic acid. According to Levenberg and Hayaishi,[2] the pterine deaminase activity in *Alcaligenes metalcaligenes* in sonic extract is 0.1 mU/mg protein when pterine-6-carboxylic acid is the substrate. However, their measurements were made at 23 °C.

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Short Communications

Studies on Electrolytic Substitution Reactions. X.[1] Anodic Trifluoroacetoxylation of Benzene and Deactivated Benzenes

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Anodic acetoxylation of aromatic compounds is limited to those substrates which are easier to oxidize than the acetic acid/sodium acetate medium.[2] Benzene derivatives containing electron-donating substituents are generally efficiently oxidized. Benzene itself is oxidized to phenyl acetate in low yield,[3] and deactivated benzene derivatives, such as benzotrifluoride or methyl benzoate, are not oxidized at all in acetic acid/sodium acetate.[4]

In trifluoroacetic acid it should be possible to oxidize even the latter compounds, since it has been shown that aliphatic hydrocarbons are oxidized in this medium,[5] which is more resistant toward oxidation than is acetic acid. It has also been reported that the cation radical intermediates in the oxidation of aromatic compounds, are more stable in trifluoroacetic acid than in less acidic media.[6–8]

We now give a preliminary report of the results from controlled potential oxidation of benzene and deactivated benzene derivatives in trifluoroacetic acid/1.0 M sodium trifluoroacetate. The reactions were carried out in a divided cell at a platinum anode until 10 % of the charge calculated for a two-electron process had been passed. This procedure was used in order to avoid further oxidation of the products.[4] Aryl trifluoroacetates were formed in all experiments and were converted into the

$$\mathrm{ArH} \xrightarrow[\substack{\mathrm{CF_3COOH}\\ \mathrm{CF_3COONa}}]{\mathrm{Pt}} \mathrm{ArOCOCF_3} \xrightarrow[\mathrm{H_2O}]{} \mathrm{ArOH} \qquad (1)$$

corresponding phenols before analysis because of their sensitivity towards moisture (eqn. 1). The results are shown in Table. 1. Current-potential curves for the solvent-supporting electrolyte system showed a steep current rise at 2.3 V *vs.* the Ag/Ag^+ reference electrode. All the substrates shown in Table 1 were oxidized at potentials well below 2.3 V. An attempt to oxidize 1,4-dicyanobenzene was unsuccessful. In this experiment no change in the current-potential curve for the solvent-supporting electrolyte system was noticed upon addition of the substrate. On the other hand, dimethyl terephthalate was converted into its hydroxy derivative in a 60 % current yield.

Table 1. Anodic trifluoroacetoxylation of aromatic compounds. Product yield and isomer distribution (observed [a] and calculated).

X in PhX	Isomer distribution Observed *o*	*m*	*p*	Calculated *o*	*m*	*p*	Current yield (%)
H							27
$COOCH_3$	51	34	15	54	16	30	65
NO_2	22	59	19	30	43	27	60
CF_3	35	47	18	50	35	15	31
$COCH_3$	54	32	14	52	17	31	67
COPh	70	18	12	54	15	31	21
CN	45	30	25	42	30	28	10

[a] The products were analyzed as the corresponding phenols.

The isomer distribution shown in Table 1 is of interest from a mechanistic point of view. It is now generally accepted that aromatic cation radicals are the primary species in the oxidation of aromatic compounds.[2,9,10] It has been suggested that the positive charge density in a ring position of an aromatic cation radical should determine the relative amount of nucleophilic attack at that position.[11–15] In the anodic acetoxylation of monosubstituted benzenes carrying an electron-donating substituent the *ortho* and *para* products predominate over the *meta* isomer, corresponding to the isomer distribution obtained in electrophilic aromatic substitution.[4] If an analogous mechanism prevails for benzene derivatives carrying an electron-withdrawing substituent one would expect the compounds shown in Table 1 to yield the *meta*

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isomer predominantly on anodic substitution. The results in Table 1 do not, however, agree with this prediction. Instead they correlate reasonably well with the isomer distribution calculated from the positive charge distribution in the cation radicals, as calculated by the INDO method.[16–18] The results are shown in Table 1 and add further support to the suggestion that the site of attack by a nucleophile is determined by the positive charge density in a particular position of the cation radical.

From a preparative point of view the reaction is promising, since it allows the ready introduction of a hydroxy group into deactivated aromatic molecules. It is also noteworthy that some oxidations produce a large proportion of the *ortho* substituted phenol and are of possibly unique preparative value in this respect. Further work of mechanistic and preparative nature will be reported later. In a recent paper Miller *et al.* report the anodic oxidation of acetophene and ethyl benzoate in trifluoroacetic acid/methylene chloride using Et_4NBF_4 as the supporting electrolyte.[19] They obtained *ortho* substituted products in yields comparable to ours, but no *meta* substituted products.

Experimental. The electrolysis cell consisted of a 20 ml water-jacketed flat-bottomed glass cylinder with a four-neck flat flange lid equipped with a platinum foil anode, a platinum wire cathode, an Ag/0.1 M $AgOCOCF_3/CF_3COOH$ reference electrode, a drying tube and a magnetic stirrer bar. The separation of the anode compartment and the cathode was achieved by a ceramic cylinder. The oxidative potential was controlled with an Amel Model 557 Potentiostat.

Electrolysis was carried out in a 1 M $NaOCOCF_3$ solution in CF_3COOH (10 ml). The aromatic substrate (0.02 mol) and neutral alumina[20] (0.5 g) were added to the anode compartment and the electrolysis was run until 0.004 F had passed. The reaction mixture was then poured into boiling water (50 ml) in order to hydrolyze the aryl trifluoroacetates formed. After cooling, the mixture was extracted with ether or methylene chloride. The organic phase was washed with water and dried. Analysis was carried out on a Perkin-Elmer Model 880 Gas Chromatograph in connection with a Model D 26 Integrator utilizing a 2 m × 0.3 cm 5 % NPGS on Chromosorb W column. Identification of the products was based on comparison with authentic samples or by the use of mass spectrometry. Yields were calculated by integration of the GLC peaks after calibration by means of authentic samples.

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Addition Reactions and Stability of Arylsulfonyl Thiocyanates

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Due to our standing interest in thiocyanates derived from organic acids,[1,2] our attention was drawn to a communication by Goerdeler and Rosenthal [3] describing the synthesis and some reactions of sulfonyl thiocyanates. A recent report by Wolf[4] confirming most of the findings of Goerdeler and Rosenthal now prompts us to report some aspects of the addition of *p*-tolylsulfonyl, *1*, and *p*-chlorophenylsulfonyl thiocyanate, *2*, to the carbon-carbon double bond and the stability of these thiocyanates. Apparently the two papers mentioned [3,4] and two patents [5,6] are the only information available on organic sulfonyl thiocyanates.**

Addition reactions to the carbon-carbon double bond. Addition of *1* to styrene gives rise to 2-phenyl-2-thiocyanatoethyl *p*-totylsulfone, *3*.[3,4] Using the method of Goerdeler *et al.*[3] we have confirmed this result and found the styrene adduct of *2* to be analogous to *3*.

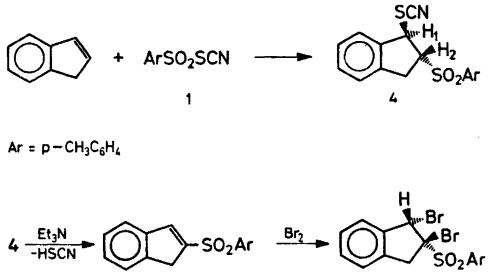
As decribed by Wolf, *3* on treatment with triethylamine undergoes a facile *trans* elimination of thiocyanic acid from the most stable conformation. We have confirmed this result and found analogous behaviour for the adduct of *2* thus giving rise to *trans*-2-phenyl-1-(arylsulfonyl)ethylene ($J = 15-16$ Hz for the alkene protons which is well within the limits reported for *trans* alkene protons, $J = 11-19$ Hz[10]).

To secure the stereochemistry of the addition reaction *1* and *2* were added to acenaphthylene and indene. By ^{1}H NMR analysis of the reaction mixture the addition reactions proved to be stereospecific.

The product from the addition of *1* to acenaphthylene exhibits two doublets at $\delta = 5.22$ and $\delta = 5.40$, $J \approx 2$ Hz, which is the value expected for *trans* coupling, while J (*cis*) $\sim 6-7$ Hz.[11-13] The corresponding values for additions of *2* were $\delta = 5.33$, $\delta = 5.48$ and $J \approx 2$ Hz securing the *trans* configuration for this product as well. The possibility of the formation of isothiocyanate derivatives was excluded by inspection of the IR spectra, which showed the characteristic SCN stretching vibration at 2160 cm^{-1}.

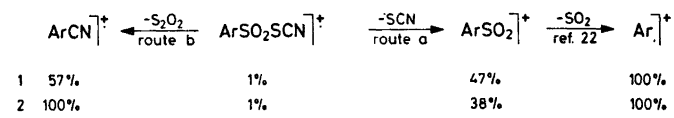
By analysis of the spin-spin coupling pattern from the ^{1}H NMR spectrum of *3* it was evident that the arylsulfonyl group exerts a more pronounced deshielding effect than does the thiocyanate group, meaning that the geminal protons adjacent to the sulfonyl group appear at higher field than the one adjacent to the thiocyanate group. Bearing this in mind, the ^{1}H NMR spectrum of the addition product of *1* and indene, exhibiting a doublet at $\delta(H_1) = 4.98$, $J_{1,2} = 4$ Hz and a multiplet around $\delta(H_2) = 4.0$, strongly support the formulation of this product as 1-thiocyanato-2-(*p*-tosyl) indane *4*. The elimination of thiocyanic acid from *4* proved much more difficult than in the case of *3*, and the ^{1}H NMR spectrum of the resulting *p*-tosylindene did not distinguish between the two possible substitution patterns (*i.e.* the *p*-tosyl group in position 1 *versus* 2). However, addition of bromine gave rise to a product, the ^{1}H NMR spectrum of which exhibits a singlet at $\delta = 6.08$ and an AB pattern with doublets centered at $\delta = 3.64$ and $\delta = 4.28$ ($J_{AB} = 16.5$ Hz). These data are consistent with the formulation *4* but exclude the isomeric 2-thiocyanato-1-(*p*-tosyl)indane structure. The assignment of the *trans* configuration in *4* is partly based on the observed slow thiocyanic acid elimination, which according to this formulation has to be a *cis* elimination. Evidently, the coupling constants of the protons in the indane system are generally of little value in assigning stereostructures in this class of compounds.[14] The limits of the reported values are $J_{1,2}$ (*cis*) $\sim 4.4-8.5$ Hz and $J_{1,2}$ (*trans*) $\sim 1-7$ Hz.[14-17] However, the observed value in *4*, $J_{1,2} = 4.0$ Hz seems to be well below the smallest reported value for a *cis* relationship and is therefore in this case taken as substantial support for the assigned stereostructure of *4*.

Thermal and photochemical stability. We have observed that solutions of arylsulfonyl thiocyanates, when left at room temperature (23–24 °C) for extended periods of time, are



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** According to Ashworth [7] arylsulfonyl thiocyanates might be formed from ethanolic solutions of arylsulfonyl chlorides and ammonium thiocyanates. The product of the reaction is described as a yellow amorphous precipitate, [7,8] insoluble in ethanol, ether, and benzene and melting higher than 230 °C.[9] Arysulfonyl thiocyanates have far lower melting points [3,4] therefore the products in question cannot be arylsulfonyl thiocyanates.



Scheme 1.

fairly stable. When a solution of *1* (5 % in CCl_4) was examined after 15 days a rough estimate based on the absorbance of the 2160 cm^{-1} and 1590 cm^{-1} bands showed that at least 90 % of the thiocyanate still remained. The same result was obtained for *2* under identical conditions (the estimate now based on the 2160 cm^{-1} and 1476 cm^{-1} bands).

During these experiments the IR spectra remained mainly unaltered except for a rather sharp absorption emerging at 1905 cm^{-1}. This band is highly characteristic of arylsulfonyl isothiocyanates.[18,19]

It is thus obvious that isomerization takes place to some extent, which is in contrast to an earlier statement by Goerdeler *et al.*[3] In thermolysis experiments at 146 °C ($Cl_2CHCHCl_2$, 12 h) compound *1* was still fairly stable, while *2* was extensively decomposed. Since arylsulfonyl isothiocyanates are thermally unstable[20,21] the possibility exists that the extensive decomposition may occur *via* the isomerization.

At irradiation with RUL 3000 Å lamps (Rayonet reactor, type RPR-208) at room temperature a fast isomerization to the corresponding arylsulfonyl isothiocyanates takes place. It is essential that the photolysis is carried out in deoxygenated solvents as a very fast photochemical reaction with oxygen was observed.

The mass spectra of sulfonyl thiocyanates are highly characteristic of this type of compound. Introduction of *1* and *2* through the direct insertion probe at 100 °C and 140 °C, respectively, gave rise to an utterly simple spectrum as shown in Scheme 1, route *a*.

Both *1* and *2* gave rise to low intensity peaks corresponding to loss of sulfur plus sulfur dioxide or S_2O_2 from the molecular ion. When the compounds were introduced through the gas inlet system (*1* at 120 °C and *2* at 140 °C) a drastic alteration in intensity distribution was noted. The compounds now degradate *via* both route *a* and *b*. In the case of *1* the intensity of the peak corresponding to loss of S_2O_2 from the molecular ion is second only to the base peak. In *2* this peak is base peak; the peak corresponding to ionized sulfur dioxide (*m/e* 64) also has gained intensity and is a major peak (80 %). These observations indicate thermal degradation reactions in the inlet system with loss of SO_2. This hypothesis was substantiated by the findings that reflux of solutions of *1* and *2* in 1,1,2,2-tetrachloroethane (b.p. 146 °C) for 12 h liberated 2.5 % and 7.9 %, respectively, of the theoretical amount of sulfur dioxide.

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Pseudomonas Cytochrome *c* Peroxidase. XI. Kinetics of the Peroxidatic Oxidation of *Pseudomonas* Respiratory Chain Components

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The steady state kinetics of cytochrome *c* peroxidase from *Pseudomonas aeruginosa* (PaCCP) has been studied by initial velocity techniques using several cytochromes *c* (550 and 555 *P. aeruginosa*; 551 *P. fluorescens*) and *Pseudomonas* azurin as electron donors and hydrogen peroxide as electron acceptor. From the initial velocity patterns a sequential mechanism with compulsory substrate-binding order is proposed for PaCCP. A comparative kinetic study of the peroxidatic oxidation of cytochrome *c*-551 (*P. aeruginosa*) by yeast cytochrome *c* peroxidase was made to evaluate the significance of electrostatic interactions in complex formation between the enzyme and substrates.

Pseudomonas cytochrome *c* peroxidase (PaCCP, cytochrome *c*:H_2O_2 oxidoreductase, EC 1.11.1.5) has been found to catalyze the peroxidatic oxidation of such bacterial respiratory chain components as cytochrome *c*-551 and azurin from *Pseudomonas*.[1] The kinetics of the peroxidatic oxidation of cytochrome *c* (551 *P. aeruginosa*) by PaCCP in buffers of different pH-values has recently been reported.[2] A sequential reaction mechanism in which both substrates add to the enzyme before either product is released was proposed for PaCCP. The present work was undertaken to differentiate between ordered and random substrate binding mechanisms.

The cytochromes used as substrates were characterized by their isoelectric points and extinction coefficients.

As in the earlier study,[2] all initial velocity measurements reported were performed in such a way that the phase of initial delay [1] was avoided, *i.e.* PaCCP was incubated with reduced substrates before the reaction was started by addition of H_2O_2.

MATERIALS AND METHODS

Pseudomonas *cytochrome c peroxidase (PaCCP)* was prepared from acetone-dried cells of *P. aeruginosa*, as previously described.[3] The ratio A_{407}/A_{280} of the preparation was 4.4 and the specific activity was 115 – 130 U/mg, measured as reported earlier.[4] The concentration of the enzyme was determined spectrophotometrically using A (1 %, 1 cm) equal to 12.1 at 280 nm.[5] The molar concentration of the enzyme was calculated using a molecular weight of 43 200.[5]

Yeast cytochrome c peroxidase (YCCP) was prepared as described previously.[6] The ratio A_{407}/A_{280} of the preparation was 1.30. The concentration of the enzyme was determined on the basis of the total hematin content, measured as pyridine ferrohemochrome according to Paul *et al.*[7]

Horse heart cytochrome c (H-cyt) was a commercial preparation from Sigma Chemical Co., Type III, 98 %, used without further purification. The extinction coefficients ε_{550}(red.) = 27.6 mM^{-1} cm^{-1} and $\Delta\varepsilon_{550}$(red.-ox.) = 19.6 mM^{-1} cm^{-1} were used for the spectrophotometric determination of the cytochrome concentration.[8]

Pseudomonas *cytochrome c-551 (Pa-cyt-551) and* Pseudomonas *azurin* were prepared from acetone-dried cells of *P. aeruginosa* by the method of Ambler [9] and Ambler and Brown.[10] The purity of the cytochrome preparations [A_{551}(red.) – A_{570}(red.)/A_{280}] was 1.12 – 1.19, and that of azurin [A_{625}(ox.)/A_{280}] 0.4. The best azurin preparation of Ambler and Brown had a purity of 0.6. Both azurin and Pa-cyt-551, however, were found to be homogeneous on disc electrophoresis. The concentration of

Pa-cyt-551 was determined spectrophotometrically applying the extinction coefficients ε_{551}(red.) = 26.9 mM^{-1} cm^{-1} and $\Delta\varepsilon_{551}$(red.-ox.) = 19.0 mM^{-1} cm^{-1}, and the concentration of azurin by using ε_{625}(ox.) = 5.1 mM^{-1} cm^{-1}.[1]

Pseudomonas *cytochromes c-550 (Pa-cyt-550) and c-555 (Pa-cyt-555)* were prepared from *P. aeruginosa*. The methods of purification were essentially similar to those described for Pa-cyt-551 by Ambler.[9] Cytochrome *c*-554,[11] which is a mixture of cytochromes *c*-550 and *c*-555, was eluted from CM-cellulose at pH 5.1 and the components were separated using either isoelectric focusing or, with slight modifications, the chromatographic purification method of Ambler and Taylor.[12] The purity of Pa-cyt-550 [A_{550}(red.) − A_{570}(red.)/A_{280}] was 1.3 and that of Pa-cyt-555 [A_{555}(red.) − A_{570}(red.)/A_{280}] 1.14. The extinction coefficients ε_{550}(red.) = 20.9 mM^{-1} cm^{-1} and $\Delta\varepsilon_{550}$(red.-ox.) = 14.9 mM^{-1} cm^{-1}, and ε_{555}(red.) = 24.6 mM^{-1} cm^{-1} and $\Delta\varepsilon_{555}$(red.-ox.) = 17.4 mM^{-1} cm^{-1} were used for the spectrophotometric determination of Pa-cyt-550 and Pa-cyt-555, respectively.

Cultivation of Pseudomonas fluorescens *(ATCC 17400)*. The bacteria were grown with stirring but without aeration, as described for *P. aeruginosa*.[3] The cells were harvested 16 h after an 8 l inoculum had been transferred to 300 l of medium in a 400 l pilot plant fermentor (Getingeverken, Getinge, Sweden).

Cytochrome c-551 of P. fluorescens *(Pf-cyt-551)* was prepared from acetone-dried cells according to the method of Ambler and Wynn.[13] The purity of Pf-cyt-551 [A_{551}(red.) − A_{570}(red.)/A_{280}] was 1.2 and the preparation was homogeneous on disc electrophoresis. The concentration of the cytochrome was determined spectrophotometrically applying the extinction coefficients ε_{551}(red.) = 29.3 mM^{-1} cm^{-1} and $\Delta\varepsilon_{551}$ (red.-ox.) = 21.9 mM^{-1} cm^{-1}.

Reduced cytochromes and azurin were prepared according to Yonetani and Ray [14] using anaerobic gel filtration on Sephadex G-25.

Hydrogen peroxide solutions were prepared from Merck Perhydrol (30 % H_2O_2). Peroxide concentration was determined enzymatically with YCCP using horse heart cytochrome *c* as substrate according to Yonetani.[8]

Measurements of reaction rates. The activities of *Pseudomonas* and yeast cytochrome *c* peroxidases were assayed spectrophotometrically by measuring the rate of peroxidatic oxidation of fully reduced substrates by the enzyme in phosphate buffer, pH 6.0, μ = 0.01 (PaCCP) or in acetate buffer, pH 6.0, μ = 0.05 (YCCP). The PaCCP-reaction was initiated by mixing 10 μl of hydrogen peroxide solution with the reaction mixture (2.0 ml), as previously described.[2] The YCCP-reaction was initiated by mixing 5 μl of suitably diluted enzyme with the reaction mixture (2.0 ml) containing varying amounts of reduced cytochrome and peroxide. The reaction was followed by recording the disappearance of electron donors or the appearance of products. All velocities were measured on a Cary 15 recording spectrophotometer with cell compartment thermostated at 25 °C. Reaction mixtures were incubated at 25 °C before the reaction was initiated. Initial velocities were determined from the slopes of the recorded lines and expressed in terms of mol reduced substrate oxidized per mol of enzyme per second.

Isoelectric focusing was carried out according to Vesterberg and Svensson [15] in a LKB 8101 (110 ml) electrofocusing column, following the LKB manual. The density gradient was produced with sucrose (0 − 50 % w/v) and the pH gradient in the range 4 − 8 (for cytochromes) and 4 − 6 (for azurin) with carrier ampholytes (LKB-Produkter AB, Stockholm) at a concentration of 1 % (w/v). When necessary, the samples were dialyzed against 1 % glycine. Electrophoresis was usually performed for 22 − 24 h at 500 V and 9 − 15 °C. The pH of the fractions was measured with a Radiometer PHM 4 c pH meter at the same temperature as the electrophoresis was run.

Disc electrophoresis was carried out in polyacrylamide gel according to the procedures of Ornstein and Davis [16] and Maurer.[17] The basic gel system No. 1a of Maurer (pH 8.9, 7 % gel) was used. The protein bands were stained with Coomassie brilliant blue G-250 by the method of Diezel *et al.*[18]

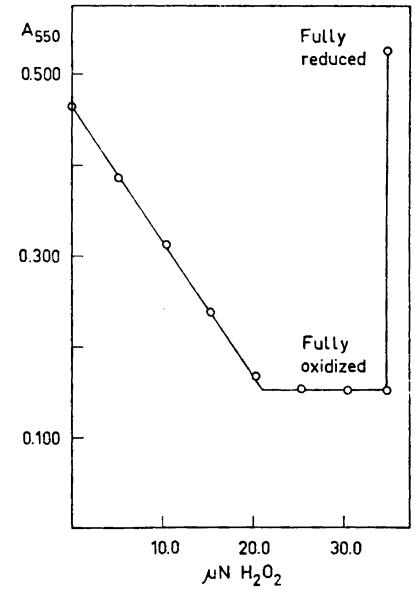


Fig. 1. Enzymatic titration of Pa-cyt-550 with H_2O_2 in the presence of 33 nM PaCCP in sodium phosphate buffer, pH 6.0, μ = 0.01 at 23 °C. Absorbances were measured with a Beckman DU-2 spectrophotometer.

Instruments. Spectrophotometric measurements were performed with a Beckman DU-2 spectrophotometer. Enzymatic activities were measured with a Cary 15 recording spectrophotometer equipped with cell compartment thermostated at 25 °C. pH was measured with a Radiometer pH meter 27 fitted with a combination glass-calomel electrode. Beckman pH 7 buffer No. 3501 was used for standardization.

Reagents were of analytical grade except those used in the cultivation medium for bacteria, which were of technical grade.

RESULTS

Properties of substrates. PaCCP was used for the determination of the extinction coefficients of Pa-cyt-550, Pa-cyt-555, and Pf-cyt-551 by enzymatic titration with standardized H_2O_2. The PaCCP preparation used showed no oxidase or catalase activity, nor did the substrates undergo significant autoperoxidation under these conditions.

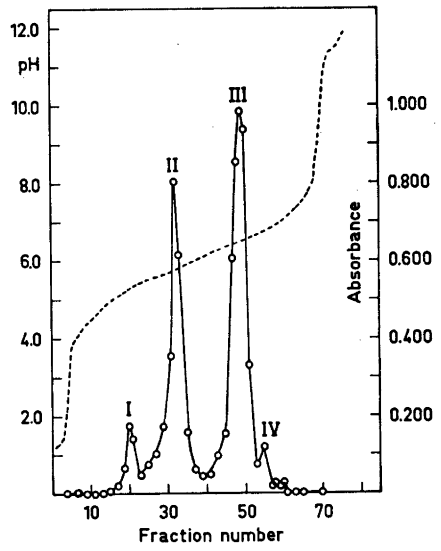


Fig. 2. Isoelectric focusing of the cytochrome c-554 "crude" preparation from *P. aeruginosa* in a pH 4 to 8 gradient at 14 °C. The experiment was carried out as described under Materials and Methods. pH is represented by the dotted line; O, absorbance at 416 nm. Fraction I represents reduced cytochrome c-555, fraction II the same cytochrome in oxidized form and fraction III oxidized cytochrome c-550. Fraction IV is due to a cytochrome (probably partly denatured) giving reduced α-peak at 553 nm.

Pa-cyt-550. Duplicate titrations were performed with Pa-cyt-550 in the presence of 0.033 μM PaCCP. The difference extinction coefficient, $\Delta\varepsilon_{550}$(red.-ox.), obtained from the slope of the titration curve was 14.9 mM^{-1} cm^{-1} in both determinations. The values of the extinction coefficient of reduced Pa-cyt-550 were 21.0 and 20.8, average 20.9 mM^{-1} cm^{-1}, while those of oxidized Pa-cyt-550 were 6.1 and 5.9, average 6.0 mM^{-1} cm^{-1}. The titration curve is shown in Fig. 1.

The isoelectric focusing of cytochrome c-554 isolated from *P. aeruginosa* [11] gave three major components (Fig. 2), one of which corresponds to Pa-cyt-550 in the oxidized form and gives the isoelectric point of 6.5.

Pa-cyt-555. The extinction coefficients of Pa-cyt-555 were determined enzymatically in duplicate. Difference extinction coefficients, $\Delta\varepsilon_{555}$(red.-ox.), of 17.3 and 17.4 mM^{-1} cm^{-1}, average 17.4 mM^{-1} cm^{-1}, were obtained from the slopes of the titration curves (Fig. 3). The values for reduced Pa-cyt-555, ε_{555}(red.), were 24.5 and 24.6 mM^{-1} cm^{-1}, average 24.6 mM^{-1} cm^{-1}, while 7.2 mM^{-1} cm^{-1} was found for oxidized Pa-cyt-555, ε_{555}(ox.), in both titrations.

The isoelectric points (Fig. 2) of reduced

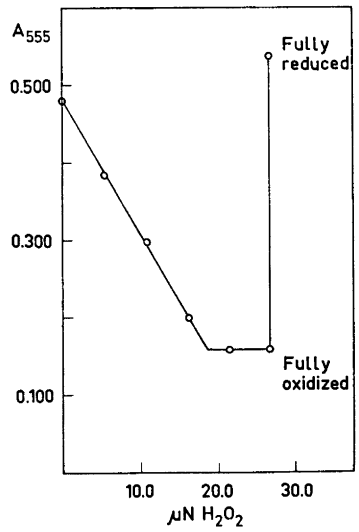


Fig. 3. Enzymatic titration of Pa-cyt-555 with H_2O_2 in the presence of 33 nM PaCCP in sodium phosphate buffer, pH 6.0, $\mu = 0.01$, at 23 °C. Absorbances were measured with a Beckman DU-2 spectrophotometer.

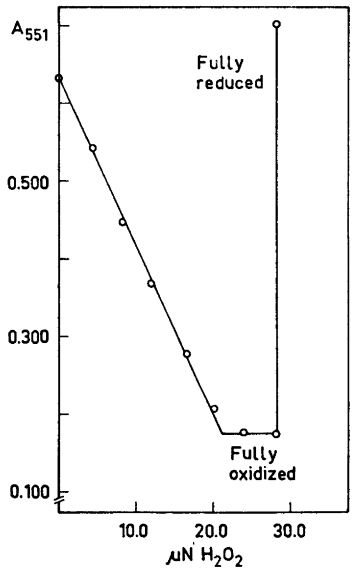


Fig. 4. Enzymatic titration of Pf-cyt-551 with H_2O_2 in the presence of 24 nM PaCCP in sodium phosphate buffer, pH 6.0, $\mu = 0.01$, at 23 °C. Absorbances were measured with a Beckman DU-2 spectrophotometer.

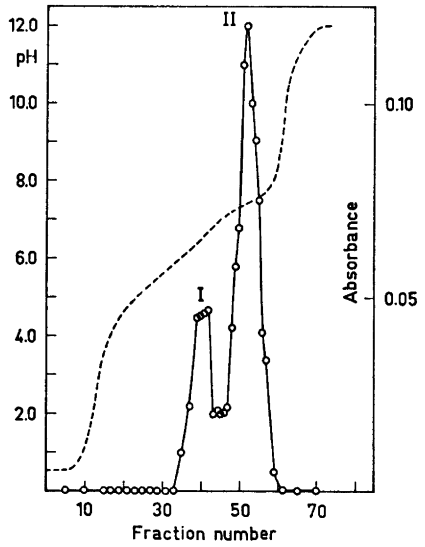


Fig. 5. Isoelectric focusing of cytochrome *c* (551 *P. fluorescens*) in a pH 4 to 8 gradient at 12 °C. Experimental details are described under Materials and Methods. O absorbance at 408 nm, pH is represented by the dotted line. Component I corresponds to the reduced and Component II to the oxidized form of the cytochrome.

and oxidized Pa-cyt-555 were 5.2 and 5.7, respectively.

Pf-cyt-551. Duplicate titrations (Fig. 4) of Pf-cyt-551 gave a difference extinction coefficient, $\Delta\varepsilon_{551}$(red.-ox.), of 21.8 and 22.0 mM^{-1} cm^{-1}, average 21.9 mM^{-1} cm^{-1}. The values for the reduced Pf-cyt-551, ε_{551}(red.), were 29.0 and 29.5, average 29.3 mM^{-1} cm^{-1}; those of the oxidized cytochrome, ε_{551}(ox.), 7.2 and 7.5 mM^{-1} cm^{-1}, average 7.4 mM^{-1} cm^{-1}.

Isoelectric focusing of Pf-cyt-551 (Fig. 5) gave the pI-values 6.7 and 7.3 for the reduced and oxidized form of the cytochrome, respectively.

Azurin. A pI-value of 5.6 (measured at 18 °C) was obtained for *Pseudomonas* azurin in the oxidized form. This is in good agreement with the literature value of 5.4.[19]

Initial velocity patterns of PaCCP. All initial rate measurements were made in sodium phosphate buffer, pH 6.0, $\mu = 0.01$. The concentrations of Pa-cyt-550 varied in the range 7 – 16 μM and hydrogen peroxide in the range 50 – 180 μM. Initial velocity measurements with Pa-cyt-555 as substrate were carried out in the concentration range 8 – 28 μM, while $[H_2O_2]$ was varied from 20 – 205 μM. The concentration range of Pf-cyt-551 was 7 – 23 μM and of H_2O_2 20 – 210 μM. Initial velocity measurements with *Pseudomonas* azurin as substrate were carried out in the concentration range 12 – 40 μM with H_2O_2 varied from 30 to 300 μM. The enzyme concentration employed (0.32 μM) was 200 times larger with horse heart cytochrome *c* than with bacterial cytochromes. The H-cyt concentration varied in the range 7 – 20 μM and H_2O_2 from 20 to 206 μM.

Within experimental error, and over the concentration ranges shown, all the plots are linear (Figs. 6, 7, 10, 11, 14, 15) and the results are therefore consistent with the initial-rate equation of Dalziel [20]

$$\frac{e}{v_0} = \phi_0 + \frac{\phi_1}{[S_1]} + \frac{\phi_2}{[S_2]} + \frac{\phi_{12}}{[S_1][S_2]} \qquad (1)$$

The secondary plots of intercepts and slopes were linear as well, and the kinetic coefficients ϕ_0, ϕ_1, ϕ_2, ϕ_{12} in eqn. 1 calculated from them are shown in Table 1. Even though the lines of the Lineweaver-Burk plots (Figs. 6 and 10) are only weakly intersecting when peroxide

Table 1. Kinetic coefficients for the peroxidatic oxidation reaction catalyzed by *Pseudomonas* cytochrome *c* peroxidase at 25 °C in sodium phosphate buffer, pH 6.0. The kinetic coefficients are those in the initial-rate equation

$$\frac{e}{v_0} = \phi_0 + \frac{\phi_1}{[S_1]} + \frac{\phi_2}{[S_2]} + \frac{\phi_{12}}{[S_1][S_2]}$$

where S_1 is taken as H_2O_2 and S_2 as the electron donor. Assuming a compulsory-order mechanism, the following relationships exist between the kinetic coefficients (Dalziel coefficients) and the rate constants: $k_3 = 1/\phi_0$; $k_1 = 1/\phi_1$; $k_2 = 1/\phi_2$; K_m of $S_2 = \phi_2/\phi_0$; K_m of $S_1 = \phi_1/\phi_0$; $K_s = k_{-1}/k_1 = \phi_{12}/\phi_2$; $k_{-1} = \phi_{12}/\phi_1\phi_2$. Rate constants refer to the reactions in Scheme 1.

Substrate	ϕ_0 s	ϕ_1 M s	ϕ_2 M s	ϕ_{12} M^2 s	ϕ_2/ϕ_0 M	ϕ_1/ϕ_0 M	ϕ_{12}/ϕ_2 M	$\phi_{12}/\phi_1\phi_2$ s^{-1}
Pa-cyt-551 [a]	1.6×10^{-3}	1.0×10^{-8}	1.4×10^{-7}	0.2×10^{-12}	8.8×10^{-5}	6.3×10^{-6}	1.4×10^{-6}	143 [b]
Pa-cyt-550	1.6×10^{-3}	1.6×10^{-8}	1.1×10^{-7}	0.8×10^{-12}	6.9×10^{-5}	1.0×10^{-5}	7.3×10^{-6}	455
Pa-cyt-555	2.0×10^{-3}	1.8×10^{-8}	1.8×10^{-7}	0.6×10^{-12}	9.0×10^{-5}	0.9×10^{-5}	3.3×10^{-6}	185
Azurin	1.4×10^{-3}	1.5×10^{-8}	1.7×10^{-7}	0.5×10^{-12}	1.2×10^{-4}	1.1×10^{-5}	2.9×10^{-6}	196
Pf-cyt-551	1.6×10^{-3}	1.3×10^{-8}	1.1×10^{-7}	0.1×10^{-12}	6.9×10^{-5}	0.8×10^{-5}	0.9×10^{-6}	70
Horse heart cytochrome	0.1	4.0×10^{-6}	3.0×10^{-5}	3.2×10^{-10}	3.0×10^{-4}	4.0×10^{-5}	1.1×10^{-5}	2.7

[a] The data for Pa-cyt-551 are from Ellfolk *et al.*[2] [b] The value of this relationship was misprinted in the earlier paper.[2]

Table 2. Kinetic coefficients for the peroxidatic oxidation reaction catalyzed by yeast cytochrome *c* peroxidase at 25 °C in sodium acetate buffer, pH 6.0. The kinetic coefficients are those in the initial-rate equation

$$\frac{e}{v_0} = \phi_0 + \frac{\phi_1}{[S_1]} + \frac{\phi_2}{[S_2]} + \frac{\phi_{12}}{[S_1][S_2]}$$

where S_1 is the peroxide and S_2 the electron donor. The following relationships exist between the kinetic coefficients and the rate constants of Scheme 1: $k_3 = 1/\phi_0$; $k_1 = 1/\phi_1$; $k_2 = 1/\phi_2$; K_m of $S_2 = \phi_2/\phi_0$; K_m of $S_1 = \phi_1/\phi_0$; $K_s = k_{-1}/k_1 = \phi_{12}/\phi_2$; $k_{-1} = \phi_{12}/\phi_1\phi_2$.

Substrate	ϕ_0 s	ϕ_1 M s	ϕ_2 M s	ϕ_{12} M^2 s	ϕ_2/ϕ_0 M	ϕ_1/ϕ_0 M	ϕ_{12}/ϕ_2 M	$\phi_{12}/\phi_1\phi_2$ s^{-1}
Horse heart [a] cytochrome *c*	3.8×10^{-4}	7.1×10^{-9}	1.7×10^{-9}	—	4.5×10^{-6}	1.9×10^{-5}	$\sim10^{-8}$	$\sim10^{0}$
Horse heart cytochrome *c*	5.5×10^{-4}	9.8×10^{-9}	2.8×10^{-9}	1.3×10^{-14}	5.1×10^{-6}	1.8×10^{-5}	4.6×10^{-6}	474
Pa-cyt-551	0.5	5.0×10^{-6}	2.1×10^{-5}	7.0×10^{-11}	4.2×10^{-5}	1.0×10^{-5}	3.3×10^{-6}	0.7

[a] Calculated from the data of Yonetani and Ray (1966).

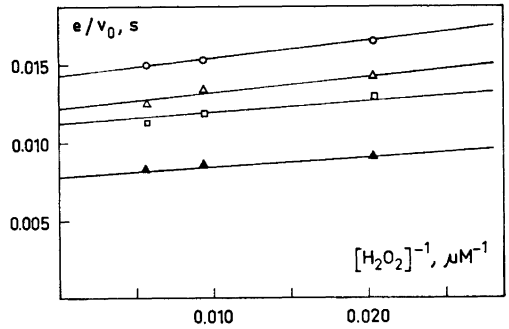


Fig. 6. Primary plots: variation of the reciprocal of the specific initial rate in sodium phosphate buffer at pH 6.0 and 25 °C with the reciprocal of the H_2O_2 concentration for several constant concentrations of reduced Pa-cyt-550 (μM): ○, 8.0; △, 10.9; □, 12.0; ▲, 16.4. The concentration of PaCCP was 1.51 nM.

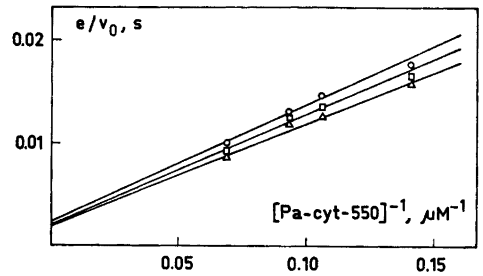


Fig. 7. Primary plots: variation of the reciprocal of the specific initial rate with the reciprocal of the Pa-cyt-550 concentration for several constant concentrations of H_2O_2 (μM): ○, 49.3; □, 108.0; △, 178.0.

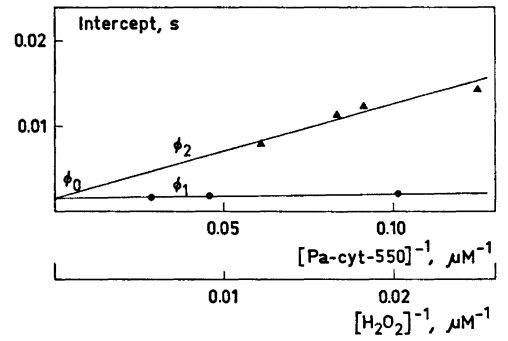


Fig. 8. Secondary plots of primary plot intercepts *versus* the reciprocal of fixed substrate concentration. Triangles and circles, respectively, represent data taken with Pa-cyt-550 and H_2O_2 as the fixed substrate. Kinetic coefficients calculated from the plot are given in Table 1.

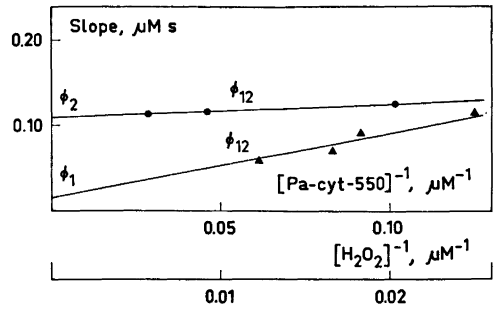


Fig. 9. Secondary plots of primary plot slopes *versus* the reciprocal of fixed substrate concentration. Triangles and circles, respectively, represent data taken with Pa-cyt-550 and H_2O_2 as the fixed substrate. Kinetic coefficients calculated from the plot are given in Table 1.

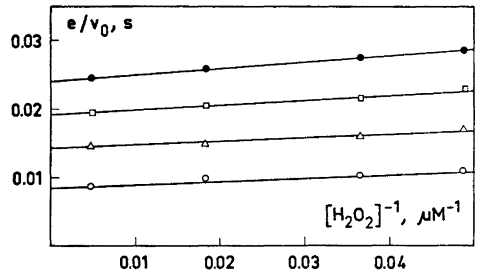


Fig. 10. Primary plots: variation of the reciprocal of the specific initial rate in sodium phosphate buffer at pH 6.0 and 25 °C with the reciprocal of the H_2O_2 concentration for several constant concentrations of reduced Pa-cyt-555 (μM): ●, 8.2; □, 11.0; △, 15.8; ○, 28.2. The concentration of PaCCP was 1.62 nM.

is the variable substrate, evidence for the presence of a ϕ_{12}-term in eqn. 1 is supplied from Slater-plots.[21] These plots of apparent K_m values against apparent maximum velocities pass through the origin in the absence of the ϕ_{12}-term, whereas in the reactions of PaCCP and YCCP Slater-plots intersect the ordinate at a positive value. Thus a sequential reaction mechanism can be proposed for these peroxidases (Scheme 1).

Initial velocity patterns of YCCP. All initial velocity measurements were made in sodium acetate buffer, pH 6.0, $\mu = 0.05$. The concentration of horse heart cytochrome *c* varied in the range 2 – 12 μM and hydrogen peroxide in the range 2 – 180 μM. The YCCP-concentration (90.5 nM) used with Pa-cyt-551 was more

$$E + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1$$

$$ES_1 + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_1S_2$$

$$ES_1S_2 \xrightarrow{k_3} E + P$$

Scheme 1.

than 300 times that employed in the H-cyt-reaction; the concentration of Pa-cyt-551 varied in the range 5 – 17 μM and H_2O_2 from 16 to 170 μM. The primary Lineweaver-Burk plots drawn from the data (Figs. 14, 15) are linear, and the kinetic coefficients in eqn. 1 could be estimated from the secondary plots of intercepts (Fig. 16) and slopes (Fig. 17). The kinetic coefficients of the YCCP-reaction are shown in Table 2.

DISCUSSION

Steady state experiments alone can distinguish between a random substrate addition mechanism and an ordered one by using Dalziel coefficients,[20] Haldane relationships,[22] and product inhibition studies.[23,24] Although product inhibition studies may resolve some mechanistic differences when used in one reaction direction only, a full kinetic diagnosis for a particular enzyme system using any of these approaches requires data from both forward and reverse reactions.

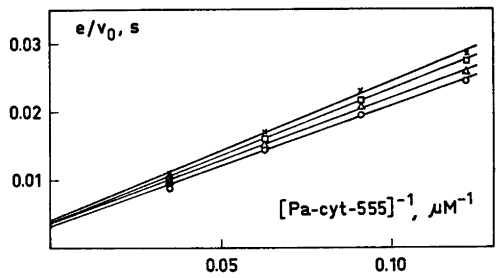


Fig. 11. Primary plots: variation of the reciprocal of the specific initial rate with the reciprocal of the Pa-cyt-555 concentration for several constant concentrations of H_2O_2 (μM): ×, 20.6; □, 27.4; △, 54.8; ○, 205.5.

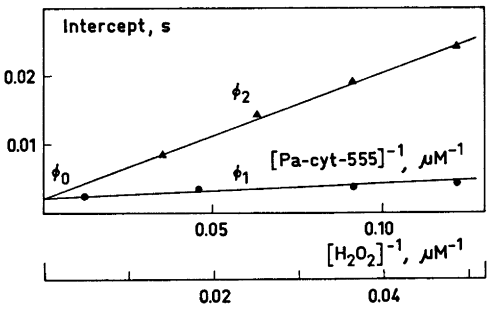


Fig. 12. Secondary plots of primary plot intercepts *versus* the reciprocal of fixed substrate concentration. Triangles and circles, respectively, represent data taken with Pa-cyt-555 and H_2O_2 as the fixed substrate. Kinetic coefficients calculated from the plot are given in Table 1.

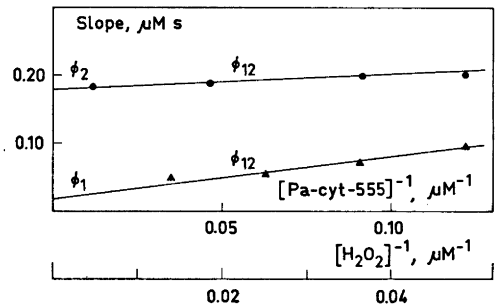


Fig. 13. Secondary plots of primary plot slopes *versus* the reciprocal of fixed substrate concentration. Triangles and circles, respectively, represent data taken with Pa-cyt-555 and H_2O_2 as the fixed substrate. Kinetic coefficients calculated from the plot are given in Table 1.

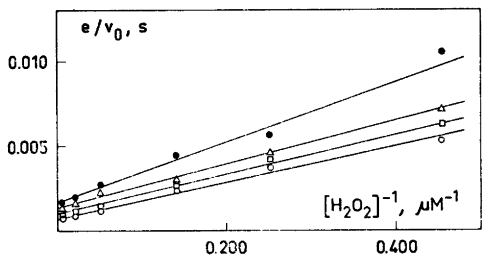


Fig. 14. Primary plots; variation of the reciprocal of the specific initial rate in sodium phosphate buffer pH 6.0 and 25 °C with the reciprocal of the H_2O_2 concentration for several constant concentrations of reduced horse heart cytochrome *c* (μM): ●, 2.1; △, 3.8; □, 8.2; ○, 12.5. The concentration of yeast cytochrome *c* peroxidase was 0.35 nM.

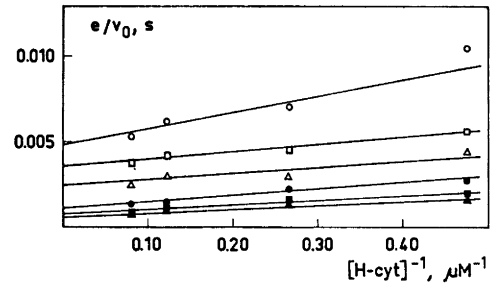


Fig. 15. Primary plots: variation of the reciprocal of the specific initial rate with the reciprocal of the horse cytochrome *c* concentration for several constant concentrations of H_2O_2 (μM): ○, 2.2;, □, 4.0; △, 7.1; ●, 19.6; ■, 49.5; ▲, 178.0.

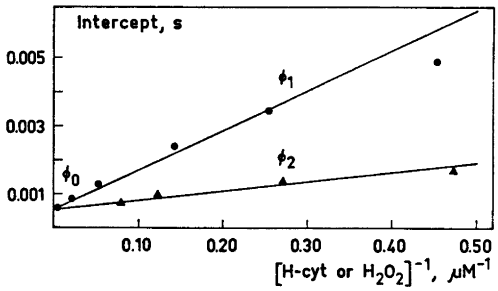


Fig. 16. Secondary plots of primary plot intercepts *versus* the reciprocal of fixed substrate concentration. Triangles and circles, respectively, represent data taken with horse heart cytochrome *c* and H_2O_2 as the fixed substrate. Kinetic coefficients calculated from the plot are given in Table 2.

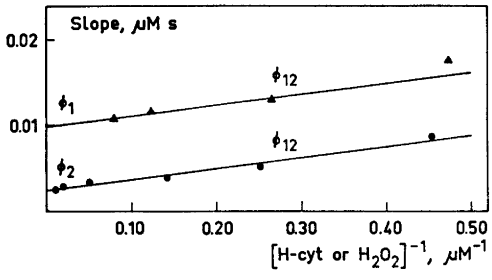


Fig. 17. Secondary plots of primary plot slopes *versus* the reciprocal of fixed substrate concentration. Triangles and circles, respectively, represent data taken with horse heart cytochrome *c* and H_2O_2 as the fixed substrate. Kinetic coefficients calculated from the plot are given in Table 2.

Intersecting initial velocity patterns led to the proposal of a sequential reaction mechanism for the peroxidatic oxidation of cytochrome *c* (551 *P. aeruginosa*) by *Pseudomonas* cytochrome *c* peroxidase.[2] Because of the "irreversible" nature of the peroxidase reaction the conventional kinetic approaches mentioned above are not conclusive. Another way of tackling the mechanistic problem of the PaCCP-reaction is to determine the kinetic coefficients with several substrates, which we have done with cytochromes *c* 550 and 555 (*P. aeruginosa*) and cyt *c* (551 *P. fluorescens*) as well as with a copper-containing protein, the blue protein or azurin from *P. aeruginosa*.

The values of ϕ_0 and ϕ_1 for the PaCCP-reaction (Table 1) with each of these substrates are almost constant, and the identity of these values is expected for the general compulsory-order mechanism.[25] Nevertheless, for a compulsory-order mechanism the ratio ϕ_{12}/ϕ_2 should also be independent of the nature of the substrate. In fact, however, the ratio varies from substrate to substrate. This variation may, at least partly, be due to the fact that the ϕ_{12}-values are very small so that the ratio cannot be calculated with certainty.

PaCCP seems to have similar reactivity with all the bacterial protein substrates used. The K_m-values of the cytochromes vary from 69 μM (Pf-cyt-551) to 90 μM (Pa-cyt-555) and the Michaelis constant for *Pseudomonas* azurin is 120 μM. These values are remarkably similar considering that the protein substrates differ widely in some molecular properties, *e.g.* isoelectric point and molecular weight. Furthermore, azurin differs from cytochromes even with respect to the prosthetic group. In contrast, horse heart cytochrome *c* is a poor substrate for PaCCP since all the kinetic coefficients are greatly increased compared to those of the bacterial electron donors. The higher value of ϕ_0 also suggests that the intramolecular reaction of the ternary complex is slower than with bacterial cytochromes.[25]

All the kinetic coefficients of the YCCP-reaction (Table 2) are substantially greater with the bacterial cytochrome Pa-cyt-551 than with horse heart cytochrome, indicating that Pa-cyt-551 is a poor substrate for YCCP. Nevertheless, both the Michaelis constant for H_2O_2 (ϕ_1/ϕ_0) and the ratio ϕ_{12}/ϕ_2 are roughly

independent of the nature of the electron donor. The constancy of the ratio ϕ_{12}/ϕ_2 agrees with a compulsory order mechanism. Because an ordered mechanism also requires the identity of the ϕ_0 and ϕ_1 values for different substrates,[25] and the bacterial cytochrome does not satisfy these criteria in the YCCP-reaction, the mechanism of the enzyme cannot be deduced from these results. The reaction mechanism of YCCP is currently a subject of some controversy. YCCP has been assumed to follow a general compulsory-order mechanism when mammalian-type cytochromes are used as substrates,[26] whereas Nicholls and Mochan,[27] working from the formation of reversible complexes between YCCP and cytochrome *c*, have recently proposed a random-addition mechanism for YCCP.

Electrostatic forces are known to be predominant in cytochrome *c* complex formation with yeast cytochrome *c* peroxidase.[27,28] Accordingly, full activity is obtained with YCCP (pI 5.2)[29] and oppositely charged basic substrates like horse heart cytochrome *c* (pI 10.05),[30] but not with the acidic Pa-cyt-551 (pI 4.7).[31] With the bacterial cytochrome, however, the selective specificity of YCCP may also affect the reaction rate. In the case of PaCCP (pI 6.7),[3] forces of an electrostatic nature seem not to be of importance in the formation of enzyme-substrate complexes with acidic or neutral cytochromes, *i.e.* Pa-cyt-551 (pI 4.7), Pa-cyt-550 (pI 6.5), and Pf-cyt-551 (pI 6.7), since reaction velocities with these different substrates are closely similar. On the other hand, the reaction velocity is markedly lowered with horse heart cytochrome *c* (Table 1). As with YCCP, the specificity of the enzyme should also be considered when explaining reduced activity. Moreover, the isoelectric points of the substrates do not necessarily reflect the charge of the contact area with the enzymes.

It seems that YCCP and PaCCP are kinetically different and cannot be directly compared with each other even if both should follow an ordered mechanism. It has to be borne in mind, too, that PaCCP, unlike YCCP, exhibits an initial delay before reacting if the enzyme is not incubated with the electron donor before addition of H_2O_2. Because of this initial delay the true nature of the PaCCP-reaction remains to be established.

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Reactions between Furfurylidenemalonic Esters and Grignard Reagents. IV. Diethyl 3-Methylfurfurylidenemalonate

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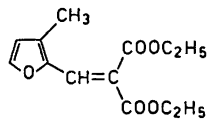
When *t*-butylmagnesium chloride reacts with diethyl 3-methylfurfurylidenemalonate, reduction, 1,4-, 1,6-, and 1,8-addition products are formed. The methyl group in position 3 of the furan nucleus does not have any blocking effect on the 1,6-addition reaction but only one product, the *trans* isomer, is formed. Methylmagnesium iodide, ethylmagnesium bromide, isopropylmagnesium bromide, and isobutylmagnesium chloride react in the same way as with diethyl furfurylidenemalonate and its 5-methyl derivative.

The rearrangements of the 1,6- and 1,8-addition products of *t*-butylmagnesium chloride and furfurylidenemalonic esters are discussed.

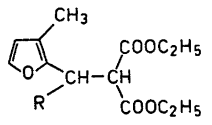
The reactions of Grignard reagents with furfurylidenemalonic ester and its 5-methyl homologue have previously been studied in this laboratory.[1–3] The main reaction in all known cases is a 1,4-addition of the Grignard reagent to the conjugated double bond system C=C–C=O. This reaction is partly suppressed by reduction of the olefinic double bond of the ester if the alkyl group of the Grignard reagent is branched at the α or β carbon atom and there is at least one hydrogen atom on the β carbon atom.[1] Benzylmagnesium chloride gives 1,4- and 1,8-additions with diethyl furfurylidenemalonate.[1] The latter reaction implies an extension of the conjugated double bond system to the furan nucleus. *t*-Butylmagnesium chloride gives 1,6- and 1,8-addition together with 1,4-addition and reduction with diethyl furfurylidenemalonate.[2] If diethyl 5-methylfurfurylidenemalonate is substituted for diethyl furfurylidenemalonate in the reaction with *t*-butylmagnesium chloride, reduction, 1,4- and 1,8-additions are observed.[3] No 1,8-addition occurs because the methyl group in position 5 of the furan nucleus of the ester apparently prevents the *t*-butyl group from entering at this position.

The reduction and 1,4-addition products, furfurylmalonic ester, 5-methylfurfurylmalonic ester, and their homologues alkylated in the β position, are, of course, quite stable while the dihydrofuran derivatives which are formed in the 1,6- and 1,8-addition reactions are unstable and rearrange prototropically in acid solution to furan derivatives. The 1,8-addition products seem to be so unstable that they rearrange immediately after their formation from the primary reaction products, which still contain magnesium.[1,2] Unlike these compounds, the 1,6-addition products are stable enough to allow their detection by combined gas chromatography/mass spectrometry.[2,3] Attempts to isolate them in a pure state have not been successful. The isolation of one rearranged 1,6-addition product, diethyl 3-*t*-butyl-5-methyl-furfurylmalonate, has, however, been achieved.[3]

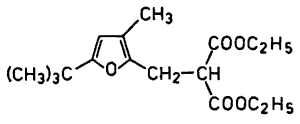
The observed blocking effect of the methyl group in position 5 of the furan nucleus of diethyl 5-methylfurfurylidenemalonate justified an examination of the reactions between *t*-butylmagnesium chloride and diethyl 3-methylfurfurylidenemalonate (I). A gas chromatographic analysis of the products of this reaction on a column containing OV-17 as the stationary phase showed that four compounds are formed. None of them rearrange in acid solution. The components of the reaction mixture were separated by preparative gas chromatography. The high temperature that had to be used caused pyrolysis to some extent but the fractions were pure enough to allow identification of the sub-



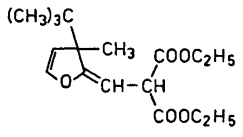
I



II



III



IV

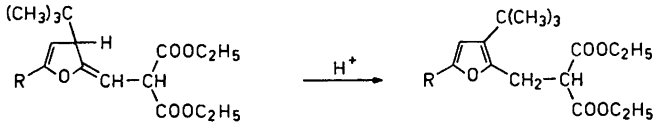
stances. The NMR and mass spectra of the fractions show that diethyl 3-methylfurfurylmalonate (II; R = H), diethyl 2,2-dimethyl-1-(3′-methyl-2′-furyl)propylmalonate [II; R = $(CH_3)_3C$], diethyl 5-*t*-butyl-3-methylfurfurylmalonate (III), and diethyl 3-*t*-butyl-3-methyl-2,3-dihydro-2-furylidenemetylmalonate (IV) are formed in the reaction. The presence of diethyl 3-methylfurfurylmalonate and diethyl 5-*t*-butyl-3-methylfurfurylmalonate was proved by comparison of the spectra with spectra of authentic samples obtained by independent syntheses.

These results show that *t*-butylmagnesium chloride gives reduction, 1,4-, 1,6-, and 1,8-additions with diethyl 3-methylfurfurylidenemalonate. The molar ratio of the products was 7:40:19:34 in the present experiments. No blocking effect of the methyl group in position 3 was observed.

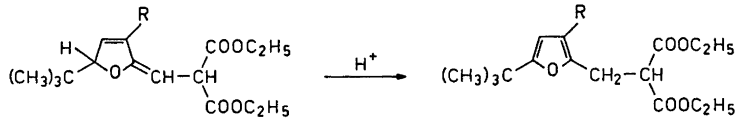
Both diethyl furfurylidenemalonate and its 5-methyl homologue give two 1,6-addition products, which are *cis* and *trans* isomers.[2,3] Diethyl 3-methylfurfurylidenemalonate gives only one 1,6-addition product. For sterical reasons, it is clear that the product is the *trans* isomer.

The structures of the 1,6-addition products (V; R = H or CH_3) of *t*-butylmagnesium chloride with diethyl furfurylidenemalonate and its 5-methyl homologue which rearrange prototropically in acid solution to 3-*t*-butylfuran derivatives (VI) have up till now been proved only by deductions based on mass spectra and the structure of one rearranged product.[2,3] Consequently, the results might have been called in question. The isolation of diethyl 3-*t*-butyl-3-methyl-2,3-dihydro-2-furylidenemethylmalonate (IV), which cannot rearrange prototropically, strongly supports the proposed structures. This fact again shows the practicability of mass spectrometry for determining structures.

A series of experiments were performed with methylmagnesium iodide, ethylmagnesium bromide, isopropylmagnesium bromide, and isobutylmagnesium chloride in order to examine whether these Grignard reagents give rise to 1,6- and 1,8-additions as well as the 1,4-addition but the results were as negative as those with diethyl furfurylidenemalonate and its 5-methyl homologue.[1,3] The reagents give reduction and 1,4-addition with diethyl 3-methylfurfurylidene-



V VI



VII VIII

malonate in the same way as in the previously reported experiments.

The unrearranged 1,8-addition products (VII; R = H or CH_3) of *t*-butylmagnesium chloride with diethyl furfurylidenemalonate and its 3-methyl homologue have not been detected directly in any of the examined cases. Their intermediate presence in the reaction mixtures has been deduced by analogy with the 1,6-addition products and from the structures of the rearranged compounds (VIII). Any plausible explanation as to why the 1,8-addition products are less stable than the 1,6-addition products has not been given.

The unrearranged addition products (V and VII; R = H or CH_3) can be considered to be vinyl ethers, the resonance energies of which are relatively low (14.1 kJ/mol in divinyl ether and 14.9 kJ/mol in ethyl vinyl ether).[4] The rearrangements imply formation of furan derivatives (VI and VIII) with high resonance energies (72 kJ/mol in unsubstituted furan [4]). Therefore, the total rate of the rearrangements ought to be high. This would explain why the primary 1,8-addition products (VII) have not been directly detected but it does not answer the question why the rearrangements of the 1,6-addition products (V) are so much slower. The reason for this retardation seems to be the steric strain in the rearranged products (VI) produced by the bulky groups in positions 2 and 3 of the furan nucleus.

A rough estimation of the steric strain in the rearranged 1,6-addition products (VI) can be performed in the following way. *cis*-Isobutyl-*t*-butylethylene can *mutatis mutandis* be compared with the compounds in question. Unfortunately, no determination of the steric strain in this hydrocarbon can be found in the literature. However, the steric strain in *cis*-*t*-butylmethylethylene (*Z*-4,4-dimethyl-2-pentene) and *cis*-di-*t*-butylethylene (*Z*-2,2,5,5-tetramethyl-3-hexene) has been calculated from the heats of hydrogenation of the *cis* and *trans* isomers. Turner, Nettleton, and Perelman obtained values in kcal/mol that on conversion give 17.9 and 39.2 kJ/mol, respectively,[5] while the values obtained by Rockenfeller and Rossini give 16.4 and 44.0 kJ/mol.[6] The steric strain of *cis*-isobutyl-*t*-butylethylene must be between these extremes and it can be concluded that the steric strain in the rearranged 1,6-addition products (VI) is large enough to explain why the rates of the rearrangements of the 1,6-addition products are retarded.

EXPERIMENTAL

3-Methylfurfural. Vilsmeyer formulation of 3-methylfuran [7] according to Kutney, Hanssen, and Nair [8] gave an oil, b.p. 69 – 71 °C/2.4 kPa. It was used in the synthesis of diethyl 3-methylfurfurylidenemalonate without removal of the small quantity of 4-methylfurfural.

5-t-Butyl-3-methylfurfural was prepared from 2-*t*-butyl-4-methylfuran [9] in the same way as 3-methylfurfural. The yield was 87 % of material boiling at 94 – 95 °C/1.05 kPa. NMR spectrum: 4 singlets; aldehyde proton at δ 9.54; H-4 proton at δ 5.92; methyl protons at δ 2.26; *t*-butyl protons at δ 1.30.

Diethyl 3-methylfurfurylidenemalonate (I) was prepared from 3-methylfurfural and diethyl malonate by the method previously used in this laboratory.[1,3] The ester, b.p. 197 °C/2.95 kPa, was purified by recrystallisation from ligroin. The yield of pure material, m.p. 70.5 – 71.5 °C, was 64 %. (Found: C 62.09; H 6.30. Calc. for $C_{13}H_{16}O_5$: C 61.90; H 6.39.) NMR spectrum and MS confirm the structure.

Diethyl-5-t-butyl-3-methylfurfurylidenemalonate was prepared from 5-*t*-butyl-3-methylfurfural and diethyl malonate by the same method. The pure ester melted at 67 – 68 °C after distillation under reduced pressure (b.p. 175 – 180 °C/2.0 kPa) and recrystallisation from ligroin. (Found: C 66.38; H 7.80. Calc. for $C_{17}H_{24}O_5$: C 66.21; H 7.85.) NMR spectrum and MS confirm the structure.

Diethyl 3-methylfurfurylmalonate (II; R = H). Diethyl 3-methylfurfurylidenemalonate (10 g) was dissolved in ethanol (150 ml) and hydrogenated at room temperature and atmospheric pressure in the presence of freshly prepared and thoroughly washed Raney nickel [10] (from 2.0 g of nickel alloy). The catalyst was filtered off and diethyl 3-methylfurfurylmalonate, b.p. 154 – 155 °C/1.9 kPa, was distilled. (Found: C 61.19; H 7.04. Calc. for $C_{13}H_{18}O_5$: C 61.41; H 7.14.) NMR spectrum and MS confirm the structure.

Diethyl-5-t-butyl-3-methylfurfurylmalonate (III) was prepared from diethyl 5-*t*-butyl-3-methylfurfurylidenemalonate in the same way as the preceding ester. The pure compound boiled at 166 °C/1.3 kPa. (Found: C 65.58; H 8.33. Calc. for $C_{17}H_{26}O_5$: C 65.78; H 8.44.) NMR spectrum and MS confirm the structure.

The reactions of diethyl 3-methylfurfurylidenemalonate with methylmagnesium iodide, ethylmagnesium bromide, isopropylmagnesium bromide and isobutylmagnesium chloride were performed on the semimicro and macro scales exactly using the previously described method.[1] The results were analogous to those obtained with diethyl furfurylidenemalonate and its 5-

methyl homologue.[1,3] Methylmagnesium iodide gave diethyl 1-(3′-methyl-2′-furyl)ethylmalonate (II; R = CH_3) and ethylmagnesium bromide diethyl 1-(3′-methyl-2′-furyl)propylmalonate (II; R = C_2H_5). Diethyl 3-methylfurfurylmalonate (II; R = H) and diethyl 2-methyl-1-(3′-methyl-2′-furyl)propylmalonate [II; R = $(CH_3)_2CH$] were formed in the experiments with isopropylmagnesium bromide. The molar ratio was 17:83. Isobutylmagnesium chloride gave diethyl 3-methylfurfurylmalonate (II; R = H) and diethyl 3-methyl-1-(3′-methyl-2′-furyl)butylmalonate [II; R = $(CH_3)_2CH-CH_2$] in the molar ratio of 22:78. Elemental analyses, NMR spectra, and MS confirm the structures.

The reactions of diethyl 3-methylfurfurylidenemalonate with t-butylmagnesium chloride. The experiments were performed using the method previously described. The reaction products were analysed by gas chromatography (column 3 mm × 1.5 m, stationary phase 1 % OV-17, nitrogen flow 28 ml/min, temperature of the oven 185 °C). Four peaks with relative retention times of 1.00 (compound A), 1.20 (compound B), 1.35 (compound C), and 1.59 (compound D) were obtained.

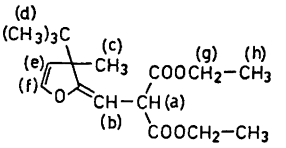
Compound A was shown to be diethyl 3-methylfurfurylmalonate (II; R = H) by comparing retention times and mass spectra (taken on a GC/MS combination instrument.

Compound B was isolated by preparative gas chromatography. Its NMR spectrum shows a simple AB quartet with the shifts at δ 3.69 and 3.48 (J = 9.0 Hz) and an AX quartet with the shifts at δ 7.02 and 5.95 (J = 1.8 Hz) as well as signals from two ester ethyl groups, one methyl, and one *t*-butyl group. These data reveal that substance B is diethyl 2-2,dimethyl-1-(3′-methyl-2′-furyl)propylmalonate [II; R = $(CH_3)_3C$]. (Found: C 65.58; H 8.24. Calc. for $C_{17}H_{26}O_5$: C 65.78; H 8.44). The MS of the substance confirms the structure.

Compounds C and D were not obtained in the pure state by preparative gas chromatography. The identity of compound C and diethyl 5-*t*-butyl-3-methylfurfurylmalonate (III) was therefore established by comparing their retention times and mass spectra.

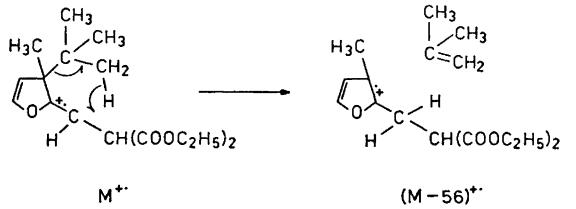
The purest sample of compound D, obtained by preparative gas chromatography, contained small quantities of compound C and diethyl 3-methylfurfurylmalonate. The latter compound is apparently a pyrolysis product of compound D. However, a rather good NMR spectrum of diethyl 3-*t*-butyl-3-methyl-2,3-dihydro-2-furylidenemethylmalonate (IV; *cf.* Table 1) is obtained if the relatively small signals in the NMR spectrum of the obtained sample arising from the impurities mentioned above are disregarded. The doublets of the protons *e* and *f* correspond to the furan protons H-4 and H-5. The fact that the signals are situated at higher field shows that the furan nucleus has reacted to form a dihydrofuran derivative. On the other hand, the chemical shifts of the protons *a* and *b* have moved to lower field as a consequence of the double bond between the β carbon atom and the dihydrofuran group.

Table 1. Chemical shift (δ) and coupling constants (J) in the NMR spectrum of compound D (diethyl 3-*t*-butyl-3-methyl-2,3-dihydro-2-furylidenemethylmalonate).



Proton	δ	Spin system	J in Hz
a	4.25	AB	9.2
b	4.68		
c	1.19		
d	0.90		
e	5.06	AB	2.9
f	6.33		
g	4.06, 4.05	A_2X_5	7.0
h	1.24, 1.22		

The mass spectrum of compound D is quite different in appearence to the spectra of the other compounds in this paper. The most important ions with high masses are found at *m/e* 310 (M^+), 254 (r.a. 11), 253 (5), 181 (35), 180 (71), and 135 (100). The formation of the ion at *m/e* 254 (=M − 56) is explained by the mechanism in Scheme 1. The ion at *m/e* 253 (=M − 57) is simply formed from the molecular



Scheme 1.

ion by splitting of the *t*-butyl group. The formation of the ion at *m/e* 181 is best explained by loss of an ethoxycarbonyl group from the ion at *m/e* 254. The type of reaction that leads to the formation of the ion at *m/e* 180 ($=M-COOC_2H_5-C_4H_9$) has previously been discussed.[2] The ion at *m/e* 135 ($= CH_3\text{-}C_4H_2O\text{-}CH{=}CH\text{-}CO^+$) seems to be a result of a stripping process: $M^+ \rightarrow$ the ion at *m/e* 180 $\rightarrow$ the ion at *m/e* $135 + \cdot OC_2H_5$.

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Binding of the Fluorescent Probes 1-Anilinonaphthalene-8-sulfonate and *N*-Phenyl-1-naphthylamine to Bacteria

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Changes in fluorescence intensities in *Pseudomonas fluorescens*, *Escherichia coli*, *Streptococcus thermophilus*, and *Bacillus subtilis* were investigated by means of the fluorescence probes (1-anilinonaphthalene-8-sulfonate and *N*-phenyl-1-naphthylamine) during a temperature programmed run.

A decrease in fluorescence intensity was observed in all investigated organisms when the temperature was elevated from 4 to about 40 °C. A strong increase in fluorescence intensity was found in gram-negative *Ps. fluorescens* and *E. coli* when the temperature was elevated over 40 °C. On the other hand, only a slight enhancement in fluorescence intensities was observed in gram-positive *Str. thermophilus* and *B. subtilis* when the lipid specific dye NPN was applied at elevated temperatures. Discontinuities in all the fluorescence temperature profiles were found between 20 and 30 °C in the case of both ANS and NPN. With ANS the fluorescence profiles turned upwards and with NPN downwards after the inflection points.

The effects of KCl, NH_4Cl, NaCl and LiCl on ANS binding differed from those detected for NPN. Saturation of probe binding was reached at lower salt concentrations in the case of NPN than ANS, but depended somewhat on temperature.

Location of the inflection points and fluorescence intensities depended on growth temperature and growth phase, as well as thermophilicity of the bacteria. When *Ps. fluorescens* and *Str. thermophilus* were cultured at critical growth temperatures (8 and 37 °C and 20 and 49 °C, respectively) the inflections between 20 and 30 °C were shifted toward higher temperatures with increasing growth temperature. Furthermore, there was a marked enhancement in both ANS and NPN fluorescence between 45 and 60 °C when growth proceeded from the lag to the stationary phase in *E. coli* and *Ps. fluorescens*. In *Str. thermophilus*, on the other hand, there was only a slight binding maximum between 45 and 60 °C; ANS binding increased with growth.

Changes in polarity and hydrophobicity in bacteria as a function of temperature are discussed.

The fluorescent dyes 1-anilinonaphthalene-8-sulfonate and *N*-phenyl-1-naphthylamine have often been used as probes of structural changes in proteins and lipids in cell constituents.[1–10] These dyes are non-fluorescent in aqueous liquids but undergo strong fluorescent enhancement in the presence of various membranes.[5] ANS reportedly binds at polar-apolar interphases within membranes that contain charged groups and NPN reflects changes in membrane lipids.[5] It has been suggested that increase in molecular rigidity rather than solvent polarity is the dominant factor in fluorescence intensity enhancement.[11] The molecular rigidity of cells changes with melting of the lipid fraction and the denaturation of proteins. Physical methods such as calorimetry,[12] X-ray diffraction,[13] and spectroscopy,[5] have been used increasingly to monitor the alterations in membranes induced by various conditions. Lipid compositions from several organisms show an increase in unsaturation with a decrease in growth temperature.[14–16] Furthermore, lipid composition may vary according to medium constituents, aeration and the growth phase.[17] In *Bacillus megaterium* NPN fluorescence has been found to increase with the growth of the bacteria, [18] reflecting the increase in lipid constituents during the growth cycle.

This paper describes changes in ANS and NPN fluorescence intensities in different microorganisms exposed to elevated temperatures. Discontinuities were observed both in ANS and

NPN fluorescence profiles in the temperature ranges 20 – 30 °C and 40 – 60 °C. Changes in growth temperatures and the physiological state of the cells resulted in shifts in the inflection points and affected fluorescence intensities.

MATERIALS AND METHODS

Test organisms. The organisms *Escherichia coli* U5/41, *Pseudomonas fluorescens* UK-1, *Streptococcus thermophilus* KQ and *Bacillus subtilis* ATCC 6633 were grown in 1 liter erlenmeyer flasks, each containing 200 – 500 ml of 0.5 % micro inoculum broth. The flasks were shaken on a rotary shaker (New Brunswick Scientific Co., G 10 Gyrotary Shaker) or aerated by aquarium pumps in water baths. Turbidity of the cultures was measured with a Klett-Summerson colorimeter, employing filter 62. The cells were harvested by centrifugation (5000 *g*, 15 min) when turbidity was about 100, and washed twice with 0.05 M phosphate buffer (pH 6.6). Samples of 20 ml were withdrawn from the different growth phases, centrifuged and washed as described above. The pellets were suspended in 0.05 M phosphate buffer (pH 6.6).

Treatment of the cells. Membrane vesicles were prepared from the cells by the method of Kaback [19] as modified by the author.[20] Samples for the fluorimetric measurements were taken after EDTA, lysozyme, and osmotic shock treatments. Freezing and thawing of the cells were carried out in 0.5 ml of 0.05 M phosphate buffer (pH 6.6). After this procedure the cell fractions were separated by centrifugation at 10 000 *g* for 15 min. Both the cell pellets and supernatants were stored for the fluorimetric measurements. The other cell samples were partly disrupted by disintegrating them for short times in a MSE Sonic Oscillator.

Fluorimetric measurements. The fluorescence was measured with a Hitachi-Perkin-Elmer MPF-2A spectrophotofluorimeter. The fluorescence values were corrected for temperature but not for the absorption of the exciting light by the free dye. The fluorimetric measurements were carried out as described earlier by Mäntsälä and Lang[8] except that a temperature-programmed run was applied. The incubation mixtures (2.5 ml) contained 0.05 M phosphate buffer (pH 6.6), 20 μM ANS or 10 μm NPN and 3.0 – 4.2 $\times$ 10^9 cells/ml. The fluorescence profiles were determined without preincubation and temperature was elevated 2 °C/min during the temperature-programmed runs.

RESULTS

Binding of ANS and NPN as a function of temperature in whole cells. Changes in fluorescence intensity in *Ps. fluorescens*, *E. coli*, *Str. thermophilus*, and *B. subtilis* were investigated in the temperature range 2 – 70 °C. Intensity decreased in all the organisms when the temperature was elevated over 5 °C. Several inflection points were observed in the fluorescence profile (Fig. 1). The first one was at 4 – 5 °C with both ANS and NPN. We can assume that this inflection point reflects crystalline-liquid phase transition or changes in

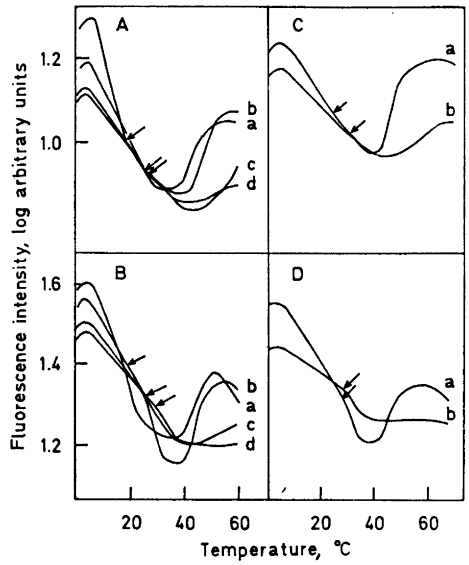


Fig. 1. Interactions of ANS and NPN with microbial cells during the temperature programmed run. The incubation mixtures of 2.5 ml contained the washed cells (2 – 5 $\times$ 10^9 cells/ml) in 0.05 M potassium phosphate buffer (pH 6.6) and the fluorescence dye (20 μM ANS or 10 μM NPN). ANS fluorescence was excited at 380 nm and emission was measured at 465 nm. Wavelengths of NPN fluorescence were 345 and 405 nm, respectively. Other experimental conditions were the same as described earlier by Mäntsälä and Lang.[8]

A. Intensities of ANS fluorescence in *Ps. fluorescens*, grown at 30 °C, a; in *E. coli*, grown at 37 °C, b; in *Str. thermophilus*, grown at 37 °C, c; in *B. subtilis*, grown at 37 °C, d.

B. Intensities of NPN fluorescense in *Ps. fluorescens*, a; in *E. coli*, b; in *Str. thermophilus*, c; in *B. subtilis*, d.

C. Intensities of ANS fluorescence in *E. coli* (a) and in *Str. thermophilus* (b) when the cells were cultured in anaerobic growth conditions

D. Intensities on NPN fluorescence in *E. coli* (a) and in *Str. thermophilus* (b) when the cells were cultured in anaerobic growth conditions.

the properties of water and water-membrane interactions. The latter suggestion seems more likely because the same effect was observed in all investigated organisms although growth at 4 °C is a property typical of *Ps. fluorescens* and requires a liquid lipid phase. Inflections between 20 and 30 °C depended on thermosensitivity of the bacteria. For example, in mesophilic *Ps. fluorescens* this inflection point was located at 20 – 22 °C, perhaps reflecting the "melting" of some lipid fraction in *Ps. fluorescens*. The inflection point was shifted toward higher temperatures in *E. coli* and *Str. thermophilus*, to 26 – 27 °C and about 28 – 30 °C, respectively. Several reports indicate that psycrophilic and mesophilic organisms contain more unsaturated fatty acids than thermophilic ones. Although the bacteria studied here are not obligate meso- or thermophiles, they exhibit different thermosensitive properties and the inflection points correlate well with the order of thermosensitivity of the bacteria. If these points represent "melting" points of the lipids, they should also correlate somehow with transport processes in the bacteria, because uptake of solutes and energy sources requires a liquid lipid phase. There are many examples in the literature indicating that initial velocities o transport are negligible below 20 – 30 °C.[5,10,21–23] As we see in Fig. 1, the inflection points are located in the same temperature range for both ANS and NPN. However, inflections were not in the same direction, for with ANS the fluorescense profiles turned upwards and with NPN downwards. These results suggest that the "melting" of lipids provides an environment with less rigidity, thus revealing more polar groups which increase ANS fluorescence intensity. Remarkable inflections were located also between 40 and 50 °C. Fluorescence intensities increased strongly especially in gram-negative *Ps. fluorescens* and *E. coli* (at about 40 – 45 °C and 42 – 50 °C, respectively). On the other hand, in gram-positive *Str. thermophilus* and *B. subtilis*, the interactions with ANS at elevated temperatures were remarkably less and only a negligible increase in fluorescence intensity at 40 – 70 °C was observed when NPN was used as the probe (Fig. 1). However, as we will see later, maximal intensities depend on the growth phase, too.

Anaerobic conditions do not markedly change the binding of the dyes in *Str. thermophilus* and *E. coli* below 30 °C (Fig. 1). Only negligible differences were observed in NPN interactions with the cells, suggesting that the hydrophobic core of the investigated bacteria is stable and that lipid synthesis is virtually independent of oxygen supply.

The effect of salts on fluorescence intensity. The exact role of the different ions remains obscure. Radda and Vanderkooi [5] have suggested that during the process of binding to membranes there is a displacement of water molecules from the hydration shells. Our results reveal that the interactions of NPN with *E. coli* and *Ps. fluorescens* are more sensitive to increased salt concentrations than the interactions of ANS (Fig. 2). Saturation levels were reached already at concentrations of 0.1 – 0.4 M NPN, whereas with ANS the maximal intensities were not reached at 1.5 M concentration. All investigated salts had about the same effect on the inter-

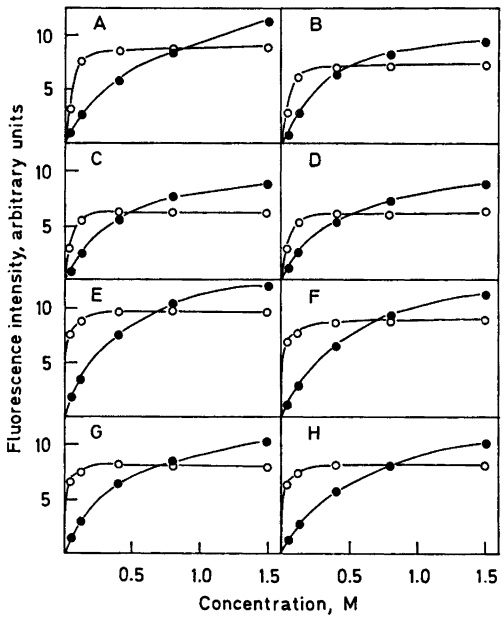


Fig. 2. Effect of various monovalent cations on ANS (●) and NPN (○) fluorescence intensities. The reaction mixtures contained 3×10^9 cells/ml and 2×10^9 cells/ml, respectively. Other experimental conditions were the same as in the legend to Fig. 1. *E. coli* A, NH_4Cl; B, NaCl; C, LiCl; D, KCl. *Ps. fluorescens* E, NH_4Cl; F, NaCl; G, LiCl; H, KCl.

actions of NPN, whereas with ANS intensities followed the order $NH_4Cl > LiCl > NaCl > KCl$.

Effect of various treatments on the interactions of the dyes with the bacteria. Several lines of evidence suggest that hydrophobic interaction between various protein components is the force holding the cell wall together. Gram-negative bacteria characteristically have a cell envelope consisting of two membranes separated by a thin peptidoglycan layer. This layer can be destroyed by lysozyme. To specify critical points in fluorescence behaviour during the programmed run of gram-negative. *E. coli* and *Ps. fluorescens*, various destructive treatments were carried out before the cells were exposed to temperature changes. After incubation with ethylenediamine tetraacetate the cells appeared to have lost both protein and lipid material, but still retained an ANS fluorescence profile similar to the untreated cells. When lysozyme was added to the incubation mixture, increases

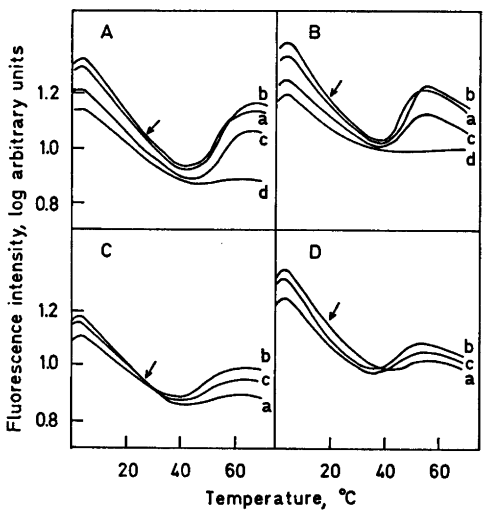


Fig. 3. Effect of various destructive treatments on ANS fluorescence intensities. Other experimental conditions were the same as in the legend to Fig. 1. A. *E. coli* no treatments, (a); 10 mM EDTA, (b); 10 mM EDTA + lysozyme (0.5 mg/ml), (c); 10 mM EDTA + lysozyme (0.5 mg/ml) + DNase (4 mg/ml) + osmotic shock, (d). B. *Ps. fluorescens* the treatments and the symbols are the same as above. C. *E. coli* freezing and thawing, twice (a); ultrasonic disintegration 10 s (b); 20 s (c). D. *Ps. fluorescens* the treatments and the symbols are the same as in Fig. C.

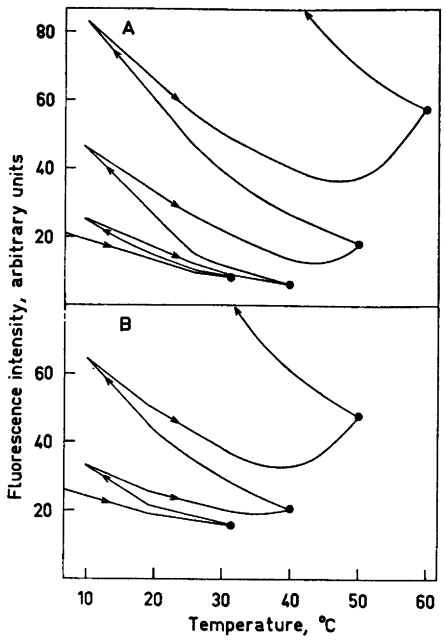


Fig. 4. Effect of stepwise temperature elevations on ANS fluorescence intensities in *E. coli* (A) and in *Ps. fluorescens* (B). The reaction mixtures (2.5 ml) contained 0.05 M phosphate buffer (pH 6.6), 20 μM ANS and 3.0×10^9 cells/ml of *E. coli* or 4.2×10^9 cells/ml of *Ps. fluorescens*. The temperature of the reaction mixtures was elevated to 32, 40, 50, and 60 °C, decreasing to 10 °C between these elevations. The arrows show the direction of the temperature change.

in fluorescence intensity were observed at 45 – 60 °C; they were not as strong as before lysozyme treatment. DNase + osmotic shock, ultrasonic or freezing and thawing treatments produced fluorescence profiles with slight or without any increases (Fig. 3). These results suggest that the increase in fluorescence intensity at 40 – 60 °C results from the irreversible denaturation of proteins. This view is further supported by experiments in which the temperature was elevated stepwise to 60 °C decreasing the temperature between these steps to 10 °C (Fig. 4). As we can see the fluorescence intensities increased strongly when temperature was decreased (arrow direction ←) and the intensities remained higher during the next elevations. When the stepwise temperature elevations reached 60 °C there was no increase

in intensity (between 40 and 60 °C) during the next elevations suggesting that the complete denaturation of proteins had occurred.

Effects of growth temperature and growth phase on fluorescence profiles. It has been reported that saturation of fatty acids increases when growth temperature is elevated.[14–16] Using NPN to probe the lipid region of the cells it was seen that the inflection point near 20 °C shifted to higher temperatures when *Ps. fluorescens* and *Str. thermophilus* were cultured at elevated temperatures. In *Ps. fluorescens* it shifted from 18 to 25 – 26 °C when the growth temperature was elevated from 8 to 37 °C (Fig. 5). The effect was the same with both NPN and ANS, although the effect of ANS was somewhat unclear. Furthermore, both NPN and ANS interacted at 40 – 60 °C more effectively

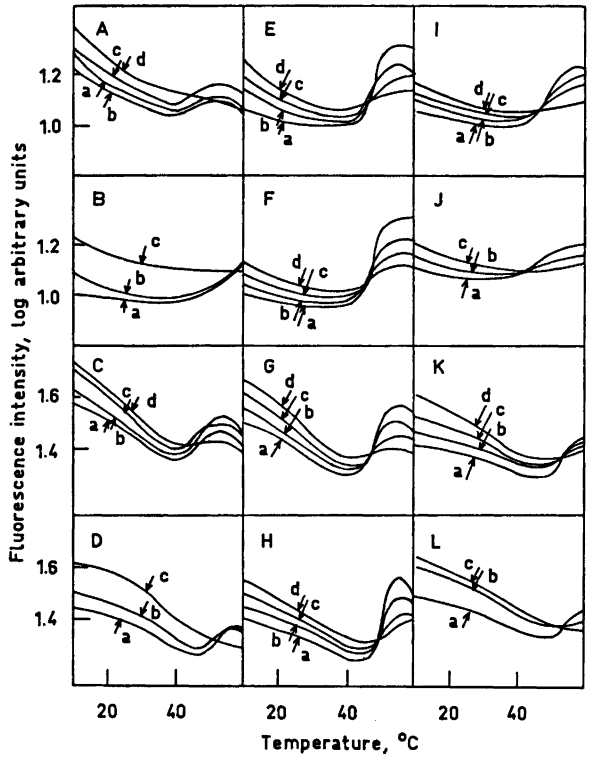


Fig. 5. Effect of growth temperature and the growth phase on fluorescence intensities. Other experimental conditions were the same as in the legend to Fig. 1.

A. ANS fluorescence intensities in *Ps. fluorescens* when the cells were cultured at 8 °C (a); at 15 °C (b); at 30 °C (c); at 37 °C (d). B. ANS fluorescence intensities in *Str. thermophilus* when the cells were cultured at 20 °C (a); at 30 °C (b); at 49 °C (c). C. NPN fluorescence intensities in *Ps. fluorescens*. The symbols are the same as in Fig. A. D. NPN fluorescence intensities in *Str. thermophilus*. The symbols are the same as in Fig. B. E. ANS fluorescence intensities in *Ps. fluorescens*. The cells were taken from the lag ($KU_{62} = 26$)* (a), from the acceleration ($KU_{62} = 38$) (b), from the logarithmic ($KU_{62} = 102$) (c) and from the stationary ($KU_{62} = 184$) (d) phases. F. ANS fluorescence intensities in *E. coli* $KU_{62} = 18$ (a); $KU_{62} = 28$ (b); $KU_{62} = 80$ (c); $KU_{62} = 161$ (d). G. NPN fluorescence intensities in *Ps. fluorescens*. The growth phases and the symbols are the same as in Fig. E. H. NPN fluorescence intensities in *E. coli*. The growth phases and the symbols are the same as in Fig. F. I. ANS fluorescence intensities in *Str. thermophilus* $KU_{62} = 18$ (a); $KU_{62} = 26$ (b); $KU_{62} = 74$ (c); $KU_{62} = 148$ (d). J. ANS fluorescence intensities in *B. subtilis* $KU_{62} = 20$ (a); $KU = 27$ (b); $KU_{62} = 94$ (c). K. NPN fluorescence intensities in *Str. thermophilus*. The growth phases and the symbols are the same as in Fig. I. L. NPN fluorescence intensities in *B. subtilis*. The growth phases and the symbols are the same as in Fig. J. *KU_{62} = Klett-reading employing filter 62.

with the cells grown at 8 °C and 15 °C than with those grown at 37 °C.

When a new medium was inoculated with the bacteria withdrawn from the stationary phase of growth, an increase in fatty acids was observed in *B. megaterium* during growth.[18] As we can see from Fig. 5, binding of NPN also increases markedly in *Ps. fluorescens* when the cells are cultured from the lag to the end of the logarithmic growth phase. These results suggest that lipid constituents increase during growth, or that denaturation of proteins or destruction of some protective structural components reveals more lipid groups. The inflection point in ANS profiles of *Ps. fluorescens* was located in the same temperature range as in NPN profiles (40 – 45 °C). Furthermore, the intensities followed the same order and were at their highest during the late growth phases. These results further support the view that the same factors are responsible for increases in ANS and NPN profiles although the components reacting with the dyes may be different. Maximal intensities were reached at the end of the exponential growth phase, being as much as 2 – 3 fold compared with the intensities at the beginning.

The profiles of *E. coli* resemble those of *Ps. fluorescens*, and as we can see from Fig. 5 a great many new groups interacting with ANS and NPN have formed during growth, or rigidity has greatly changed due to the denaturation of proteins. The effects are remarkable, suggesting that, for *E. coli* 40 to 45 °C are critical temperatures at which partial destruction of cells occurs.

Fluorescence profiles of *Str. thermophilus* differ somewhat from those of *Ps. fluorescens*. Inflection points shifted from 24 – 25 to 30 – 33 °C when the growth temperature was elevated from 20 to 49 °C (Fig. 5). Furthermore, maximal intensities increased and shifted toward higher temperatures when growth proceeded from the lag to the stationary phase. However, only slight increases in fluorescence intensities were observed during the temperature-programmed run when NPN was used as the fluorescent probe. The fluorescence profiles of *B. subtilis* (Fig. 5) resemble those of *Str. thermophilus*, though the first inflection point was located somewhat earlier (at 24 – 25 °C).

DISCUSSION

The factors responsible for heat resistance in intact bacteria and membranes have been extensively studied by measuring the differences in chemical composition produced by heat treatments.[24–28] Anilinonaphthalene dyes, which become highly fluorescent in a hydrophobic environment, have also proved to be useful indicators of alterations in cell constituents. There are several examples in the literature of ANS binding to membrane proteins as well as to lipids, making fluorescence data difficult to interpret. We used both ANS and the lipid specific dye NPN as fluorescence probes, because differences in the fluorescence intensities may reveal specific alterations in the protein and lipid constituents. It has been reported that the increased rigidity of the environment enhances fluorescence intensity.[29,30] This agrees well with our results, and a steeper decrease in NPN fluorescence intensity after the inflection point (between 20 and 30 °C) can result from the "melting" of lipids.

One of the most important physiological functions of cytoplasmic membranes is to transport solutes. Shechter *et al.*[23] have reported that the initial rates of sugar and amino acid transport exhibit sharp activity changes with respect to temperature at 15 to 20 °C and optima at 45 to 50 °C. By means of fluorescence they found two inflections to be functions of temperature. One inflection point was correlated with the transition in transport activity that occurred at around 20 °C and the other at above 35 °C. They explained the first transition as due to the "melting" of paraffin chains of membrane phospholipids and they linked the second one to abrupt increase in passive membrane permeability. On the other hand, "melting" of lipids may allow ANS to transport to an environment with more binding groups. It is also possible that "melting" of lipids changes the conformation of proteins and thus the environment of the binding sites. Trauble[31] found a considerable enhancement of ANS fluorescence when one of the characteristic transition temperatures of lipids was reached. He attributed this enhancement to a "dispersing" of the polar groups and a greater flexibility of the CH_2-chains of the lipid, thus creating more binding sites for the dye.

Low growth temperatures shifted the inflection points a few degrees downwards in *Ps. fluorescens* and *Str. thermophilus*. On the other hand, no considerable shift to lower or higher temperatures was observed when these bacteria and *E. coli* were cultured in aerobic or anaerobic conditions. It has been reported earlier that relative amounts of unsaturated acids increase with decreasing growth temperature in *Ps. fluorescens*.[16] The results of Cullen *et al.*[16] as well as our own suggest that, although *Ps. fluorescens* is an aerobe and *E. coli* and *Str. thermophilus* are facultative anaerobes, they all have a rather constant lipid composition independent of oxygen supply. This view agrees well with the results of Erwin and Bloch [32] who found that all these organisms synthesize unsaturated acids by an anaerobic pathway.

Melchior *et al.*[12] have demonstrated by means of a calorimeter two inflection points of heat absorption in the membranes of *Mycoplasma laidlawi*. The first one is due to reversible transition in the lipid component and the second to an irreversible denaturation of the protein component. The second inflection point in our investigations is likely due to irreversible protein denaturation, too, because when the temperature was elevated by stepwise heating and then returned to the initial temperature, alterations in the cells were partly reversible until 35 °C, after which the fluorescence intensity steadily increased (Fig. 4). Furthermore, preincubations of the bacterial cells at elevated temperatures resulted in irreversible increases in fluorescence intensity.[8] The effective temperatures were also between 40 and 60 °C depending on the thermosensitivity of the bacteria. Although the inflection points of fluorescence intensity (between 35 and 60 °C) followed the order of bacterial thermosensitivity, the presence or absence of the peptidoglycan layer may affect the fluorescence profile too. When this layer was removed by EDTA-lysozyme treatment the intensities of ANS and especially NPN were considerably reduced.

These results suggest that liquid-crystalline phase transitions as well as membrane damages of the bacterial cells change the rigidity of the environment of naphthalene dyes; thus these dyes can be applied to monitor phase transitions and the starting points of bacterial destructions.

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The Preparation and Dynamic Stereochemistry of Oxyarsoranes Containing Five- and Six-membered Ring Systems

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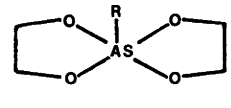
The exchange reactions of a series of acyclic oxyarsoranes with pinacol and 2,2-dimethyl-1,3-propanediol are reported. The general result of these reactions is the formation of five- or six-membered cyclic oxyarsoranes. Mixing of the arsorane $R-As(OCH_3)_4$ ($R=OCH_3$, CH_3, Ph) with the diol compound in molar ratio 1:2 leads to the quantitative formation of a spiro-arsorane. The same components in molar ratio 1:1 form the monocyclic arsorane quantitatively only in the case where the diol is pinacol and $R=OCH_3$. When $R=CH_3$ and Ph a mixture of products is obtained, including the spiro and probably also the monocyclic compound. It is concluded that the bis-ring compound is thermodynamically more stable than the mono-ring compound whether the ring is five- or six-membered. On the other hand, the spiro arsoranes derived from pinacol are generally more stable than those derived from 2,2-dimethyl-1,3-propanediol. The synthesized oxyarsoranes have been studied by variable temperature NMR in the temperature range -100 °C to 30 °C and the results interpreted in terms of pseudorotation processes. Comparison with available data on phosphorus compounds shows that there is close correspondence between the kind of pseudorotation processes undergone by analogous compounds of arsenic and phosphorus.

In a previous paper [1] we reported the preparation and variable temperature NMR spectra of oxyarsoranes of the type $R_nAs(OCH_3)_{5-n}$,

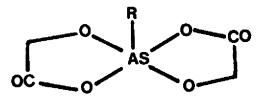
1 $n=1$ $R=OCH_3$
2 $n=1$ $R=CH_3$
3 $n=1$ $R=Ph$
4 $n=2$ $R=CH_3$
5 $n=2$ $R=Ph$
6 $n=3$ $R=Ph$

The main emphasis of the present work has been to investigate the extent to which these compounds participate in exchange reactions with 1,2- and 1,3-diols. If cyclic oxyarsoranes resulted, we were interested in examining the intramolecular bond bending processes in these compounds, using NMR as a tool.

While there is no report in the literature on cyclic oxyarsoranes containing six-membered rings (although synthesis of such compounds has been attempted),[2] oxyarsoranes of types I and II have been known for a long time.[3,4] These very stable spirocyclic compounds are



I



II

generally made by allowing 1 mol of the appropriate arsonic acid to react with 2 mol of the 1,2-dihydroxy compound, the water formed being removed either by performing the reaction in acetic anhydride or by azeotropic distillation from a proper solvent, *e.g.* benzene.[2]

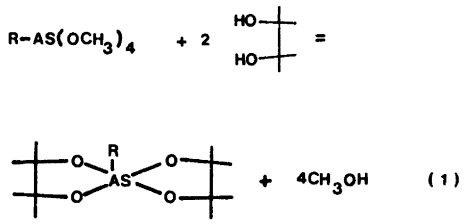
The role of pseudorotation processes in I, II,[5,6] and analogous phosphorus compounds [7,8] has been studied by variable temperature NMR. In the present work such studies are extended to monocyclic derivatives and to spirooxyar-

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soranes containing six-membered rings. The results obtained are discussed in relation to the data on corresponding phosphorus compounds recently reported by Denney and co-workers.[9]

RESULTS AND DISCUSSION

Exchange reactions involving 1,2-diols. The reaction of one mol of *1*, *2*, or *3* with 2 mol of pinacol leads to the formation of spiroarsoranes according to reaction (1). The occurrence of this

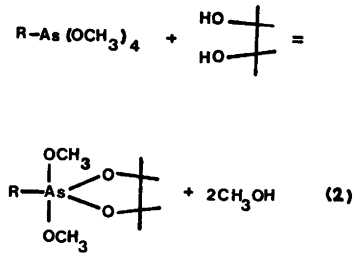


7 R = OCH_3
8 R = CH_3
9 R = Ph

reaction is not surprising when the known stability of these compounds is taken into account. However, the reaction above opens a new route to their preparation.

While the reaction between *1* and ethylene glycol gave the spirocyclic compound, the reaction between *1* and catechol resulted in a green solution which gradually darkened and polymerized. A mixture of *2* and catechol in molar ratio 1:2 led, however, to the quantitative formation of the spiro-bis(1,2-phenylenedioxy)methylarsorane.

The reaction of *1*, *2*, and *3* with pinacol in 1:1 ratio takes a route which differs for each arsorane. *1* reacts quantitatively according to eqn. 2 (R = OCH_3).



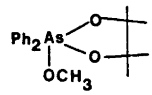
10 R = OCH_3

The product was thermally stable and could be distilled at reduced pressure.

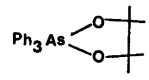
2 and *3* also undergo the above reaction, (eqn. 2) (R = CH_3 and Ph), but this is clearly not the only one taking place. In the case of *2* for instance, the NMR spectrum of the reaction mixture shows four signals in the As – CH_3 region. Thus there are at least four compounds present which contain the As – CH_3 group. However, both in the case of *2* and *3*, the spiroarsoranes *8* and *9* are observed (a pair of singlets in the C – CH_3 region). It thus appears that the bis-ring compounds are relatively more stable than the mono-ring compounds when R = CH_3 or Ph than is the case when R = OCH_3.

Clearly, steric hindrance plays a role with respect to what extent spiro and monocyclic compounds are formed when mixing $RAs(OCH_3)_4$ and a 1,2-diol. Thus while *1* and pinacol in 1:1 ratio gave the monocyclic compound *10* in quantitative yield, *1* and ethylene glycol in the same ratio resulted in a mixture of mono- and bis ring compounds.

Compounds *11* and *12* were prepared by allowing *5* and *6*, respectively, to undergo exchange reaction with pinacol.

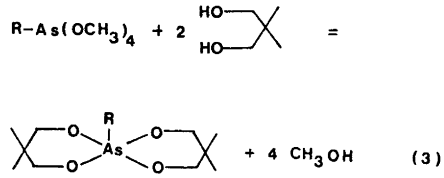


11



12

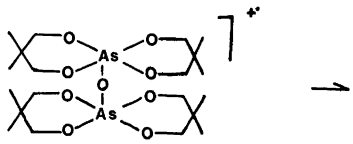
Synthesis of oxyarsoranes derived from 2,2-dimethyl-1,3-propanediol. The reactions of *1*, *2*, and *3*, respectively, with neopentyl glycol in molar ratio 1:2 yields the spirooxyarsoranes according to eqn. 3.



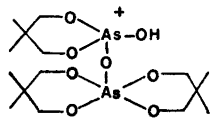
13 R = OCH_3
14 R = CH_3
15 R = Ph

While *14* and *15* could be distilled at reduced pressure, *13* was converted into a high boiling substance which solidified when left at room temperature. $As(OCH_3)_5$ was the only compound which could be isolated from the reaction mixture by distillation.

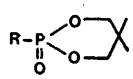
A possible structure for the nonvolatile compound is given by III. This structure is consistent with the finding that its ^1H NMR spectrum in deuteriochloroform solution shows two relatively broad singlets in the ratio 2:3 in the OCH_2- and $C-CH_3$ region, respectively. The presence of bis-ring units is also supported by an abundant ion at $m/e=279$ in its mass spectrum. Other abundant ions in the high mass region were observed at $m/e=489$, 403, 387, 301. The absence of a molecular ion is in accordance with what is found for other oxyarsoranes.[10] The ion at $m/e=489$ can be rationalized by the fragmentation



III



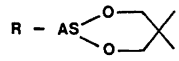
This fragmentation of the ring residue is of the same type as observed in arsoranes *14* and *15*[11] and in phosphorus compounds of the type[12]



It should be noted that the tendency of trivalent arsenic compounds to dimerize by forming an $As-O-As$ bond has been known for a long time.[13] However, the structure of the isolated compound has yet to be verified.

The reaction of *1*, *2*, and *3* with 2,2-dimethyl-1,3-propanediol in molar ratio 1:1 did not result in a single compound. From NMR it could be concluded that several species were formed. The formation of monocyclic derivatives probably occurs to some extent, but they are in every case accompanied by others, even under very mild reaction conditions. In the case of *2* the spiro compound could be clearly detected from the NMR spectrum of the reaction mixture. The reaction mixtures resulting from *1* and *3* also gave NMR spectra consistent with the presence of the respective spiro-oxyarsoranes.

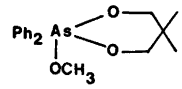
Preparation of monocyclic compounds derived from 2,2-dimethyl-1,3-propanediol was attempted by treating the trivalent arsenic compounds *16* – *18* with bromine and sodium methoxide.



16 R = Cl
17 R = CH_3
18 R = Ph

Distillation of the reaction mixtures obtained from *17* and *18* again lead to the isolation of the bis-ring oxyarsoranes. $As(OCH_3)_5$ was the only product isolated from the reaction of *16* with $Br_2/NaOCH_3$.

Compound *19* was formed by allowing *5* to react at room temperature with equivalent amounts of 2,2-dimethyl-1,3-propanediol.

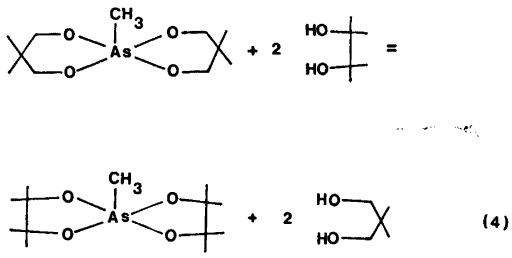


19

Relative stability of oxyarsoranes. The synthetic results seem to indicate that the spiro compounds, whether derived from pinacol or 2,2-dimethyl-1,3-propanediol are thermodynamically more stable than their acyclic analogues. Treatment of the spiro arsoranes with methanol did not lead to reversion to the acyclic compounds.

The greater stability of five-menbered cyclic oxyarsoranes as compared to those containing six-membered rings was clearly illustrated by the ^1H NMR spectrum of a deuteriochloroform solution of *14* and pinacol in molar ratio 1:2. The change in the NMR spectrum with time

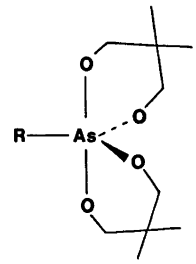
was in complete accordance with the exchange reaction shown in eqn. 4. This reaction went to completion within approximately 30 min. The main driving force for the reaction is probably a combination of crowding in the six-membered oxyarsoranes and the favourable angular situation present in oxyarsoranes containing five-membered rings.



NMR spectra of oxyarsoranes derived from 2,2-dimethyl-1,3-propanediol. ^{1}H spectral data at 25 °C for compounds *13* – *15* are given in Table 1. In all cases equivalency of O – CH_2- and C – CH_3 protons are observed. Whether the R substituent is apically or equatorially situated, a rigid trigonal bipyramidal structure would require that their respective NMR signals should be more complicated than simple singlets. The NMR results at room temperature can therefore be explained by assuming that these compounds undergo pseudorotation processes at a rate which is fast on the NMR time scale.

Cooling to −60 °C (deuteriochloroform) or −100 °C (methylene chloride or carbon disulfide) did not change the NMR spectrum of *13* essentially. A broadening of all three signals was, however, observed. *14* and *15* underwent qualitative changes on cooling. The C – CH_3 signal broadened and split into two signals of equal intensity. Similarly the methylene group split into a quartet.

The results obtained on compounds *14* and *15* mean that pseudorotation processes at these low temperatures are slow enough to be detected by NMR. The NMR spectra are consistent with the structure expected on grounds of electronegativity, *i.e.* the one in which the R group is in an equatorial position, IV.



IV

This structure would predict a NMR spectrum in which the methyl groups appear as two singlets and the methylene protons as two AB quartets. If overlapping of the AB quartets is assumed (a situation which must be considered very likely), the NMR results are in agreement with the structure pictured by IV.

The free energy of activation for the pseudorotation process leading to equivalence of the methyl groups can, knowing the coalescence temperature and the chemical shift difference between the two signals at low temperature, be estimated by means of the Eyring equation.[14] Using the values of T_c and $\Delta\nu$ listed in Table 1, the free energy of activation was found to be 11.9 kcal mol^{-1} and 12.8 kcal mol^{-1} for *14* and *15*, respectively.

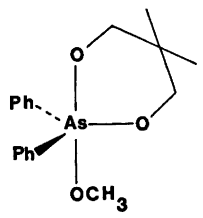
Table 1. ^{1}H NMR data [a] for oxyarsoranes derived from 2,2-dimethyl-1,3-propanediol.

Compound	δ (ppm downfield from internal TMS) [b] O – CH_2	C – CH_3	O – CH_3	As – CH_3	As – Ph	$\Delta\nu$(C – CH_3) [c] (Hz)	T_c [d] (°C)	$\Delta G^{\ddagger}$ [e] (kcal mol^{-1})
13	3.82	0.97	3.63					
14	3.86	0.99		2.02		8.4	−45	11.9
15	3.82	0.96			7.55 7.95	14.4	−24	12.8
19	3.89	0.93	3.17		7.42 7.80	45.0	+23	14.6

[a] Obtained on a JEOL JNM-C-60H spectrometer. [b] In deuteriochloroform solution at 25 °C. [c] Measured at −55 °C. [d] Coalescence temperature for ring methyl groups. [e] Free energy of activation at T_c.

The fact that compound *13* shows equivalence of methyl groups down to −100 °C can be interpreted in terms of a rather small free energy of activation for the relevant pseudorotation process. In contrast to what is the case for *14* and *15*, the R group in compound *13* is strongly electronegative. From the electronegativity rule[8] it can therefore be concluded that a transition state in which the R group adopts an apical position, is energetically more favourable in the case of *13* than in *14* and *15*. Consequently, if similar chemical shift differrences are assumed between the methyl groups, the coalescence temperature for *13* should be lower than for *14* and *15*. The failure of compound *13* to show a ^{1}H NMR spectrum in which the signals split up at low temperatures can alternatively be due to accidental identity of chemical shifts. The possibility of fast exchange of methoxy groups, catalyzed by traces of water, should also be considered.

NMR data for compound *19* are listed in Table 1. At 25 °C a singlet is observed for the methyl groups as well as for the methylene groups. Cooling caused the methylene signal to split into two signals of equal intensity, while the methyl signal remained a singlet, although broad. The NMR results are compatible with the most probable trigonal bipyramidal structure for the compound, namely the one in which the maximum number of oxygen substituents are apically situated, V.



V

In this structure the two methylene groups are non-equivalent while the methyl groups are equivalent. A similar NMR behaviour has already been noted for $Ph_2As(OCH_3)_3$.[1] This compound showed a low temperature NMR spectrum which was in agreement with a trigonal bipyramidal structure having the apical positions occupied by methoxy groups. The free energy of activation for the relevant pseudorotation process undergone by *19* was found to be 14.6 kcal mol^{-1}.

It should be noted that the NMR results from this investigation are very similar to those obtained by Denney and co-workers on analogous phosphorus compounds.[9] They found for example that while the ^{1}H NMR spectrum of the phosphorus ethoxy analogue of *13* did not change on cooling down to −65 °C, coalescence was observed for the analogues of *14* and *15*. Similarly, at −65 °C the NMR spectrum of the phosphorus-ethoxy analogue of *19* showed two distinct doublets for the methylene hydrogens and a singlet for the methyl groups. The correspondence in NMR behaviour between these closely related compounds of arsenic and phosphorus strongly indicate that the same type of structures are involved in the pseudorotation processes which they undergo.

NMR of oxyarsoranes derived from pinacol. The ^{1}H NMR spectra of compounds *7* – *9* have previously been studied by Goldwhite[5] and by Casey and Mislow.[6] At room temperature the ring methyl groups appeared as a pair of singlets in the case of *8* and *9*, while a single peak was observed for *7*. These results are in disagreement with what to expect from the energetically more favoured TBP configuration. According to the rules found valid for pentacoordinated phosphorus compounds, this configuration will be the one in which each of the five-membered rings is in an equatorial-apical position and at the same time the most electronegative substituents in apical positions. In the structure predicted from these rules, VI, there will be four different environments for the methyl groups. It should be noted that no further splitting of signals was observed on cooling down to −100 °C. On the other hand, the doublet found for *8* and *9*, collapsed to a singlet at higher temperatures (about 160 °C). As first pointed out by Goldwhite the pair of methyl signals observed for *8* and *9* can be rationalized by the occurrence of the pseudorotation process shown in Fig. 1.

The equivalency of methyl signals at higher temperatures was explained by assuming the

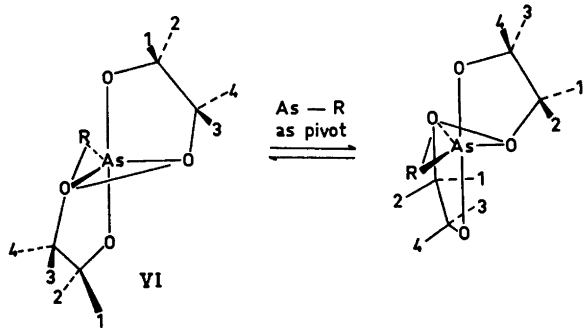
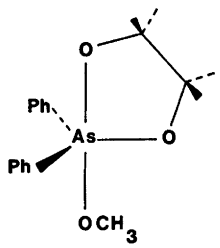


Fig. 1. Pseudorotation process.

existence of two additional successive pseudorotation processes.[5]

The free energies of activation reported by Casey and Mislow for compounds *8* and *9*, 21.8 kcal mol^{-1} and 22.8 kcal mol^{-1}, respectively, are very much larger than those obtained for the corresponding 2,2-dimethyl-1,3-propanediol derivatives. This behaviour is very similar to what observed for analogous compounds of phosphorus.[6] The effect can be explained, as pointed out by Holmes,[16] in terms



of different degree of strain in the transition state associated with the pseudorotation process leading to NMR equivalency of chemical shifts. As the transition state for this process requires diequatorial ring oxygens, it follows that less strain is to be expected in compounds containing a six-membered ring as compared to a five-membered one.

The observation that the NMR spectrum of *7* shows a singlet both at low and high temperature was explained by Goldwhite in terms of rapid interchange of ligands. However, Casey and Mislow attributed this observation to accidental isochrony of the ring methyl groups.[6]

In connection with the present work we were able to verify the latter explanation by utilizing the lanthanide chemical shift reagent Eu(fod)$_3$.[15] The addition of this compound to a carbon tetrachloride solution of *7* resulted in two downfield shifted singlet absorptions for the ring methyl groups. Presumably, the effect was due to a pseudocontact shift caused by the europium reagent upon complexation with the oxygens in *7*.

NMR data for compounds *10* and *12* are given in Table 2. No splitting of ring methyl signals was observed on cooling down to −100 °C in methylene chloride or carbon disulfide solution. The expected structure for these compounds would be the one in which the five-membered ring spans an apical-equatorial position, thus leading to two types of methyl groups. However, the methyl groups would be interchanged by a pseudorotation with the As−OCH$_3$ and As−Ph axis, respectively, as pivot. If this process is fast on the NMR time

Table 2. ^{1}H NMR data for oxyarsoranes derived from pinacol.

Compound	δ (ppm downfield from internal TMS)[a]					
	C−CH$_3$		O−CH$_3$	As−CH$_3$	As−Ph	
7	1.27		3.78			
8	1.19	1.24		2.03		
9	1.03	1.31			7.41	8.10
10	1.25		3.77			
11	1.14		3.37		7.38	8.00
12	1.11				7.34	7.64

[a] In deuteriochloroform solution at 25 °C.

scale, equivalency of ring signals will result. It may be recalled that this pseudorotation process is of the same type as the one discussed above for explaining the two methyl signals observed for *8* and *9*.

The variable temperature spectrum of compound *11* is similar to that observed in the case of *19*, Table 2. At room temperature the ring methyl groups appear as a singlet. Cooling results in gradually broadening and finally splitting into two signals. These observations indicate that the expected structure, VII, at low temperatures has a life time long enough to be detected by NMR. From the coalescence temperature (−39 °C) and the chemical shift difference between the ring methyl groups (18.2 Hz at −55 °C), the free energy of activation for the process leading to equivalency of methyl groups was estimated to be 11.8 kcal mol^{-1}.

Denney and co-workers have prepared a number of oxyphosphoranes derived from ethylene glycol.[9] The variable temperature NMR behaviour is analogous to what has been observed for the oxyarsoranes. Compound *11* is, however, an exception as the NMR spectrum of the phosphorus-ethoxy analogue was essentially unchanged on cooling to −60 °C.

Recently, an X-ray structure determination of bis-ring oxyphosphoranes derived from catechol and the analogous dithiol, initiated an alternative interpretation of the variable temperature NMR spectra obtained for spirocyclic phosphorus compounds.[16] It was shown that the NMR data obtained by Denney and co-workers could be interpreted in terms of a square pyramidal structure. From what was said above regarding the similarity in spectral behaviour between analogous compounds of phosphorus and arsenic, it is evident that the variable temperature NMR spectra obtained for the spiro oxyarsoranes can be interpreted with reference to a square pyramidal geometry for the molecule. It should be mentioned, however, that X-ray studies on cyclic oxyphosphoranes similar to the oxyarsoranes under discussion points to TBP geometry in the former compounds.[17,18] In view of these results we have interpreted the ^1H NMR spectra on the basis of a trigonal bipyramidal structure. Presumably, further work will better define the factors determining the configuration of these compounds.

EXPERIMENTAL

Instrumental. The NMR spectra were recorded on a JEOL JNM-C-60H spectrometer. Mass spectra were obtained on a JEOL JMS-D100 instrument.

Syntheses

The acyclic arsoranes *1*, *2*, *3*, *5*, and *6* were prepared as described previously.[19,1]

General procedure for the exchange reaction between an acyclic oxyarsorane and a diol. To a carbon tetrachloride or methylene chloride solution of the acyclic arsorane was added the appropriate amount of the dihydroxy compound. The reaction mixture was left for a few hours or overnight at room temperature and the volatile components thereafter pumped off at 1 mmHg. The residue was dissolved in a proper solvent and examined by NMR. In the cases where the residue was a solid or thermally stable liquid the following physical data were obtained:

10 (b.p. = 75 °C/1 mmHg)
12 (m.p. = 93 − 96 °C)
14 (b.p. = 104 °C/2 mmHg)
15 (b.p = 131 °C/0.1 mmHg)

An attempt to distill compound *13* led to the isolation of $As(OCH_3)_5$ and a fraction boiling at 110 °C/1 mmHg. However, most of the reaction mixture (about 80 % by volume) was left in the distillation flask. On cooling it turned into a wax-like solid. Recrystallisation from petroleum ether resulted in a hygroscopic material. Its ^1H NMR spectrum in deuteriochloroform solution showed two relatively broad singlet absorptions, at δ 0.09 and 3.95.

Preparation of 16, 17 *and* 18. These 1,3,2-dioxarsenanes were prepared according to well known methods[20] and purified by fractionation under vacuum.

Reaction of 16, 17 *and* 18 *with bromine/sodium methoxide.* To a methanol solution containing 0.02 mol of *16* was added 0.06 mol of sodium methoxide and thereafter dropwise 0.02 mol of bromine. After the volume of the reaction had been reduced the formed salt was separated by filtration. Distillation afforded a fraction boiling at 40 − 41 °C/1 mmHg. Its ^1H NMR spectrum in deuteriochloroform solution showed a single absorption at δ 3.74. On this basis the compound was identified as $As(OCH_3)_5$. The distillation residue, about 50 % by volume, was not subjected to further analysis.

Similarly, distillation of the filtrate resulting from reacting 0.0312 mol of *17* with 0.0624 mol of sodium methoxide and 0.0312 mol of bromine

led to the isolation of a fraction with boiling point 104 °C/2 mmHg. Its ^{1}H NMR spectrum was identical to the one obtained for compound *14*.

From 0.0353 mol of *18* was as described above obtained a filtrate which was distilled at reduced pressure. A fraction boiling at 120 °C/0.01 mmHg which had the same NMR characteristics as compound *15* was isolated.

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Intermediates in the Penicillic Acid Biosynthesis in *Penicillium cyclopium*

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Feeding experiments using ^{14}C-labelled precursors demonstrate the following sequence of the biological formation of penicillic acid in *Penicillium cyclopium:* orsellinic acid→2-*O*-methylorsellinic acid →1,4-dihydroxy-6-methoxy-2-methylbenzene→6-methoxy-2-methylbenzoquinone(1,4)→penicillic acid.

Penicillic acid was isolated already in 1913 by Alsberg and Black.[1] They also established that penicillic acid was toxic to various laboratory animals. The toxicity of penicillic acid has been confirmed by several research groups and its natural occurrence in moldy corn, *e.g.* in blue-eye diseased corn has been demonstrated by Ciegler and Kutzman.[2]

Penicillic acid is synthesized by a large number of fungi. Mosbach [3] has shown that the biological formation of penicillic acid in *Penicillium barnensee* proceeds *via* orsellinic acid which in itself is derived from acetate-polymalonate. Later Bentley and Keil [4] have established in a series of incorporation experiments with *P. cyclopium* the participation of malonate in penicillic acid biosynthesis which is in accordance with its formation from orsellinic acid. Recently Al-Rawi *et al.*[5] using penicillic acid obtained from tritium labelled acetate in a feeding experiment with *P. cyclopium* confirmed with ^{3}H NMR spectroscopy the origin of penicillic acid.

It is obvious that there are several metabolic steps with the possible involvement of defined intermediates in the transformation of orsellinic acid into penicillic acid. In order to investigate the details of this reaction sequence the following conceivable intermediates were synthesized labelled with ^{14}C: 2-*O*-[$^{14}C_4$methylorsellinic acid, 1-*O*[^{14}C]methylorcinol,6-[^{14}C]methoxy-2-methylbenzoquinone(1,4), 1,4-dihydroxy-6-[^{14}C]methoxy-2-methylbenzene, 3-hydroxy-6-[^{14}C]methoxy-2-methylbenzoquinone(1,4), 2,5,6-trihydroxy-3-[^{14}C]methoxy-toluene. These ^{14}C-labelled substances were used in feeding experiments with submerged cultures of *P. cyclopium*. In most cases the labelled substrate was added after 74 h growth of the culture when the penicillic acid production just had started. The efficiency of the incorporation of radioactivity into penicillic acid is shown in Table 1.

2-*O*-Methylorsellinic acid is taken up and transformed into penicillic acid by the organism with remarkable efficiency (50 %). The incorporation of the decarboxylated substance, *O*-methylorcinol, is appreciably lower (17 %). It could be explained by differences in abilities to penetrate the cell membrane but it could also reflect the occurrence of separate catabolic pathways both leading to penicillic acid. 6-Methoxy-2-methylbenzoquinone(1,4) as well as the corresponding hydroquinone give rise to penicillic acid, the quinone being significantly more efficient as a precursor than the hydroquinone. Labelled methoxy-methylbenzoquinone could also be isolated from the culture when radioactive 2-*O*-methylorsellinic acid was fed to the organism. The two labelled compounds carrying a further hydroxyl group in 3 position do not function in our experiments as precursors of penicillic acid. These results suggest a sequence of intermediates from orsellinic acid to penicillic acid as described in Fig. 1.

All attempts to demonstrate the presence of

Table 1. Incorporation of radioactive precursors into penicillic acid.

^{14}C-Substrate	Added dpm × 10^{-3}	Penicillic acid dpm × 10^{-3}	Incorporation %
2-*O*-[$^{14}CH_3$]orsellinic acid	80	40	50
1-*O*-[$^{14}CH_3$]orcinol	120	20	17
6-*O*-[$^{14}CH_3$]-2-CH_3-benzoquinone(1,4)	600	162	27
1,4-DiOH-6-*O*-[$^{14}CH_3$]-2-CH_3-benzene	400	20	5
3-OH-6-*O*-[$^{14}CH_3$]-2-CH_3-benzoquinone(1,4)	800	nil	–
2,5,6-TriOH-3-*O*-[$^{14}CH_3$]toluene	800	nil	–

a 2-*O*-methylorsellinic acid decarboxylase in the organism have so far failed. It makes the suggested decarboxylation of 2-*O*-methylorsellinic acid coupled with a hydroxylation reaction, with a mechanism resembling the action of salicylate-1-monooxygenase,[6] very likely. Thus the transformation of *O*-methylorcinol to 6-methoxy-2-methylbenzoquinone(1,4) would not be a part of the main route to penicillic acid.

The natural occurrence of 1,4-dihydroxy-6-methoxy-2-methylbenzene was demonstrated in a short term experiment by feeding the organism with [$^{14}CH_3$] methionine. After 40 min of incubation 20 % of the labelling from methionine was found to be incorporated into the hydroquinone derivative.

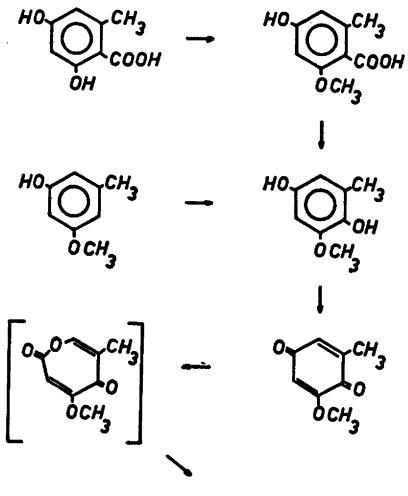


Fig. 1. Metabolic pathway of penicillic acid formation.

The Baeyer-Villiger type of oxidation in the ring opening reaction in the last step of penicillic acid biosynthesis has been confirmed in a cell free system. The enzymic reaction requires besides oxygen also NADPH indicating a monooxygenase system. The reaction is stimulated by the presence of FMN and Fe^{3+}.

A report on the isolation of the enzyme and on its properties will be published separately.

EXPERIMENTAL

Culture conditions. Penicillium cyclopium NRRL 1888 was grown in 500 ml conical flasks on a shake table (2.5 cm stroke, 240 rpm) at 28 °C. Each flask contained 150 ml of Raulin-Thom medium supplemented with diammonium tartrate as described by Bentley *et al.*[4]

Preparation of radioactive precursors

O-[^{14}C]*Methylorcinol.* This substance was synthesized by refluxing 1.0 mmol of anhydrous orcinol with 1.0 mmol of $^{14}CH_3I$ and 1.5 mmol K_2CO_3 in anhydrous acetone for 8 h. After acidification with diluted H_2SO_4 the monomethyl ether of orcinol was separated from the mixture by steam distillation. As some unreacted orcinol followed the methyl ether in the distillation the methyl ether was further purified by fractionation in a counter current distribution apparatus using diethyl ether and 0.5 M Na_2CO_3 respectively as phases. Yield of *O*-(^{14}C)methylorcinol 29 %, m.p. 133 – 134 °C.

2-O-[^{14}C]*Methylorsellinic acid.* This compound was prepared in two different ways.

Method A. Ethylorsellinate synthesized according to Gaucher and Shepherd [7] was treated with an equivalent amount of ethyl chloroformate in aqueous NaOH. The radioactive isotope was introduced by reacting the obtained carbomethoxy derivative with $^{14}CH_3I$ as described above. The 2-*O*-[^{14}C]methylorsellinic acid was set free by treatment of the ethyl ester of the

carbomethoxy derivative as described by Fischer and Hoesch.[8] Radioactive yield 41 %, m.p. 175–177 °C (dec.).

Method B. 4-Carbomethoxyorcylaldehyde was methylated by treatment with $^{14}CH_3I$ as described above. After oxidation of the aldehyde with aqueous $KMnO_4$ the carbomethoxy group was removed from the acid as described in method A. Radioactive yield 27 %, m.p. 175–177 °C (dec.).

6-[^{14}C]Methoxy-2-methylbenzoquinone(1,4) and 1,4-dihydroxy-6-[^{14}C]methoxy-2-methylbenzene. The phenolic group of 2-nitro-3-hydroxytoluene was methylated with $^{14}CH_3I$ as described above. Reduction of the nitro group with hydrogen at atmospheric pressure and Raney-Ni as catalyst yielded quantitatively the amine. Oxidation of the amine with Fremy's salt as described by Teuber and Hasselbach [9] gave radioactive 6-methoxy-2-methylbenzoquinone(1,4), radioactive yield 52 %, m.p. 155–156 °C. The corresponding hydroquinone was obtained by reduction of the quinone with $Na_2S_2O_4$.

3-Hydroxy-6-[^{14}C]methoxy-2-methylbenzoquinone and 2,5,6-trihydroxy-3-[^{14}C]methoxytoluene. The benzoquinone derivative was prepared from 6-[^{14}C]methoxy-2-methylbenzoquinone(1,4) *via* the triacetate as described by Anslow and Raistrick.[10] Yield 48 %. 2,4,6-Trihydroxy-3-[^{14}C]methoxytoluene was synthesized by reduction of the quinone with $Na_2S_2O_4$.

^{14}C-Incorporation experiments

In the feeding experiments generally 1–4 mg of the synthesized radioactive precursors dissolved in a small volume of ethanol were added to the organism grown for 72 h. After another 24 h of incubation penicillic acid was extracted with ethyl acetate from the acidified culture medium. The ethyl acetate solution was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. After redissolving the residue in a small volume of ethyl acetate penicillic acid crystallized on addition of hot light petroleum.

The radioactive penicillic acid was recrystallized to constant specific radioactivity. When *O*-[^{14}C]methylorcinol and 6-[^{14}C]methoxy-2-methylbenzoquinone(1,4) were used as labelled precursors the radioactivity in the penicillic acid was localized to the methoxy group by splitting the molecule with phenylhydrazine and distilling off the formed methanol. The methanol was purified with preparative gas chromatography.

In one experiment a 72 h old culture was exposed for 40 min to 1.25 μCi (0.37 mg) of [$^{14}CH_3$]methionine. The culture filtrate was acidified and extracted with ethyl acetate. The ethyl acetate solution was washed with aqueous $NaHCO_3$ and water. Thin layer chromatography on silica gel (Kieselgel, G. Merck) of an aliquot of the organic phase demonstrated the presence of 1,4-dihydroxy-6-methoxy-2-methylbenzene (R_F 0.23). As solvent was used the organic phase of the mixture chloroform-methanol-water-formic acid (250:24:25:1, by vol.)

To the remaining ethyl acetate solution was added after air-oxidation 15 mg of non-labelled 6-methoxy-2-methylbenzoquinone(1,4). The evaporated residue was repeatedly crystallized to constant specific radioactivity from diethyl ether-light petroleum.

Of the added methionine 20 % was incorporated into the hydroquinone derivative.

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Selective Aliphatic Hydrogen Exchange in 2,4,6-Triisopropyltoluene

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2,4,6-Triisopropyltoluene has been treated with deuteriated trifluoroacetic acid in carbon tetrachloride. In addition to the exchange of the aromatic hydrogens, the hydrogen atoms of the methyl groups in the isopropyl group at position 4 were exchanged. The isopropyl groups at positions 2 and 6 were unaffected, as were the methine hydrogens of all the isopropyl groups. A mechanism for the observed aliphatic hydrogen exchange is proposed. Several other compounds were treated with the same reagent, but no aliphatic hydrogen exchange was observed.

In order to exchange the aromatic hydrogens of 2,4,6-triisopropyltoluene for deuterium, this compound was treated with deuteriated trifluoroacetic acid. The desired exchange occurred, but in addition an exchange of some of the aliphatic hydrogens was also observed. The present paper deals with the last-mentioned exchange reaction for which a mechanism is proposed. The behaviour of several other compounds under the actual experimental conditions is also reported.

EXPERIMENTAL

Mass spectrometric determinations were performed on an AEI MS 902 instrument (at the Department of Medical Biochemistry, University of Göteborg) under the following conditions: electron energy 70 eV, accelerating voltage 8 kV and emission 100 μA. For the mass spectrometric analyses of 2,4,6-tri-*tert*-butyltoluene, *p*-cymene, 2-bromo-1,3,5-triisopropylbenzene, and 2,4-dibromo-1,3,5-triisopropylbenzene an LKB 9000 instrument (at the Department of Medical Chemistry, University of Göteborg) fitted with a gas chromatograph was used. The electron energy was 70 eV. The intensities of the peaks of the mass spectrograms are reported in parentheses as percentages of the base peak, and only the most abundant peaks are reported together with the parent peaks and the isotope peaks corresponding to them. For the NMR analyses Varian A 60 and XL 100 instruments (the latter at the Chemical Centre, University of Lund) were used. The chemical shifts are reported in ppm downfield from tetramethylsilane as internal standard. Gas chromatographic analyses were performed on a Perkin Elmer 900 instrument using columns packed with SE-30 silicon gum rubber.

Deuteriated trifluoroacetic acid with an isotopic purity of 99.5 % ($d_0 = 1.535$) obtained from CIBA was used. The deuteriated sulfuric acid used was CIBA 95 – 98 %, density 1.86×10^3 kg m^{-3}, > 99 % deuterium. Deuterium oxide with an isotopic purity of 99.75 % obtained from CIBA was used.

The following compounds were analytical grade commercial products and were used without further purification: carbon tetrachloride, *p*-cymene, and 1,3,5-triisopropylbenzene (P). Other commercial products were all of reagent grade purity.

2,4,6-Triethyltoluene (MeE) and *1,3,5-triethyl-2,4-dimethylbenzene* (Me_2E) were prepared according to a reported method.[1] *1,3,5-Tri*-tert-*butylbenzene* (B) was prepared according to Baas *et al.*[2] and *2,4,6-tri*-tert-*butyltoluene* (MeB) was prepared according to Myhre *et al.*[3]

2,4,6-Triisopropyltoluene (MeP) was prepared by lithium tetrahydridoaluminate reduction of 2-chloromethyl-1,3,5-triisopropylbenzene, which had been obtained by chloromethylation of 1,3,5-triisopropylbenzene with chloromethyl methyl ether. A similar preparative method has previously been reported.[1] The product was pure according to gas chromatography.

Physical properties of 2-chloromethyl-1,3,5-triisopropylbenzene. Mp. 26 – 28 °C, b.p. 119 °C at 2 – 3 Torr. NMR: δ 6.91 (singlet, 2 H, aromatic), 4.65 (singlet, 2 H, CH_2Cl), 3.28 (heptet, $J = 7.2$ Hz, 2 H, CH), 2.84 (heptet, $J = 7.2$ Hz, 1 H, CH), 1.28 (doublet, $J = 7.2$ Hz, 12 H, CH_3), 1.24 (doublet, J 7.2 Hz, 6 H, CH_3).

Physical properties of MeP. MS: 41 (24), 43 (47), 55 (19), 57 (28), 69 (18), 71 (18), 81 (10),

83 (13), 85 (10), 97 (15), 105 (10), 119 (10), 175 (19), 203 (100), 204 (19), 218 (44.1). 219 (9.1). NMR: δ 6.82 (singlet, 2 H, aromatic), 3.15 (heptet, J 7.2 Hz, 2 H, CH), 2.78 (heptet, J7.2 Hz, 1 H, CH), 2.20 (singlet, 3 H, CH_3), 1.20 (doublet, J 7.2 Hz, 18 H, CH_3).

3-Bromo-2,4,6-triisopropyltoluene (BrMeP) was prepared by bromination of 2,4,6-triisopropyltoluene with molecular bromine in dimethylformamide according to a method previously described.[4] MS: 43 (72), 128 (15), 129 (17), 131 (18), 143 (12), 159 (14), 281 (100), 282 (18), 283 (99), 284 (21), 296 (79.5), 297 (15.3), 298 (78.5), 299 (5.1). NMR: δ 6.92 (singlet, 1 H, aromatic), 4.00 (heptet, J 7.2 Hz, 1 H, CH), 3.48 (heptet, J 7.2 Hz, 1 H, CH), 3.13 (heptet, J 7.2 Hz, 1 H, CH), 2.33 (singlet, 3 H, CH_3),1.38 (doublet, J 7.2 Hz, 6 H, CH_3), 1.21 (doublet, J 7.2 Hz, 12 H, CH_3).

2-Bromo-1,3,5-triisopropylbenzene (BrP) and *2,4-dibromo-1,3,5-triisoprobylbenzene* (Br_2P) were prepared from 1,3,5-triisopropylbenzene according to a method previously described.[4] Mass spectrum for BrP: 43 (62), 115 (17), 117 (15), 128 (18), 129 (17), 145 (13), 239 (15), 241 (15), 267 (100), 268 (19), 269 (98), 270 (16), 282 (43.0), 283 (9.4), 284 (42.8), 285 (7.7). Mass spectrum for Br_2P: 43 (58), 105 (18), 128 (23), 129 (20), 141 (14), 143 (12), 305 (12), 345 (44), 347 (100), 349 (43), 360 (23.8), 361 (6.1), 362 (47.6), 363 (8.5), 364 (22.7).

1,3,5-Triisopropyl-2,4-dimethylbenzene (Me_2P) was prepared from 3-bromo-2,4,6-triisopropyltoluene by treatment first with butyllithium and then with iodomethane. The method has been described by Myhre.[3] Mass spectrum for Me_2P: 41 (25), 43 (37), 119 (12), 133 (14), 147 (16), 217 (100), 218 (18), 232 (32.2), 233 (5.5).

Hydrogen exchange reactions

A. To a solution of 2,4,6-triisopropyltoluene (50 mg, 0.23 mmol) in 1 ml of carbon tetrachloride, 2 ml (26.6 mmol) of deuteriated trifluoroacetic acid was added. The reaction flask was closed in order to avoid contamination with moisture and kept at 40 °C for 60 h. The solvent and the trifluoroacetic acid were then distilled off at reduced pressure. The procedure was repeated three times. The residue, which was found to be pure according to gas chromatography, was then analyzed by means of NMR and mass spectrometry. The product was identified as 3,5-dideuterio-2,6-diisopropyl-4-(1,1,1,3,3,3-hexadeuterio-2-propyl)-toluene (MeP-d_8). Mass spectrum of the product: 41 (10), 43 (21), 49 (12), 177 (60), 183 (26), 207 (62), 208 (75), 211 (100), 226 (69.0), 227 (11.9). NMR: δ 3.15 (heptet, J 7.2 Hz, 2 H, CH), 2.76 (broad singlet, 1 H, CH), 2.21 (singlet, 3 H, CH_3), 1.21 (doublet, J 7.2 Hz, 12 H, CH_3).

B. MeP-d_8 was also obtained when MeP (15 mg, 0.07 mmol) was treated with an excess of deuteriated trifluoroacetic acid (4 ml, 53.2 (mmol) in 1 ml of carbon tetrachloride for 48 h. After 12 h an aliquot was withdrawn, and according to mass spectrometry only two types of molecules had been formed. One of them was 3,5-dideuterio-2,4,6-triisopropyltoluene (MeP-d_2) and the other was MeP-d_8. These compounds had been formed in about equal amounts. It might be noted that no random deuterium distribution occurred among the hydrogen atoms capable of undergoing exchange.

C. Exchange experiments were carried out using the concentrations described under A, once under a nitrogen atmosphere and with solutions freed from oxygen and once under an oxygen atmosphere and with solutions treated with oxygen. The reaction times were in all cases 12 h. By means of mass spectrometry the products were identified as mixtures of MeP, MeP-d_2 and MeP-d_8 The compositions of the mixtures were the same in both cases.

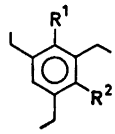
D. The influence of peroxides and of radical inhibitors on the rate of the aliphatic hydrogen exchange was determined. The experimental conditions described under A were used, and after 12 h the reaction products were analyzed by means of mass spectrometry and the relative amounts of MeP, MeP-d_2, and MeP-d_8 were determined. It was found that addition of dibenzoyl peroxide had a marked accelerating influence on the rate of exchange of the aliphatic protons, and it was also found that if the MeP was freed from peroxides (chromatographed on alumina with hexane as eluent) prior to the experiment, the exchange was slowed down. Radical inhibitors like pyrocathecol and iodine had no influence on the rate of exchange, whereas hydroquinone had a marked decelerating influence.

E. Treatments with deuteriated trifluoroacetic acid under the same conditions as used in A were carried out with 1,3,5-triisopropyl-2,4-dimethylbenzene, 2-bromo-1,3,5-triisopropylbenzene, 2,4-dibromo-1,3,5-triisopropylbenzene, 3-bromo-2,4,6-triisopropyltoluene, 1,3,5-triisopropylbenzene, 2,4,6-triethyltoluene, 1,3,5-triethyl-2,4-dimethylbenzene, and *p*-cymene. For none of these compounds could an exchange of aliphatic protons be detected by mass spectrometry. The aromatic protons were exchanged in all compounds except 2-bromo-1,3,5-triisopropylbenzene, 2,4-dibromo-1,3,5-triisopropylbenzene, and *p*-cymene.

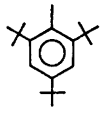
When the same experiment was performed with 2,4,6-tri-*tert*-butyltoluene, the compound underwent a partial decomposition as found by Myhre.[3] The product mixture was analyzed by means of combined gas chromatography and mass spectrometry. In the remaining 2,4,6-tri-*tert*-butyltoluene (amounting to *ca.* 10 %) only the aromatic protons were exchanged.

F. 2,4,6-Triisopropyltoluene (100 mg, 0.46 mmol) was added to a mixture of deuteriated sulfuric acid (9.0 ml, 170 mmol) and deuterium oxide (2 ml, 100 mmol). The reaction mixture was stirred at room temperature for 60 h and was then worked up as previously

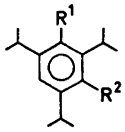
described.[1] According to NMR and mass spectrometry the product consisted of a mixture of 2,4,6-triisopropyltoluene, 3,5-dideuterio-2,4,6-triisopropyltoluene (MeP-d_2), 3,5-dideuterio-2,6-diisopropyl-4-(1,1,1,3,3,3-hexadeuterio-2-propyl)-toluene (MeP-d_8), 3,5-dideuterio-2-isopropyl-4,6-bis(1,1,1,3,3,3-hexadeuterio-2-propyl)-toluene (MeP-d_{14}) and 3,5-dideuterio-2,4,6-tris (1,1,1,3,3,3-hexadeuterio-2-propyl)-toluene (MeP-d_{20}). The two *ortho* isopropyl groups reacted more slowly than the *para* isopropyl group as is evident from the fact that the only species with eight deuterons was the isomer MeP-d_8, identified in section A. As observed in the previous experiments, no partial deuteriation of the methyl groups in an isopropyl group had occurred. The experiment was repeated with 1,3,5-triisopropylbenzene, but, in this case, exchange was found only in the aromatic positions.



$R^1 = CH_3$, $R^2 = H$	MeE
$R^1 = R^2 = CH_3$	Me_2E



Me B



$R^1 = R^2 = H$	P
$R^1 = CH_3$, $R^2 = H$	MeP
$R^1 = R^2 = CH_3$	Me_2P
$R^1 = Br$, $R^2 = H$	BrP
$R^1 = R^2 = Br$	Br_2P
$R^1 = Br$, $R^2 = CH_3$	BrMeP

DISCUSSION

When MeP is treated with deuteriated trifluoroacetic acid in carbon tetrachloride the aromatic protons are exchanged for deuterons as expected. In addition, the protons of the two methyl groups of the isopropyl group in the 4-position are also exchanged. The methine proton of that isopropyl group is not exchanged, however, and the product is MeP-d_8.

When BrP, Br_2P, and *p*-cymene undergo the treatment in question no protons at all are exchanged. When MeE, Me_2E, P, Me_2P and BrMeP are treated as described above, they undergo exchange of the aromatic protons, but all the aliphatic protons are retained.

The compound MeB undergoes decomposition under the actual conditions, but if the reaction is stopped before all the starting material is consumed, only the aromatic protons are exchanged in the remaining MeB.

The observations made may be interpreted in the following way. BrP, Br_2P, and *p*-cymene are not activated enough so as to undergo an electrophilic aromatic hydrogen exchange under the actual conditions. BrP and MeP have different electronic properties but should sterically be quite similar. Their different behaviour indicates that there is a correlation between the ability to undergo aliphatic hydrogen exchange and the reactivity towards electrophiles. The same conclusion can be drawn from a comparison of MeP and *p*-cymene.

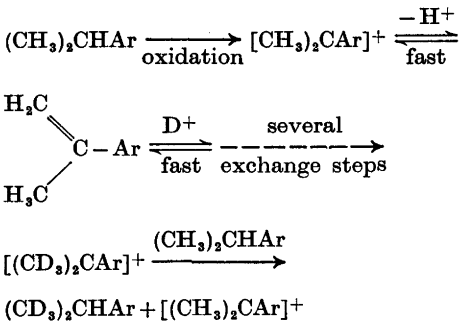
From a comparison between MeE, MeP, and MeB, it can be concluded that it is only in an isopropyl group that the methyl hydrogens can exchange under the experimental conditions used. Ethyl and *tert*-butyl groups are left unaffected, as are the methyl groups directly attached to the benzene rings.

When the behaviour of P, MeP, and Me_2P is compared it is found that not only the electronic properties, but also the steric ones are of importance for the aliphatic hydrogen exchange. P does not exchange any aliphatic hydrogens but MeP does. MeP ought to be more reactive towards electrophiles than P and their difference concerning aliphatic hydrogen exchange is thus consistent with the above-mentioned correlation. Me_2P, on the other hand, does not exchange aliphatic hydrogens. This may be due to steric hindrance since all the isopropyl groups of Me_2P have at least one methyl group in an *ortho* position. In the case of MeP it is only the isopropyl group without an *ortho* substituent that undergoes exchange.

The aliphatic hydrogens of BrMeP are unaffected, but the aromatic hydrogens do ex-

change. This behaviour may be due either to steric hindrance or to the electronic properties of the substrate.

Since it was found that dibenzoyl peroxide had a catalytic effect on the exchange of the aliphatic protons in MeP, the mechanism in Scheme 1 seems to be the most likely one for that reaction.



Scheme 1. Ar denotes a 3,5-diisopropyl-4-methylphenyl group.

A similar mechanism has been suggested by Setkina *et al.*[5] for hydrogen exchange occurring when benzene derivatives containing condensed polymethylene rings were treated with deuteriated sulfuric acid in nitrobenzene. The same result was also obtained when these compounds were treated with deuteriated trifluoroacetic acid[6] and when hexaethylbenzene was treated with a mixture of deuteriated sulfuric acid and deuteriated trifluoroacetic acid.[7]

In the proposed mechanism the substrate is oxidized and a tertiary carbocation is formed. For maximum stabilization of this ion the two methyl groups directly attached to the positive carbon of the side-chain should lie in the plane of the benzene ring. This explains the selectivity of the aliphatic hydrogen exchange (the isopropyl groups without *ortho* substituents are much more reactive than the others). For steric reasons, the isopropyl groups which are next neighbours to other substituents cannot become coplanar with the benzene ring and lose the methine proton as easily as those without such substituents. The carbocation undergoes a rapid hydrogen exchange which occurs *via* an olefin. This step must be faster than the step leading to the deuteriated MeP since only hexadeuteriated or completely nondeuteriated isopropyl groups were observed. Molecules with no methyl deuterons and those with six (or even twelve or eighteen) methyl deuterons were thus found together if the reaction time was short enough. See cases B and F in the Experimental section. The step leading from the carbocation to the product is thought to be a hydride-ion transfer from a methine position of another molecule, and thus the reaction is expected to be of the chain type.

The proposed mechanism explains why there is a correlation with the ability to undergo aliphatic hydrogen exchange and the reactivity towards electrophiles. The intermediate carbocation that is formed in the aliphatic hydrogen exchange has the positive charge delocalized in a way that resembles the delocalization of the positive charge in the intermediate cyclohexadienyl cation formed during electrophilic aromatic substitution. For a discussion of similar cases, see Ref. 8.

Since it is well-known that the methine protons of isopropyl groups attached to aromatic rings can be easily oxidized (one example is the oxidation of cumene [9,10]), the first step in the proposed mechanism seems quite probable.

When strong sulfuric acid was used as the medium, it was indeed possible to exchange the methyl hydrogens of the isopropyl groups *ortho* to the methyl group attached to the ring in MeP. This observation may be explained as a consequence of the more severe conditions in this case, as compared with the case of trifluoroacetic acid. The two *ortho* isopropyl groups underwent exchange more slowly than the *para* group.

Hydrogen exchange in the α-positions of polyalkylated aromatic hydrocarbons has been reported on treatment with trifluoroacetic acid.[11] No such exchange could be detected in the present investigation.

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Chemical Composition of Angelica Root Oil

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The volatile constituents of angelica root separated by extraction with etherpentane and by extraction with alcohol-water were investigated by means of glass capillary and preparative gas chromatography, IR, UV, NMR and mass spectrometry. 16 monoterpene hydrocarbons, 13 sesquiterpene hydrocarbons, 12 monoterpene alcohols, 4 oxygenated sesquiterpenes, 11 esters, 3 lactones, 7 aliphatic carbonyl compounds and 4 aromatics were identified. Twenty additional compounds identified were present only in the alcoholic sample. These consisted of ethyl ethers of monoterpene alcohols, ethyl esters of long-chain fatty acids, and acetals. The relative proportion of many compounds was found to depend on the method used to isolate the essential oil.

Angelica archangelica L. is a herbaceous plant cultivated mostly in Middle Europe. Steam distillation of the roots yields 0.1 – 1 % of essential oil.[1] Dried angelica root and its derivatives (the essential oil, extracts, distillates) find extensive use in flavour formulations for alcoholic beverages, such as vermouths, bitters, benedictine and chartreuse-type liqueurs and liquors like gin.[2]

In the early investigations of angelica root oil, reviewed by Gildemeister and Hoffmann,[1] α-pinene, α-phellandrene, borneol, pentadecanolide, and 2-methyl butyric acid were found. Klouwen and ter Heide [3] confirmed the identity of pentadecanolide by its IR spectrum and developed a gas chromatographic method for its quantitative determination.

Gas chromatographic studies of the monoterpene hydrocarbons in angelica root oil have been carried out by Klouwen and ter Heide [4] and by Baerheim Svendsen and Karlsen.[5] The former report the following monoterpene hydrocarbons, identified by retention times: α-pinene, β-pinene, 3-carene, sabinene, α-phellandrene, β-phellandrene, limonene, terpinolene, myrcene, *cis*-ocimene, *trans*-ocimene, and *p*-cymene. Baerheim Svendsen and Karlsen found camphene, fenchene, α-terpinene, and γ-terpinene as minor constituents. Klouwen and ter Heide also reported the presence of borneol, carvacrol, bornyl acetate, carvotanacetone, carvone, pulegone, dihydrocarvone, and furfural.

The aims of this study were to obtain a more detailed knowledge of the volatile constituents of angelica root and to elucidate the actual aroma composition of an alcoholic angelica distillate used to flavour liquors. It is known that treating a herb with ethanol can affect the composition of the volatile oil.[6] Consequently two samples were studied, one isolated by extraction with ether-pentane followed by steam distillation and second prepared as an alcoholic distillate by Alko's flavour distillery.

EXPERIMENTAL

Apparatus. Analytical gas chromatography was performed using glass capillary columns on a Hewlett-Packard 7620 A instrument equipped with FID and a glass inlet splitter. Of the two capillary columns (0.28 mm I.D. × 25 m) used one was coated with SP-1000 and the other with OV-101. Peak areas were measured with an Infotronics CRS-11 NSB/42 integrator. For preparative GLC the same instrument with TC detector and 4 mm I.D. × 3 m glass columns packed with 5 % LAC-2R-446 or 5 % OV-101 on 70/80 mesh Chromosorb G AW DMCS were used. 6 mm × 3 m stainless steel columns packed with 10 % FFAP or 15 % Apiezon L on 60/80 mesh Chromosorb W AW were used for some separations. Fractions were collected in 1 mm × 20 cm glass tubes cooled with dry ice.

IR spectra of pure samples were recorded as films between KBr pellets on a Perkin-Elmer

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521 spectrophotometer equipped with a beam condenser. UV spectra were measured in ethanol on an Opton PMQ II spectrophotometer. NMR spectra were determined on a Varian A-60 spectrometer in Department of Chemistry, Helsinki University of Technology, or Fourier transformation spectra on a JEOL JMM PFT 100 in Department of Organic Chemistry, University of Helsinki. CCl_4 or $CDCl_3$ was used as the solvent and TMS as an internal standard. The mass spectra were determined in Department of Medical Chemistry, University of Helsinki, on a Varian CH 7 combined GLC-MS instrument coupled with Spectro System 100 MS computer. A 2 mm I.D. × 2 m SE-30 glass column was used in the gas chromatograph.

Materials and procedure. Angelica root oil was isolated by extracting 600 g of the ground herb (purchased from G.H. Worlée & Co m.b.H, Germany) with etherpentane (2:1) for two days in a Soxhlet-type extractor. After removing the solvent, the residue was distilled with steam to separate the volatiles. The distillate was extracted with pentane and, after drying over anhydrous Na_2SO_4, pentane was distilled off to leave 1.4 g of volatile oil.

The alcoholic angelica distillate was prepared in Alko's flavour distillery by percolating the herb with an ethanol-water mixture and distilling the percolate under reduced pressure. For analysis the essential oil was separated from the alcoholic solution by extracting with pentane as described previously.[6]

4 Litres of the alcoholic flavour distillate was distilled to 1.5 l under reduced pressure to concentrate the less volatile aroma fraction. Higher boiling compounds were extracted with pentane from the residue.

The steam distilled oil as well as the samples prepared from the alcoholic distillate were fractionated by column chromatography on silica gel as described earlier [6] to obtain a hydrocarbon fraction and a fraction of oxygenated compounds. These were further fractionated by preparative GLC using the LAC-2R-446 column. Analysis of the subfractions with one or both of the capillary columns showed all of them to be mixtures of several compounds. Pure compounds for determination of spectra were obtained by rechromatographing these subfractions on the OV-101 column. A third preparative column was occasionally needed.

Some esters of monoterpene alcohols were hydrolysed using 10 % alcoholic KOH at room temperature to aid the identification.

Relative amounts of components were determined gas chromatographically from the peak areas without using response factors.

RESULTS AND DISCUSSION

The gas chromatogram of angelica root oil obtained by extraction with ether-pentane followed by steam distillation (sample A) is shown in Fig. 1, and the chromatogram of the pentane extract from the alcoholic angelica root distillate (sample B) in Fig. 2. In Table 1 are presented the compounds identified with their percentage contribution and identification methods with references to literature spectra. The percentages calculated from peak areas are only approximate because of the use of the injection splitter and some incomplete separations.

GLC with capillary columns showed that the hydrocarbon fraction of both samples contained

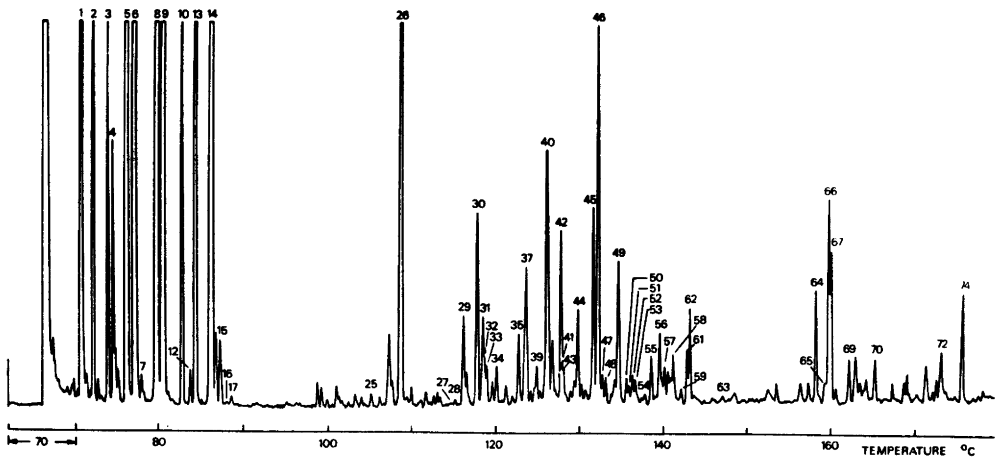


Fig. 1. Gas chromatogram of sample A on SP-1000 glass capillary column (0.28 mm I.D. × 25 m). Helium flow 0.7 ml/min. Temperature program: 4 min at 70 °C, 2 °C/min 70 – 215 °C.

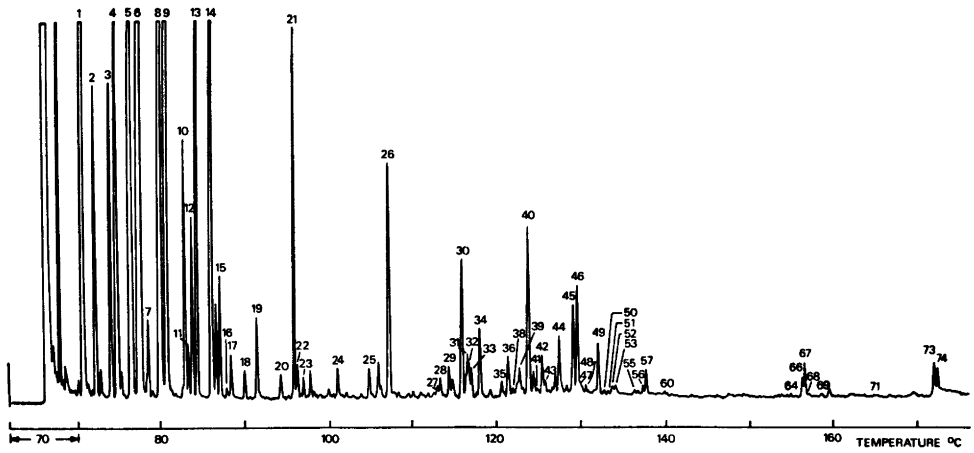


Fig. 2. Gas chromatogram of sample B on SP-1000 glass capillary column.

the same components in approximately the same relative proportions. The 16 monoterpene hydrocarbons identified, all of which had been found before, constituted about 82 % of sample A and 89 % of sample B. The main components were α-pinene, 3-carene, α-phellandrene, β-phellandrene, limonene, and *p*-cymene.

The sesquiterpene hydrocarbons formed about 7 % of sample A and about 4 % of sample B. For identification they were isolated from the high boiling fraction of the alcoholic distillate. The compounds identified were α-copaene, β-copaene, α-muurolene, γ-muurolene, δ-cadinene, germacrene-D, caryophyllene, α-humulene, β-farnesene, β-bisabolene, β-elemene, ar-curcumene, and cuparene.

In the fractions containing the oxygenated compounds, which constituted about 11 % of sample A and about 7 % of sample B, striking differences were found between the two samples. In sample A terpene alcohols proved to be the principal constituents. From monoterpenes, *trans*-verbenol (the most abundant alcohol), *cis*-verbenol, *cis*- and *trans*-2-*p*-menthen-1-ol, *cis*- and *trans*-piperitol, *cis*- and *trans*-sabinene hydrate, linalool, terpinenol-4, and *trans*-carveol were identified.

Three additional alcohols were found, which, according to spectral evidence were derivatives of the phellandrenes. One of them (peak 46) had IR absorptions at 3390 and 1125 cm^{-1}, corresponding to a tertiary OH group and at 3020, 820, 790, 770, and 730 cm^{-1}, which were attributed to a trisubstituted and a *cis*-disubstituted double bond. The compound had a UV absorption maximum at 263 nm, showing conjugation of the double bonds. The mass spectrum gave a molecular weight of 152, clearly corresponding to the formula $C_{10}H_{16}O$, so that a ring besides the two double bonds should be present. The structure derived from 1,5-*p*-menthadiene giving 262 nm for the calculated λ_{max},[7] is compatible with these data. The base peak at *m/e* 59 suggests that the position of the hydroxyl is at C-8. Accordingly, this compound was tentatively identified as α-phellandrene-8-ol. The mass spectrum of component 56 displayed the molecular peak at *m/e* 152 and base peak at *m/e* 92. The IR spectrum revealed the presence of secondary or primary OH (3360, 1035 cm^{-1}), *gem*-dimethyl (1382, 1365 cm^{-1}), terminal methylene (3070, 888 cm^{-1}) and a *cis*-disubstituted double bond (3020, 798, 760 cm^{-1}). The double absorption at 1635 and 1595 cm^{-1} implied conjugation of the double bonds, which was confirmed by UV spectrum (λ_{max} 232 nm). The most reasonable structure to fit these data seems to be 1(7),2-*p*-menthadiene with hydroxyl in the 5- or 6-position. The proposed structures give 232 nm for the calculated λ_{max}. The third alcohol (peak 59), which had a similar IR spectrum and almost identical mass spectrum to those of 56, is evidently the diastereomer of this compound.

Table 1. Compounds identified in angelica root oil obtained by extraction with ether-pentane followed by steam distillation (sample A) and in a pentane extract of alcoholic angelica root distillate (sample B).

GC peak	Compound	Percentage		Basis for identification
		Sample A	Sample B	
	Hydrocarbons			
1	α-Pinene	24.0	25.4	GLC, MS[a], IR[a]
1	α-Thujene	+	+	MS[11]
2	Camphene	1.30	1.15	GLC. MS[a]
3	β-Pinene	1.25	1.22	GLC, MS[a]
4	Sabinene	0.76	3.17	GLC, MS[a]
5	3-Carene	10.1	10.8	GLC, MS[a], IR[a]
6	α-Phellandrene	7.6	13.9	GLC, MS[a], IR[a]
6	Myrcene			GLC, MS[a], IR[a]
7	α-Terpinene	0.07	0.29	GLC, MS[a]
8	Limonene	13.2	12.5	GLC, MS[a], IR[a]
9	β-Phellandrene	10.0	10.7	IR[12], MS[13]
10	*cis*-Ocimene	1.25	1.03	MS[13]
12	γ-Terpinene	0.11	0.69	GLC, MS[a]
13	*trans*-Ocimene	2.68	2.38	MS[13], IR[12]
14	*p*-Cymene	9.8	5.48	GLC, MS[a], IR[a]
15	Terpinolene	0.16	0.46	GLC, MS[a]
26	α-Copaene	1.91	1.10	MS[14], IR[15]
31	β-Copaene	0.40	0.25	IR[16]
32	Caryophyllene			MS[14], IR[a]
33	β-Elemene	0.90	0.04	MS[14], IR[15]
36	β-Farnesene	0.15	0.21	MS, IR[15]
40	α-Humulene	0.5	0.4	MS[14], IR[15]
43	γ-Muurolene	0.04	0.03	IR[17]
44	Germacrene-D	0.26	0.30	IR[18]
45	β-Bisabolene	0.72	0.42	IR[15]
46	α-Muurolene	1.2	0.5	IR[17]
49	δ-Cadinene	0.51	0.24	MS,[14] IR[15]
52	ar-Curcumene	0.06	0.03	IR[15]
57	Cuparene	0.10	0.08	MS, IR[15]
	Alcohols			
25	*trans*-Sabinene hydrate	0.01	0.14	MS, IR[19]
27	*cis*-Sabinene hydrate	+	0.03	MS, IR[19]
28	Linalool	0.01	0.05	MS[a], IR[a]
29	*cis*-2-*p*-Menthen-1-ol	0.31	0.14	MS, IR[10]
34	Terpinenol-4	+	0.31	MS[a], IR[a]
35	*trans*-2-*p*-Menthen-1-ol	0.24	0.09	MS, IR[10]
40	*cis*-Verbenol	0.1	0.05	MS, IR[a]
41	*trans*-Piperitol	+	+	MS[20]
42	*trans*-Verbenol	0.64	0.19	MS, IR[a]
46	α-Phellandrene-8-ol	0.4	0.1	MS, IR, UV
47	*cis*-Piperitol	0.05	+	MS[20], IR[10]
58	*trans*-Carveol	0.11		MS, IR[10]
61	*m*-Cymene-8-ol	0.19	+	MS, IR
62	*p*-Cymene-8-ol	0.31	+	MS[22], IR[10]
65	Cuminyl alcohol	0.02		MS, IR[10]
66	α-Copaene-11-ol	0.46	0.05	MS[23], IR, NMR[23]
69	Elemol	0.18	+	MS, IR[24]
72	β-Eudesmol	0.21		MS, IR[25]
	Esters and lactones			
30	Bornyl acetate	0.73	0.69	MS[26], IR
37	*trans*-Verbenyl acetate	0.46	0.02	MS, IR[a]

Table 1. Continued.

39	*trans*-Pinocarvyl acetate	0.17	0.18	MS, IR, H[b]
48	*cis*-Carvyl acetate	0.05	0.02	MS,[26] IR[a]
50	Bornyl isovalerate	0.07	0.01	MS, IR[a]
51	*trans*-Carvyl acetate	0.03	0.01	MS[26], IR[a]
53	*trans*-Verbenyl 2-methyl-butyrate	0.04	0.02	MS, IR, H
55	*trans*-Verbenyl isovalerate	0.17	0.02	MS, IR, H
17	Isoamyl isovalerate	0.02	0.02	GLC, MS[27]
	Heptyl isovalerate[c]	+	+	MS
	Octyl isovalerate[c]	+	+	MS[a], IR[a]
38	Ethyl caprate		0.02	MS, IR[a]
60	Ethyl laurate		0.01	MS, IR[a]
67	Ethyl myristate		+	MS, IR
71	Ethyl pentadecanoate		+	MS, IR
73	Ethyl palmitate		0.19	GLC, MS[a], IR[a]
67	Tridecanolide	0.45	0.13	MS, IR, NMR
74	Pentadecanolide	0.37	0.14	GLC, MS[a], IR[a]
	Ethyl stearate[c]		+	GLC, MS, IR[a]
	Ethyl oleate[c]		+	GLC, MS, IR[a]
	Ethyl linoleate[c]		0.17	GLC, MS, IR[a]
	Ethyl linolenate[c]		+	GLC, MS, IR[a]
	Heptadecanolide[c]	+		MS
	Ethers			
11	*trans*-4-Ethoxy-thujane		0.19	MS[6], IR[6]
17	*cis*-4-Ethoxy-thujane		0.12	MS[6], IR[6]
18	*cis*-4-Ethoxy-2-pinene		0.10	MS, IR
19	*cis*-1-Ethoxy-2-*p*-menthene		0.33	MS[6], IR[6]
20	*trans*-3-Ethoxy-1-*p*-menthene		0.09	MS[6], IR[6]
21	*trans*-4-Ethoxy-2-pinene		1.58	MS, IR, NMR
22	*trans*-1-Ethoxy-2-*p*-menthene		0.12	MS, IR
23	4-Ethoxy-1-p-menthene		0.06	MS[6], IR[6]
24	*cis*-3-Ethoxy-1-p-menthene		0.15	MS[6], IR[6]
	Carbonyl compounds			
16	Octanal	0.01	0.03	GLC, MS[28]
	Nonanal[c]	+	+	MS[28]
63	Tetradecanal	0.02		MS[28], IR
	Pentadecanal	+		MS
54	Cuminyl aldehyde	0.02	+	MS, IR[29]
	2-Nonanone[c]		+	MS
	2-Decanone[c]	+		MS
40	Cryptone	0.5	0.4	MS[30], IR[30]
	Others			
	1,1-Diethoxyhexane[c]		+	MS[31]
	1,1-Diethoxyoctane[c]		+	MS[31]
64	Humulene monoxide	0.41	0.01	MS, IR[32]

[a] Spectrum of an authentic sample. [b] Hydrolysis. [c] Retention time was not measured on the capillary column.

Several sesquiterpene alcohols, distributed from peak 66 to 72, were detected, although many of them remained unidentified. Peak 66 was composed of two sesquiterpene alcohols one of which was identified as α-copaene-11-ol. Compounds represented by peaks 69 and 72 were identified as elemol and β-eudesmol, respectively.

Most of the above alcohols were also found in sample B, although generally in smaller relative proportions, whereas the presence of their ethyl ethers proved to be a characteristic feature of the alcoholic angelica distillate. The most abundant oxygen-containing compound (peak 21) in sample B was identified as the ethyl ether of *trans*-verbenol (*trans*-4-

ethoxy-2-pinene). Its IR spectrum had absorptions at 3020, 1647, 1380, 1365, 1080, 810, and 770 cm^{-1} corresponding to a double bond, a *gem*-dimethyl group and an ether linkage. The NMR spectrum showed signals at δ 0.84 and 1.33 (each 3 H, singlets) due to two methyls at quaternary carbons, at δ 1.70 (3 H, doublet of doublets, J 1.6 and 1.6 Hz) and 5.30 (1 H, multiplet) corresponding to a methyl on a trisubstituted double bond at δ 3.79 (1 H, multiplet) due to a methine proton adjacent to oxygen and at δ 1.13 (3 H, triplet, J 7 Hz) and 3.43 (2 H, quartet, J 7 Hz) from an ethoxy group. M^+ at m/e 180 corresponds to the formula $C_{12}H_{20}O$. Consequently, because only one double bond was found the compound should be bicyclic. These data are consistent with a structure such as 4-ethoxy-2-pinene. The choice between the possible stereoisomers was made by comparing the chemical shift of the C-9 methyl protons with that of *cis*-verbenol (δ 1.07) and *trans*-verbenol (δ 0.87).[8] A smaller component (peak 18) with similar IR and mass spectra to those of component 21 was concluded to be *cis*-4-ethoxy-2-pinene. In addition to these, the ethyl ethers of the sabinene hydrates, the piperitols, the 2-*p*-menthen-1-ols and terpinenol-4, which were previously identified from a sweet marjoram distillate,[6] were found. According to mass spectra some of the sesquiterpene alcohols were also present as ethyl ethers.

The ethyl esters of caproic, lauric, myristic, pentadecanoic, palmitic, stearic, oleic, linoleic, and linolenic acid formed another group of compounds found only in sample B. Both these esters and the ethyl ethers are evidently formed during the preparation of the alcoholic aroma distillate.

Esters of monoterpene alcohols were notable constituents in both samples, bornyl acetate being the most abundant oxygenated component in sample A. *trans*-Verbenyl acetate, *cis*- and *trans*-carvyl acetate and bornyl isovalerate were also found. Component 39 was identified as *trans*-pinocarvyl acetate. Its mass spectrum gave a molecular weight of 194. The IR spectrum showed absorptions at 1735 and 1240 cm^{-1} typical for acetates and at 3070, 1655, and 885 cm^{-1} corresponding to a terminal methylene. *trans*-Pinocarveol was obtained on hydrolysis. The components giving rise to peaks 53 and 55 were identified as *trans*-verbenyl 2-methyl-butyrate and *trans*-verbenyl isovalerate respectively. The mass spectra of both displayed the molecular peak at m/e 236 and prominent peaks at m/e 57, 85, and 119. Both compounds had IR absorptions at 3030, 1650, 780, and at 1735 and about 1180 cm^{-1} corresponding to a trisubstituted double bond and an ester function respectively. They gave *trans*-verbenol on hydrolysis. The identification of the acid parts was based on the comparison of absorptions in the 1300 – 1100 cm^{-1} region of the IR spectra of the esters. Besides these terpene esters, isoamyl isovalerate, heptyl isovalerate, and octyl isovalerate were found in both samples.

The musk-like odour of angelica root oil is generally attributed to the lactone of 15-hydroxypentadecanoic acid. Besides pentadecanolide, three macrocyclic lactones were found in the samples studied. The mass spectrum of compound 67 showing M^+ at m/e 212, base peak at m/e 55 and peaks for M – 18, M – 36 and M – 60 fragments at the high mass end, closely resembled that of pentadecanolide. The IR spectrum exhibited strong absorptions at 1733 (C=O) and 1240 cm^{-1} (C–O). The NMR spectrum had signals at δ 4.14 (2 H, doublet of doublets, J 4 and 5.5 Hz) due to methylene protons adjacent to oxygen, at δ 2.39 (2 H, doublet of doublets, J 5 and 6 Hz) corresponding to methylene protons adjacent to a carbonyl group, and broad signals due to methylene protons at δ 1.3 – 1.8. The absence of signals corresponding to methyl protons revealed that the compound has a ring structure with no side chain. The obvious molecular formula, $C_{13}H_{24}O_2$, allows one ring in addition to a carbonyl double bond. Accordingly, compound 67 was identified as tridecanolide. A minor component, peak 68, had very similar IR spectrum to that of tridecanolide. The mass spectrum gave a molecular weight of 226. The fragmentation pattern showed marked similarity to that of the other lactones, but some anomalous peaks were also present, *e.g.* m/e 153, 171. It remained unclear whether these were caused by impurities or some structural difference. A trace compound from sample A was identified as heptadecanolide. Its mass spectrum had molecular peak at m/e 268 and the fragmentation pattern was analogous to that of pentadecanolide.

Carbonyl compounds constituted less than 1 % of both samples. Nevertheless, cryptone was one of the main components in the oxygen-containing fraction.[5] The other aliphatic carbonyl compounds identified were octanal, nonanal, tetradecanal, pentadecanal, 2-nonanone, and 2-decanone. 1,1-Diethoxy hexane and 1,1-diethoxy octane were detected in sample B, showing that the aldehydes are present partly as acetals in the alcoholic distillate.

Three aromatic compounds, cuminyl aldehyde, cuminyl alcohol and *p*-cymene-8-ol, were conclusively identified. The mass spectrum of compound 61 closely resembled that of *p*-cymene-8-ol, displaying M^+ at *m/e* 150 and the base peak at *m/e* 43. The IR spectrum had absorptions at 3400 and 1165 cm^{-1} corresponding to a tertiary OH, and at 1605 and 1487 cm^{-1} for the aromatic nucleus. The out-of-plane bending absorptions at 773 and 703 cm^{-1} suggested *meta*-substitution. Accordingly, compound 61 was tentatively identified as *m*-cymene-8-ol. The mass spectrum of peak 70 was almost identical with that published for carvacrol.[9] The IR spectrum showed absorptions corresponding to a phenolic OH (3380, 1250 cm^{-1}), a *gem*-dimethyl group (1380, 1360 cm^{-1}) and a 1,2,4-substituted aromatic ring (1615, 1585, 1500, 860, 810 cm^{-1}). The spectrum, however, differed from those published for carvacrol and thymol.[10] It seems likely that the compound is one of the other isomers with the same substituents in the 1, 2, and 4 positions. Besides the compounds discussed above, capillary gas chromatography revealed more than hundred minor components in the samples.

The results show that the method used to isolate the angelica root oil has a substantial effect on its chemical composition. Quantitative differences between the two samples were observed in the fractions of oxygenated compounds. Furthermore, the alcohol extraction was found to produce artefacts by esterification, etherification and acetal formation.

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Synthesis of *p*-Isothiocyanatophenyl 3-*O*-(3,6-Dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranoside

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The synthesis of the disaccharide derivative Tyv*p* $\xrightarrow[\alpha]{1\to3}$ D-Man*p* $\xrightarrow[\alpha]{1}$ *p*-C_6H_4–N=C=S (XVI, title compound) is described. The key step is reaction of di-*p*-nitrobenzoyl 3,6-dideoxyhexosyl bromide VI with protected mannoside VIII using the Helferich glycosylation procedure. The disaccharide derivative XVI reacts with free amino groups in proteins to give disaccharide-protein antigens with the same specificity as *Salmonella* serogroup D_1 O-factor 9 (*e.g. S. typhi*). These disaccharide-protein conjugates have previously been found to be promising for specific diagnosis and possibly for immunization.

As part of our efforts [1–4] directed towards the preparation of disaccharide-protein conjugates having disaccharide or oligosaccharide moieties with serospecific *Salmonella* O-factors,[5] we now report the synthesis of a 3-*O*-tyvelosyl-α-D-mannopyranoside having a functional group in the mannoside aglycon useful for attachment of the disaccharide to proteins. Such synthetic antigens are of potential use for diagnosis and prophylactic procedures.[4]

In the present work 2,4-di-*O*-*p*-nitrobenzoyl α-tyvelosyl bromide (VI) was prepared and condensed with *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (VIII) in acetonitrile using mercuric cyanide as a promotor.[6]

Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (I) [7] was treated with *N*-bromosuccinimide [8,9] to give the 6-bromo-4-*O*-benzoyl derivative II which upon catalytic

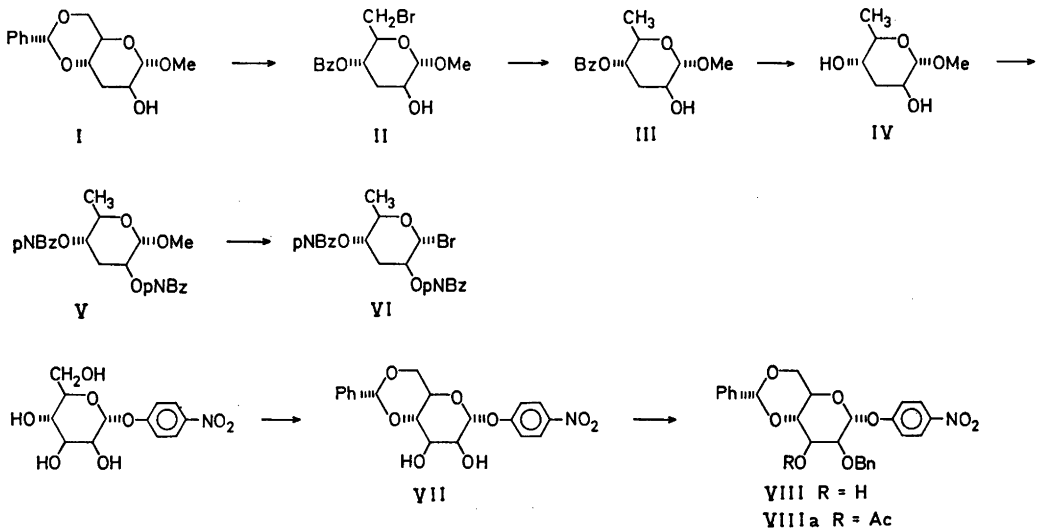


Table 1. Selected NMR shifts (in ppm downfield from internal TMS) and coupling constants

Substance	H-1	H-3	H-4	H-5	H-6 in C-CH_3	Other H-6
II	4.70 $J_{1,2}$<1	1.8–2.4 (2 H)m	5.30 sext $J_{4,3e}$5.5 $J_{4,3a}$ 10 $J_{4,5}$ 10	–	–	3.55d $J_{5,6}$5
III	4.59 $J_{1,2}$<1	1.9–2.35 (2 H)m	5.15 sext $J_{4,3e}$ 5.5 $J_{4,3e}$ 10 $J_{4,5}$ 10	–	1.28d $J_{5,6}$6.5	–
V	4.79 $J_{1,2}$<1	2.15–2.6 (2 H)m	–	4.17 oct $J_{4,5}$ 10 $J_{5,6}$ 6.5	1.36d $J_{5,6}$ 6.5	–
VI	6.55 $J_{1,2}$<1	2.35–2.85 (2 H)m	–	4.34 oct $J_{4,5}$ 10 $J_{5,6}$ 6	1.40d $J_{5,6}$ 6	–
VIII	5.60 $J_{1,2}$ 1.5	–	–	–	–	–
VIIIa	5.63 $J_{1,2}$ 1.5	5.52 $J_{3,2}$ 3 $J_{3,4}$ 9.5	–	–	–	–
VIIIa [a]	5.76 $J_{1,2}$ 1.5	6.28 $J_{3,2}$ 3 $J_{3,4}$ 9.5	–	–	–	–
IX	5.72 [b] $J_{1,2}$ 1.5	2.2–2.6 [c] (2H)m	–	–	1.38d $J_{5,6}$ 6.5	–
X	5.61 $J_{1,2}$ 1.5 [b] 5.00 $J_{1,2}$ 1.5 [c]	1.7–2.3 [c] (2 H)m	–	–	1.28d $J_{5,6}$ 5.5	–
XI	5.69 [b] $J_{1,2}$ 2	1.85–2.25 [c] (2 H)m	–	–	1.23d $J_{5,6}$ 6	–
XIII [b]	5.56 [b] $J_{1,2}$ 1.5	1.85–2.25 [c] (2 H)m	–	–	1.23d $J_{5,6}$ 6.5	–
XIV	5.51 [b] $J_{1,2}$ 1.5	1.85–2.3 [c] (2 H)m	–	–	1.21d $J_{5,6}$ 6	–
XVI	5.61 [b] $J_{1,2}$ 2	1.7–2.2 [c] (2 H)m	–	–	1.28d $J_{5,6}$ 5.5	–

[a] VIIIa With 0.2 mol $Eu(DPM)_3$ per mol sugar added. [b] Mannosyl residue. [c] Tyvelosyl residue.

(in Hz). Solvent: $CDCl_3$ throughout, except for XIV and XVI for which CD_3OD was used.

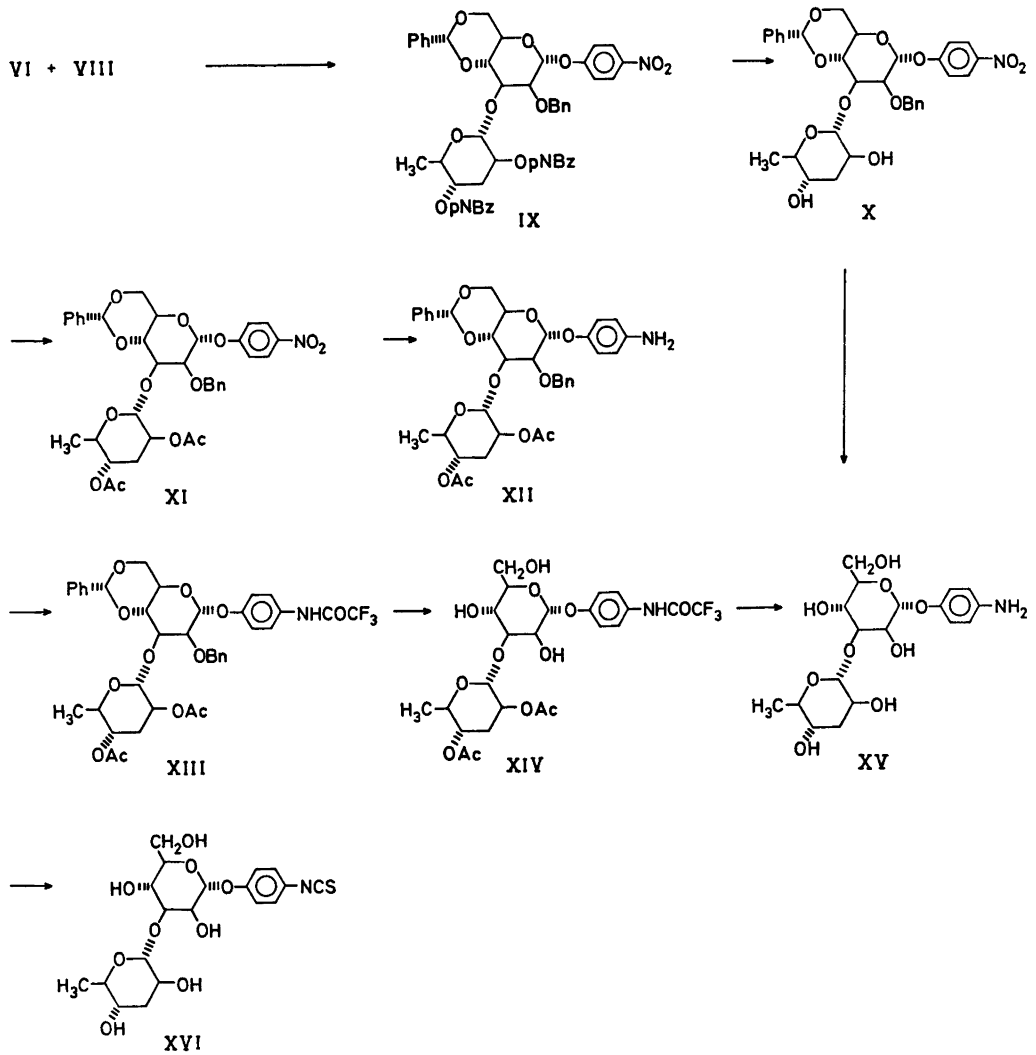
$OCOCH_3$	OCH_3	$PhCH_2$	PhCH	$pNO_2C_6H_4CO$	$pNO_2C_6H_4O$	pCF_3CONH C_6H_4	Other aromatic
–	3.50s	–	–	–	–	–	7.3–8.2 (5 H)
–	3.44s	–	–	–	–	–	7.3–8.2 (5 H)
–	3.52s	–	–	8.0–8.4 (8 H)	–	–	–
–	–	–	–	–	–	–	–
–	–	4.81s	5.57s		7.06d 8.16d $J_{H,H}$ 9	–	7.2–7.7 (10 H)
2.06s	–	4.73s	5.59s	–	7.10d 8.18d $J_{H,H}$ 9.5	–	7.25–7.6 (10 H)
2.50s	–	4.90s	5.72s	–	7.07d 8.18d $J_{H,H}$ 9.5	–	7.25–7.6 (10 H)
–	–	4.97s	5.67s	8.05–8.4 (8 H)	7.0–7.7 (2 H) 8.05–8.4 (2 H)	–	7.0–7.7 (10 H)
–		4.83s 4.86s	5.56s	–	7.06d 8.17d $J_{H,H}$ 9	–	7.2–7.6 (10 H)
2.03s 2.07s	–	4.91s	5.64s	–	7.09d 8.20d $J_{H,H}$ 9.5	–	7.25–7.6 (10 H)
2.03s 2.08s	–	4.87s	5.62s	–	–	7.04d 7.52d $J_{H,H}$ 9	7.2–7.5 (10 H)
2.06s 2.13s	–	–	–	–	–	7.19d 7.58d $J_{H,H}$ 9	–
–	–	–	–	–	–	Signals centered at 7.21 (2 H) m and 7.42 (2 H) m	–

[d] N–H 8.48 ppm (s).

hydrogenation (to give III), followed by debenzoylation (to give IV) and treatment with *p*-nitrobenzoyl chloride in pyridine afforded V in 35 % yield from I. Treatment of the glycoside V with hydrogen bromide in dichloromethane gave 2,4-di-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexopyranosyl bromide (VI) which was used directly in the glycosylation step described below.

p-Nitrophenyl α-D-mannopyranoside was treated with benzaldehyde in formic acid as described by Buchanan and Schwarz for the corresponding methyl mannoside.[10] The 4,6-*O*-benzylidene derivative VII, obtained in 36 % yield, was monobenzylated with benzyl bromide and silver oxide in dimethyl formamide [11,12] giving, after chromatography using silica gel, *p*-nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (VIII) in 56 % yield. The structure of VIII was shown by acetylation of the remaining free hydroxyl group and examination of the product (VIIIa) by NMR. The large (9.5 Hz) and small (3 Hz) coupling observed for the hydrogen attached to the acetoxylated carbon demonstrates that this hydrogen is axial (*a*/*e* coupling to H-2, *a*/*a* coupling to H-4). This was confirmed by NMR in the presence of tris(dipivaloylmethanato)-europium (Table 1).

Condensation of VIII with the 3,6-dideoxy-glycosyl bromide VI in acetonitrile in the



presence of mercuric cyanide,[6] afforded the protected disaccharide IX in 54 – 70 % yield. *p*-Nitrobenzoyl groups were removed from IX by treatment with barium oxide in methanol. Further deprotection of the disaccharide and conversion to the *p*-aminophenyl glycoside XV by catalytic hydrogenation proved unsatisfactory because the nitro group was converted to an amino group and thereafter hydrogenolysis proceeded very slowly, with a maximum yield of XV from IX of only 10 %. We therefore proceeded by a more circuitous route. The disaccharide derivative X was acetylated (XI), the nitro group of XI was converted to an amino group by brief catalytic hydrogenation, then the amino group of XII was tri-fluoroacetylated giving XIII. Catalytic hydrogenation of XIII proceeded in good yield to give XIV which upon treatment with methanolic ammonia afforded XV. This more indirect route has the added attraction of crystalline intermediates (XI, XIII, and XIV). Treatment of the *p*-aminophenyl glycoside XV with thiophosgene [14] afforded the title compound XVI in a 44 % yield from IX.

Although the above synthetic scheme may appear lengthy, the good yields in each step and the relative ease of purification of the intermediates have permitted the synthesis of the disaccharide derivative IX in decagram quantities. The compound IX is stable and suitable for storage. Further conversions into the title compound XVI, in particular the conversion of XIV into XVI, have therefore only been carried out on a small scale, producing small quantities of XVI required for disaccharide-protein conjugates as directed by the needs of the immunological part of our studies.

EXPERIMENTAL

General methods. Melting points are corrected. Concentrations were performed at reduced pressure and a bath temperature below 40 °C. Optical rotations (*c* 0.5 – 1.0) were recorded at room temperature using a Perkin-Elmer 141 instrument. IR spectra were recorded in KBr discs using a Perkin-Elmer 257 instrument. NMR spectra were recorded with a Varian A-60 A instrument. Analytical TLC was performed on precoated silica gel F_{254} plates (Merck). Sulfuric acid was used as spray reagent. Separations on a scale less than 5 g were performed using Merck's prepacked silica gel columns; for larger-scale separations silica gel (Merck) of particle size 0.040 – 0.063 mm was used. GLC-MS was performed using Perkin-Elmer 900 and 270 instruments and an ECNSS-M column (3 % on silanized Gas-Chrom Q) for separation of components prior to recording mass spectra (manifold temperature 200 °C, ionization potential 70 eV, ionization current 60 μA, temperature at the ion source chamber 120 °C).

NMR spectra recorded for all new compounds were in agreement with the postulated structures. Pertinent NMR parameters are shown in Table 1.

Methyl 4-O-benzoyl-6-bromo-3,6-dideoxy-α-D-arabino-hexopyranoside (II). *N*-Bromosuccinimide (0.88 g) was added to a suspension of methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (I, 1.12 g) and barium carbonate (3 g) in carbon tetrachloride (80 ml).[8,9] The mixture was refluxed with stirring for 2 h, diluted with chloroform and filtered. The filtrate was shaken with water and the organic phase dried over sodium sulfate, filtered and concentrated to a colourless amorphous powder (1.5 g). TLC (chloroform-methanol 20:1) showed the presence of major component, which was isolated by preparative TLC using the same solvent (or by column chromatography on silica gel in other preparations) to yield pure I (1.03 g). Material thus purified had $[\alpha]_D$ +96° (chloroform) (Found: C 48.9; H 5.01; O 23.0; Br 23.3. $C_{14}H_{17}O_5Br$ requires: C 48.7; H 4.96; O 23.2; Br 23.2).

Methyl 4-O-benzoyl-3,6-dideoxy-α-D-arabino-hexopyranoside (III). A solution of the 6-bromo compound II (3.7 g) in methanol (100 ml) containing 10 % palladium on carbon (0.5 g) and excess triethylamine (5 ml) was hydrogenated at 410 kPa and room temperature until no more hydrogen was consumed. Filtration and concentration gave a residue which was dissolved in dichloromethane. The dichloromethane solution was extracted with saturated aqueous sodium hydrogen carbonate and then water. After drying over sodium sulfate and filtering, the solution was concentrated to a syrup (2.61 g) which was used without further purification in the next step. An aliquot was purified by TLC (chloroform-methanol 100:5) to give a syrup, $[\alpha]_D$ +111° (chloroform) (Found C 63.1; H 6.94; $C_{14}H_{18}O_5$ requires: C 63.1; H 6.81).

Methyl 3,6-dideoxy-α-D-arabino-hexopyranoside (IV). The above 4-benzoate (47 g) was dissolved in methanol (500 ml). Sodium (0.95 g) was added and the solution refluxed for 1 h. TLC (chloroform-methanol 20:1) showed that no more starting material remained. The reaction mixture was neutralized with solid carbon dioxide, concentrated, suspended in water and the aqueous phase shaken twice with hexane (150 ml) and concentrated to dryness. The product was extracted with ethyl acetate; the combined ethyl acetate extracts were filtered and concentrated to a syrup (26.6 g), which

was chromatographically pure (above solvent) and indistinguishable from an authentic sample of IV by NMR and by GLC-MS on the derived alditol acetate.

*Methyl 3,6-dideoxy-2,4-di-*O*-(p-nitrobenzoyl)-*α*-D-arabino-hexopyranoside (V).* Methyl tyveloside (IV, 2.41 g) was dissolved in pyridine (150 ml) and cooled to 0 °C. *p*-Nitrobenzoyl chloride (8.28 g) was added with stirring. The reaction mixture was kept at 0 °C for 1 h and then at room temperature for 7 h. The solution was poured with stirring into ice-water (200 ml). After stirring for 15 min the aqueous mixture was extracted with chloroform. The combined chloroform extracts were shaken with, in turn, ice-cold 0.25 M aqueous sulfuric acid, saturated aqueous sodium hydrogen carbonate and water, dried over sodium sulfate and concentrated to a syrup (5.13 g) which crystallized from methanol, m.p. 139 – 141 °C; $[\alpha]_{578}$ – 59° (chloroform). (Found: C 55.0; H 4.56; N 6.20. $C_{21}H_{20}N_2O_{10}$ requires C 54.8; H 4.38; N 6.09).

*3,6-Dideoxy-2,4-di-*O*-(p-nitrobenzoyl)-*α*-D-arabino-hexopyranosyl bromide (VI).* A solution of the above glycoside V (2.0 g) in dichloromethane (60 ml) was cooled to 0 °C and then saturated at this temperature with hydrogen bromide. The reaction was monitored by TLC (toluene-ethyl acetate 4:1).
After 2 h at 0 °C, when no starting material remained, the solution was concentrated to a syrup (2.2 g) which was used directly in the next step. The NMR spectrum was compatible with the presumed structure (Table 1). *

p-*Nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (VIII).* *p*-Nitrophenyl α-D-mannopyranoside was converted into the 4,6-benzylidene derivative as described by Buchanan and Schwarz for the corresponding methyl mannoside.[10] The benzylidene compound VII had m.p. 116 – 117 °C, $[\alpha]_D$ +196° (chloroform) and a satisfactory elemental analysis. It was converted into the monobenzyl derivative VIII by treatment with approximately 1 equivalent of benzyl bromide and silver oxide in dimethyl formamide.[11,12] The product VIII was obtained in a 56 % yield and had m.p. 118 – 120 °C, $[\alpha]_D$ +118° (chloroform) and a satisfactory elemental analysis. Details of the preparation of the two above compounds will be communicated in a separate paper.[13] An aliquot of VIII was converted into the 3-*O*-acetyl derivative VIIIa by acetylation at room temperature with acetic anhydride and pyridine. The usual work-up afforded VIIIa, the NMR spectrum of which is described in Table 1.

p-*Nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(3,6-dideoxy-2,4-di-*O*-p-nitrobenzoyl-α-D-arabino-hexopyranosyl)-α-D-mannopyranoside (IX).* The hexosyl bromide VI (prepared from 25 g of V and used immediately) was dissolved in acetonitrile (62.5 ml) and the solution added over a period of 1 h to a mixture of VIII (25 g) and mercuric cyanide (9.25 g) in acetonitrile (62.5 ml) while the mixture was stirred at room temperature in an atmosphere of dry nitrogen. After the addition was completed, stirring was continued for 3 h. The reaction mixture was diluted with chloroform (600 ml). The chloroform solution was shaken with saturated aqueous sodium hydrogen carbonate and then water, dried over magnesium sulfate, filtered and concentrated to a syrup (51 g). Column chromatography on silica gel (toluene-ethyl acetate 8:1) yielded chromatographically homogeneous IX, R_F 0.65 (TLC, same solvent) (27 g) $[\alpha]_{578}$ +42° (chloroform). In another experiment on the 2 g scale, and otherwise as described above, a 70 % yield of pure IX was obtained.

*p-Nitrophenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,4-di-*O*-acetyl-3,6-dideoxy-α-D-arabino-hexopyranosyl)-α-D-mannopyranoside (XI).* The above disaccharide derivative IX (4 g) in methanol (1000 ml) containing barium oxide (2 g) was refluxed for 1 h. The mixture was concentrated to a syrup which was taken up in ethyl acetate (300 ml) and filtered. The filtrate was concentrated and purified by silica gel column chromatography (toluene-ethyl acetate 1:2) to yield chromatographically pure X, R_F 0.56 (TLC in the same solvent system) (2.45 g) $[\alpha]_{578}$ +125° (chloroform). X (2.25 g) was acetylated with acetic anhydride (15 ml) in pyridine (20 ml) overnight at room temperature. The product was concentrated and excess reagents removed by repeated codistillation with toluene. The diacetate XI was obtained as crystals from ethanol, m.p. 100 – 105 °C $[\alpha]_{578}$ +146° (chloroform). (Found C 62.2; H 5.65; N 1.97. $C_{36}H_{39}O_{13}N$ requires: C 62.3; H 5.67; N 2.02.

p-*Trifluoroacetamidophenyl-3-*O*-(2,4-di-*O*-acetyl-3,6-dideoxy-α-D-arabino-hexopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (XIII).* The above disaccharide derivative XI (2.40 g) was hydrogenated at room temperature and atmospheric pressure in ethyl acetate (150 ml) using Adam's catalyst (0.25 g). When the hydrogen comsumption had ceased, trifluoroacetic anhydride (2.5 ml) and pyridine (6 ml) were added. The solution was kept at 60 °C for 30 min, filtered and concentrated. The residue was dissolved in toluene (200 ml) and shaken with water, in order to remove pyridinium salts. The organic layer was dried over magnesium sulfate, filtered and concentrated to yield XIII (2.63 g) which crystallized from ethanol, m.p. 166 – 167 °C $[\alpha]_{578}$ +113° (chloroform) (Found: C 60.0; H 5.26; N 1.95; F 7.39. $C_{38}H_{40}NO_{12}F_3$ requires C 60.1; H 5.31; N 1.84; F 7.50).

p-*Trifluoroacetamidophenyl 3-*O*-(2,4-di-*O*-acetyl-α-D-arabino-hexopyranosyl)-α-D-mannopyranoside (XIV).* The above disaccharide derivative XIII (1.64 g) in 95 % ethanol

* *Added in proof.* A crude sample had $[\alpha]_{578}$ +23° (chloroform).

(60 ml) and tetrahydrofuran (40 ml) was hydrogenated at room temperature and atmospheric pressure using 10 % palladium on charcoal (1.5 g). When hydrogen consumption ceased, the catalyst was removed by filtration and the filtrate concentrated to dryness. The crude XIV (1.20 g) thus obtained crystallized and was recrystallized from ethyl acetate-diethyl ether to give the pure substance m.p. 228–230 °C (dec.). $[\alpha]_{578}$ +127° (methanol). (Found: C 49.8; H 5.31; N 2.55; F 9.94. $C_{24}H_{30}NO_{12}F_3$ requires: C 49.6; H 5.20; N 2.41; F 9.80).

p-*Isothiocyanatophenyl* 3-O-*(3,6-dideoxy-α-*D-arabino-*hexopyranosyl)-α-*D-*mannopyranoside (XVI)*. The above disaccharide derivative XIV (56 mg) was dissolved in saturated ammoniacal methanol (10 ml) in a capped serum bottle under nitrogen and allowed to stand at room temperature for 12 h. The solution was concentrated to a syrup which contained one component (XV) only (TLC, chloroform-methanol 3:1). The syrup was dissolved in 80 % aqueous ethanol (10 ml). The pH was adjusted to about 8 by adding barium carbonate and kept at this level by adding more barium carbonate as required throughout the reaction. After addition of thiophosgene (0.05 ml) the mixture was stirred for 1.5 h. Filtration and concentration gave a syrupy compound which was purified by TLC (chloroform-methanol 3:1) to give pure XVI (25 mg), $[\alpha]_D$ +180°. IR showed a broad absorption band centered at 2130 cm^{-1}.

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α-*N*-Benzoylarginine-2-naphthylamide Hydrolase (Cathepsin B1 ?) from Rat Skin. II. Purification of the Enzyme and Demonstration of Two Inhibitors in the Skin

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A thiol-activated, α-*N*-benzoylarginine-2-naphthylamide (BANA) hydrolyzing enzyme was purified from rat skin by ammonium sulfate precipitation, gel filtration, and DEAE cellulose chromatography. In DEAE cellulose chromatography the enzyme was fractionated into two multiple forms (preparations I and II). The activity of undiluted preparation II, but not that of preparation I, was increased, when the enzyme was preincubated at pH 4, at 55 °C. Simultaneously, the isoelectric point of preparation II was shifted to that of preparation I, i.e., from 6.2 to 7.5. Activated preparation II behaved in DEAE cellulose chromatography as preparation I. Molecular weights of the enzymes of both preparations were 27 000, and pH optima were at pH 5.8 and 7.0, for BANA and leucine-2-napthylamide (Leu-NA), respectively. The BANA and Leu-NA hydrolyzing enzymes could not be separated by gel filtration, DEAE, CM, or Amberlite IRC-50 chromatography, isoelectric focusing, or analytical polyacrylamide gel electrophoresis. Two inhibitors of BANA hydrolase were demonstrated by gel filtration in the salt precipitated skin extract. The activities of the BANA hydrolase preparations did not increase linearly with increasing enzyme concentration, with the exceptions of activities of preparation I and acid-activated preparation II. The role of the inhibitors in the nonlinearity of the activity/enzyme concentration curves is discussed.

In a previous report some properties of α-*N*-benzoylarginine-2-naphthylamide (BANA) hydrolyzing enzyme from crude extract of rat skin was presented.[1] The enzyme resembled cathepsin B1 purified from other tissues in that it hydrolyzed BANA optimally at pH 5.8 in the presence of thiol activators (dithiothreitol, EDTA, and KCN). The activity/enzyme concentration curve of the extract that was obtained by plotting the BANA hydrolase activity of the extract against enzyme concentration, was unlinear. This suggested the presence of some inhibitor in the skin extract. In the present report the purification of the skin BANA hydrolase is described and evidence for the existence of two inhibitors of the enzyme in the rat skin is presented.

MATERIALS AND METHODS

Reagents. α-*N*-Benzoyl-DL-arginine-2-naphthylamide hydrochloride (BANA) and pepsinogen were from Fluka A. G., α-*N*-benzoyl-L-arginine amide hydrochloride hydrate (BAA) and papain (for biochemical purposes) were from Merck A. G., glycyl-L-arginine-2-naphthylamide (Gly-Arg-NA) was from Fox Chem. Co., and dithiothreitol (DTT) from Calbiochem. L-Leucine-2-naphthylamide (Leu-NA), L-arginine methylester (Arg-ME), ficin (crude), bromelain (grade II), bovine trypsin (type III), bovine ribonuclease A (type I A), soybean trypsin inhibitor (SBTI, type II S), bovine serum albumin (crystallized) and hemoglobin (Hb, type II) were from Sigma Chem. Co. A cathepsin C preparation was partially purified by Sephadex G-200 gel filtration from the rat skin extract, as an enzyme hydrolyzing Gly-Arg-NA, at pH 5.0, in the presence of thiol activators.

Enzyme assays. The hydrolysis of BANA, Leu-NA, Hb, and BAA was estimated as described previously.[1] Arg-ME was assayed as described for benzoylarginine ethyl ester, except that Britton-Robinson buffer, pH 5.8, containing 0.6 mM DTT, 3 mM EDTA, and 3 mM KCN was used.[1] The hydrolysis of Gly-Arg-NA was assayed in a similar way as that of BANA, but the pH of the buffer was 5.0. Pepsinogen was

assayed by the method of Herriot [2] and ribonuclease according to McDonald.[3]

Inhibition of BANA hydrolase by rat skin inhibitors was determined by mixing 0.1 ml of enzyme (purified preparation AI, see "Results"), 0.1 ml of Britton-Robinson buffer, pH 5.8,[1] containing 0.6 mM DTT, 3 mM EDTA, and 3 mM KCN. To this mixture 0.1 ml of inhibitor solution was added and the solution was allowed to stand at 37 °C for 10 min. Finally, 0.1 ml of 5 mM BANA in 20 % (v/v) methanol was added and the reaction mixture was incubated for 30 min at 37 °C. After incubation, 0.4 ml of 4-dimethylaminobenzaldehyde reagent was added, solutions were allowed to stand for 30 min at room temperature, and the optical density was measured at 450 nm.[1] Optical density values were substracted from those of controls, which contained water instead of inhibitor. The inhibition of cathepsin C was assayed in a similar manner with Gly-Arg-NA as a substrate, at pH 5.0. Inhibition of papain, ficin, and bromelain (0.1 mg/ml) were tested with BANA using Gomori's tris-maleate buffer,[4] pH 7.6, containing 1.2 mM DTT, 3 mM KCN, and 3 mM EDTA. The inhibition of trypsin was assayed as described by Junnila *et al.*[5]

Protein determination. The method of Lowry [6] was used for pooled samples, but during the chromatographic procedures the absorbance at 280 nm was measured with an Isco UA-2 monitor.

Purification procedure. Extraction of the enzymes from the skin was performed as described earlier.[1]

Precipitation with ammonium sulfate. The extract (735 ml) was saturated to 50 % with solid ammonium sulfate at 0 °C and centrifuged for 10 min at 16 000 *g*, at + 4 °C. The precipitate was discarded, and the supernatant was saturated to 80 % with ammonium sulfate. After centrifugation, the precipitate was dissolved in 50 ml of 10 mM sodium phosphate buffer, pH 7.5.

Treatment at pH 4. The concentrated enzyme solution (56 ml) was adjusted to pH 4.0 by adding 0.5 M HCl solution at 0 °C. The solution was centrifuged at + 1 °C, at 16 000 *g*, for 10 min, the inactive sediment was discarded, and the supernatant was adjusted to pH 6.0 with 0.5 M NaOH.

Sephadex G-100 gel chromatography. The acid treated enzyme solution (57 ml) was applied to a 5 × 90 cm Sephadex ® G-100 column at + 4 °C. The column was equilibrated with a 10 mM sodium phosphate buffer, pH 7.5. Fractions of 8 ml were collected at a flow rate of 60 ml/h (Fig. 1), Two protein peaks, responsible for the hydrolysis of BANA, were fractionated. The first one was eluted at V_0 and was discarded. The second peak, of greater activity, was pooled (fractions Nos. 104 – 116) and was subjected to a DEAE cellulose chromatography.

First DEAE cellulose chromatography. A 2.6 × 28 cm column of DEAE cellulose (Whatman DE-32, pretreated following the instructions of the manufacturer) was equilibrated with a 10 mM sodium phosphate buffer, pH 7.5. The pooled enzyme preparation from the Sephadex G-100 chromatography (100 ml) was applied to the column and unabsorbed proteins were eluted with 100 ml of the buffer. The flow rate was 60 ml/h and the fraction volume 5 ml. Absorbed proteins were eluted using a linear salt gradient in the buffer (0 – 0.5 M NaCl, total volume of the gradient was 800 ml). The BANA hydrolase was fractionated into two main peaks (Fig. 2). The first was eluted with the equilibrating buffer and the second with a weak (48 mM) sodium chloride solution. The first peak (fractions Nos. 19 – 31) and the second peak (fractions Nos. 47 – 57) were pooled separately and called preparations I and II, respectively.

Treatment of preparations I and II at pH 4, at 55 °C, increased the BANA hydrolase activity of undiluted preparation II, but not the activity of preparation I (Fig. 3). The fact that preparation I was not further activated suggested that it might be an activation form of preparation II. Following this, one half of preparation II was adjusted to pH 4.0 at + 4 °C by adding 0.5 M HCl solution and was incubated for 20 min at 55 °C. Both acid treated and nontreated portions of the enzyme were dialyzed in 10 mM sodium phosphate buffer, pH 7.5, at + 4 °C, and subjected to a rechromatography on DEAE cellulose.

Second DEAE cellulose chromatography. Two identical columns of DEAE cellulose with dimensions of 2.6 × 25 cm were equilibrated with 10 mM sodium phosphate buffer, pH 7.5. The acid treated and nontreated portions of preparation II were adjusted to the same volume (30 ml) with the buffer and were applied simultaneously using different channels of a peristaltic pump. Unabsorbed proteins were eluted with 50 ml of the buffer (per column). A linear salt gradient (0 – 0.4 M NaCl, total volume 600 ml) was formed in the same mixer and was divided between the two columns *via* different channels of the pump. The flow rates were 30 ml/h and the fraction volumes 4.5 ml. The chromatography of the nontreated preparation II was called DEAE-N and that of the acid treated preparation II DEAE-A (Fig. 4). Elution profiles from the DEAE-N chromatography were similar to those from the first DEAE chromatography. A small peak (NI) was eluted with the equilibrating buffer and a more active peak (NII) at the 0.046 M NaCl concentration. In the DEAE-A chromatography the unabsorbed peak (AI) was much more active than that eluted with NaCl solution (AII). The second peak from the DEAE-N chromatography (fractions Nos. 27 – 33) and the first peak from the DEAE-A chromatography (fractions Nos. 8 – 18) were pooled separately and called preparations NII and AI,

Table 1. Summary of the purification procedure of rat skin BANA hydrolase. The activity values are extrapolated to infinite dilution. Values in parentheses represent the activities of undiluted enzyme preparations.

Purification step	Volume ml	Protein mg	Activity nmol/min mg	Activity nmol/min	Purific. coefficient	Relative yield %
Extract	735	2250	1.18 (0.22)	2616 (485)	1	100
Ammonium sulfate fractionation	56	795	1.90 (0.07)	1510 (56)	1.6	58
Treatment at pH 4	57	467	3.3	1541	2.8	59
Sephadex G-100	100	56.1	22.8 (6.4)	1279 (361)	19	49
1. DEAE cellulose						
Preparation I	51	1.27	44.8	56.9	38	2
Preparation II	57	3.02	224.5 (63.6)	678.3 (192)	190	26
2. DEAE cellulose						
Preparation AI	48	0.58	400.8	233	339	9
Preparation NII	30	0.93	291.9 (85.2)	272 (79)	247	10

respectively. The purification procedure is summarized in Table 1.

CM cellulose chromatography. A 2.6 × 25 cm column of CM cellulose (Whatman CM-32, pretreated following the instructions of the manufacturer) was equilibrated with a 10 mM sodium citrate buffer, pH 4.8. The sample (25 ml of pooled BANA hydrolase from a Sephadex G-100 chromatography) was dialyzed in the same buffer and the elution was performed using a linear gradient of NaCl in the buffer (0 – 0.5 M NaCl, total volume 800 ml). The flow rate was 60 ml/h and the fraction volume 4.5 ml.

Amberlite IRC-50 chromatography. A 2.6 × 30 cm column of Amberlite® IRC-50 (BDH, standard grade) was equilibrated with 90 mM sodium citrate buffer, pH 5.3. The sample (25 ml of pooled BANA hydrolase from a Sephadex G-100 chromatography) was dialyzed in the same buffer and the chromatographic run was performed as described by Otto.[7]

Isoelectric focusing. Sucrose gradient in a LKB Ampholine® 8100 column was used.[8] The ampholine concentration was 1 % and the pH range 3 – 10. The potential difference was 300 V during the first 3 h and 800 V during the next 45 h.

Polyacrylamide gel electrophoresis. Electrophoresis was performed according to Smith *et al.*[9] using a 7.5 % gel in a tris-borate buffer, pH 8.6. Gels were stained with Coomassie Brilliant Blue (Brilliant Blue G-250, Sigma Chem. Co.).[10] In the determination of the activities of BANA and Leu-NA hydrolyzing enzymes, the gels were sliced to pieces of 2 mm. Proteins were extracted from the pieces with 0.3 ml of Britton-Robinson buffers, pH 5.8 or 7.0, containing thiol activators,[1] for BANA and Leu-NA, respectively. Extractions were performed for 4 h at +4 °C. Then 0.2 ml of extract was taken and 0.1 ml of substrate solution added, the mixture was then incubated for 2 h at 37 °C, and the degree of hydrolysis was determined by adding 0.4 ml of 4-dimethylaminobenzaldehyde reagent.[1]

Molecular sizes were determined by gel chromatography on a Sephadex G-100 column according to Andrews.[11] The dimensions of the column were 1.5 × 90 cm and a 10 M sodium phosphate buffer, pH 7.5, containing 0.1 M NaCl, was used at a flow rate of 5 ml/h, collecting fraction volumes of 2 ml. The column was standardized with 1 mg of Blue Dextran (Pharmacia Fine Chemicals, M. W. 2 000 000), 2 mg of bovine serum albumin (M. W. 68 000), 0.2 mg of pepsinogen (M. W. 40 400), 0.2 mg of soybean trypsin inhibitor (SBTI, M. W. 23 700), and 0.1 mg of ribonuclease A (M. W. 13 700). The standards and the BANA hydrolase preparations were applied to the column consecutively in 2 ml of the buffer; the BANA hydrolase was placed between pepsinogen and SBTI.

RESULTS

Purification of the enzyme. The extraction of BANA hydrolase from rat skin was performed as described earlier.[1] About 72 % of the total activity of the homogenate was recovered in the extract. The specific activity of the extract was increased about five-fold, when the extract was diluted and the activity extrapolated

to infinite dilution (see Table 1). This suggested presence of dissociable inhibitors in the extract.

In the ammonium sulfate fractionation, 9 % of the total BANA hydrolase activity and 42 % of the total proteins of the extract was recovered in the precipitate in 50 % saturation. The precipitate in 80 % saturation contained 58 % of the enzyme activity and 35 % of the total proteins of the extract. The specific activity was increased 1.6 times, as compared to the activity of the extract. The main part of inhibitor activity of the extract was precipitated together with BANA hydrolase (see Fig. 8), and a marked increase of specific activity was noticed after dilution of the enzyme solution. Dialysis of the enzyme solution in 10 mM sodium phosphate buffer, pH 7.5, increased specific activity of undiluted enzyme 2.2 times, but had no effect on BANA hydrolase activity when the enzyme was highly diluted. Dialysis was not used in the purification procedure, because it did not increase the specific activity of pooled BANA hydrolase after Sephadex G-100 gel chromatography.

Treatment of the ammonium sulfate fractionated enzyme solution at pH 4, at 0 °C, caused precipitation of some inactive proteins, and after centrifugation, increased the specific activity of the BANA hydrolase slightly. The specific activity of the enzyme was increased 2.8 times and the yield was 59 %, as compared to the activity of the extract.

In Sephadex G-100 gel chromatography two protein peaks, responsible for the hydrolysis of BANA, were found (Fig. 1). The first one was eluted at V_0 and was discarded. The second peak, of greater activity, hydrolyzed BANA, Leu-NA, and Arg-ME. The maximal activity obtained with the above substrates was found in the fraction No. 106 ($V_e/V_0 = 1.66$). The highly active Leu-NA hydrolyzing enzyme which in the preliminary Sephadex G-100 chromatography [1] was eluted in V_0 had been eliminated during ammonium sulfate and acid precipitations. The principal BAA hydrolyzing activity (cathepsin B2) was separated from the BANA hydrolyzing enzyme, but a smaller peak was found in the same elution volume as the BANA hydrolase (fraction No. 106). The hemoglobin hydrolyzing enzyme (cathepsin D) was incompletely separated from the BANA hydrolase. When the fractions of BANA hydrolase activity

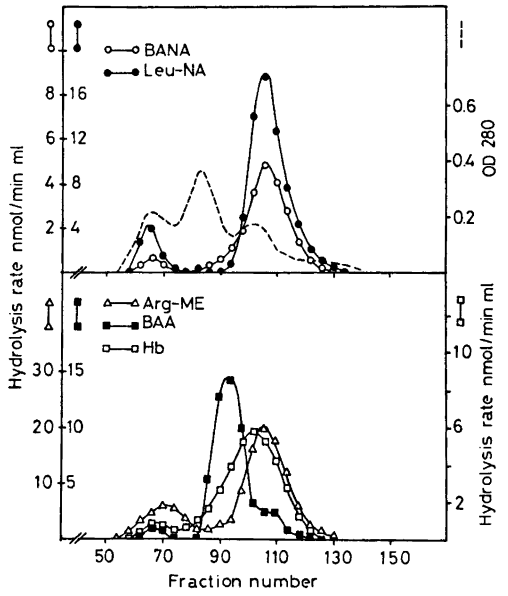


Fig. 1. Sephadex G-100 chromatography of the acid treated ammonium sulfate fraction (50–80 % saturated). Details are in the text.

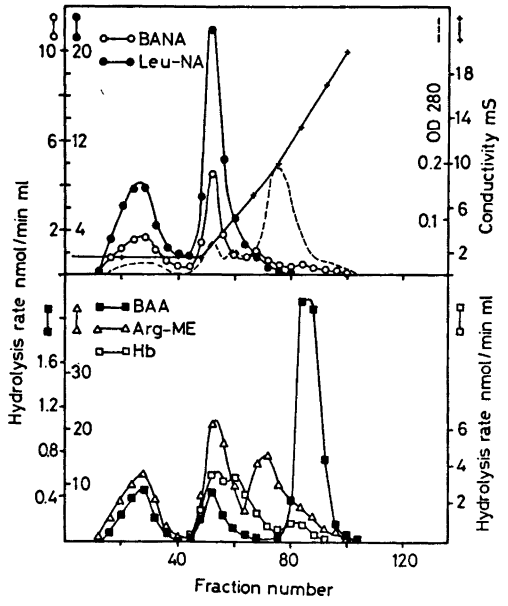


Fig. 2. First DEAE cellulose chromatography of the pooled preparation from the Sephadex G-100 chromatography. Details are in the text.

were pooled, the majority of the hemoglobin hydrolase activity was included. This pooled preparation gave a purification coefficient of 19 and a yield of 49 %, as compared to the activity of the extract. The majority of BANA hydrolase inhibiting activity was separated from the enzyme during the gel chromatography (see Fig. 8) but the specific activity of BANA hydrolase in the pooled preparation was still increased by diluting the enzyme.

In the first DEAE cellulose chromatography the BANA hydrolase from the Sephadex G-100 gel chromatography was fractionated into two main peaks (Fig. 2). The enzymes in both peaks hydrolyzed BANA, Leu-NA, and Arg-ME, and the second contained an enzyme that hydrolyzed hemoglobin. The main BAA hydrolyzing enzyme (cathepsin B2) was eluted at a high salt concentration (fraction No. 84). Arg-ME was also hydrolyzed by an enzyme (fraction No. 72) that was separate from the major BANA hydrolyzing enzymes. The pooled enzyme of the first BANA hydrolase peak was called preparation I and the enzyme of the second peak preparation II, respectively. Preparation I had a 38-fold increase in specific activity and preparation II a 190 fold increase, in comparison with the specific activity of the extract. The relative yields were 2 % for preparation I and 26 % for preparation II, in comparison to the total activity of the extract. The specific activity of preparation I was independent on the enzyme concentration, but the specific activity of preparation II was increased when the enzyme concentration was lowered by dilution.

Treatment of preparation II at pH 4. At all stages of purification, lowering the pH to 4.0 and preincubation of the enzyme at 55 °C increased the activities of undiluted enzymes. When preparations I and II were preincubated at pH 4, at 55 °C, only the activity of preparation II was increased (Fig. 3). Its BANA and Leu-NA hydrolyzing activities were increased in the same proportion, while the hemoglobin hydrolyzing enzyme was slightly inactivated. The fact that the acid treatment did not increase the BANA hydrolase activity of preparation I suggested that preparation I might be an activated form of preparation II. In order to study if the chromatographic behavior of preparation II is changed after acid treatment to

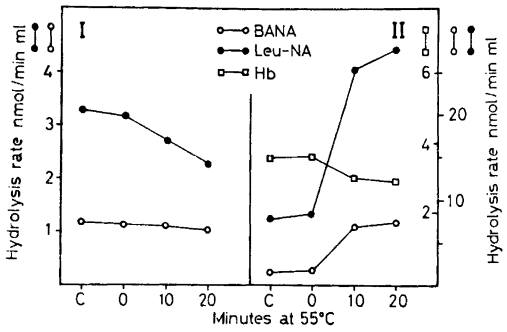


Fig. 3. Effect of treatment at pH 4, at 55 °C, on the activity of undiluted preparations I (left) and II (right) obtained after the first DEAE chromatography. C = control (nontreated enzymes).

resemble the behaviour of preparation I, one half of preparation II was treated at pH 4, at 55 °C. Both acid treated and nontreated portions were subjected to an additional chromatography on DEAE cellulose.

Second DEAE cellulose chromatography. The chromatograms obtained from nontreated

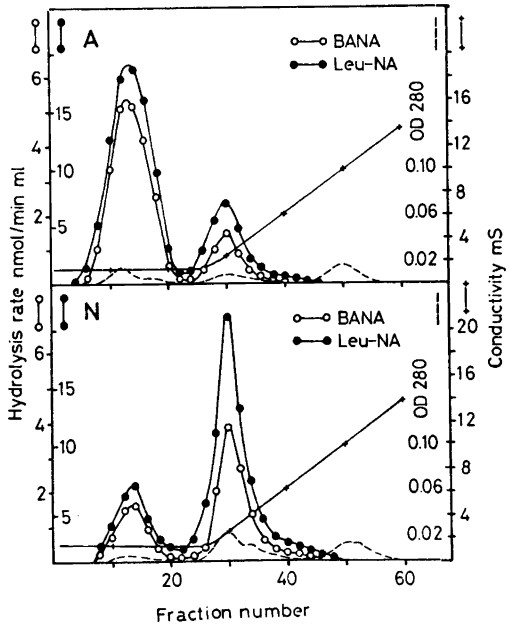


Fig. 4. Second DEAE cellulose chromatography. In run A the sample was the acid treated preparation II and run N was of nontreated preparation II, from the first DEAE chromatography. Details are in the text.

(DEAE-N) and acid treated (DEAE-A) portions of preparation II are in Fig. 4. The elution profiles from the DEAE-N chromatography were similar to those of the first DEAE chromatography. A small BANA hydrolase peak (NI) corresponding to preparation I was eluted with the equilibrating buffer and a more active peak (NII) corresponding to preparation II at the 46 mM NaCl concentration. In the DEAE-A chromatography the unabsorbed peak (AI) was much more active than that eluted with NaCl solution (AII). The increase of the unabsorbed, preparation I-like peak after the acid treatment of preparation II suggests that preparations I, NI, and AI are derivatives of preparation II.

Equal amounts of BANA hydrolase were applied to DEAE-N and DEAE-A columns and approximately the same amounts of proteins should be obtained by summation of proteins in BANA hydrolase preparations from each charomatography. In DEAE-N chromatography the sum of proteins in preparations NI and NII was 1.23 mg and in DEAE-A chromatography the sum of proteins in preparations AI and AII was 1.01 mg. These results are in agreement with the hypothesis that preparations AI and NI are formed from preparation II, but loss of some protein as a consequence of the acid treatment is noticed.

The enzyme preparations NI, NII, AI, and AII hydrolyzed BANA, Leu-NA, BAA, and Arg-ME, but hemoglobin was hydrolyzed only by an enzyme in preparations NII and AII. This suggests that the hemoglobin hydrolyzing enzyme is quite distinct from the BANA hydrolase. The specific activity of the diluted BANA hydrolase preparation NII was 292 nmol/min mg and the activity of preparation AI 401 nmol/min mg, which gave purification coefficients of 247 and 339, respectively, as compared to the activity of the extract. The relative yield of preparation NII was 10 % and that of preparation AI 9 %, as compared to the total activity of the extract. The activity of preparations NI and AI increased linearly with enzyme concentration, while activities of preparations NII and AII did not. The complete purification procedure is summarized in Table 1.

Additional purification efforts. At all stages of purification those fractions hydrolyzing BANA also hydrolyzed Leu-NA to an even greater extent (×4.5). This is interesting, since cathepsin B1 preparations that hydrolyse Leu-NA are usually regarded as impure. The separation of Leu-NA and BANA hydrolyzing activities of pooled preparations from Sephadex G-100 chromatography was attempted by CM cellulose chromatography, Amberlite IRC-50 chromatography,[7] isoelectric focusing in a sucrose gradient,[8] and analytical polyacrylamide gel electrophoresis.[9] Two peaks of enzyme activity were demonstrated using CM cellulose chromatography, gel electrophoresis, and isoelectric focusing and they corresponded to preparations I and II of the DEAE chromatography. Both peaks obtained by these methods hydrolyzed BANA and Leu-NA. In isoelectric focusing the peak with a pI of 6.2 corresponded to preparation II and that with a pI of 7.5 to preparation I. The peak with a pI of 6.2 was activated at acidic pH treatment and its activity did not increase linearly with enzyme concentration. In Amberlite IRC-50 chromatography, which according to Otto [7,12] separates cathepsin B1 and the Leu-NA hydrolyzing enzymes of beef spleen, BANA and Leu-NA hydrolyzing enzymes were eluted together with the equilibrating buffer (90 mM sodium citrate buffer, pH 5.3).

No purification method attempted was able to separate the BANA and Leu-NA hydrolyzing activities.

Molecular weight. The molecular weights of the purified preparations were estimated using a calibrated Sephadex G-100 column. The results obtained with preparation NII are given in Fig. 5. The same approximate molecular

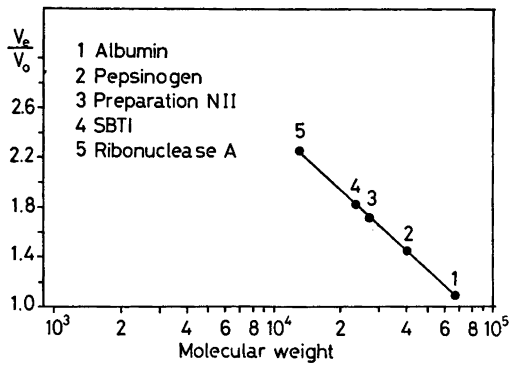


Fig. 5. Estimation of the molecular weight of preparation NII after gel filtration on Sephadex G-100. Details are in the text.

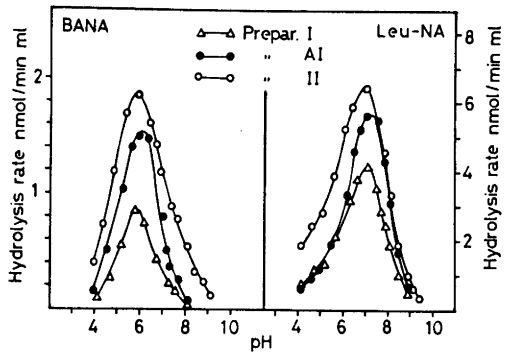


Fig. 6. Effect of pH on the hydrolysis of BANA and Leu-NA using the purified preparations I, AI, and NII. The buffer was Britton-Robinson universal buffer.[1]

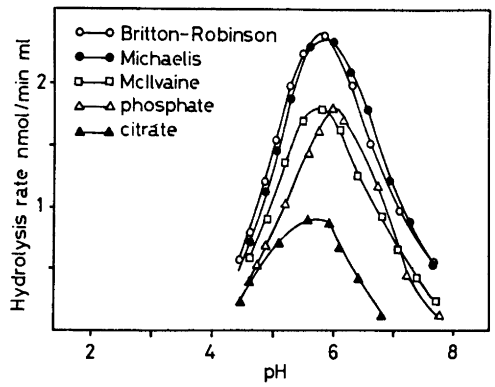


Fig. 7. Effect of pH on the BANA hydrolase activity of preparation II in various buffers. Britton-Robinson buffer=acetate-phosphate-borate,[1] Michalis' buffer = veronal-acetate,[4] McIlvaine buffer = citrate-phosphate buffer.[4] Sørensen's citrate [4] and phosphate [4] buffers were also used.

weight of 27 000 was obtained for all preparations.

Optimal pH. BANA was hydrolyzed optimally at pH 5.8 and Leu-NA at pH 7.0 by the purified preparations I, AI, and NII (Fig. 6). The hydrolysis rate of BANA in various buffers is presented in Fig. 7. Britton-Robinson buffer [1] and Michaelis' veronal-acetate buffer [4] gave the best activities. Sørensen's phosphate buffer [4] and McIlvaine's citrate-phosphate buffer [4] gave activities that were 20 % lower than the activities in Britton-Robinson buffer. In the Sørensen's citrate buffer [4] the activity was 60 % less than in the Britton-Robinson buffer.

Demonstration of inhibitors. The activity of

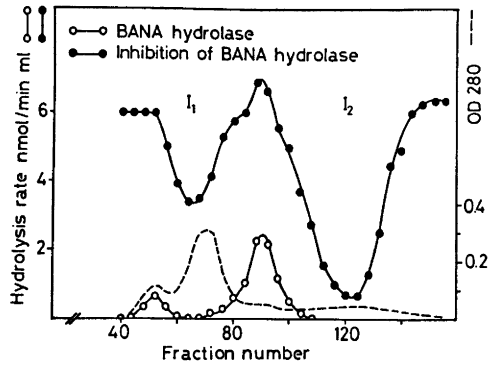


Fig. 8. Demonstration of inhibitors by Sephadex G-100 gel chromatography in the acid treated ammonium sulfate fraction. A sample of 60 ml was applied to a 5 × 70 cm column at 4 °C and the elution was performed with a 10 mM sodium phosphate buffer, pH 7.5. Fractions of 9 ml were collected at a flow rate of 60 ml/h.

BANA hydrolase from various purification stages did not increase linearly with increasing enzyme concentrations, excepting activities of the DEAE cellulose preparations I, NI, and AI. The presence of inhibitors that might have caused the nonlinearity of the activity/enzyme concentration curves was studied by fractionating the various pools obtained during the purification procedure by Sephadex G-100 gel chromatography and measuring the inhibitory capacity of the chromatographic fractions on BANA hydrolase. Two inhibitors were clearly demonstrated in the extract and acid treated ammonium sulfate fraction, but pooled BANA hydrolase preparations from the Sephadex G-100 and DEAE chromatographies did not contain measurable amounts of inhibitor activity. In Fig. 8, the fractionation of inhibitors in the acid treated ammonium sulfate fraction is presented. The first inhibitor eluted in fractions Nos. 62 – 80 was called I_1 and the second inhibitor in fractions Nos. 102 – 128 was called I_2. The inhibitory activity of I_2 was greater than that of I_1. They were also found to inhibit the thiol proteinases papain, ficin, bromelain, and cathepsin C, but not trypsin. Either inhibitor may be as a minor contaminant of the BANA hydrolase in preparations II and NII. Disappearance of the nonlinearity of the activity/enzyme concentration curve during acid treatment may account for irreversible dissociation of these inhibitors.

DISCUSSION

The fairly simple purification procedure used revealed that in the rat skin there were at least two cathepsin B1 like enzymes hydrolyzing BANA optimally at pH 5.8. The number of multiple forms of cathepsin B1 in various mammalian tissues varies greatly; one form in rat liver,[13] three forms in sheep thyroid gland,[14] five forms in bovine spleen,[15,16] and six forms in human liver [17] have been noticed. The molecular weight of the rat skin BANA hydrolase preparations was about 27 000. This is of the same magnitude as those obtained for cathepsin B purified from various other tissues.[14–14,16–20]

The rat enzymes hydrolyzed BANA optimally at pH 5.8. This corresponded to the pH-optima of other cathepsin B1 preparations (pH 5.5 – 6.0). An exception is rat liver BANA hydrolase, which has a pH-optimum of 7.0.[13] The pH-optimum for the hydrolysis of Leu-NA by the rat skin BANA hydrolase was at pH 7.0. Snellman,[18] who is the only worker to have reported Leu-NA hydrolysis by cathepsin B1, obtained from calf liver, has reported the same optimum pH of 7.0. Phosphate ions that are known to inhibit bovine spleen and sheep thyroid cathepsin B1 preparations,[12,14,21] also inhibited the rat skin enzyme to a minor degree. In addition, a marked inhibition of the rat skin BANA hydrolase was noticed in a citrate buffer.

The treatment of preparation II at pH 4, at 55 °C, for 20 min transformed the enzyme to a form that is very similar to preparation I. After the acid treatment, the isoelectric point of preparation II was changed from 6.2 to 7.5, which is the pI obtained with preparation I, and the chromatographic behaviour of the enzyme became similar to that of preparation I. The activity of acid-treated preparation II increased linearly with enzyme concentration, as did the activity of preparation I, but not that of preparation II.

The mechanism of activation of preparation II remains unclear. One probable hypothesis is that the hemoglobin hydrolyzing cathepsin D contaminant that has a pH optimum of 4.3, destroyed some acidic inhibitory peptide during incubation at pH 4, at 55 °C. However, the possibility that the BANA hydrolase molecule itself is modified during the acid treatment has not been excluded.

The purified preparations hydrolyzed classical substrates of cathepsin B1, like BANA and BAA. In addition, substrates with a free α-amino group, like Leu-NA and Arg-ME were hydrolyzed even better than BANA. The ratio Leu-NA/BANA was 4.5 and Arg-ME/BANA 6.7, for preparation II. This aminopeptidase-like activity is worth of noticing since pure cathepsin B1 preparations from other tissues do not hydrolyze Leu-NA.[12–14,17,22] The early preparation of Snellman [18] from calf liver was found to exhibit some Leu-NA hydrolyzing activity. This was strongly criticized by Barrett and Poole,[23] McDonald *et al.*,[24] and Otto,[12] and it was later admitted by Sylvén and Snellman [25] to be caused by a contaminating aminopeptidase. In the rat skin, BANA and Leu-NA hydrolyzing activities seemed to be tightly bound together. They were not separated by gel filtration, DEAE, CM, or Amberlite IRC-50 chromatography, isoelectric focusing or analytical polyacrylamide gel electrophoresis. In addition, treatment of preparation II at acidic pH caused similar changes in the BANA and Leu-NA hydrolyzing activites: The activity of undiluted enzyme tested against both substrates increased in the same proportion and the isoelectric point shifted from 6.2 to 7.5; neither activity was retained on the DEAE cellulose and after the acid treatment hydrolysis of both substrates increased linearly with increasing enzyme concentrations.

The nonlinearity of the activity/enzyme concentration curve of the extract suggested the presence of inhibitors of BANA hydrolase in the skin. Two inhibitors were indeed separated from the extract, as well as from the acid treated ammonium sulfate fraction, by Sephadex G-100 chromatography. Approximate molecular weights of these inhibitors were 74 000 for I_1 and 13 000 for I_2. The low molecular weight suggests that I_2 is probably similar to the inhibitor purified by Udaga and Hayashi [26] from rabbit skin with a healing Arthus inflammation. Lazarus and Dingle [27] have also demonstrated the inhibitory activity of rabbit skin extract on human cathepsin B1. Human liver cathepsin B1 is also inhibited by α_2-macroglobulin and IgG, but not with haptoglobin,[28] which according to Snellman and Sylvén inhibits proteolytic activity of calf liver cathepsin B1.[29] Later Snellman and Sylvén

found that mouse haptoglobin itself was not inhibitory but it contained an inhibitory polysaccharide with a molecular weight of 900.[30] This inhibitor may be dissociated from haptoglobin by a treatment with hemoglobin, or low pH. I_1 preparations from rat skin are also known to contain polysaccharides.[31] Relationships between I_1 and haptoglobin remain to be elucidated.

The rat skin inhibitors I_1 and I_2 inhibited thiol proteinases, such as BANA hydrolase, cathepsin C, papain, ficin, and bromelain. Since trypsin was not inhibited, the rat skin inhibitors appear to be group specific and polyvalent against thiol proteinases in a similar manner as inhibitors of serine proteinases.

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Binding of a Nitroxyl to Radiation-induced DNA Transients in Repair and Repair Deficient of *E. coli* K-12

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Binding of tritiated 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl (^{3}H-TAN) to radiation-induced DNA-transients in *E. coli* K-12 strains AB 1157 and JO 307 rec A uvr A has been studied under *in vivo* conditions. After irradiation the cells were washed and resuspended in growth medium and left overnight at 37 °C. Within an uncertainty of about 10 %, no effect of repair could be detected on the yield of TAN bound to DNA for any of the strains. During the period after resuspension. TAN or fragments of TAN leaked out of the irradiated cell samples. This leakage may be attributed to semi-permanent association between TAN and radiation-induced radicals within the cell.

The relevance of different interactions between TAN and transients in DNA is discussed.

I. INTRODUCTION

Nitroxyl free radicals, like 2,2,6,6-tetramethyl-piperidone-*N*-oxyl (TAN), 2,2,6,6-tetramethyl-4-piperidinol-*N*-oxyl (TMPN), and norpseudopelletierine-*N*-oxyl (NPPN) are of considerable interest because of their ability to sensitize anoxic cells to ionizing radiation. Despite intense research, however, the mechanism of their sensitizing effect is still mainly unknown.

It is suggested that these compounds act by interaction with radiation induced damages in vital cellular molecules, of which DNA is assumed to be the most important.[1] In our laboratory we have therefore studied interactions between nitroxyls and radiation induced radicals in DNA and DNA-components. By pulse radiolysis we have demonstrated a correlation between the reactivity of the three nitroxyls mentioned above towards OH-induced radicals in DNA-bases and nucleosides, on one hand, and the sensitizing ability of the nitroxyls on the other.[2,3] Furthermore, *in vitro* experiments have revealed that TAN is able to react with OH-induced DNA-transients in two ways, (1) by an oxydation-reduction reaction yielding reduced TAN (TAN-H), and (2) by binding.[4] Both these reactions can occur even when TAN is added several minutes after DNA is exposed to radiation, illustrating that the DNA radicals responsible for the interaction have a considerable lifetime, at least under *in vitro* conditions.

Similar experiments were performed *in vivo* where TAN was added to cell suspensions at different times prior to or after irradiation.[5] DNA was thereafter extracted and the relative yields of TAN bound to DNA determined. The investigation showed that TAN rapidly penetrated the cellular membrane. Even when TAN was added 2 s after irradiation we found a significant degree of binding. In the latter case, however, no sensitization was observed. Thus, there is no simple correlation between total TAN-binding to DNA and anoxic sensitization.

In order to elucidate further the relevance for sensitization of TAN-binding to DNA, we have in this work studied the influence of recombination and excision repair on the degree of binding. Since post-replication excision of the damage is suggested to be the last step in recombinational repair,[6] both repair mecha-

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nisms result in elimination of damaged fragments from the DNA helix. The release of DNA fragments complexed with TAN may therefore provide some information as to whether the cell considers TAN bound to DNA as a serious damage or not.

2. MATERIALS AND METHODS

2.1. Bacterial strains and growth conditions. *E. coli* K-12, strains AB 1157 and JO 307 were used for the experiments. The wild-type strain AB 1157 has both excisional and recombination repair capability, whilst the strain JO 307 is deficient in these repair mechanisms, due to mutations in the rec (A-13) and uvr (A-6) loci.

A single colony of the actual strain was transferred to YET broth, containing 5 g yeast extract, 10 g bacto-tryptone (both from Difco Laboratories, Detroit, Michigan), and 10 g NaCl per liter (pH 7) and incubated overnight. Next morning the suspension was mixed with the double volume of a YET glucose medium, which in addition to the components mentioned above, contained 2 g D(+)-glucose per liter. The culture was incubated under aeration until mid log phase in the mixed medium. Finally, the cells were washed and resuspended in phosphate buffer, pH 7, containing 0.13 M NaCl. The concentration was then about 10^{10} cells per ml.

2.2. Chemicals. TAN was synthesized by catalytic oxydation of the corresponding amine according to the method of Rozantsev,[7] with some minor modifications. The amine (HCl salt) was obtained from Fluka AG, Basel. The tritium labeling was performed by proton exchange from tritiated water of high specific activity (Amersham, Buckinghamshire, U.K.). The stability of the label was checked by measuring the activity in recrystallized TAN after 24 h in water solution, pH 7, at room temperature. No loss of activity was observed.

All other chemicals were of the highest purity commercially obtainable, and were used without purification.

2.3. Experimental procedure. The mixing and flushing setup has been presented earlier (Wold and Brustad [5]), 1 ml samples of bacterial suspension in test tubes, and 1 ml aliquots of 2×10^{-3} M TAN (~ 150 μCi tritium activity) in glass syringes were continuously flushed with highly purified nitrogen gas (less than 6 ppm O_2, Norsk Hydro A/S, Oslo) for at least 30 min before exposure. The TAN solution was transferred to the bacterial suspension under maintenance of anoxia 2 min before exposure. 1 min after irradiation, air was admitted to the exposed mixture. All solutions were kept at ice temperature during the experiment.

The test mixture was irradiated with 4 MeV electrons from a modified AEI-linear accelerator. The dosimetry was performed with the modified Fricke dosimeter system.[8] The oxygen saturated dosimeter solution, contained in a glass tube, was irradiated simultaneously with the test solution.

After irradiation, the test samples were washed 4 – 5 times in phosphate buffer in order to reduce the activity caused by unbound TAN. Then, the cells were resuspended in YET glucose medium.

After centrifugation, the cells were resuspended in cold 5 % trichloroacetic acid (TCA), washed several times with nearly boiling ethanol to remove lipids, and again resuspended in cold TCA. The ^{3}H-activity in this final supernatant was checked and used as a measure for the "background activity". The nucleic acid fraction (mainly DNA [5]) was extracted from the residue by 5 % TCA at 95 °C. After centrifugation, 1 ml samples of the supernatant were counted in a scintillation counter.

3. RESULTS

3a. Radiation induced binding of TAN in E. coli *K-12, strains AB 1157 and JO 307.* When a test solution of TAN and *E. coli* K-12, strain AB 1157 is exposed to 40 krad and treated as described in the previous paragraph, the DNA extract has an activity corresponding to about one TAN molecule per 20 000 nucleotides. In comparison, the frequency of single strand breaks under anoxic conditions is of the same magnitude for this dose.[9]

In order to find whether the extent of binding of TAN to DNA is changed by repair processes, we compared (1) the binding in the repair proficient strain AB 1157 with that in the repair deficient strain JO 307, and (2) the binding in normal and heat-treated cells (10 min at 52 °C) for the repair proficient strain. This treatment is assumed to inactivate some enzymes necessary for repair.[10] Since (a) the radiation affects the survival ratios of the two strains differently because of different sensitivity, (b) usually, different DNA concentrations resulted in extraction from the two irradiated strains, and (c) the heat treatment seems to result in leakage of proteins from the cells (leading to pronounced foaming when flushing), a small difference in TAN binding may be caused by effects other than repair. Therefore, we also compared the two strains with regard to binding of TAN to DNA in intact cells after repair relative to that in heat-treated cells.

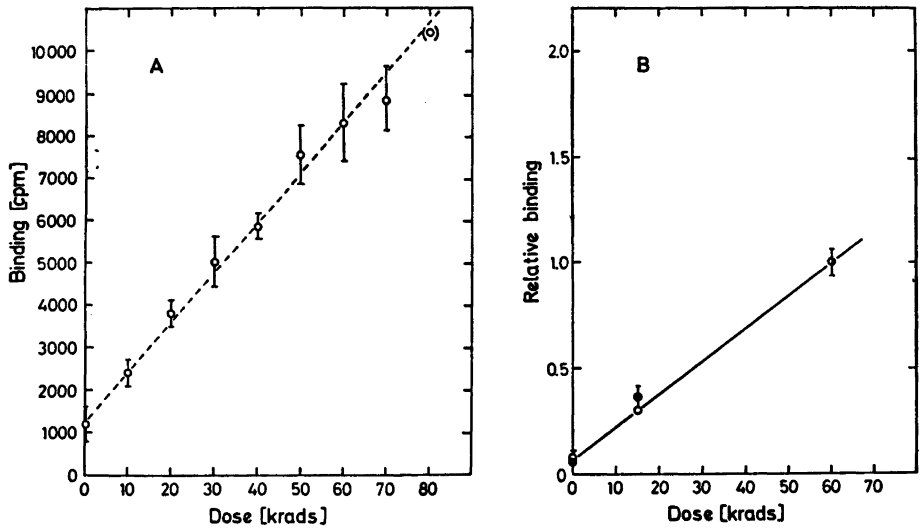


Fig. 1. Binding of ^{3}H-TAN to DNA in *E. coli* K-12 strain AB 1157 as a function of dose. The cells were washed, resuspended in growth medium and left overnight at 37 °C. Next morning, DNA was extracted and measured for ^{3}H-TAN activity.
A. Cells incubated under conditions permitting minimal cell division (see text). No air flushing. Standard error indicated as vertical lines.
B. ○, Same as A. ●, Cells incubated in sufficient medium for 2 – 3 divisions. Air flushing. Standard error for the most uncertain value at each dose indicated as vertical lines. Because of diverging growth conditions during the repair period, resulting in different quantities of DNA, the extraction efficiency is not the same for the cases on Fig. 1B. The values for 60 krad are therefore normalized to 1.0.

Due to the features mentioned above, the results of the different comparisons suffer from a considerable uncertainty. The present conclusion, however, must be that there is no significant reduction in binding level in the repair proficient strain as compared to that in the repair deficient strain.

3.2. Dose-binding curves. As a further check for the ability of repair mechanisms to remove DNA-bound TAN, dose-binding curves were determined for the repair proficient strain. Cell death, or lacking ability for cell division is assumed to be caused by insufficient repair of demages in DNA. Although some repair may occur even at the highest doses at which most cells are "killed", the repair failure ought to be more pronounced at high than at low doses. For recombination repair, post-replication excision is assumed to be the last step in the repair process.[6] This post-replication excision may be blocked if replication and recombination are not completed because of substantial radiation-induced DNA damage. One would therefore expect an even more pronounced repair failure at high doses for recombination than for excision repair. Thus, for both mechanisms a deviation from the linear dose-binding relationship is expected if bound TAN is removed as a result of repair.

Fig. 1A shows the curves for strain AB 1157. The curve is for repair under conditions permitting very little cell division. (The cells were resuspended to a concentration of about 10^9 ml^{-1} without air flushing). In such a highly concentrated suspension about half of the cells in an unirradiated control will die during the 18 h incubation period. Since repair seems to be completed within about 70 min,[11] most cells should have a survival period long enough to excise the damage.

The highest dose in Fig. 1A corresponds to about 1 % survival, while about 80 % of the cells survive the lowest dose applied (unpublished data for lower concentrated cell suspensions).[12]

Fig. 1B shows the importance of sufficient nutrition for cell division during repair. Binding is measured for a high and a low survival ratio

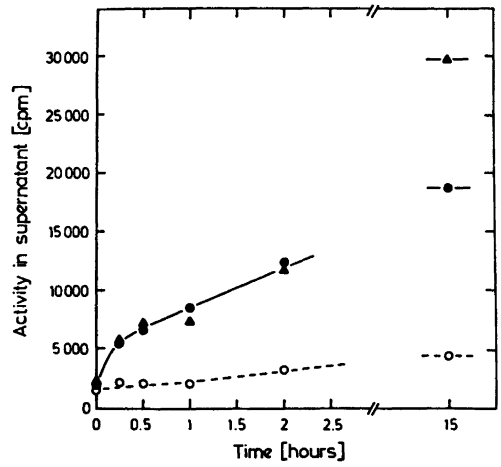


Fig. 2. ^{3}H-TAN activity in growth medium as function of time since suspension. The cells (strain AB 1157) were washed free from unbound ^{3}H-TAN before suspension. O, Unirradiated cells. ●, Cells irradiated with 25 krad in anoxia. ▲, Cells heat-treated (see text) before irradiation (25 krad).

under two different nutritious conditions during the repair period, (a) for medium sufficient for 2–3 cell divisions, and (b) for the growth medium mentioned above. None of the curves show significant deviations from linearity. We have, therefore, no evidence for excision of TAN-DNA fragments. The experiments presented in this section are, however, not sensitive enough to detect low yields of TAN excision.

3.3. TAN leakage from irradiated cells. When irradiated mixtures of TAN and bacteria have been washed several times with buffer, TAN or TAN fragments containing the tritium label continues to leak out, even at room temperature. After 15 h the total activity of the leakage is of the same magnitude as the TAN level in the DNA extracts. TAN also leaks out of unirradiated cells, but not to the same extent.

The feature is demonstrated in Fig. 2, where the leakage is measured from normal and heat treated cells (15 min at 60 °C) of strain AB 1157. All samples were washed 4 times with buffer to remove unbound TAN. At different times after resuspension in growth medium, the samples were centrifuged, and aliquots of the supernatant measured for ^{3}H activity. The cells were immediately afterwards resuspended in the same solvent and again stored. During the first hour of storing the samples were kept at room temperature. Thereafter, the samples were incubated at 37 °C. Thus, much of the leakage occurred at a temperature resulting in reduced biological activity of the cell. During the two first hours, normal cells and heat treated cells (denaturated repair enzymes [10]) yield nearly identical leakage. The leakage is therefore not likely caused by enzymatic repair mechanisms inside the cell.

A possible reason for the leakage is that some TAN may be semipermanently associated with radiation-induced radicals in the cell. With time and warming (to 37 °C) part of this association may break, and free TAN or TAN bound to small molecular debris separates. The proposed association must be much weaker than the covalent binding between TAN and DNA transients reported by Brustad *et al.*[4] since the covalent binding was not affected by heating to 80 °C for 15 min. An alternative explanation is that some of the bound TAN molecules are destroyed, leading to the liberation of the tritium activity. In both cases, the leakage of activity is a consequence of an interaction between TAN and transients within the cell.

The high final level for the inactivated cell sample on Fig. 2 may be caused by cell destruction, permitting rather big molecules and cell debris with bound TAN to separate.

3.4. Investigation of TAN bound to DNA in cells of strain JO 307 after low doses. Strain JO 307 is more sensitive to ionizing radiation than strain AB 1157. We therefore investigated TAN binding and leakage at 800 rad, a dose yielding about the same survival (approximately 10 %, unpublished for low concentrated suspensions [12]) as that caused by the medium dose used in the work on AB 1157. A very low, but probably significant yield of TAN bound to DNA was detected. The leakage of TAN from irradiated cells was only slightly higher than that from unirradiated cells.

Despite this small yield of binding and leakage, TAN has a marked sensitizing effect as regards cell survival of strain JO 307 cells at the applied dose.

4. DISCUSSION

A variety of investigations have led to the conclusion that DNA is a vital target for radiation killing. As a consequence, sensitization is assumed to be connected with some kind of interaction between the sensitizer and radiation-induced damages in DNA. Emmerson [13] has demonstrated clearly that the presence of TAN during irradiation modifies DNA in a way that leads to reduced survival for the cell. He irradiated a donor strain of *E. coli* K-12 in presence and absence of TAN. After conjugation, he found that the sensitizer reduced the 1/e survival dose by a factor of 7.5. Since DNA was the only part of the irradiated donor that was transferred to the recipient, the work establishes that TAN exerts its effect, at least partly, on DNA. Further, Rupp, Zipser, von Essen, Reno, Prosnitz and Howard-Flanders [11] have shown by alkaline sedimentation that DNA synthesized in cells newly irradiated in presence of TAN sediments more slowly that in cells irradiated without sensitizer. In repair proficient strains, DNA synthesized one hour later had nearly recovered its normal sedimentation pattern, while the DNA from repair defective cells maintained the slow sedimentation pattern.

Thus, it seems clear that TAN in some way interacts with irradiated DNA. Two different reactions between TAN and DNA-damages are known, a reduction-oxydation reaction and covalent binding.[4] The binding has gathered most interest, partly because of the assumed resemblance to fixation of DNA damages by oxygen.[14,15] The enzymatic excision of the damage is then suggested to work in a similar way as that for UV-induced thymine dimers.

Some other features, however, can hardly be explained from such a model. TAN sensitized Chinese hamster cells even when washed out of the sample before exposure.[16] In contrast, no pre-irradiation effect of TAN was discovered for *E. coli* K-12 strain AB 2463.[12] In an earlier work on *E. coli* cells we have demonstrated that TAN can be bound to DNA to a nearly full extent on admixture 0.2 s before irradiation, and that a significant degree of binding occurred on post-exposure admixture.[5] Cell sensitization, however, requires the presence of TAN during irradiation, and is very small when the interval between admixture and irradiation is as short as 0.2 s.

The present work does not prove that TAN-DNA fragments are excised from DNA as a result of enzymatic repair processes. As mentioned above, the experimental techniques employed are rather insensitive, and 10 % TAN excision can escape detection. It seems, however, clear that the bulk of TAN-DNA complexes is not excised. This means, alternatively, that (a) total TAN binding does not represent a serious damage, (b) total TAN binding is harmful, but the enzymatic repair mechanisms are not able to handle this kind of damage, (c) part of the binding, say 10 %, is lethal, but can be excised, while the rest is harmless and ignored by the cell.

Experiments that will make detection of small amounts of excised TAN possible are now in progress.

Leakage of TAN or TAN fragments from irradiated cells indicates breaking of some kind of radiation-induced association between the nitroxyl and cell components. The present work does not reveal where TAN is associated, but because of the high reactivity of the compound, it seems reasonable to assume association to a lot of different structures and molecules within the cells, including DNA. Even if such an association is not permanent, it may be strong enough to represent a lethal damage.

It may seem strange to explain sensitization by covalent binding or by some weaker association, since sensitization occurs even when the interaction with the sensitizer is negligible, as is the case for strain JO 307 at low doses. On the other hand, this particular strain is very sensitive to radiation even in the absence of a sensitizer. This general sensitivity may well cause a susceptibility even to weak interactions with the sensitizer. This might for instance be the case if most primary hits in DNA were sublethal. The effect of the interaction with the sensitizer could then be to transfer the sublethal damages to lethal ones. Such a transfer is expected to be more serious for strain JO 307 than for strain AB 1157, due to the deficiency in the enzymatic repair mechanisms of the former strain.

The red-ox process between TAN and DNA transients as reported by Brustad, Jones and

Wold [4] may result in fixation of radiation damage, for instance by the mechanism proposed by Adams and Cooke.[17] In *in vitro* experiments we observed a reaction with irradiated DNA even when TAN was added several minutes after irradiation. Such a long-lived post-irradiation effect on survival is not known for any cell strain. Furthermore, van Hemmen, Mauling and Bleichrodt [18] were unable to detect any inactivation of biologically active DNA by TAN admixture 1.5 ms after irradiation of DNA.

At present, none of the simple models for radiation sensitization seem to yield a satisfactory explanation of all observations. It may be necessary to consider models including other cell components than DNA in order to arrive at a complete understanding of sensitization. One such hypothesis has been presented, where sensitization is ascribed to influence of the sensitizer upon radiation-induced reactions between cell components and DNA.[19]

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Intermolecular Hydride Transfer Reactions. II. Reinvestigation of the Reaction between Flavylium Perchlorate and Dimethylaniline

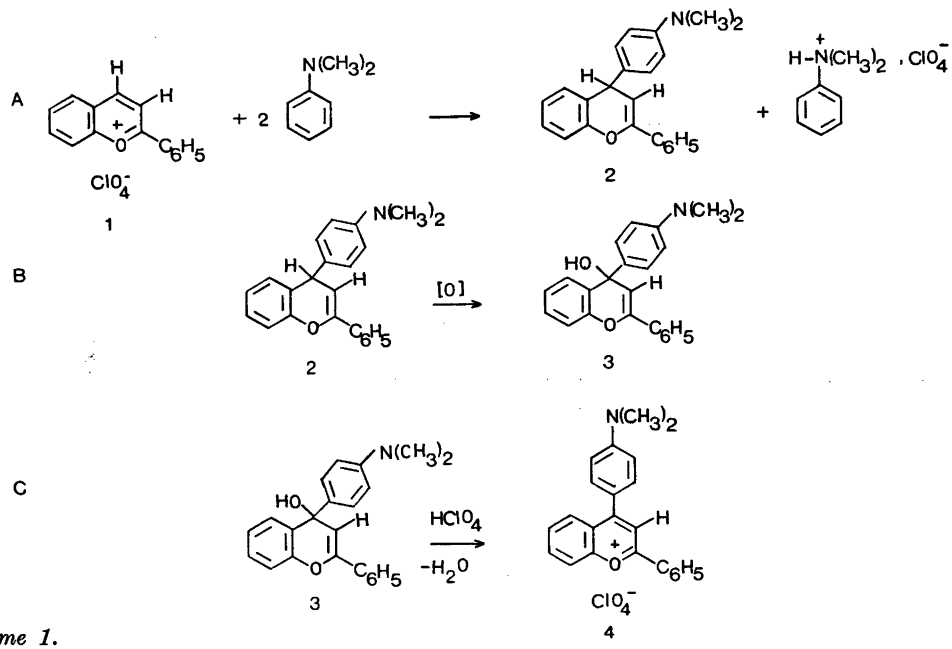
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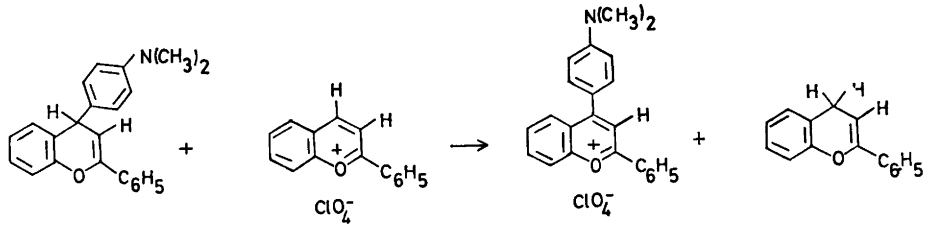
Reaction of flavylium perchlorate with dimethylaniline in ether solution produced 4-(*p*-dimethylaminophenyl)flav-2-ene. Intermolecular hydride transfer between the 4-substituted flavene and unreacted flavylium salt with formation of 4-(*p*-dimethylaminophenyl)-flavylium perchlorate and the parent flav-2-ene was observed. In addition, 3-(flav-2-ene-4-yl)-2-methoxyflavane was formed when the reaction was carried out in methanol solution.

Recently the reactions of 2,6-dimethoxycarbonylpyrylium perchlorate with dimethylaniline, anisole, and toluene have been studied and it was shown that the 2,6-dimethoxycarbonyl-4*H*-pyranyl group was introduced into the *para* postion of these benzene derivatives.[1] The 4*H*-pyranyl group was further oxidized to the corresponding pyrylium cation by an intermolecular hydride transfer reaction. The 2,6-dimethoxycarbonylpyrylium cation acted as hydride acceptor and was reduced to the 2,6-dimethoxycarbonyl-4*H*-pyran in this process.

The present work deals with the nature of the reaction between flavylium perchlorate (*1*) and dimethylaniline (DMA). This reaction



Scheme 1.



Scheme 2.

was first studied by Shriner and Shotton whose conclusions are summarized in Scheme 1.[3]

Addition of flavylium perchlorate (*1*) to an ether solution of DMA produced the flavene derivative *2* and the hydroperchlorate salt of DMA (step A). The flavene *2* was further oxidized by air to the carbinol *3* (step B). The carbinol *3* yielded with perchloric acid the flavylium salt *4* (step C). The reaction which was carried out in presence of air, gave a mixture of the products mentioned above. The evidence supporting the conclusion that carbinol *3* is an intermediate in the oxidation of the flavene *2* to the flavylium salt *4* was based on the following observations. A dark coloured precipitate was collected from the reaction mixture of *1* with DMA in ether solution. The flavene *2* was isolated from the filtrate. The dark coloured material was treated with 5 % sodium carbonate and ether. The flavylium perchlorate *4* was obtained by filtration of this mixture and furthermore the carbinol *3* was isolated from the ether soluble fraction.

It is not clear whether the isolated carbinol *3* resulted from air oxidation of *2* or from reaction of flavylium salt *4*, present in the coloured material, with sodium carbonate solution. The transformation of *4* to the carbinol *3* with base has been demonstrated by Shriner and Shotton.[3]

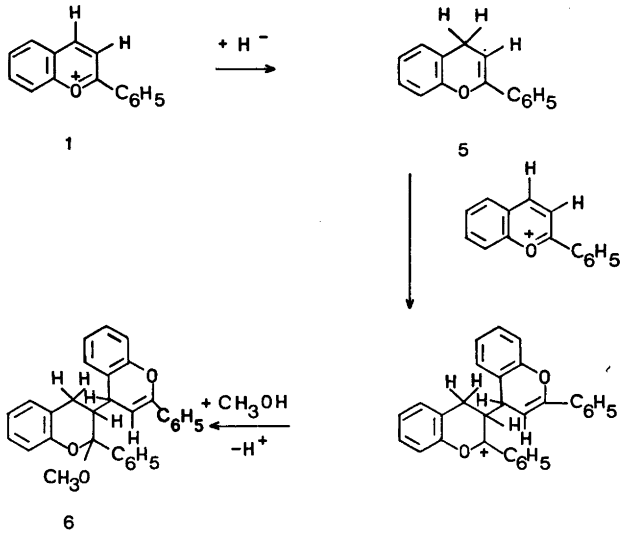
In addition to the sequence proposed by Shriner and Shotton (Scheme 1), the flavylium salt *4* could arise by a second possible route. An intermolecular hydride transfer reaction between uncreated flavylium salt *1* and flavene *2* would result in the formation of *4* and the parent flav-2-ene (*5*) (Scheme 2).

In search of the reduction product 5, flavylium perchlorate (*1*) was reacted with DMA in ether. The ether insoluble material was removed by filtration. Gas chromatography and ^{1}H NMR analysis of the filtrate verified the formation of flavenes *2* and *5*. The latter two flavenes were synthesized by alternative procedures for purposes of identification. Compound *5* was not reported by Shriner and Shotton. The intermolecular hydride transfer reaction depicted in Scheme 2 was further demonstrated in a separate experiment.

Equimolecular amounts of the flavene *2* and flavylium perchlorate were allowed to react for 24 h in acetonitrile solution. Treatment with ether yielded a crystalline product, which was identified as the flavylium salt *4* (80 %). The etheral solution consisted mainly of flavene *5* with traces of unreacted *2* as shown by GLC and ^{1}H NMR analysis. These results suggest that the formation of *4* from the reaction of *1* with DMA proceed *via* the steps shown in Schemes 1A and 2.

The reaction of flavylium perchlorate *1* with DMA has also been investigated by Blackburn *et al.*[3] using methanol as solvent. The flavylium salt *4* reportedly precipitated from the reaction mixture. The constituents of the remaining solution were not commented on. On repeating this reaction it was verified that the main component in the precipitated material was the flavylium salt *4*. However, careful extraction of the collected solide material with ether resulted in the isolation of the flavane derivative *6* (m.p. 202 °C) (Scheme 3). The remaining methanol solution was examined by GLC and ^{1}H NMR analysis. First of all the substitution product *2* and the flavene *5* could be identified Furthermore resonances at δ 5.2 (doublet, J 4.5 Hz) in the ^{1}H NMR spectrum could, as shown below, be attributed to the diastereomers of the isolated flavane derivative *6*.

Reynolds *et al.*[4] have reported that reduction



Scheme 3.

of the flavylium cation (1) with sodium borohydride in methanol resulted in formation of the flavane derivative *6*.

The reaction sequences used to explain the formation of *6* were similar to those depicted in Scheme 3. While the borohydride reduction of *1* yielded the parent flav-2-ene (*5*) the redox reaction depicted in Scheme 2 explains the formation of this compound in the reaction of *1* with DMA.

The reaction sequence in Scheme 3 is supposed to yield four stereoisomers provided a selective addition to the double bond of flavene (*5*). Repetition of the reduction of *1* with sodium borohydride in methanol resulted in the isolation of the isomers (m.p. 203 °C) reported by Reynolds *et al.*[4] and in addition their methanol soluble diastereomers (m.p. 163 °C).

^{1}H NMR resonance signals of the β-flavenyl proton of the methanol soluble isomers (m.p. 163 °C) showed up as a doublet at δ 5.2 (J = 4.5 Hz) consistent with signals observed by ^{1}H NMR analysis of the methanol fraction from the reaction of *1* with DMA as mentioned above. Signals at δ 3.0 (s, 3 H and m, 4 H) were also compatible.

The observations described in this paper do not rule out the possibility of a reaction path in which air oxidation plays an important part in formation of *4* from flavene *2*. However, it seems possible that the main path is through the intermolecular hydride transfer process depicted in Scheme 2.

It should be pointed out that Shriner and Shotton [2] reported that addition of acid to an air oxidized solution of flavene *2* resulted in the formation of the flavylium salt *4*.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian A-60A instrument. A Perkin-Elmer F11 instrument equipped with an OV-17 column was used for the GLC analysis. The mass spectra were run on a AEI MS902 with a PDP11 computer.

Flavylium perchlorate and dimethylaniline in ether. Flavylium perchlorate [5] (7.65 g, 0.0025 mol) was added to a solution of dimethylaniline (6.05 g, 0.05 mol) in ether with vigorous stirring. After 3 h the insoluble material was collected. Crystallization from methanol yielded 1.9 g of 4-(*p*-dimethylaminophenyl)flavylium perchlorate (*4*) m.p. 246 °C. ^{1}H NMR spectrum in TFA: δ 3.63 [s, $N(CH_3)_2$], 7.5–8.7 (m, 14 arom. H).

The filtrate was evaporated and the residual oil was examined by GLC and ^{1}H NMR spectroscopy. Flav-2-ene (*5*) and 4-(*p*-dimethylaminophenyl)flav-2-ene (*2*) were identified together with unreacted dimethylaniline.

Flav-2-ene (*5*) which was synthesized by the reduction of flavylium perchlorate with lithium-aluminium hydride [6] and 4-(*p*-dimethylaminophenyl)flav-2-ene (*2*) synthesized from flavylium perchlorate and *p*-dimethylaminophenyl lithium,[2] were used as reference substances.

^{1}H NMR spectrum of flav-2-ene (*5*) in $CDCl_3$: δ 3.5 (d, 2 H_4), 5.4 (t, H_3, $J_{3,4}$ 4 Hz) 6.8 – 7.8 (m, 9 arom. H). ^{1}H NMR spectrum of 4-(*p*-dimethylaminophenyl)flav-2-ene (*2*) in $CDCl_3$: δ 2.88 [s, $N(CH_3)_2$], 4.72 (d, H_4, $J_{3,4}$ 4.5 Hz), 5.58 (d, H_3) 6.6 – 7.9; (m, 13 arom. H).

*Flavylium perchlorate and 4-(*p*-dimethylaminophenyl)flav-2-ene (2) in acetonitrile.* To a solution of flavylium perchlorate (0.306 g, 0.001 mol) in acetonitrile (10 ml) was added 4-(*p*-dimethylaminophenyl)flav-2-ene (*2*) (0.327 g, 0.001 mol) with stirring. After 24 h ether was added and the precipitated material was collected by filtration. Crystallization from methanol yielded 4-(*p*-dimethylaminophenyl)flavylium perchlorate (*4*), m.p. 248 °C (83 %). The filtrate was evaporated and GLC and ^{1}H NMR analyses of the residual oil showed almost pure flavene (*5*). The product was contaminated with 4-(*p*-dimethylaminophenyl)flav-2-ene (*2*).

Flavylium perchlorate and dimethylaniline in methanol. To a solution of dimethylaniline (1.21 g, 0.01 mol) in methanol (50 ml) was added flavylium perchlorate (3.06 g, 0.01 mol) with stirring. After 12 h at 25 °C the reaction mixture was filtered. The collected solid material was triturated with 250 ml ether and filtered. This yielded 0.68 g of 4-(*p*-dimethylaminophenyl)flavylium perchlorate (*4*), m.p. 248 °C. Evaporation of the ether fraction yielded 0.25 g of 3-(flav-2-ene-4-yl)-2-methoxyflavene (*6*) m.p. 202 °C.

The remaining methanol solution from above was concentrated and the residual oil was examined by GLC and ^{1}H NMR spectroscopy Flav-2-ene (*5*), 4-(*p*-dimethylaminophenyl)flav-2-ene (*2*) and diastereomers of the 3-(flav-2-ene-4-yl)-2-methoxyflavanes (*6*) were identified from comparison with authentic samples.

Diastereomers of 3-(flav-2-ene-4-yl)-2-methoxyflavane (6). A suspension of flavylium perchlorate (5.0 g, 0.016 mol) in methanol (100 ml) was stirred and sodium borohydride (1 g) was added in small portions. After 0.5 h, 2.2 g of a white solid was collected (m.p. 203 °C). ^{1}H NMR spectrum in $CDCl_3$: δ 2.90 (s, OCH_3), 2.0 – 2.7 (m, 2 H, methylene), 2.9 – 3.6 (m, 2 H, methine), 4.68 (doublet; H_β-flavenyl, $J_{\beta,\gamma}$ 6 Hz), 6.5 – 7.9 (m, 18 arom. H). The filtrate was concentrated and diluted with water. The precipitated material was collected and after fractionated crystallization a white crystalline compound was obtained (m.p. 163 °C). Molecular weight by MS: Found 446.1887. Calc. for $C_{31}H_{26}O_3$: 446.1882. ^{1}H NMR spectrum in $CDCl_3$: (δ): 3.0 (s, OCH_3), 2.4 – 2.8 (m, 2 H, methylene), 3.1 – 3.5 (m, 2 H, methine) 5.15 (d. H_β-flavenyl, $J_{\beta,\gamma}$ 4.5 Hz), 6.6 – 7.9 (m, 18 arom. H).

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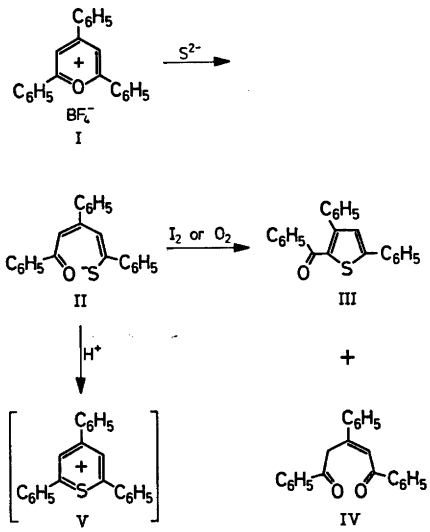
Short Communications

Formation of 2-Benzoyl-3, 5-diphenylthiophene and 2-Benzoyl-3,5-diphenylfuran from 2,4,6-Triphenylpyrylium Ion

CHRISTIAN L. PEDERSEN

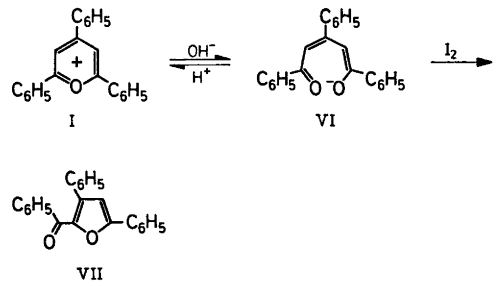
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The reaction between 2,4,6-triarylpyrylium salts and sodium sulfide is a well known method for the preparation of the analogous thiopyrylium salts.[1–4] Addition of an aqueous solution of sodium sulfide to the triphenylpyrylium salt I dissolved in acetone gives an intensely coloured solution, possibly due to formation of the anion II.[1] Upon addition of acid, II cyclizes to the thiopyrylium ion V (Scheme 1).



We found that the anion II was readily converted to 2-benzoyl-3,5-diphenylthiophene (III) when treated with mild oxidizing agents, *e.g.* air or iodine. The latter is more conveniently used owing to better yield and considerably shorter reaction time. Besides compound III small amounts of the so-called pseudobase IV was formed.* The structure of compound III was established by comparison (IR, mixed melting point) with a sample prepared independently.[5]**

On the basis of this result, it seemed interesting to examine whether this type of reaction could be extended to include preparation of other heterocyclic systems. While treatment of the 2,4,6-triphenylpyrylium ion with weak oxygen bases (*e.g.* acetate or hydrogen carbonate) gave the pseudobase IV [6,7] treatment with hydroxide ion or carbonate ion gave a red-coloured solution due to formation of the anion of the pseudobase VI[1,6,8]. This anion was similarly, although more slowly, converted to the furan VII (Scheme 2) when oxidized with



iodine. The structure of the product was established by comparison (IR, mixed melting point) with a sample prepared by benzoylation of 2,4-diphenylfuran [9] (see experimental). Furthermore, its spectroscopic features (see experimental section) support the proposed structure. In spite of these findings, attempts to prepare the selenophene and the pyrrole (the latter by oxidation of the monoimine [10] of the pseudobase IV) by an analogous procedure, failed.

The conversion of pyrylium salts to furans has been described previously. Thus, 2,4,6-trialkylpyrylium salts were oxidized with hydrogen peroxide to 2-acyl-3,5-dialkylfurans [11,12] and

* Compound IV was identical with a sample prepared from pyrylium salt I and sodium hydrogen carbonate.[6]

** All compounds showed satisfactory elemental analysis.

hydrolysis of 2,3,4,6-tetraphenylpyrylium perbromide gave 2-benzoyl-3,4,5-triphenylfuran.[13] Similar ring contractions were observed by oxidation of other heteroaromatic cations with manganese dioxide,[14,15] *e.g.* the oxidation of the parent thiopyrylium ion to 2-thiophenecarboxaldehyde. However, the thoroughly investigated 2,4,6-triphenylpyrylium ion, from which many heterocyclic systems are available,[4,16,17] has not previously been converted to a furan and a thiophene.

Experimental. Preparative layer chromatography (PLC) was performed on 20 × 100 cm plates with a 2.5 mm thick layer of silica gel (Merck $PF_{254}{}_{+366}$). The plates were developed 1 – 2 times with benzene.

Pyrylium salt. This was prepared according to the method of Lombard and Stephan.[7]

Preparation of 2-benzoyl-3,5-diphenylthiophene (III) by oxidation with iodine. A mixture of 2,4,6-triphenylpyrylium tetrafluoroborate (1.00 g, 2.52 mmol) in acetone (50 ml) and sodium sulfide nonahydrate (1.21 g, 5.04 mmol) in water (10 ml) was stirred for ½ h. Iodine (2.00 g, 7.88 mmol) was then added, and stirring was continued for 2 min. After addition of chloroform, excess iodine was removed by extraction with dilute sodium thiosulfate. The organic phase was separated and dried over sodium sulfate. After partial evaporation of the chloroform, the solution was separated by PLC into (1) 2-benzoyl-3,5-diphenylthiophene (III) (0.48 g ~56 %, m.p. 103 – 104 °C (methanol), Ref. 5 96.5 – 98 °C), IR (in KBr) 1625 cm^{-1} (C=O), ^1H NMR (60 MHz, $CDCl_3$) only aromatic protons at δ 7.8 – 7.0, UV (96 % ethanol) λ_{max} at 248 and 342 nm with log ε 4.26 and 4.14, respectively, and (2) 1,3,5-triphenyl-1,5-pentenedione (IV) (0.08 g ~9 %).

Preparation of 2-benzoyl-3,5-diphenylthiophene (III) by oxidation with air. 2,4,6-Triphenylpyrylium tetrafluoroborate (1.00 g, 2.52 mmol) dissolved in acetone (50 ml) was mixed with sodium sulfide nonahydrate (1.21 g, 5.04 mmol) in water (10 ml). After stirring for ½ h acetone (50 ml) was added, and air was sucked through the mixture for 22 h. The reaction mixture was then worked up as described above into (1) 2-benzoyl-3,5-diphenylthiophene (III) (0.38 g ~44 %) and (2) 1,3,5-triphenyl-1,5-pentenedione (IV) (0.08 g ~9 %).

Preparation of 2-benzoyl-3,5-diphenylfuran (VII). A mixture of 2,4,6-triphenylpyrylium tetrafluoroborate (1.00 g, 2.52 mmol) in acetone (50 ml) and aqueous potassium carbonate (5.0 ml, 1 M) was stirred for 2 h. Iodine (2.00 g, 7.88 mmol) was added and stirring was continued for 16 h. The reaction mixture was then worked up as described above yielding 2-benzoyl-3,5-diphenylfuran (0.43 g ~53 %, m.p. 117 – 118 °C (methanol)), IR (in KBr) 1640 cm^{-1} (C=O), ^1H NMR (60 MHz, $CDCl_3$) δ 8.0 – 7.1 (m, 15 H), 6.90 (sharp s, 1 H), UV (96 % ethanol) λ_{max} at 267 and 346 nm with log ε 4.59 and 4.43, respectively.

Preparation of 2-benzoyl-3,5-diphenylfuran (VII) from 2,4-diphenylfuran. 2,4-Diphenylfuran (0.80 g, 3.6 mmol) was dissolved in carbon disulfide (8.0 ml). Benzoyl chloride (0.60 g, 4.3 mmol) and aluminium chloride (0.60 g, 4.5 mmol) were added and the reaction mixture was stirred for 48 h. It was then poured into water and the organic material was extracted with chloroform. After drying the chloroform solution over sodium sulfate and partial evaporation, the resulting solution was separated by PLC into (1) starting material (0.29 g) and (2) 2-benzoyl-3,5-diphenylfuran (0.67 g ~90 % from reacted starting material).

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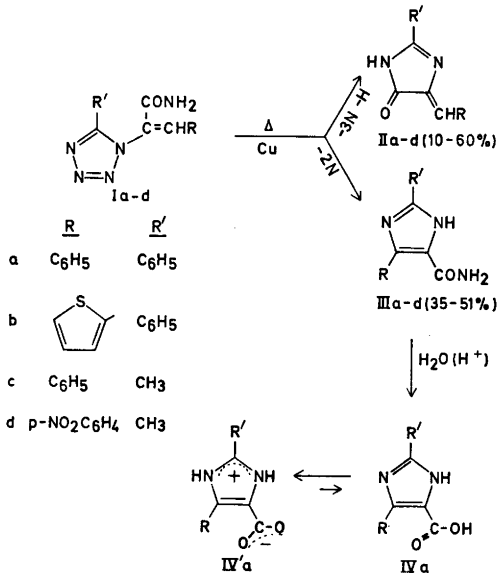
Preparation of Some 2,4-Disubstituted Imidazole-5-carboxamides by Thermolysis of β-Substituted α-(1-Tetrazolyl)acrylamides

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In a previous paper [1] it was described that the β-substituted α-(1-tetrazolyl)acrylamides I by copper catalyzed thermolysis were transformed into the unsaturated 5-imidazolones II by an overall loss of three nitrogen atoms and one hydrogen atom. Further it was mentioned that another, unidentified, type of compound was simultaneously formed by an overall loss of two nitrogen atoms. In the present paper it is shown that the latter compounds are 2,4-disubstituted imidazole-5-carboxamides III, and a modification of the thermolytic procedure is given which forms a convenient synthetic route to these compounds. No 4-alkylated, 4-arylated or 4-heteroarylated imidazole-5-carboxamide has to our knowledge been reported in the literature.

4-Aminoimidazole-5-carboxamide (AICA), which is a purine precursor, and some analogous compounds, *e.g.* 4-chloroimidazole-5-carboxamide, enhance the carcinostatic activity of 8-azaguanine, presumably because these compounds are inhibitors of guanine deaminase.[2-4] Furthermore antitumor activity of some 4-(3-dialkylated-triazeno)imidazole-5-carboxamides has been detected.[5-7] These findings suggest that the 2,4-disubstituted imidazole-5-carboxamides III might be taken into consideration as potential anticancer agents.



The imidazole-5-carboxamides III were formed in optimum yields (35–51 %) by heating the compounds I with copper powder *in vacuo* under the conditions summarized in Table 1. The intermediately formed imidazole-copper-complexes [8] were decomposed with ethanol, and the amides III were isolated from the ethanolic extract by preparative layer chromatography.

The amide IIIa was hydrolyzed by refluxing with 6 N hydrochloric acid. The acid IVa thus obtained was identical (melting point and IR spectrum) with 2,4-diphenylimidazole-5-carboxylic acid prepared by alkaline hydrolysis of the corresponding ethyl ester, which had been formed by action of ammonium acetate on ethyl α-benzoyloxybenzoylacetate according to Strzybny *et al.*[9] The IR, ^{1}H NMR and mass spectrum of IIIa were recorded. Using these data the structures of the analogous compounds IIIb–d could be established by spectroscopy supported by elemental analyses.

The acid IVa exists predominantly as the zwitterion IV'a as indicated by the IR spectrum.[10] This is in accordance with the structure found for imidazole-5-carboxylic acid.[11]

Unsuccessful attempts were made to prepare IIIa from the corresponding ethyl ester by treatment with 30 % aqueous ammonia.

Experimental. Recording of IR spectra and ^{1}H NMR spectra, determination of melting points and microanalyses were performed as previously described.[1] The mass spectra were obtained with an AEI MS 902 mass spectrometer operating at 70 eV.

2,4-Disubstituted imidazole-5-carboxamides III. General procedure. A mixture of the tetrazolylacrylamide I [1] (4 mmol) and 10 % by weight of copper powder (BDH Ltd., Laboratory reagent, reduced by hydrogen) was heated under reduced pressure (12 mmHg) for 2–15 min at 197–230 °C. The mixture was extracted with several portions of boiling ethanol, and the ethanol was evaporated under reduced pressure. From the residue the imidazole-5-carboxamide III was isolated by preparative layer chromatography on 1 mm silica gel PF_{254} (Merck) plates using benzene–ethyl acetate 1:1 (in the case of IIIa and b) or benzene–ethanol 8:2 (in the case of IIIc and d) as eluent. Experimental and analytical data are given in Table 1.

IR spectra of IIIa–d were recorded and found in accordance with data published for other primary amides:[10] 2900–3600 cm^{-1} (m) (broad band with one submaximum about 3450 cm^{-1} (N–H, free) and another, very broad submaximum about 3150 cm^{-1} (N–H, bonded)), 1670 cm^{-1} (s) (amide I, C=O) and 1590–1610 cm^{-1} (s) (amide II, mostly NH_2). Intensities are indicated as s (strong), m (medium), or w (weak).

Table 1. Preparation of 2,4-disubstituted-5-imidazolecarboxamides IIIa – d by copper catalyzed thermolysis of the β-substituted α-(1-tetrazolyl)acrylamides Ia – d.

Starting material M.p. °C	Reaction conditions Temp. °C	Time min	Product	Yield[a] %	Recryst. from[b]	M.p. °C	Formula	Analyses C, H, N, S
Ia 180–182	197–203	15	IIIa[c]	51	A	217–219	$C_{16}H_{13}N_3O$	Found: 72.65; 5.10; 16.07 Calc.: 72.98; 4.98; 15.96
Ib 207–208	207	2	IIIb	38	B	82–85	$C_{14}H_{11}N_3SO$	Found: 62.50; 4.60; 15.76; 11.51 Calc.: 62.45; 4.12; 15.61; 11.88
Ic 204–212	207	10	IIIc	37	C	194–197	$C_{11}H_{11}N_3O$	Found: 65.57; 5.66; 20.85 Calc.: 65.57; 5.51; 20.88
Id 250–253	230	2	IIId[d]	35	B	207–210	$C_{11}H_{10}N_4O_3 \cdot \frac{1}{2}H_2O$	Found: 52.05; 4.19; 21.80 Calc.: 51.76; 4.35; 21.95

[a] Yields calculated after isolation by preparative layer chromatography. [b] A, dry toluene; B, dry xylene-light petroleum; C, dry toluene-light petroleum. [c] Crystallizes from benzene-ethyl acetate with ½ mol of water. M.p. 197–210 °C. (Found: C 70.55; H 5.00. N 15.20. Calc. for $C_{16}H_{13}N_3O.\frac{1}{2}H_2O$: C 70.60; H 5.18; N 15.44). [d] Dried *in vacuo* at 180 °C over P_2O_5 for 3 h. Decomposes slowly above 180 °C without releasing water.

^{1}H NMR spectra (DMSO-d_6) were recorded for IIIa, c and d. IIIa: δ 8.3–7.9 (m, 4 aromatic *o*-protons), 7.7–7.0 (m, 6 aromatic *m*- and *p*-protons and 3 NH protons). IIIc: δ 8.0–7.7 (m, 2 aromatic *o*-protons), 7.5–6.9 (m, 3 aromatic *m*- and *p*-protons and 3 NH protons), 2.33 (s, 3 methyl protons). IIId: δ 8.26 (s, 4 aromatic protons), 8.2–7.0 (broadened signal, 3 NH protons), 2.38 (s, 3 methyl protons). By deuteration of IIIa, c, and d with D_2O approximately two NH protons were exchanged.

Mass spectra were recorded for IIIa and c. The spectra display fragmentation patterns which are in accordance with data published for primary amides [12] and for imidazoles.[12,13] Prominent peaks in the mass spectra of IIIa and IIIc, respectively: Molecular ion (M) (*m/e* 263 (100 %) and *m/e* 201 (100 %); M–H (42 resp. 46 %), M–NH_2 (23 resp. 38 %), M–NH_3 (40 resp. 41 %), M–$CONH_2$ (8 resp. 3 %), M–$CONH_3$ (23 resp. 4 %), R'CNCCO (14 resp. 43 %), R'CNC (36 resp. 5 %), R'CNH (26 resp. 19 %). Ions common for IIIa and IIIc: C_6H_5CCN (36 resp. 9 %), C_6H_5CNH (26 resp. 15 %), C_6H_5C (42 resp. 23 %), $CONH_2$ (8 resp. 6 %).

2,4-Diphenylimidazole-5-carboxylic acid (IVa). A solution of IIIa (0.3 mmol) in 10 ml of 6 N hydrochloric acid was refluxed for 2 h. After cooling to room temperature a reddish precipitate was removed, and the pH of the solution was adjusted to about 3 by means of sodium hydroxide (30 %). The carboxylic acid IVa precipitated was filtered and washed with several portions of water. Yield 75 %. M.p. (decomp.) 204–206 °C (3 °C/min), 210–213 °C (5 °C/min). (Lit.[9] m.p. (decomp.) 209–210 °C). (Found: N 10.43. Calc. for $C_{16}H_{12}N_2O_2$: N 10.61). IR spectrum: 1600 (s) and 1380 cm^{-1} (s) (COO^-); 3200–1800 cm^{-1} (m) (broad band with submaxima at 1950, 2600, 2800 and 2900 cm^{-1}) (NH^+); 3600 cm^{-1} (m) (OH, free), 3400 cm^{-1} (m) (NH, free), 1720 cm^{-1} (w-m) (C=O in COOH).

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Shaking with Aqueous Sodium Hydrogen Sulfite; a Simple Procedure for Removing Some Norbornene Impurities

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When preparing nortricyclyl compounds *via* homoallylic rearrangement of norbornenyl starting materials [1–3] or by addition of appropriate reagents to norbornadiene,[4–6] undesired norbornenyl compounds often remain or form. Also hydrogenation of norbornenyl compounds to norbornyl analogs may be imperfect. Procedures for removing these norbornene impurities have been presented, *e.g.* shaking with aqueous potassium permanganate [5] or with aqueous silver nitrate,[6] and precipitation with nitrosyl chloride in chloroform.[4] A cheap, gentle and as yet new procedure is now being reported: shaing with aqueous sodium hydrogen sulfite, which seems best suitable for removing norbornenones and norbornenols.

It is generally known that sodium hydrogen sulfite easily forms addition compounds with aldehydes and ketones if they are not sterically hindered (reversible reactions) and it adds fairly easily at the carbon-carbon double bond conjugated with the carbon-oxygen double bond or with the carbon-nitrogen triple bond (irreversible reactions).[7,8] Simple olefinic double bonds generally react with hydrogen sulfite poorly.[7] However, the reaction between the hydrogen sulfite ion and 2-norbornenols seems to be fairly rapid, but 2-norborneols and 3-nortricyclanols do not react under the same conditions.

Disappearance rates of some substituted norbornenes were studied by shaking *ca.* 2 mmol of the substrate and an equal amount of an internal standard (2-norborneols and 3-nortricyclanols or corresponding acetates) in 20 ml ether with 50 ml of 1 M aqueous sodium hydrogen sulfite at room temperature. If the reaction was not complete in half an hour, shaking by hand was replaced by magnetic stirring. The ether phase was analyzed by GLC (an FFAP column). The following rough disappearance times (at least 99 % of the substrate had reacted) were measured: 2-norbornenone 10 min, *endo*-2-norbornenol 5 min, *exo*-2-norbornenol 30 min, 2-methyl-*endo*-2-norbornenol 5 min, 2-methyl-*exo*-2-norbornenol 40 min, *endo*- and *exo*-2-norbornenylmethanols 10 – 15 min, 2-methyl-*endo*- and 2-methyl-*exo*-2-norbornenylmethanols a few hours. The corresponding acetates also reacted, but much more slowly (10 – 100 h), and the disappearance times of norbornene and norbornadiene were very long (100 – 1000 h). The sequence of disappearance times does not evidently follow the sequence of rates of the homogeneous reactions in the aqueous solutions owing to differences in partitions of the substrates between the ether and aqueous phases. Measurements of rates of the homogeneous reactions for some compounds are under way.

Addition of a free-radical inhibitor, *e.g.* hydroquinone (4 mmol),[8] totally prevents the reaction of the alcohols studied or at least retards it very markedly. Addition of sulfuric acid (final pH ≈ 0) or sodium hydroxide (final pH ≈ 14) into the aqueous phase after the reaction has proceeded to completion does not liberate the alcohols. Thus, the sulfuric atom of the hydrogen sulfite anion (or sulfite anion) [8] attacks the double bond of the substrate *via* a free-radical mechanism producing substituted norbornanesulfonate anions, which dissolve easily in the aqueous phase.

Addition of hydroquinone retarded the reaction of 2-norbornenone, but did not stop it. About 10 % of the substrate remained unreacted (measured 18 h after the reaction had started). About two thirds of the initial ketone was regenerated by addition of sodium hydroxide. In the absence of hydroquinone, addition of alkali did not liberate any of the completely reacted 2-norbornenone. Thus, in the case of the unsaturated ketone the irreversible, mostly free-radical, attack at the carbon-carbon double bond and the reversible ionic attack at the carbon-oxygen double bond compete. The latter reaction also occurred in the case of 2-norbornanone (hydroquinone had no effect on its disappearance rate) leading to equilibrium (*ca.* 30 % of the substrate was left after 19 h). The release of 2-norbornanone occurred quantitatively by addition of alkali. Thus, shaking with sodium hydrogen sulfite also removes irre-

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versibly 2-norbornenone from its mixtures, *e.g.* with 2-norbornanone or 3-nortricyclanone, but the saturated ketones which have disappeared from the ether phase can be regenerated by addition of alkali or acid and by shaking a second time.

Shaking by hand is, however, not a proper procedure for removing great quantities of unsaturated substrates, because the reaction seems to be highly exothermic and may be violent. In these cases mechanical stirring in a flask fitted with a reflux condenser is preferred. Addition of fresh hydrogen sulfite into the flask may be necessary at times owing to the escape of sulfur dioxide.

It has been recently reported that also some acyclic alkenols react with hydrogen sulfite.[9,10]

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Chemical Relaxation Studies on the System Liver Alcohol Dehydrogenase, NADH and Imidazole*

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Several years ago, Theorell and Czerlinski conducted experiments on the system of horse liver alcohol dehydrogenase, reduced nicotinamide adenine dinucleotide and imidazole, using the first version of the temperature jump apparatus with detection of changes in fluorescence. These early experiments were repeated with improved instrumentation and confirmed the early experiments in general terms. However, the improved detection system allowed to measure a slight concentration dependence of the relaxation time of around 3 ms. Furthermore, the chemical relaxation time was smaller than the one determined earlier (by factor 2). The data were evaluated much more rigorously than before, allowing an appropriate interpretation of the results.

The observed relaxation time is largely due to rate constants in an interconversion of ternary complexes, which are faster than three (of the four) dissociation rate constants, determined previously by Theorell and McKinley-McKee.[1,2] This fact contributed to earlier difficulties of finding any concentration dependence. However, the binding of imidazole to the binary enzyme-coenzyme complex can be made to couple kinetically into the interconversion rate of the two ternary complexes.

The observed signal derives largely from the ternary complex(es). A substantial fluorescence signal change is associated with the observed relaxation process, suggesting a relocation of the imidazole in reference to the nicotinamide moiety of the bound coenzyme. Nine models are considered with two types of coupling of pre-equilibria (none-all). Quantitative evaluations favor the model with two ternary complexes connected by an interconversion outside the four-step (bimolecular) cycle. The ternary complex outside the cycle has much higher fluorescence yield than the one inside. The interconversion equilibrium is near unity for imidazole. If it would be shifted very much to the side of the "dead-end" complex (as in isobutyramide?!), stimulating action could not take place.

Previously, we investigated thoroughly the chemical relaxation spectrum of the system of horse liver alcohol dehydrogenase (L-ADH) with nicotinamide adenine dinucleotide (NAD^+) and ethanol, observing primarily the slowest relaxation time over a wide range of reactant concentrations and pH.[3] Only indirect evidence was obtainable on the rate of interconversion among ternary complexes. We, therefore, decided to study the previously investigated chemical relaxation of the ternary complex between L-ADH, reduced nicotinamide adenine dinucleotide (NADH) and imidazole, projecting a possible correlation of this interconversion to the one expected in the previously mentioned system. No such correlation could be made, as the concentration dependence of the observed relaxation time was not particularly large. However, the equilibrium characteristics of the ternary complexes allowed some interesting mechanistic conclusions. Specifically, imidazole was selected as it forms a ternary complex of comparatively high fluorescence yield.

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Furthermore, imidazole is known to stimulate the action of liver alcohol dehydrogenase. Finally, equilibrium and steady state kinetic data had already been determined by Theorell and McKinley-McKee [1,2] and were utilized in this investigation.

METHODS

Horse liver alcohol dehydrogenase was obtained from Boehringer (prepared according to the method of Dalziel [4]). The commercial preparation was centrifuged and dialyzed as described by Taniguchi *et al.*[5] The concentration of active sites in the stock solutions was determined as described by Theorell and Yonetani,[6] utilizing the ternary complex with pyrazole and NAD^+. In all cases was the binding capacity of the enzyme 100 % relative to its concentration determined spectrophotometrically. Anhydrous imidazole, Grade III, was obtained from Sigma Chemical Company and used without further purification. Phosphate buffers were prepared from the sodium salts as obtained from Baker Chemical Co., "Baker Analyzed" grade. Experiments were run at a constant ionic strength of 0.1 M and at 23.5 °C to coincide with the conditions established by Theorell and co-workers.[1,2,5-7] The pH was determined with a Radiometer pH meter, Model 26. The temperature jump apparatus used was based on the general coaxial design of Czerlinski,[8] following changes in fluorescence. As previously reported,[9] the excitation filter was a Kodak Glass filter type 18A, the emission filter a Kodak Wratten filter type 2B.

Considerable improvement of the signal-to-noise ratio has been obtained compared to the original instrument. The principal improvement was accomplished through an electronic refinement described recently.[10] Also, the General Electric BH6 Mercury arc lamp was replaced by the PEK107 Mercury arc lamp, selected for high performance (in conjunction with a Model 6274A hp/Harrison Power Supply). The employed power supply was operated either in the "constant current" mode, or in the "constant power" mode to further stabilize the energy output. It should be mentioned that the detection system still showed fluctuations of around 0.25 % of the total signal in a time range of 1 s, thus limiting the precision of repeat traces.

The oscilloscope traces were evaluated with Berman's computer program [11] for simulation, analysis and modeling, SAAM 23. Actual coordinate values for a given trace were obtained directly in digital form through the use of a Biomation 802 transient recorder, connected *via* a Dijiscan interface to a teletypewriter (ASR33) paper tape punch. The paper tape information was transferred onto disk for further evalution by computer programs. Up to 1024 points were compressed to a data matrix, not containing more than 50 abscissa-values: a typical section is shown in Fig. 1. A subordinate program extended these data (adding initial parameter estimates), to have them conform to the input format for SAAM 23 (executed in batch). Some more recent experiments were evaluated interactively from a teletypewriter, using a special fitting program.[12]

.2000	12.02133
.5000	26.91733
.8000	43.64267
1.1000	48.60800
1.4500	51.94000
1.8500	66.05200
2.2500	63.70000
2.6500	72.32400
3.1000	80.12480
3.6000	86.71040
4.1000	87.49440
4.6000	95.49120
5.1500	93.94933
5.7500	103.22667
6.3500	102.18133
6.9500	108.58400
7.6000	105.50400
8.3000	108.64000
9.0000	113.12000
9.7000	109.76000
10.4500	110.44600
11.2500	111.13200
12.0500	107.60400
12.8500	110.44600

Fig. 1. Listing of one data set for input into the SAAM 23 program. The first column gives values for the independent observable (time in ms). The second column denotes the dependent observable (signal change in mV). The data shown refer to chemical relaxation measurements on a mixture, consisting of 18 μN enzyme, 10 μN NADH, 5 mM imidazole, phosphate buffer of ionic strength 0.1, pH 8.0.

Program SAAM 23 returned the following value for the reciprocal of the chemical relaxation time: 0.383 ± 0.222 ms^{-1}.

The initial evaluation is based on the presumption of an exponential decay curve, $\Delta I(t) - \Delta I_1 \exp(-t/\tau_1) + \Delta I_0$ with $\Delta I(t)$ representing the signal change observed as a function of time (t). The ΔI_j, represent equilibrium signal changes, and τ_1 the chemical relaxation time constant. Time constant τ_1 is a function of the concentrations of L-ADH, NADH and imidazole. The quantitative relation depends upon the mechanistic details assumed by the investigator(s). After it became apparent (see discussion) that a monomolecular interconversion is associated with the ternary complex, two alternate schemes were analyzed further. Three models were considered for every scheme. Independent of earlier results from steady state kinetic experiments, two conditions were considered: no coupling-in of pre-equilibria (all other steps are much slower than the ones under consideration), full coupling-in of pre-equilibria (all independent other steps are much faster than the ones under consideration).

RESULTS

The experimental details were designed on the basis of earlier equilibrium studies of Theorell and McKinley-McKee.[1] Values for various equilibrium and rate constants were taken from the literature and are summarized in Table 1. The table shows the notations and subscripting for the equilibrium and rate constants, used in this paper. The direction of the flux for the dissociation rate constants is indicated directly in the column headings for the rate constants and refers also to the reaction step, described by the equilibrium constant in that column. The subscripting is unconventional in rapid chemical kinetics but introduced here for facilitating the needed transition to a generalized treatment of kinetic data. Positive and negative subscripts are frequently used in steady state kinetic treatments. However, the dissociation rate constants do not necessarily have negative subscripts. The equilibrium constants employed in the design of these experiments are graphically presented in Fig. 2. Specific relationships were derived (see Appendix I) to express the experimental apparent dissociation constants in

Table 1. Dissociation and rate constants[a] in the system of horse liver alcohol dehydrogenase (E), reduced nicotinamide adenine dinucleotide (R) and imidazole (I).

pH	Equil const. μM K_1	K_2	K_3	K_4
6	4200	2.01	470	0.23
7	3500	2.03	550	0.31
8	3600	2.20	680	0.41
9	3000	2.91	670	0.65
10	—	—	—	5[b]
	Rate const. s^{-1} k_{-1}	k_{+2}	k_{+3}	k_{-4}
	ERI→ ER+I	EIR→ EI+R	EI→ E+I	ER→ E+R
7	24.5	20.3	≥257	3.1
9	9	22	≥319	4.9

[a] Refs. 1,2; see especially pages 1846–1854; k_{+3} (pH 9) was computed under assumption that k_{-3} is not dependent upon pH, then $k_{+3}(\text{pH } 9) = k_{+3}(\text{pH } 7)\ K_{E,I}(\text{pH } 9)/K_{E,I}(\text{pH } 7)$.
[b] Ref. 18: In PO_4 $\mu = 0.1 + 0.1$ M glycine.

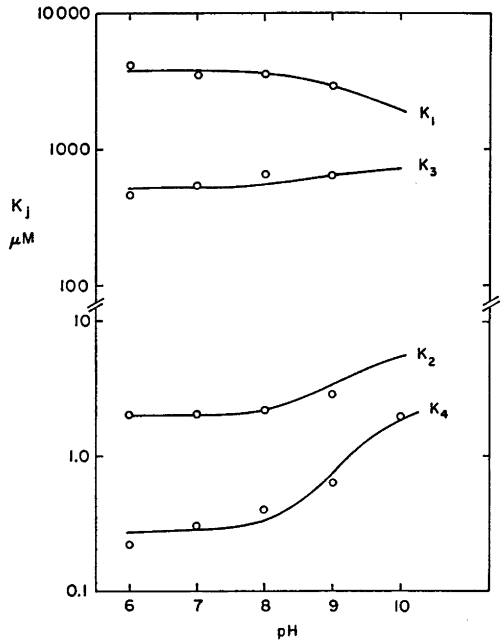


Fig. 2. Apparent dissociation constants K_1, K_2, K_3, and K_4 of binary and ternary complexes in the reaction of liver alcohol dehydrogenase with NADH and imidazole. The experimental points are those of Theorell and McKinley-McKee.[2] The theoretical curves are based on Scheme I of Appendix I with the following parameter values: $p\underline{K}_{-1} = 8.60$, $p\underline{K}_{-2} = 9.55$, $p\underline{K}_{-3} = 8.75$, $p\underline{K}_{-4} = 9.20$, $\underline{K}_{EH,R} = 9.28\ \mu M$, $\underline{K}_{EH,I} = 525\ \mu M$, $\underline{K}_{EHI,R} = 2\ \mu M$. The pH- independent constants are defined by the reaction scheme of Fig 3.

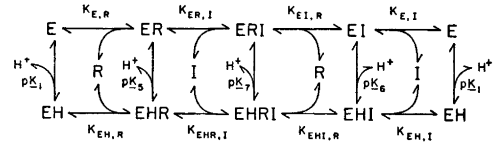


Fig. 3. Simplified reaction scheme to describe the pH-dependence of the dissociation constants in the reaction of horse liver alcohol dehydrogenase with NADH and imidazole. Values of specific constants are presented in the captions to Fig. 2. The reaction cycle involving free enzyme E and free reduced coenzyme R was introduced previously.[2] The remaining cycles are equivalent to mechanistic "interpolations". Values for the various dissociation constants are chosen such that the data of Theorell and McKinley-McKee are optimally approximated by them over the whole experimental pH-range. See Appendix I for quantitative relations.

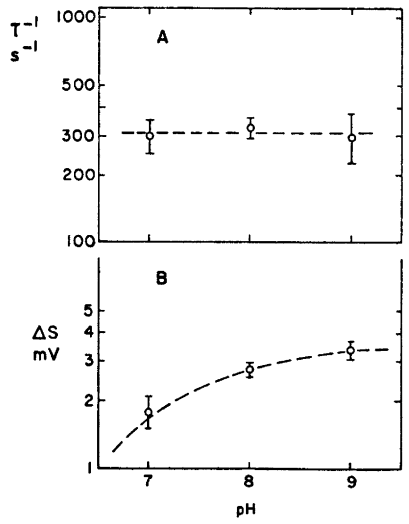


Fig. 4. The reciprocal of the chemical relaxation time as a function of pH in part A and the signal amplitude as a function of pH in part B of this figure. Conditions: 9.5 μN enzyme, 9.5 μM NADH and 1 mM imidazole; 25 °C final temperature, ionic strength 0.1 by phosphate (and a small amount of glycine). The curve indicates the "best fit" (no equation). Ordinate "bars" represent the total range of several repeat experiments. The ternary complex contributes most to the fluorescence.

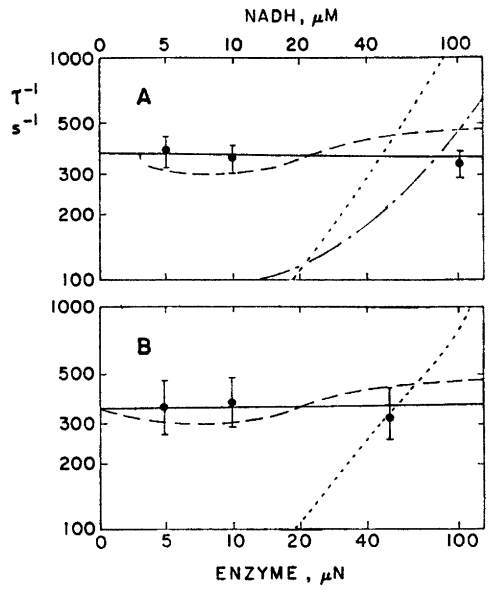


Fig. 5. The inverse chemical relaxation time as a function of the concentration of NADH (part A) and of enzyme (part B). The imidazole concentration was 5 mM and the concentration of the nonvarying component 10 μN; pH 7.0, ionic strength 0.1, 25 °C final temperature. The various theoretical curves are defined as follows: dotted for model *b*, full for model *e* (pos. root), dashed for model *d* (pos. root), dashed-dotted for model *a*. The dash-dotted curve in Fig. 5B is located outside the limits of the figure (below 100 s^{-1}). See Table 2.

terms of pH-independent constants. The underlying reaction scheme is shown in Fig. 3. One obtains theoretical curves which are shown in Fig. 2.

The results from the evaluation of the relaxation processes, utilizing the program SAAM 23, are summarized in Fig. 4 for pH-dependence of the inverse relaxation time and of the fluorescence equilibrium signal change. The horizontal line in Fig. 4A and the curve in Fig. 4B correspond to "best interpolations" without a specific model implied. Fig. 5. summarizes the measurements of the reciprocal relaxation time for coenzyme- and enzyme-dependence at pH 7. The curves here and in subsequent figures correspond to specific mechanistic models, which will be elaborated on under DISCUSSION. The experimental data are repeated in Fig. 6 with the curves referring to additional mechanistic models. The dependence of the observed reciprocal relaxation time upon the concentration of imidazole is presented in three different figures, namely in Fig. 7 for pH 7, in Fig. 8 for pH 8 and in Fig. 9 for pH 9. The various models tested are distributed over parts A and B of these three figures, showing their extent of fit to the experimental observations. Fig. 10B shows the reciprocal relaxation time plotted *versus* the imidazole concentration at three levels of NADH-concentration. Curves are here only inserted for a small number of mechanistic models. Fig. 10 A repeats the data of Fig. 7 and 8 with the theoretical curves referring to a combined evaluation (see below). To ascertain the concentration dependence at extreme concentration conditions, a large number of repetitions were necessary because of the low signal-to-noise ratio. Even then, the final value for the reciprocal of the relaxation time constant is only

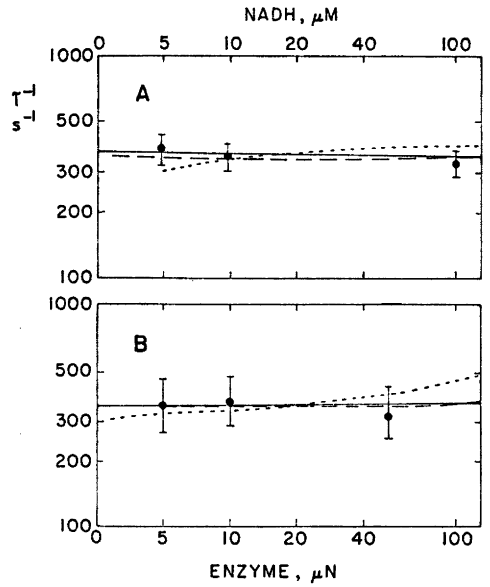


Fig. 6. Continuation of Fig. 5 with full line identical to full line in Fig. 5, the dashed line refers to Model *d*, the dotted one to Model *c*, for other details see Table 2.

accurate to ± 20 % at the concentration limits, to ± 10 % in between.

The most pronounced dependence of the reciprocal relaxation time upon the concentration of imidazole was found at pH 8. This pH seemed thus the optimal one for observing the concentration dependence. Subsequent experiments at increased coenzyme concentration (33, 166 and 330 μM NADH) did not allow to improve the extent of the dependence

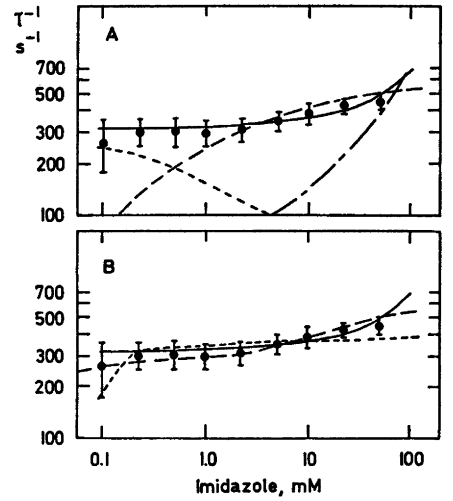


Fig. 8. The inverse chemical relaxation time as a function of the total concentration of imidazole at pH 8; 18 μN enzyme, 10 μN NADH, ionic strength 0.1, 25 °C final temperature. The theoretical curves are defined for Fig. 8A as in Fig. 5 and for Fig. 8B as in Fig. 6.

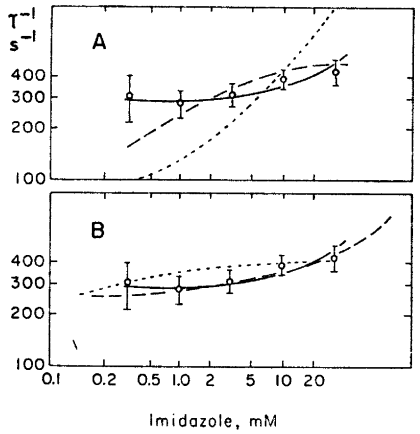


Fig. 7. The inverse chemical relaxation time as a function of the concentration of imidazole at pH 7.0 18 μM enzyme, 10 μN NADH, ionic strength 0.1, 25 °C final temperature. The various theoretical curves are defined for Fig. 7A as in Fig. 5 and for Fig. 7B as in Fig. 6.

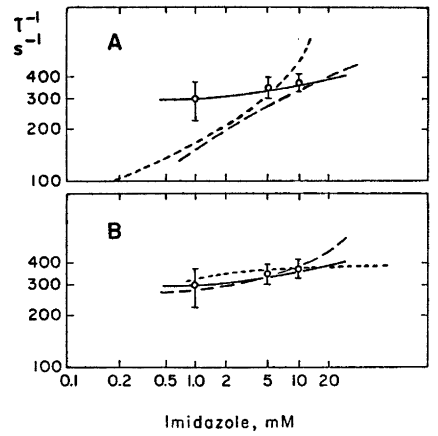


Fig. 9. The inverse chemical relaxation time as a function of the total concentration of imidazole at pH 9; 18 μN enzyme, 10 μN NADH, ionic strength 0.1, 25 °C final temperature. The theoretical curves are defined for Fig. 9A as in Fig. 5, and for Fig. 9B as in Fig. 6.

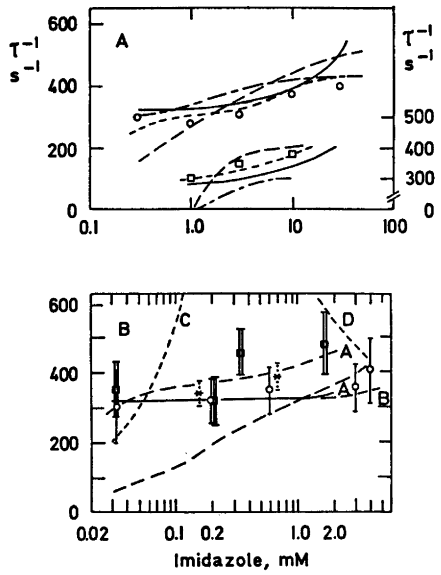


Fig. 10. The inverse chemical relaxation time as a function of the imidazole concentration. Fig. 10B refers to pH 8.0, 11.2 μN enzyme, phosphate buffer of ionic strength 0.1, 25 °C final temperature. Vertical "bars" refer to 33 μM NADH, vertical "double-bars" to 330 μM NADH and the two asterisks to 166 μM NADH. The various theoretical curves are defined as follows: full curve A with 33 μM NADH, dashed extension B of full curve A with 330 μM NADH, both for Model *d* (see Table 2). Dashed curve A refers to 330 μM NADH, dashed curve B to 33 μM NADH, both for Model *d*. Dotted curve C refers to 330 μM NADH and Model *b*, dotted curve D to 33 μM NADH and Model *a* (equivalent curve to 330 μM is off scale, meaning: above 600 s^{-1}). Other models have been tested, but fit even less. Fig. 10A. refers to fixed NADH at 10 μM, enzyme at 18 μN, phosphate buffer of ionic strength 0.1, 25 °C final temperature. The squares refer to pH 7.0 and the right scale, the circles to pH 8.55 and the left scale. The imidazole-dependence and the pH-dependence were fitted together, using the rate constants from the fit at pH 8.0 as initial estimates. The final values for the constants are insignificantly different from the initial estimates. The full theoretical curves refer to Model *f* (see Table 2) the dashed ones to Model *e*, the dotted ones to Model *d* and the dash-dotted ones to Model *a*. The curves for Model *b* are in shape similar to those of Model a, but deviate further from the experimental points than the dash-dotted curve does. The curves for Model *c* are outside the limits of the figure.

of the inverse relaxation time upon imidazole concentration, see Fig. 10A. However, these experiments clearly established the dependence of the relaxation time upon the concentration of NADH. This latter dependence transcends the assumption of only two steps competing in time range. A more rigorous analysis becomes then necessary, reserved for a specialized paper.[13]

Experiments were also conducted on mixtures of horse L-ADH with NAD^+ and ethanol in the presence of imidazole at pH 7.0. No influence of imidazole upon the reciprocal of the slowest relaxation time was observed, except at very high concentrations. Preliminary reports on the results were given at two meetings[14,15] Early experiments had not revealed a concentration dependence.[16]

DISCUSSION

1. Mechanistic principles. Already Theorell and McKinley-McKee [2] wrote the reaction of liver alcohol dehydrogenase, E, reduced nicotinamide adenine dinucleotide, R, and imidazole, I, as a four-step-cycle. The ternary complex ERI could be formed from E by two paths, one proceeding *via* ER, the other one *via* EI. Such a four-step cycle may be considered to be present in many enzyme reactions, equilibrating before attainment of the steady state. The heavy arrows in Fig. 11A notate an enzyme catalyzed isomerization reaction with competitive inhibition. The ternary complex ESI does not exist in ideal competitive inhibition, but may play a role in inhibitions of mixed character.

If one considers letter "I" to denote coenzyme, the system of Fig. 11B may be considered. An ideal ordered mechanism (heavy arrows) causes ES not to exist in the system. In any random (or even mixed) order mechanism, binary complex ES will have to be considered. A four-step-cycle of the described kind may thus be expected to appear quite commonly in enzyme kinetics.

One may now consider the time range of the equilibration of the four-step-cycle. The concentration dependence is expected to be considerable.[17] As only a small concentration dependence was found, presence of a monomolecular interconversion becomes highly prob-

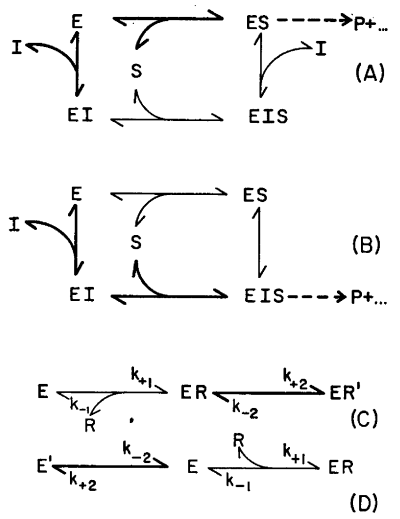


Fig. 11. Four reaction schemes are shown. The first two (A and B) relate to steady state kinetics, the last two (C and D) to chemical relaxation. Model A emphasizes competitive inhibition, but permits path *via* short-lived intermediate EI. Model B emphasized an ordered two-substrate reaction (S and I are substrates), but permits path *via* shortlived ES. Model C shows fast bimolecular step with slow isomerization of binary complex. Model D shows fast bimolecular step with slow isomerization of free enzyme. The dependence of the inverse relaxation time upon the concentration of reactant R is quite different for the two models C and D and will be used in the text to pinpoint the location of the observed isomerization.

able. Either one of the four enzyme containing components may in principle exist in two isomeric forms. However, specific patterns in the concentration dependence of the relaxation time may eliminate some choices. To illustrate the available patterns, two simple two-step-mechanisms are considered, namely those of Fig. 11 C and D. Case D shows two forms of free enzyme. The fast relaxation time, τ_1, is associated with the bimolecular step and the slow relaxation time, τ_2, with the interconversion step: $\tau_1 \ll \tau_2$.

If the time range of τ_2 is observed, the bimolecular reaction step is in pre-equilibrium and one obtains in case D (see eqn. (3−51) of Czerlinski [17]).

$$\frac{1}{\tau_2} = k_{-2} + k_{+2}\frac{[\mathrm{E}]+[\mathrm{R}]}{K_1+[\mathrm{E}]+[\mathrm{R}]} \qquad (1)$$

Similarly, one obtains in case C (see eqn. (3-57) of Czerlinski [16]):

$$\frac{1}{\tau_2} = k_{-2} + k_{+2}\frac{K_1+[\mathrm{E}]}{K_1+[\mathrm{E}]+[\mathrm{R}]} \qquad (2)$$

The rate constants are defined as shown in Fig. 11 C and D. If "Y" denotes a generalized component, equilibrium concentrations are indicated by [Y] and analytical concentrations by $C_\mathrm{y}°$. The equilibrium constant K_1 is defined by:

$$K_1 = \frac{k_{-1}}{k_{+1}} = \frac{[\mathrm{E}][\mathrm{R}]}{[\mathrm{ER}]} \qquad (3)$$

One may design the experiments such that:

$$C_\mathrm{R}° \gg C_\mathrm{E}° \qquad (4)$$

These analytical concentrations are defined by:

$$C_\mathrm{R}° = [\mathrm{R}]+[\mathrm{ER}]+[\mathrm{ER'}] \qquad (5)$$

$$C_\mathrm{E}° = [\mathrm{E}]+[\mathrm{E'}]+[\mathrm{ER}]+[\mathrm{ER'}] \qquad (6)$$

With $[\mathrm{E'}]\equiv 0$ for case C and $[\mathrm{ER'}]\equiv 0$ for case D. Condition (4) simplifies eqns. (1) and (2) to

$$(1/\tau_2)_\mathrm{C} = k_{-2} + k_{+2}C_\mathrm{R}°/(C_\mathrm{R}° + K_1) \qquad (7)$$

$$(1/\tau_2)_\mathrm{D} = k_{-2} + k_{+2}K_1/(K_1 + C_\mathrm{R}°) \qquad (8)$$

One realizes quickly that the concentration dependence of τ^{-1} upon $C_\mathrm{R}°$ is different for the two cases: Eqn. (7) shows an increase of τ_2^{-1} with increasing $C_\mathrm{R}°$ and with asymptotic approach of the limit $k_{-2}+k_{+2}$. Eqn. (8), applying to case D, shows in contrast to eqn. (7) a decrease of τ_2^{-1} with increasing $C_\mathrm{R}°$ with the value approaching asymptotically k_{-2}. This difference in the concentration dependence is crucial for differentiating among alternate mechanisms.

Returning to the pre-equilibration of the four-step-cycle in Fig. 11A, one may now consider four possibilities for isomerization, associated with either one of the four enzyme-containing components. One may further assume in zero approximation that bimolecular steps are rapid in comparison to monomolecular ones *and* that bimolecular steps adjoining monomolecular interconversions determine the concentration dependence (remote bimolecular steps do not contribute to the concentration dependence; any such contributions may in fact be treated as higher order corrections,

cf. eqn. (3-212) of Czerlinski [17]). The four possibilities show distinctly different dependencies of τ^{-1} *vs.* the analytical concentrations.

α. Isomerization of E. Case D applies with regard to inhibitor I *and* with respect to substrate S. The concentration dependence of eqn. (8) applies in both instances (R and I are equivalent).

β. Isomerization of ES. Case C (and thus eqn. (7)) applies with respect to component S and case D (and thus eqn. (8)) with regard to component R.

γ. Isomerization of ESI. Case C applies with regard to inhibitor I *and* with respect to substrate S. The concentration dependence of eqn. (7) applies thus in both instances.

δ. Isomerization of EI. This case shows a behaviour, which is inverted to case *β*: eqn. (7) applies with respect to component R and eqn. (8) with respect to component S.

The above discussion reveals that unique patterns of concentration dependence may be found. To what extent the underlying assumptions are fulfilled, will have to be investigated separately in every case. Our experimental results show the dependence of case *γ*: an isomerization among ternary complexes is thus most probable.

2. Mechanistic modeling

Although the small concentration dependence of the observed relaxation time speaks against assuming only a four-step-cycle, an exhaustive analysis of the four-step-cycle should be conducted nevertheless. Three specific models of the four-step-cycle will be considered, outlined in Fig. 12 *a*, *b*, *c*. Further details on these models will be discussed after the introduction of the nine basic models.

Even if a monomolecular interconversion is associated with the ternary complex, three different types of coupling of individual steps

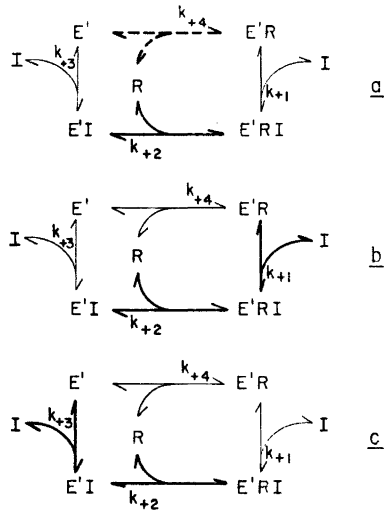


Fig. 12 Illustration of three four-step bimolecular cycles, used in the kinetic analysis. Heavy double-arrows indicate competing time ranges, which are considered to be possibly associated with the observed relaxation process. Model *a* describes the two steps with coenzyme-binding governing the time range of observation and the two steps involving Inhibitor I considered in pre-equilibrium. Only one relaxation process could be measured: the third relaxation time constant. Model *b* refers to the competition of the steps with k_{+1} and k_{+2}. Although k_{+4} is indicated as ordinary step it is ignored in the kinetic evaluation because of its established small contribution. Model *c* describes the steps with k_{+2} and k_{+3} kinetically competing.

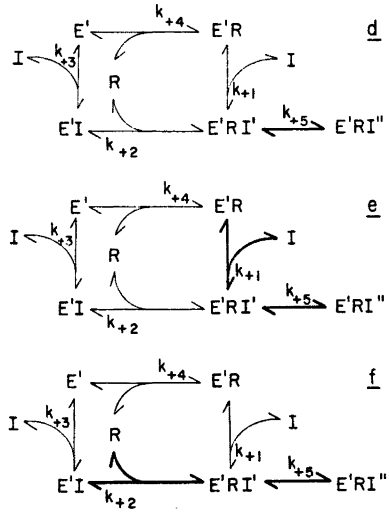


Fig. 13. Refers to models which involve the isomerization of a ternary complex to take place in a side-branch from a four-step bimolecular reaction cycle. Model *d* considers only one step governing the observed process, involving rate constants k_{+5} and k_{-5}. Model *e* considers the kinetic competition of two steps, namely those with the isomerization and with the binding of inhibitor involving rate constant k_{+1}. Model *f* refers to the kinetic competition of the isomerization with the adjoining bimolecular reaction step with rate constant k_{+2}.

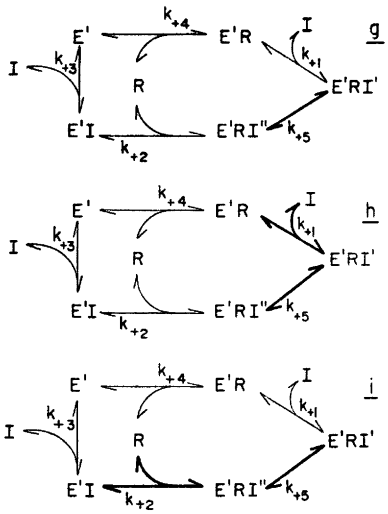


Fig. 14. Definition of reaction cycles of five steps with the interconversion among two ternary complexes being part of the five step cycle. Model *g* considers only the isomerization step being in the time range of the observation. Model *h* considers the adjoining binding step with inhibitor competing kinetically with the isomerization of ternary complexes. Model *i* considers the adjoining binding step with R (NADH) as kinetically competing with the isomerization of the two ternary complexes. The step with k_{+4} is considered as too slow to contribute significantly to the observed relaxation process.

have to be considered. The three types are shown in Fig. 13 as models *d*, *e* and *f*. The differentiation among these models derives also from the fact that only one relaxation process could be measured; no dependencies of other relaxation times are accessible.

The isomer of the ternary complex was shown outside a four-step-reaction cycle. However, two ternary complexes may also be considered as part of a five-step-reaction cycle. Differentiation of three types of coupling of individual steps leads to models *g*, *h* and *i* of Fig. 14.

Most of the nine models show two heavily drawn double-arrows. This notation refers to kinetically competing reaction steps. The system may be reduced to not less than two differential equations of the general type:

$$\frac{\mathrm{d}\Delta\xi_1}{\mathrm{d}t} = a_{11}\Delta\xi_1 + a_{12}\Delta\xi_2 \qquad (9)$$

$$\frac{\mathrm{d}\Delta\xi_2}{\mathrm{d}t} = a_{21}\Delta\xi_1 + a_{22}\Delta\xi_2 \qquad (10)$$

In these equations, the Greek symbol ξ_j refers to the "extent of the j-th reaction" and is associated with only one reaction step (concentrations may be involved in more than one reaction step: all components in model *a* participate in two reaction steps). Specific expressions for the a_{ij} will be given later on, where appropriate. One of the authors used $\mathrm{d}\xi$ recently in another kinetic study.[18] A general theory with programming implenentation on a digital computer is in preparation.[13]

The coefficients in (9) and (10) are utilized to compute the two reciprocal relaxation time constants (see eqns. (3-26) to (3-28) of Ref. 17):

$$1/\tau_{1,2} = \tfrac{1}{2}(a_{11}+a_{22})\ [1 \pm (1-b)^{1/2}] \qquad (11)$$

$$\text{with } b = \frac{4(a_{11}a_{22}-a_{12}a_{21})}{(a_{11}+a_{22})^2} \qquad (12)$$

The positive sign in front of the square root in eqn. (11) applies for subscript 1, the negative sign for subscript 2.

If only one heavy arrow is shown in a scheme, that step is considered alone with all remaining steps coupling in as pre-equilibria. If two heavy arrows are shown, two conditions for the remaining steps are considered. The remaining steps are either too slow to be included, or they are treated as coupled pre-equilibria. If the effects of coupled pre-equilibria are small, they may be undetectable below the error of the date and then appear as "too slow".

Expression for the a_{ij} are generally different for the various models. They will therefore now be discussed individually. Several special relations will be dicussed, as the various models are treated.

Model *a* considers the four-step cycle with those steps in pre-equilibrium, which involve the binding of imidazole, I. The cyclic sequence of the reaction steps makes one of the four steps dependent upon the others. A four-step cycle of the type of Model *a* can thus only have three relaxation time constants. Expressions for reciprocal relaxation times usually consist of only two terms. However, the slowest reciprocal relaxation time in a four-step cycle with one step dependent upon the

other three shows the reciprocal slowest relaxation time with four individual terms. The general expression was already discussed several years ago (see eqn (3-261) of Czerlinski [17]).

The following four equilibrium constants are defined with R = NADH and I = imidazole.

$$K_1 \equiv \frac{k_{-1}}{k_{+1}} = \frac{[E'R][I]}{[E'RI]} \quad (13)$$

$$K_2 \equiv \frac{k_{-2}}{k_{+2}} = \frac{[E'RI]}{[E'I][I]} \quad (14)$$

$$K_3 \equiv \frac{k_{-3}}{k_{+3}} = \frac{[E'I]}{[E'][I]} \quad (15)$$

$$K_4 \equiv \frac{k_{-4}}{k_{+4}} = \frac{[E'][R]}{[E'R]} \quad (16)$$

The Principle of Total Reversibility [19] applies to any reaction cycle, giving here:

$$\prod_{j=1}^{4} K_j = 1 \quad (17)$$

Eqn. (17) causes one of the prior equations to become dependent. Three equations for the analytical (total) concentrations are needed for the computation of equilibrium concentrations for all species:

$$C_E^\circ = [E'] + [E'R] + [E'RI] + [E'I] \quad (18)$$

$$C_E^\circ = [R'] + [E'R] + [E'RI] \quad (19)$$

$$C_I^\circ = [I] + [E'I] + [E'RI] \quad (20)$$

There are now six independent equations for the six unknown equilibrium concentrations. A subroutine for the computation of the six equilibrium concentrations with these six equations was described recently.[20]

The expression for the slowest relaxation time of this system was previously presented (eqn. (3-261) of Czerlinski [17]) and is repeated here with proper substitution:

$$1/\tau = k_{-4}\beta + k_{+2}\beta' + k_{+4}([E'] + [R]\alpha) + k_{-2}([E'I] + [R]\alpha') \quad (21)$$

The coefficients α, α', β, and β' were defined previously (eqns. (3-244), (3-245), (3-252), and (3-253) of Czerlinski [17]), but subsequently rearranged and are presented here, utilizing the symbols of model *a*. The following important relation exists among pairs of coefficients:

$$\alpha' = 1 - \alpha \quad (22)$$

$$\beta' = 1 - \beta \quad (23)$$

Furthermore:

$$\alpha = \left[1 + \frac{[I]([E'R] + [E'] + [I] + K_1)}{K_1([E'] + K_3^{-1}) + K_3^{-1}([E'R] + [I])}\right]^{-1} \quad (24)$$

$$\beta = \left[1 + \frac{[I]([E'R] + [E'] + [I] + K_3^{-1})}{K_1([E'] + [I]) + K_3^{-1}([E'R] + K_1)}\right]^{-1} \quad (25)$$

Eqn. (21) represents the exact solution for a "wide" separation of τ from any faster process.

The above equations contain equilibrium concentrations which are computed from pH dependent equilibrium constants and analytical concentrations numerically in a computer program, as described previously.[20] The pH-dependent dissociation constants are computed from pH-independent constants, as outlined in Appendix I. A numerical analysis revealed that the step with rate constant k_{+4} and k_{-4} contributes only a small part to the relaxation process. Under extreme conditions, the contribution barely approaches 10 %. As this amount is within the experimental error, one may consider the step with rate constant k_{+4} as too slow to be significant for the observed relaxation process. This means that the four-step cycle could be treated as a three-step sequence, involving forward rate constants k_{+1}, k_{+2} and k_{+3}.

Model *b* considers the mechanistic competition of the steps with the forward rate constant k_{+1} and k_{+2}. Eqn. (11) has to be applied together with the definitions

$$a_{11} = k_{+2} + k_{-2}([E'I] + [R]\alpha) \quad (26)$$

$$a_{22} = k_{-1} + k_{+1}([E'R] + [I]) \quad (27)$$

$$\alpha = ([E'] + [I])/([E'] + [I] + k_3^{-1}) \quad (28)$$

The terms a_{12} and a_{21} are omitted as they contain one of the two terms of the expressions for a_{ii}. Quite generally that term of the two-term expression has to be taken, the rate constant of which describes the flux *away* from the adjoining mechanistically competing step.

Model *c* represents a mechanistic competition between the reaction steps with rate constant k_{+2} and k_{+3}. Eqn. (11) thus has to be used again together with the coefficients:

$$a_{11}=k_{+2}\,\beta+k_{-2}([E'I]+[R] \quad (29)$$

$$a_{22}=k_{+3}+k_{-3}([I]+[E']) \quad (30)$$

$$\beta=([E'R]+[I])/([E'R]+[I]+K_1) \quad (31)$$

The expressions for a_{12} and a_{21} determined as described in the prior paragraph for model *b*.

Model *d* has no mechanistically competing steps, all reaction steps are considered in pre-equilibrium. The system of equations may thus be reduced to only one differential equation and the relaxation time constant becomes:

$$\tau^{-1}=k_{-5}+\gamma\beta k_{+5} \quad (32)$$

$$\gamma=([E'I]+[R])/([E'I]+[R]+K_2^{-1}) \quad (33)$$

although the expressions may be written in several ways, the shown one would reveal any concentration dependence most appropriately.

Model *e* shows two mechanistically competing steps, namely those with forward rate constants k_{+1} and k_{+5}. Eqn. (11) thus has to be applied together with

$$a_{11}=k_{-5}+k_{+5}\frac{[E'I]+[R]\gamma}{[E']+[R]\gamma+K_3^{-1}} \quad (34)$$

and the expression given in eqn. (27) plus a_{12} and a_{21} as described above.

Model *f* shows the alternate type of mechanistic competition with forward rate constants k_{+5} and k_{+2} involved. Eqn. (11) again has to be used together with

$$a_{11}=k_{-5}+\beta k_{+5} \quad (35)$$

$$a_{22}=k_{+2}+k_{-2}([E'I]+\alpha[R]) \quad (36)$$

Model *f* completes one class of three models.

The addition of the monomolecular interconversion requires a redefinition of K_1 and K_2 for the kinetic evaluations *only*. The redefinition derives from the new constant

$$K_5\equiv\frac{k_{-5}}{k_{+5}}=\frac{[E'RI]}{[E'RI'']} \quad (37)$$

One may add the definition:

$$[E'RI]=[E'RI']+[E'RI''] \quad (38)$$

A "prime" may be used to define the "kinetic" (or "reduced) equilibrium constants.

$$K_1'=\frac{[E'R][I]}{[E'RI']}=K_1(1+K_5^{-1}) \quad (39)$$

$$K_2'=\frac{[E'RI']}{[E'R][I]}=K_2/(1+K_5^{-1}) \quad (40)$$

Equilibrium constants K_1 and K_2 in eqns (31) and (33) will have to be replaced by K_1' and K_2' from eqns (37) and (38) for models *d*, *e* and *f*.

Model *g* shows only one reaction step to be considered in the time range of observation. The system of equations may therefore be reduced to one differential equation with the reciprocal relaxation time given by

$$\tau^{-1}=\gamma k_{+5}+\beta k_{-5} \quad (41)$$

Model *h* shows two mechanistically competing steps, involving k_{+1} and k_{+5}. Eqn. (11) applies with the expression

$$a_{11}=k_{-5}+k_{+5}\frac{[E'I]+[R]\alpha}{[E'I]+[R]\alpha+K_3^{-1}} \quad (42)$$

and eqn. (27) for a_{22} plus a_{12} and a_{21} as described above.

Model *i* shows two mechanistically competing steps, namely those with forward rate constants k_{+5} and k_{+2}. Thus, eqn. (11) applies with the definitions

$$a_{11}=K_{-5}+\beta+K_{+5} \quad (43)$$

while for eqn. (36) applies: a_{12} and a_{21} are used as described earlier.

Models *g*, *h* and *i* involve K_2' for K_2', as defined in eqn. (37). However, K_2' is differently defined and a new expression has to be used, namely:

$$K_1'=\frac{[E'RI'']}{[E'I][R]}=K_2/(1+K_5) \quad (44)$$

This new kinetic dissociation constant is to be used in the expressions for models *h* and *i*.

All the above equations apply to the limiting case of pre-equilibration of those steps, which are not mechanistically competing. The other limiting case refers to the condition that the not mechanistically competing steps are too slow for having any effect in the time range of observation. This condition is obtained computationally by

$$\alpha=\beta=\gamma=1.0 \quad (45)$$

There is one additional restriction in reference to the use of eqn. (45) for model *e*: eqn. (35)

Table 2. Survey of models employed.

Model	Relevant eqns.	No pre-equilibria assumed Fitted values (in s^{-1}) for a_{11}-term	a_{22}-term	a Fit *	With pre-equilibria assumed Fitted values (in s^{-1}) for a_{11}-term	a_{22}-term	b Fit *
a	(22)	–	–		$k_{+3}=14.1$ β $k_{-2}=K_2k_{+2}$	$k_{+4}=2.63$** $k_{-4}=K_4k_{+4}$	– – –
b	(11, 26, 27)	$k_{+1}=5.03$ α $k_{-1}=K_1k_1$	$k_{+2}=131.6$ $k_{-2}=K_2k_{-2}$ **	–	$k_1=5.5$ α $k_{-1}=k_1K_1$	$k_{+2}=97$ $k_{-2}=K_2k_2$ **	– –
c	(11, 29, 30)	$k_{+2}=263$ β $k_{-2}=K_2k_{+2}$	$k_{+3}=331$ β $k_{-3}=K_3k_{+3}$	– –	$k_{+2}=25$ $k_{-2}=K_2k_{+2}$	$k_{+3}=351$ β $k_{-3}=K_3k_{+3}$	– –
d	(32)	–	–		$k_{+5}=217$ β $k_{-5}=77$	(also in pre-equil.)	– –
e	(11, 27, 34)	$k_{+5}=177$ α $k_{-5}=125$	$k_{-1}=14.4$ $k_{+1}=k_{-1}/K_1'\phi$	+ + +	$k_{+5}=234$ β $k_{-5}=79$	$k_{-1}=14.6$ $k_{+1}=k_{-1}/K_1'\phi$	–
f	(11, 35, 36)	$k_{+5}=179$ α $k_{-5}=161$	$k_{+2}=0.15$ $k_{-2}=K_2'k_{+2}$ α	+ +	$k_{+5}=202$ β $k_{-5}=220$	$k_{+2}=0.19$ β $k_{-2}=K_2'k_{+2}$	–
g	(41)	–	–		$k_{+5}=243$ β $k_{-5}=670$	(also in pre-equil.)	–
h	(11, 27, 42)	$k_{+5}=177$ β $k_{-5}=125$	$k_{-1}=24.4$ $k_{+1}=k_{-1}/K_1'\phi$	+	$k_{+5}=138$ $k_{-5}=191\phi$	$k_{-1}=13.6$ $k_{+1}=k_{-1}/K'\phi$	+
i	(11,30,43)	$k_{+5}=183$ α $k_{-5}=163$	$k_{+2}=0.90$ $k_{-2}=K'_2k_{+2}$ α	+ +	$k_{+5}=207$ β $k_{-5}=206$	$k_{+2}=0.15$ β $k_{-2}=K_2'k_{+2}$	– –

* "Fit" takes into account all accessible parameters, including errors of parameter values, magnitude of "Sum-squares of residuals" and the visual agreement of theoret. curves with the data, covering a total of *18* data values for every entry in the table. The entries in this column refer to the fitted values listed to the left with the following definitions: + + + indicates the "best of all fits." + + indicates a reasonably good fit; any entry of "+ +" aside of "+ + +" does not allow the model with "+ + +" to be labeled "uniquely fitting." + indicates a fit which cannot be completely ruled out as referring to a possible model. A large number of additional data from suitably extended experiments could possibly provide further information on the ultimate validity of this model. – indicates a fit which would rule out the underlying model from further consideration. – – indicates a bad fit. – – – indicates the worst of all fits.

** The value refers to a bimolecular rate constant in the units M^{-1} s^{-1}.

β standard errors are about half the size of the given parameter-values.

α standard errors are of the size of the given values.

ϕ The computer program returned errors for this rate constant, which are substantially larger than those returned for the rate constants k_{+5} and k_{-5}. To express these errors properly, an undimensioned number ϕ has to be used, which is both a factor in a product and a divisor in a quotient *with* a parameter value χ. If $(\phi-1) \ll 1$, it is directly related to the standard error ε by: $\varepsilon=(\phi-1)\chi$, Where ϕ is actually listed, it is of the order of 5 to 10 (applies directly *only* to the equilibrium constant in the expression).

ought to be employed in place of eqn. (34). This replacement is obvious from the inspection of the factor of k_{+5} in eqn. (34), which describes a pre-equilibrium. As all models and underlying mathematics have now been described, the equations may be employed in data fitting programs.

3. Evaluation of goodness of fit. The experimental observable, like the reciprocal relaxation time, may be generally defined as λ_i*. The corresponding computed value, which is dependent upon the independent variables C_E°, C_R°, C_I° and pH, is similarly defined by λ_i°. One may then form the following relation for all points, considered in the fitting process:[21]

$$\sigma_m = \sum_i (\lambda_i^* - \lambda_i^\circ)^2 \qquad (46)$$

The fitting program reduces σ_m to a minimum for all points included in the fitting procedure. Subscript m refers to the model number. Comparisons are made between different sets of data, including also a consideration of standard errors of the parameters. The results of

the least squares fits are shown in Figs. 5 to 10. An overall evaluation is presented in Table 2, indicating how well individual models can be brought into agreement with the experimental results. Model *e* fits best, if no pre-equilibration is assumed. However, the data will not allow to define Model *e* as unique, as the fit for Models *f* and *i* can not be labelled poor enough in reference to Model *e*. It is quite possible that three reaction steps compete mechanistically in the time range of observation. However, there are then too many adjustable kinetic parameters, the precise determination of which is not justified by the available data. It is doubtful that the relaxation time can be measured over a sufficiently broad range of concentration, to permit consideration of three mechanistically competing steps. However, concentration ranges may be found where some concentration dependence upon both imidazole and coenzyme can be detected.

The measured relaxation process is associated with a substantial fluorescence signal change. This signal change is interpreted as largely due to a change in fluorescence yield, as the weakly fluorescing ERI′ converts to the highly fluorescing ERI″ (the "dead-end complex" in models *d*, *e* and *f*). Imidazole thus rearranges in the ternary complex in a way, which diminishes vibrational energy losses from the electronically excited nicotinamide moiety of bound NADH. The equilibrium constant of the interconversion among the two ternary complexes is close to 1.0. If the equilibrium would be shifted to the weakly fluorescing complex ERI′ (by an inhibitor related to imidazadole or by a change in the solution medium), stimulation of the overall kinetics would be further increased to eventually reach a limit of factor 2. On the other hand, if an inhibitor should favor formation of the highly fluorescing compound ERI″, the fast equilibration among the two ternary complexes would remove the dissociating ERI′ from the reaction path and the stimulatory effect upon the overall reaction would diminish and may even disappear completely. Certainly, one should search for effectors, which stimulate the ethanol metabolism further. More data on different inhibitors have to be collected, to more precisely define the structure of the optimal stimulator.

It should be mentioned that the "Fits" in column a of Table 2 are generally significantly better than the "Fits" in column b. This is to be expected as all values for k_{+5} and k_{-5} exceed 100 s^{-1}, while the remaining adjoining rate constants k_{-1} and k_{+2} are below 25 s^{-1} (compare also the corresponding steady state rate constants in Table 1).

The results of Table 2 will now be compared with the earlier ones summarized in Table 1. To make a comparison with data from steady state kinetic experiments feasable, one has to establish quantitative relations between the model for steady state kinetics and the model for chemical relaxation experiments. Such relations were considered previously.[22] Two situations were discussed there: One reactant in a bimolecular reaction is present in two forms, only one of which participates in the reaction; the product in a bimolecular reaction (the binary complex) is present in two forms, only one of which is directly involved in the bimolecular reaction step. The latter case applies here. If a rate constant with a "prime" denotes the one from steady state kinetics, one equates on the basis of model *e*: $k_{+1}' = k_{+1}$ and $k_{-2}' = k_{-2}$. The reverse rate constants are with the definitions of model *e*:

$$k_{-1}' = k_{-1}/(1 + K_5) \quad (47)$$

$$k_{+2}' = k_{+2}/(1 + K_5) \quad (48)$$

These equations imply $k_{+5} \gg k_{-1}, k_{+2}'$ which is fulfilled with sufficient approximation. As k_{+2} is not kinetically competing with k_{+5} in the observable time range for model *e*, eqn. (48) can not be applied. One obtains from Table 2 with eqn. (37): $K_5 = 0.706$ and thus with eqn. (47) $k_{-1}' = 8.47$ s^{-1}. This value is rather close to the corresponding value, listed in Table 1 for pH 9. The agreement between results from steady state kinetics and those from chemical relaxation kinetics is thus quite good.

APPENDIX I

The pH-dependence of the apparent equilibrium constants. Fig. 3 of this paper presents an elaborate reaction scheme, containing both protonated and unprotonated enzyme forms. This scheme may be directly derived from the results of Theorell and McKinley-McKee [1]

and implies the assumption of only one active-site-linked proton. The captions to Fig. 3 define the set of constants, which has to be used to obtain the proper pH-dependent constants. The latter are defined in terms of the constants in Figure 3 by the four equations:

$$K_4 = \frac{([EH]+[E])[R]}{[EHR]+[ER]} = K_{EH,R}\frac{1+\underline{K}_1[H^+]}{1+\underline{K}_5[H^+]} \quad (A\text{-}1)$$

$$K_1 = \frac{([EHR]+[ER])[I]}{[EHRI]+[ERI]} = K_{EHR,I}\frac{1+\underline{K}_5/[H^+]}{1+\underline{K}_7/[H^+]} \quad (A\text{-}2)$$

$$K_3^{-1} = \frac{([EH]+[E])[I]}{[EHI]+[EI]} = K_{EH,I}\frac{1+\underline{K}_1/[H^+]}{1+\underline{K}_6/[H^+]} \quad (A\text{-}3)$$

$$K_2^{-1} = \frac{([EHI]+[EI])[R]}{[EHRI]+[ERI]} = K_{EHI,R}\frac{1+\underline{K}_6/[H^+]}{1+\underline{K}_7/[H^+]} \quad (A\text{-}4)$$

The apparent dissociation constants are also defined in eqns. (13) to (16).

The "best" values for the various dissociation constants are given in the caption to Figure 2. The constants $\underline{K}_2^{-1}$ and $\underline{K}_{EHI,R}$ are *not* independent of the other constants. The apparent constants can be determined with the values in the mentioned table for any pH in the range $6.0 \leq$ pH ≤ 10.0 (values for the upper limit are extrapolated and therefore more speculative than values below this limit). The value for $p\underline{K}_6$ represents a "lower limit"; if $\underline{K}_6$ is decreased, both $\underline{K}_{E,I}$ and $\underline{K}_{EI,R}$ would change to satisfy the condition among dissociation constants in a symmetric reaction cycle. Fig. 2 shows the agreement between experimental points and theory for a given set of pH independent constants.

The pH-dependent equilibrium constants are computed in a separate subroutine, labelled "THEØR", whenever a change in the value of the pH is detected by the main program. The new constants are then used for the computation of equilibrium concentrations of all components and for the computation of the relaxation times, according to the various models.

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The Molecular Structure of Pyruvaldehyde-1-oxime Determined by Gas Phase Electron Diffraction

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The molecular structure of pyruvaldehyde-1-oxime (oximinoacetone) has been determined by gas phase electron diffraction. The molecule exists in the *syn* s-*trans* form and there is no evidence of deviation from heavy atom coplanarity. C_{3v} symmetry was assumed for the methyl group. The root mean-square amplitudes of vibration and the perpendicular amplitude correction coefficients were calculated from an approximate valence force field. The following values were found for the more important bond distances and angles:

$R(C-CH_3)=1.513(6)$ Å, $R(C=O)=1.212(3)$ Å, $R(C-C)=1.479(4)$ Å, $R(C=N)=1.260(4)$ Å, $R(N-O)=1.382(4)$ Å, $\angle CCC=118.4(3)°$, $\angle CCO=118.4(4)°$, $\angle CCN=120.5(5)°$ and $\angle CNO=112.3(4)°$.

Values in parentheses are standard deviations obtained by the least-squares method and they are not corrected for systematic errors. The parameters are consistent with the R_α structure.

Pyruvaldehyde-1-oxime (oximinoacetone) can exist in two isomeric forms because of the C=N bond. Throughout this article the configurational isomers will be denoted by the prefix *syn* and *anti*, in which the hydroxyl group is in *cis* and *trans* position to the methine proton, respectively. In this oxime with conjugated double bonds one can expect restricted rotation around the central C–C bond. This gives in addition conformational isomerism. Under the assumption of a planar NCCO skeleton four different forms can be distinguished (Fig. 1).

The compound has been subjected to configurational [1–6] and conformational studies. [5] The fact that only one isomer is known hindered the assignment of the configuration. Kimura *et. al.*[2] inferred by means of infrared spectroscopy that intramolecular hydrogen bonding, which is only possible in the *anti* isomer could be excluded. Mosher *et al.*[3] came to the opposite conclusion. Attempts to interprete the magnitude of the chemical shifts of the methine and hydroxyl protons also led to contradictory results.[2,3] The *syn* form was mentioned by Somin and Gindin,[4] who used the stereospecific geminal spin-spin coupling of $^{15}N=CH$. Pigenet *et. al.*[5] concluded from dipole moment measurements and the results of their ^1H NMR work, that the *syn* s-*trans* form is the most likely one.

Until now, no structural studies in the gas phase have been published. The aim of this work is to elucidate the molecular structure and to give a contribution to the discussion about the preferred forms of α,β-unsaturated oximes.[5,7]

Besides, the investigation seemed interesting in connection with the studies on the length of the central C–C single bond in conjugated systems like glyoxal,[8] acrolein,[8,9] and butadiene,[8,10] which indicate a dependence on the electronegativities of the substituents to which the carbon atoms are doubly bonded.

EXPERIMENTAL AND CALCULATION PROCEDURE

Pyruvaldehyde-1-oxime was obtained from Schuchardt and was purified by repeated sublimation (m.p. 67–68 °C).

Diffraction photographs were taken on the Oslo apparatus.[11] The nozzle temperatures were about 85 °C and the electron wavelength was 0.064580 Å, corresponding to an acceler-

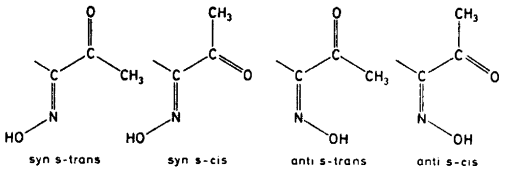


Fig. 1. The four forms of pyruvaldehyde-1-oxime with a planar heavy atom skeleton.

ating voltage of about 36 kV. Five and three plates, for which the camera distances were 480.56 and 200.56 mm, respectively, were selected for the structure investigation.

The plates were photometered and the data processed in the usual way.[12] The 48 cm data covered a range from $s = 1.50$ to $s = 18.75$ Å^{-1} with $\Delta s = 0.125$ Å^{-1} and the 20 cm data from $s = 6.25$ to $s = 40.75$ Å^{-1} with $\Delta s = 0.25$ Å^{-1}. Scattering factors were calculated for 35 kV electrons using the partial wave method.[13] The calculations were based on Hartree-Foch potentials.[14] The backgrounds were drawn on the leveled intensity curves and the molecular intensities obtained were modified by $s/|f_N'||f_C'|$. Background adjustment on the single curves was achieved by comparing the modified experimental intensities with the intensities calculated for the final model. The modified experimental intensities were scaled and averaged according to the methods described by Seip *et al.*,[15] giving a set of data for each of the camera distances. The average correlation coefficient and the standard deviations at each point were calculated. A non-diagonal weight matrix [15] was applied in the final least-squares refinements. The non-diagonal elements in the weight matrix were -0.6 and 0.11 for the 48 cm and -0.6 and 0.115 for the 20 cm data. The constants w_2, s_1, w_3 and s_2 for the diagonal part of the weight matrix [11] were 4.5, 5.5, 0.047, and 10.0 and 1.0, 11.0, 0.014, and 25.0, respectively. The curves were given equal weight with respect to each other.

The molecular intensities, together with the differences between experimental and theoretical intensities are represented in Fig. 2. The

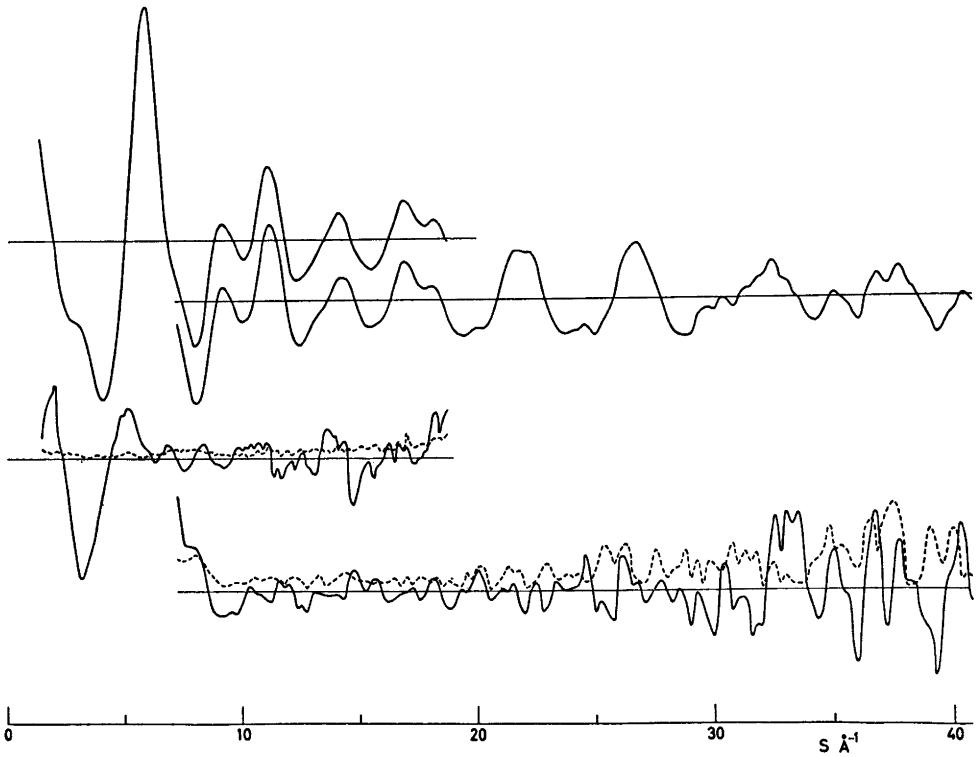


Fig. 2. The two upper curves represent the $s/|f_N'||f_C'|$-modified experimental intensities of pyruvaldehyde-1-oxime for the 48 cm camera distance from $s = 1.50$ to $s = 18.75$ with $\Delta s = 0.125$ Å^{-1} and for the 20 cm distance from $s = 6.25$ to $s = 40.75$ with $\Delta s = 0.25$ Å^{-1}. The two lower curves are the differences between the experimental and calculated intensities for the parameters given in colum C of Tables 1 and 2 (solid curves) and the standard deviations of the averaged intensities (dashed curves). The standard deviations and the differences were multiplied by a factor of 5.

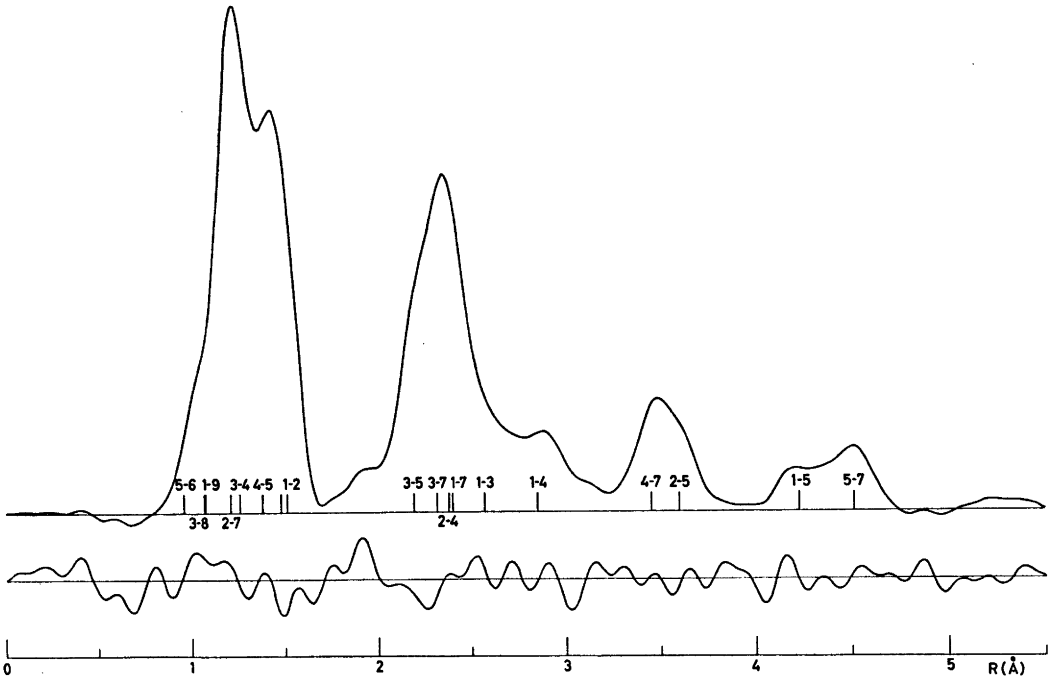


Fig. 3. Experimental radial distribution function for pyruvaldehyde-1-oxime and the differences between experimental and calculated functions. The differences were multiplied by a factor of 5. For the experimental function, calculated intensities were added inside $s = 6$ Å^{-1} and the intensities were multiplied by a damping function of (exp $-0.0015\ s^2$) before the Fourier inversion.

large differences at small s-values for the 48 cm data could not be removed by a smooth background correction. In fact, the weighting scheme does not reflect the experimental uncertainties in this region. It gives a very little weight below $s = 5.0$ Å^{-1}.

Radial distribution functions (Fig. 3) were calculated by Fourier inversion of the experimental and theoretical intensity curves after multiplication with an artificial damping function of exp $(-0.0015s^2)$. For the experimental function, calculated intensities for the final model were added inside $s = 6.0$ Å^{-1}.

STRUCTURE ANALYSIS

With parameters obtained from related compounds radial distribution functions were calculated for the four forms with a planar heavy atom skeleton (Fig. 1). The agreement between experimental and theoretical functions was very poor for the forms with the *anti* configuration. There were significant differences in the region with short non-bonded distances from 2.1 to 2.8 Å. In addition a peak is definitely apparent at ~4.5 Å in the experimental function, which could not be obtained with reasonable values for bond distances and angles.

After adopting the *syn* configuration it was more difficult to decide whether there is a conformational equilibrium and which conformation is preferred. Most of the peaks in the radial distribution function are composite and with the initial parameters no basic differences between the two models could be detected. Preliminary least-squares refinements made the features of the models more distinct. Converged solutions for the s-*cis* model showed substantial disagreement between experimental and theoretical radial distribution functions and unreasonable values for the parameters. The refinements for the s-*trans* model gave directly better results and a satisfying agreement with the experimental data was obtained. Therefore, the *syn* s-*trans* form with a planar heavy atom skeleton and C_{3v} symmetry for the methyl group was chosen as

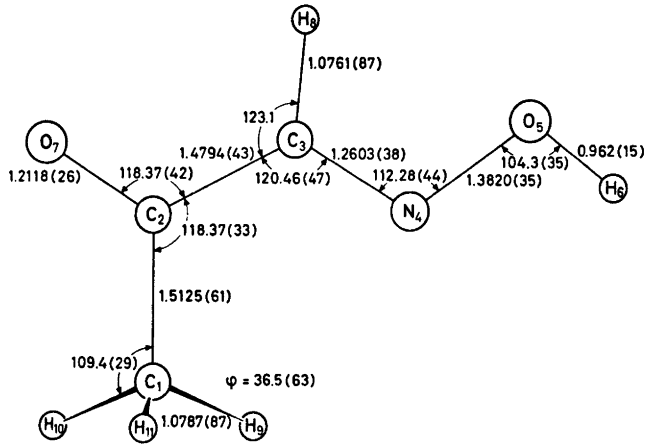


Fig. 4. The numbering of the atoms for pyruvaldehyde-1-oxime and final parameters from column C of Table 1. The bond distances are given in Å and the angles in degrees. The numbers in parenthesis are standard deviations, not corrected for systematic errors. ϕ is the dihedral angle $\phi(H_9C_1C_2C_3)$.

the final model. The atom numbering is shown in Fig. 4.

The mean amplitudes of vibration (u) and the perpendicular amplitude correction coefficients (K) at 85 °C were computed from an approximate valence force field using a modified [16] computer program by Gwinn.[17] The structural parameters used in this calculation are listed in column A of Table 1. Most of the force constants were transferred from related compounds such as acetone [18] and acetaldoxime.[19] Some force constants were adjusted to get a better fit between observed and calculated frequencies. Since only frequencies higher than 835 cm^{-1} have been reported [20] the torsional force constants had to be guessed. (The final force field data are available upon request.)

The results of three least-squares refinements are given in Tables 1 and 2; in Table 2 are also included the computed u- and K-values. For refinement A the given structure parameters correspond to R_a distances, while for refinement B and C R_α distances are given. To the approximation of small vibrations the two types of distances are related by [21]

$$R_e + \langle \Delta z \rangle = R_a + u^2/R - K \equiv R_\alpha$$

For A the model was satisfied by the R_a distances, while in B and C the R_α distances fulfilled the geometry. The differences between the R_a distances $C_1 - C_2$ and $C_2 - C_3$ of the model, Δ_{C-C}, was in A and B set equal to zero and not varied.

Several attempts were made to refine Δ_{C-C} together with as many parameters as possible. By excluding the u-values of the bonded distances and fixing the $C_2C_3H_8$ angle on the value obtained in B, the results of C with a lower $V'PV$ were obtained. As expected some of the parameters were strongly correlated. The following terms of the moment matrix of the parameters were large

$$\varrho[R_{C_1C_2}, \Delta_{C-C}] = -0.96;$$
$$\varrho[R_{C_2N_4}, \angle 3,4,5] = -0.81$$
$$\varrho[R_{N_4O_5}, \angle 3,4,5] = -0.74;$$
$$\varrho[R_{C_2N_4}, u_{N_4O_7}] = 0.76$$

In all the three refinements the u-values of distances involving hydrogen atoms were fixed on the computed values. The u-values which could be refined together with the geometrical parameters are given with standard deviations. To reduce the number of independent parameters, some of the u-values were collected in groups; the u-values belonging to the same group got the same least-squares shifts and the same standard deviations. The experimental u-values for the bonded distances given without standard deviations in Table 2, were refined without varying the geometrical parameters and starting from computed values.

Table 1. Least-squares results for the distances, angles and scale factors of pyruvaldehyde-1-oxime.

Distances in Å and angles in degrees. In A the structure parameters correspond to R_a distances, and for B and C R_α distances are given. For A the geometry was satisfied by the R_a distances, while in B the corresponding R_α distances fulfilled the geometry. The split parameter Δ_{C-C} was defined by $\Delta_{C-C} \equiv R_{a\,1-2} - R_{a\,2-3}$, and R_{1-2} and Δ_{C-C} were kept as independent parameters. In A and B Δ_{C-C} was set equal to zero and not varied. For B $\Delta_{C-C} = 0$ gives a difference of -0.0011 between the R_α distances from computed u- and K-values of Table 2.

For C Δ_{C-C} was varied and the geometry was satisfied by the R_α distances. These R_α distances are given.

The same weighting scheme including offdiagonal elements was applied in all the three refinements (see text). $K(48)$ and $K(20)$ are the scale factors for the data of the 48 and 20 cm camera distances.

The u-values for the same three refinements are given in Table 2.

	A	B	C
R_{1-2}	1.4951(17)	1.4930(17)	1.5125(61)
R_{2-3}		1.4941(17)	1.4794(43)
Δ(C−C)	0.0	0.0	0.0331(98)
R_{2-7}	1.2139(30)	1.2104(33)	1.2118(26)
R_{3-4}	1.2649(47)	1.2593(50)	1.2603(38)
R_{4-5}	1.3839(39)	1.3789(38)	1.3820(35)
R_{5-6}	0.985(15)	0.958(16)	0.962(15)
R_{3-8}	1.0838(87)	1.0725(88)	1.0761(87)
R_{1-9}	1.0938(87)	1.0751(88)	1.0787(87)
Δ(C−H)	0.01		
∠1,2,3	118.76(42)	118.54(39)	118.37(33)
∠3,2,7	117.14(35)	117.63(36)	118.37(42)
∠2,3,4	119.47(54)	120.26(55)	120.46(47)
∠3,4,5	111.91(54)	112.37(54)	112.28(44)
∠4,5,6	102.8(33)	105.1(36)	104.3(35)
∠2,3,8	122.9(53)	123.1(50)	123.1
∠2,1,9	109.9(31)	111.9(34)	109.4(29)
∠ϕ	37.6(63)	35.1(63)	36.5(63)
$K(48)$	99.19(242)	98.16(239)	98.13(202)
$K(20)$	103.16(498)	101.23(492)	102.02(428)
$V'PV \times 10^{-3}$	10.69	10.62	10.50

The difference between the R_a distances between C_1-H methyl and C_3-H_8 was assumed to be 0.01 Å.

In the earlier refinements the methyl group was kept fixed in a position with one of the hydrogen atoms eclipsed with respect to the carbonyl oxygen atom. However, refinement of the torsional angle about the C_1-C_2 bond improved the agreement between experimental and theoretical radial distribution functions and $V'PV$ decreased with 6 %. Although the dihedral angle $\phi(H_9C_1C_2C_3)$ was found to have a large standard deviation it was decided to include this parameter in the final refinements.

In order to test the assumption of a planar heavy atom skeleton, the torsional angle about the C_2-C_3 bond was refined during different stadia in the analysis together with the most important parameters.

The atomic coordinates consistent with the refinement C are given in Table 3.

DISCUSSION

The result is that pyruvaldehyde-1-oxime exists mainly in the *syn* s-*trans* form. The agreement between the calculated and experimental radial distribution functions indicates that the concentration of any additional conformers present must be small. This is in agreement with the suggestions of Somin and Grindin[4] and Pignet *et al.*[5] based on NMR and dipole moment measurements.

The final test on the torsional angle about

Table 2. Least-squares results for the mean amplitudes of vibration for pyruvaldehyde-1-oxime, including u- and K-values calculated from an approximate valence force field at 85 °C.

Values for the root mean-square amplitudes of vibration, $u = \langle \Delta z^2 \rangle^{\frac{1}{2}}$ in Å, and the perpendicular amplitude correction coefficients, $K = (\langle \Delta x^2 \rangle + \langle \Delta y^2 \rangle)/2R$ in Å are given. The experimental u-values were obtained in refinement A, B, and C of Table 1, and the values with standard deviations were varied in these refinements. Some of the u-values were refined in groups. These have the same standard deviations.

Distance	u(Å) A	B	C	Calc.	K(Å)
1 – 2	0.0492(41)	0.0477(41)	0.0462	0.0488	0.0043
2 – 3				0.0485	0.0031
2 – 7	0.0406	0.0409	0.0407	0.0393	0.0065
3 – 4	0.0419	0.0422	0.0420	0.0406	0.0054
4 – 5	0.0493(41)	0.0470(41)	0.0463	0.0489	0.0069
5 – 6				0.0706	0.0339
3 – 8				0.0770	0.0195
1 – 9(10,11)				0.0779	0.0027
1 – 3	0.0744(85)	0.0748(85)	0.0738(67)	0.0665	0.0026
1 – 4	0.1108(109)	0.1123(110)	0.1128(93)	0.1051	0.0022
1 – 5	0.1123(80)	0.1107(79)	0.1099(77)	0.1089	0.0005
1 – 7	0.0584(34)	0.0595(29)	0.0569(39)	0.0619	0.0028
2 – 4	0.0608(34)	0.0619(29)	0.0593(39)	0.0645	0.0022
2 – 5	0.0749(60)	0.0733(59)	0.0762(67)	0.0684	0.0021
3 – 5	0.0496(40)	0.0492(38)	0.0491(37)	0.0605	0.0047
3 – 7	0.0594(34)	0.0605(29)	0.0579(39)	0.0626	0.0039
4 – 7	0.0678(49)	0.0667(48)	0.0697(53)	0.0649	0.0015
5 – 7	0.0893(44)	0.0884(45)	0.0889(43)	0.0799	0.0005

the $C_3 - C_8$ bond gave the value 0.5° with a standard deviation of 7° starting from the results of refinement C, thereby justifying our assumption of a planar heavy atom skeleton.

The value for the difference between $C_1 - C_2$ and $C_2 - C_3$ obtained in refinement C is of the order of three times its standard deviation. However, all the independent parameters could not be varied in this refinement and the difference is probably on the border of being significant.

The torsional angle $H_9C_1C_2C_3$ was included in the least-squares refinements to improve the agreement with the experimental data. This value should be interpreted as the single position best representing the thermal average of all the possible methyl group positions.

Then we regard the results of refinement C of Tables 1 and 2 as our final estimate of the structure. This result is also included in Fig. 4.

Several bond distances and bond angles have values as expected from related compounds. The $C_1 - C_2$ (1.513 Å) and $C_2 - O_7$ (1.212 Å) distances (R_α-values) agree quite well with the corresponding distances in acetone, [22] 1.517 Å and 1.211 Å, respectively (R_g-values). The $N_4 - O_5$ (1.382 Å) and $C_3 - N_4$ (1.260 Å) distances are somewhat shorter than those in formaldoxime,[23] found by microwave spectroscopy (1.408 Å and 1.276 Å, respectively). The length of the central C – C bond is consistent with a conjugated system and almost equals the one in acrolein.[8,9] The angles $C_1C_2C_3$ and $O_7C_2C_3$ are equal (118.4°) which is surprising

Table 3. Atomic coordinates (Å) for pyruvaldehyde-1-oxime corresponding to the R_α-structure of refinement C of Table 1.

Atom	x	y	z
C1	0.0	0.0	0.0
C2	0.0	1.5125	0.0
C3	1.3016	2.2156	0.0
N4	2.3801	1.5635	0.0
O5	3.4901	2.3868	0.0
H6	4.2365	1.7802	0.0
O7	−1.0133	2.1771	0.0
H8	1.3909	3.2880	0.0
H9	0.8193	−0.3589	−0.6030
H10	−0.9318	−0.3589	−0.4080
H11	0.1125	−0.3589	1.0110

because usually only the angle opposite to the double bond is smaller than 120°. The reason for this can be found in the steric interactions between the hydrogen atoms of the methyl group, the carbonyl oxygen atom and the C_3 atom.

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Ring-opening Reactions of Heterocyclic Metalorganics. V. On the Metalation of 2,5-Dimethylthiophenes and Related Compounds with Alkyllithium-*N*,*N*,*N*′,*N*′-tetramethylethylenediamine Complexes

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The reaction of 2,5-dimethylthiophene with alkyllithium-*N*,*N*,*N*′,*N*′-tetramethylethylenediamine complexes has been studied. After reaction with carbon dioxide, varying relative amounts of 3-carboxy-5-methyl-2-thienylacetic acid and 2,5-dimethyl-3-thiophenecarboxylic acid were formed, depending upon the solvent. A maximum yield of 16 % of the former acid was obtained when hexane was used as solvent. The low yield was shown to depend to some extent upon ring-opening of the intermediate lithium derivatives.

3-Carboxy-2-methyl-5-thienylacetic acid was synthesized and shown not to be formed in the above reaction.

In the reaction of 3,4-dichloro-2,5-dimethylthiophene with ethyllithium-TMEDA complex followed by carbon dioxide, 4-chloro-2,5-dimethyl-3-thiophenecarboxylic acid and 4-chloro-5-methyl-2-propyl-3-thiophenecarboxylic acid are formed in the relative proportions 3:4, but in low total yield. Methods for the authentic synthesis of these two acids have been worked out.

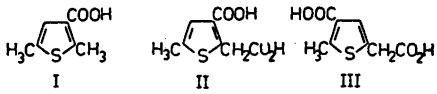
The reaction of tetramethylthiophene with the metalating agent gave a low yield of 3,4,5-trimethyl-2-thienylacetic acid. A large number of highly unsaturated compounds was also formed, giving clear evidence for the ring-opening ability of 2-thenyllithium derivatives. A method for the synthesis of 2,4,5-trimethyl-3-thienylacetic acid has been worked out.

The only preparatively useful case of lateral metalation was that of 1,2,5-trimethylpyrrole, which gave 1,5-dimethyl-2-pyrrylacetic acid in a 38 % yield.

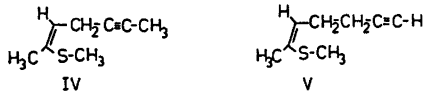
It has been found that complexes between alkyllithium derivatives and certain diamines such as *N*,*N*,*N*′,*N*′,-tetramethylethylenediamine (TMEDA) or 1,4-diazabicyclo[2,2,2]octane (DABCO) metalate faster than alkyllithium derivatives in hexane and ether [1–3] and also metalate substrates such as benzene [1] and toluene [1] (to yield benzyllithium) which are not attacked by ethereal alkyllithium derivatives.

We were therefore interested in studying the reaction of 2,5-dimethylfuran, 2,5-dimethylthiophene, and 1,2,5-trimethylpyrrole and some of their derivatives with these alkyllithium-amine complexes, in order to find out if metalation occurs, and if so, whether it occurs on the methyl group or in the 3-position. These compounds do not react with ethereal alkyllithium derivatives. A recent short communication by Meth-Cohn *et al.*[4] has prompted us to publish our hitherto obtained results as they in part do not agree with those obtained by these authors. We found that treatment of 2,5-dimethylthiophene with ethereal ethyllithium-TMEDA followed by reaction with carbon dioxide gave in low yield a mixture of two acids in the proportions 1:3. By silylation and combined GLC-mass spectrometry the minor component was identified as 2,5-dimethyl-3-thiophenecarboxylic acid (I), which was confirmed by comparison with an authentic sample. The molecular weight of the silyl derivative of the major component (344), which had the longer retention time, indicated it to be a diacid and to have the composition $C_8H_8O_4S$. This compound could be obtained pure by recrystallization from meth-

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I II III



IV V

anol-chloroform-hexane (2:20:10), m.p. 217–219 °C. Its NMR spectrum indicated it to be 3-carboxy-5-methyl-2-thienylacetic acid (II). The main argument was the presence of the characteristic long-range coupling [5] of 1 Hz between the thiophene hydrogen and the methyl group. The compound with m.p. 219 °C has been claimed [4] to be 3-carboxy-2-methyl-5-thienylacetic acid (III).

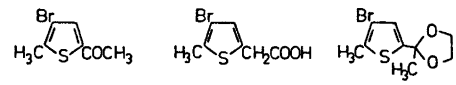
We could prove that this assignment is erroneous by the synthesis of authentic III (*cf.* below) and careful GLC analysis using its di-trimethylsilyl derivative, which clearly showed that this compound was not formed in the metalation of 2,5-dimethylthiophene.

We could increase the yield of acidic products from about 8 % to 16 % by using butyllithium-TMEDA in hexane. The same yields were obtained by Meth-Cohn *et al.*[4] GLC analyses of the silylated mixture showed three components in the proportions 2:8:92. The latter two were the trimethylsilyl derivatives of I and II, respectively, while the minor component with the shortest retention time was not identified. No traces of the trimethylsilyl derivative of III were found. Through recrystallization from the solvent system mentioned above, II was easily obtained pure. However, in spite of many attempts to use different reaction times and temperatures and also other amines such as DABCO, the yield of acidic products could not be increased. One of the reasons for this became apparent, when the reaction mixture from the treatment of 2,5-dimethylthiophene with ethereal ethyllithium-TMEDA was reacted with methyl iodide. GLC analysis of the neutral fraction showed the presence of at least ten components. The major component (80 %) was 2,5-dimethylthiophene. The IR spectrum of the residue after evaporation of this compound showed absorptions at 3280 (≡C–H), 2210 (C≡C) and 2080 cm^{-1} (C≡C). The ^1H NMR spectrum (CCl_4) of the crude product showed adsorptions at δ 3.1 (≡C–*H*, CH_2–C≡C) and 5.25–5.70 (C=CH–) and several lines in the methyl region. No absorptions were found in the aromatic region.

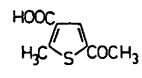
Combined GLC-MS gave the relative percentages, retention times, and molecular weights presented in the experimental part. The component with a molecular weight of 140 could have the structure IV or V.

The three components with the molecular weight 154 are most probably different *C*-methylated derivatives of IV and V, while the three components with the molecular weight 168 are dimethylated derivatives. It is also possible that some fully alkylated thiophenes such as tetramethylthiophene or trimethyl-ethylthiophene are present. No attempts were made to isolate any of the compounds and prove their structure. However, it is evident from these results that metalation is relatively slow and that the lithiated derivatives ring-open in a way similar to that recently demonstrated for 3-thienyllithium derivatives.[6–8] The different products observed can be formed by methylation before or after the ring-opening (after prior lithiation). It is also obvious that the presence of I (obtained upon carbonation) can not be taken as binding evidence that metalation first occurs in the 3-position and then in the 2-methyl group, as it is known that thenylic metal organic reagents react with carbon dioxide under allylic rearrangement.[9] Nor do these experiments give any definitive evidence for ring-opening of 2-thenyllithium derivatives (however, *cf.* below).

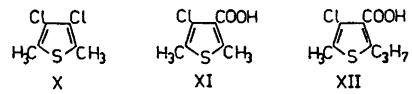
The synthesis of authentic III caused some trouble. Starting from the known 5-acetyl-3-bromo-2-methylthiophene (VI),[10] we could prepare methyl 3-bromo-2-methyl-5-thienylacetate in 65 % yield using acid-catalyzed reaction with



VI VII VIII



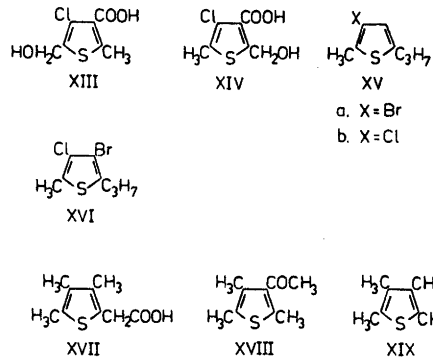
IX



X XI XII

thallium(III) nitrate in methanol according to Taylor, Swann and McKillop.[11] Alkaline hydrolysis then gave 3-bromo-2-methyl-5-thienylacetic acid (VII). However, attempts to prepare III by halogen-metal exchange using two or three equivalents of butyllithium failed. A complex mixture of products was obtained upon reaction with carbon dioxide, in part due to metalation of the CH_2 group in 3-bromo-2-methyl-5-thienylacetic acid, as evidence for the presence of a thienylmalonic acid in the product was found. However, it was possible to prepare III by introducing the carboxyl functions in opposite order. The ketone VI was acetalized to give 2-(3-bromo-2-methyl-5-thienyl)-2-methyl-1,3-dioxolane (VIII), which upon halogen-metal exchange with butyllithium at −70 °C followed by reaction with carbon dioxide and hydrolysis gave 5-acetyl-2-methyl-3-thiophenecarboxylic acid (IX). Treatment of IX with thallium nitrate in methanol followed by alkaline hydrolysis of the methyl ester gave III. In order to obtain more information on the possibility of lateral metalation and the stability of 2-thenyllithium derivatives, we studied the reaction of 2,5-dimethyl-3,4-dichlorothiophene (X) with ethyllithium-TMEDA.

The synthesis of X caused also some trouble. As early as 1937, Steinkopf found that direct chlorination of 2,5-dimethylthiophene with chlorine in carbon tetrachloride led mainly to tar formation.[12] We also had no success with chlorination with *N*-chlorosuccinimide in acetic acid, which also led to tar formation. Starting from tetrachlorothiophene, prepared by the reaction of hexachlorobutadiene with sulfur,[13] we obtained 3,4-dichloro-2,5-dilithiothiophene by treatment with butyllithium according to Gilman *et al.*,[14] which upon reaction with dimethyl sulfate gave X in 72 % yield. However, the reaction of X with ethereal ethyllithium-TMEDA led first to halogen-metal exchange followed by side-chain lithiation of the intermediate 4-chloro-2,5-dimethyl-3-thienyllithium, as reaction with carbon dioxide gave 4-chloro-2,5-dimethyl-3-thiophenecarboxylic acid (XI) and 4-chloro-5-methyl-2-propyl-3-thiophenecarboxylic acid (XII) in low yield, and in the approximate proportions 3:4. These acids were not separated. From the neutral phase, 60 % of starting material was recovered. The proportions and structures of the acids were determined by combined GLC-mass spectrometry of the trimethylsilylated derivatives and from the NMR spectrum of the crude product, which showed the presence of the propyl grouping, and by comparison with authentic samples. The acid XI was prepared by chlorination of 2,5-dimethyl-3-thiophenecarboxylic acid with *N*-chlorosuccinimide in acetic acid. Unexpectedly, this chlorination did not proceed cleanly, but also yielded a chloro-hydroxymethyl-methylthiophenecarboxylic acid, which could be either 4-chloro-5-hydroxymethyl-2-methyl-3-thiophenecarboxylic acid (XIII) or 4-chloro-2-hydroxymethyl-5-methyl-3-thiophenecarboxylic acid

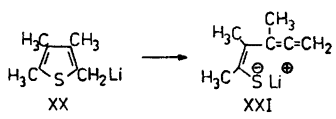


(XIV) according to NMR spectra and analyses. This compound was obtained in good yield when two equivalents of *N*-chlorosuccinimide were used. Obviously chlorination at one of the two possible thenylic positions takes place, after which the thenylchloride derivative is hydrolyzed during work up. Reaction of 3-bromo-2-methyl-5-propylthiophene [15] (XVa) with butyllithium at −70 °C followed by hexachloroethane [16] gave the corresponding chloro derivative (XVb), which was brominated with bromine in acetic acid to yield 3-bromo-4-chloro-5-methyl-2-propylthiophene (XVI). Halogen-metal exchange of the crude brominated product with butyllithium followed by reaction with carbon dioxide gave authentic XII.

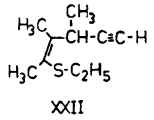
The trimethylsilyl derivatives of authentic XI and XII had the same retention times (GLC) and mass spectra as the trimethylsilyl derivatives of the acids formed in the metalation experiments. The acid XII was most probably formed by coupling of the thenyllithium derivative with ethyl chloride formed in the halogen-

metal exchange or ethylbromide from the ethyllithium preparation.

We also prepared tetramethylthiophene by dichloromethylation of 2,5-dimethylthiophene to give 3,4-dichloromethyl-2,5-dimethylthiophene followed by reduction with lithium aluminium hydride.[17] Reaction of tetramethylthiophene with ethereal ethyllithium-TMEDA gave after reaction with carbon dioxide only 5 % of 3,4,5-trimethyl-2-thienylacetic acid (XVII). The structure followed from the fact that this acid was not identical with 2,4,5-trimethyl-3-thienylacetic acid (XIX), prepared through acetylation of 2,3,5-trimethylthiophene followed by transformation of the 4-acetyl derivative (XVIII) by the thallium nitrate method.[11] If the lithiated mixture was reacted with excess ethyl bromide, a neutral phase was obtained which GLC analysis showed to consist of two major components besides at least 15 minor ones. One of the major components (60 %) was starting material, while the second one, according to combined GLC-mass spectroscopy, had a molecular weight of 168. Due to its instability it was not possible to obtain this compound pure by distillation *in vacuo* or chromatographic methods. The IR spectrum of the crude product, however, had absorptions at 3300, 2080, and 2100 cm^{-1}, indicating the presence of acetylenic compound(s), including terminal triple bonds. It seems therefore quite clear that the 2-thenyl-



XX XXI



XXII

lithium derivative (XX) ring-opens primarily to the allene (XXI), which in the presence of ethyllithium can be isomerized to an acetylene. Under these conditions, alkylation by ethyl bromide can occur on sulfur to give the compound with the molecular weight of 168, which could have a structure such as (XXII). A similar ring-opening of 3-methyl-2-benzo[b]-thenylmagnesium chloride was reported by Gaertner,[18] and of some 2-lithiomethylthiazoles by Meyers *et al.*[19]

We also attempted the metalation of 2,5-dimethylfuran and 1,2,5-trimethylpyrrole with butyllithium-TMEDA. With the former compound no defined acidic products were obtained, while the metalation of 1,2,5-trimethylpyrrole with butyllithium-TMEDA in hexane gave a 32 % yield of 1,5-dimethyl-2-pyrrylacetic acid. The acid was identified by its NMR spectrum and had the same melting point as a sample prepared in another way.[20] From the neutral phase most of the unreacted starting material could be recovered. In this case apparently no complications due to ring-opening occurred. However, prolonged reaction time did not increase the yield.

EXPERIMENTAL

3-Carboxy-5-methyl-2-thienylacetic acid (II). (a) To 165 ml (0.10 mol) of 0.63 M ethereal ethyllithium, 11.6 g (0.100 mol) of TMEDA was added and 10 min later 11.2 g (0.100 mol) of 2,5-dimethylthiophene all at once (yellow precipitate). After 2 h reflux the reaction mixture was poured onto solid carbon dioxide in ether. The mixture was acidified with 5 N HCl and the aqueous phase was extracted with ether. The combined ether phases were then extracted with 2 N NaOH. Acidification of the basic water solution gave 1.55 g of an acid (~8 %), a sample of which was dissolved in ether and silylated with BSA. Combined GLC-MS analysis showed the presence of two components with the relative percentages 25:75, and with *m/e* 228 and 344, respectively. The most abundant component was the least volatile one. By mixing the above sample with authentic silylated 2,5-dimethyl-3-thiophenecarboxylic acid (I) it was shown after GLC analysis (SE 30, 2.0 m, 150 – 200 °C, 4 °C/min) that the most volatile component was the trimethylsilylester of I. The most abundant component had a shorter retention time than that of the authentic silylated 3-carboxyl-2-methyl-5-thienylacetic acid (III), as shown by a "mixing" analysis (as above). (Retention times 9.5 and 10.6 min for the silylated II and III, respectively, on the above column).

The pure title acid (II), m.p. 217 – 219 °C was obtained through recrystallization from a mixture of methanol, chloroform, and hexane (2:20:10). IR: C=O 1700 and 1640 cm^{-1}. NMR (DMSO-d_6): δ 7.04 (4-H, q, 1 H); 2.38 (5-CH_3, d, 3 H); 4.06 (2-CH_2, s, 2H). $J_{4-H,5-CH_3}$ 1.1 Hz. [Found: C 47.8; H 4.18; S 15.9. Calc. for $C_8H_8O_4S$. (M.wt. 200.21): C 48.00; H 4.03; S 16.01].

(b) To 143 ml (0.200 mol) of 1.4 M butyllithium in hexane, 23.2 g (0.200 mol) of TMEDA was added. After 10 min, 11.2 g (0.100 mol) of 2,5-dimethylthiophene was added rapidly. A yellow precipitate was formed and the reaction mixture was allowed to stand at room temperature (~20 °C) for 2 h, whereupon it was poured onto solid carbon dioxide in ether. The carbonated mixture was extracted with 1 M NaOH (3 × 100 ml) and the collected water portions were extracted with ether. After acidification of the water phase it was again extracted with ether (3 × 50 ml). A sample of this ether phase was silylated with BSA and analysed by GLC (SE 30, 2,0 m, 150–200 °C, 4 °C/min). Three components were found in the proportions (retention times in min) 2(2.6): 8(2.9): 90(9.5). The latter two were identical with those in experiment (a). The first one was not identified. No traces of compounds with longer retention times were found.

After evaporation of the ether, 3.2 g of the crude acid mixture was collected; yield 16 %. (17 % was reported in Ref. 4, but no details were given).

Ring-opening experiment on 2,5-dimethylthiophene with ethyllithium-TMEDA. To 130 ml (0.100 mol) of 0.77 M ethereal ethyllithium, 11.6 g (0.100 mol) of TMEDA was added. To this mixture, 11.2 g (0.100 mol) of 2,5-dimethylthiophene was added in a rapid stream. The reaction mixture was refluxed for 2 h (a yellow precipitate was formed), whereupon 42.6 g (0.300 mol) of methyl iodide was added. Reflux was maintained for another hour, after which the mixture was hydrolyzed with water. The ether phase was washed with 2 N HCl and water to neutral reaction. GLC of the dried ether phase showed 10 components, of which the starting material amounted to about 80% (OV 17 3 %, 1.9 m, 80–190 °C, 4 °C/min). Evaporation left 3.0 g of crude product not containing 2,5-dimethylthiophene. IR: 3280, (≡C–H), 2210, (C≡C), and 2080 cm^{-1} (C≡C). NMR (CCl_4) of the crude product: δ 3.1 (≡C–H and/or $-CH_2-C\equiv C$); 5.25–5.70 (m, C=CH–) and several lines in the methyl region. There were no absorptions in the aromatic region. A combined GLC-MS analysis of the crude mixture gave the following data: (BDS 10 %, 2.0 m, 125 °C).

Peak No.	1	2	3	4	5	6	7	8	9
Rel. %	22	27	18	10	7	2	5	2	6
Ret. time (min)	7.4	8.2	9.0	9.5	11.5	12	13	14	15
m/e	140	154	154	154	168		168		168

Peaks No. 6 and 8 were too small to give reliable mass numbers. For possible structures, see discussion. No attempts were made to isolate any of the compounds.

5-Acetyl-3-bromo-2-methylthiophene (VI). From 35.0 g (0.250 mol) of 2-acetyl-5-methylthiophene, 85 g (0.64 mol) of $AlCl_3$ and 43 g (0.27 mol) of bromine, 38.2 g (70 %) of the title compound was obtained. $B.p._{11}$ 142–148 °C [lit.[10] 71 % $b.p._{10}$ 140–144 °C].

5-Acetyl-2-methyl-3-thiophenecarboxylic acid (IX). 5-Acetyl-3-bromo-2-methylthiophene (VI), 8.67 g (0.0396 mol), was acetalized with 20 ml of ethylene glycol and 0.25 g of *p*-toluenesulfonic acid in refluxing toluene (150 ml) with water separation (18 h). The reaction mixture was poured onto a saturated $NaHCO_3$ solution and washed with water. After drying and evaporation, the residue was distilled, giving the acetal (VII) contaminated with ~10 % of the starting material according to GLC analysis; $b.p._1$ 95–105 °C, 5.86 g (56 %). The acetal, 5.26 g (0.0200 mol), was dissolved in 50 ml of ether and cooled to −70 °C, after which 16 ml (0.020 mol) of 1.25 M butyllithium in hexane was added dropwise. When the addition was complete, the reaction temperature was allowed to rise to −30 °C (the warming-up took ~1/2 h), and the reaction mixture was poured onto solid carbon dioxide in ether. The organic layer was extracted with 3 × 50 ml of 2 M NaOH solution. The water phase was acidified with conc. HCl and heated to boiling. After filtration, the solution was left overnight at room temperature whereupon 1.3 g of white crystals was filtered off and the filtrate was evaporated to half of its volume, which gave another 1.5 g of the acid; yield 76 %. Recrystallization from water gave the pure acid, m.p. 167–168 °C. NMR (DMSO-d_6): δ 8.03 (4-H, s, 1 H); 2.53 (2-CH_3 or $COCH_3$, s, 3 H), 2.72 ($COCH_3$ or 2-CH_3, s, 3 H). [Found: C 52.7; H 4.35; S 17.5. Calc. for $C_8H_8O_3S$ (M.wt. 184.21) C 52.16; H 4.38; S 17.41].

3-Carboxy-2-methyl-5-thienylacetic acid (III). A mixture of 500 mg (2.71 mmol) of 5-acetyl-2-methyl-3-thiophenecarboxylic acid (IX), 1.25 g (2.82 mmol) of thallium trinitrate trihydrate and 1.0 ml of perchloric acid (70 %) in 15 ml of methanol was stirred for 18 h at room temperature. The white precipitate (thallium mononitrate) was filtered off and the filtrate diluted with 100 ml of water. The water phase was extracted with 3 × 30 ml of chloroform and the collected organic portions were extracted with 3 × 30 ml 2 M NaOH. The basic water solution was left at room temperature for 1 h and was then acidified. Thus, 280 mg (52 %) of crude III was collected, m.p. ~222 °C. Recrystallization from methanol:water (8:2) gave the pure acid, m.p. 224–226 °C. A silylated sample of the crude product showed only one peak with a retention time of 10.6 min. on the gas chromatogram (SE 30, 2.0 m, 150–200 °C, 4 °C/min). Combined GLC-MS gave the mass number 344 which corresponds to the bis-trimethylsilylester of the title compound (calc. for $C_{14}H_{24}O_4SSi_2$ 344). NMR (DMSO-d_6): δ 7.17 (4-H, broad s, 1 H); 2.67 (2-CH_3, s, 3 H); 3.78 (5-CH_2, broad s, 2 H). IR: C=O 1700 and 1640 cm^{-1}. The IR spectrum was only slightly different from that of 3-carboxy-5-methyl-2-

thienylacetic acid (II). [Found: C 48.4; H 4.12; S 16.0. Calc. for $C_8H_8O_4S$ (M.wt. 200.21): C 48.00; H 4.03; S 16.01].

3-Bromo-2-methyl-5-thienylacetic acid (VII). A mixture of 12.3 g (0.0562 mol) of 5-acetyl-3-bromo-2-methylthiophene (VI), 27.1 g (0.0610 mol) of thallium trinitrate trihydrate and 28 ml of perchloric acid (70 %) in 140 ml of methanol was stirred at room temperature for 12 h, whereupon the precipitate was filtered off and the filtrate diluted with 300 ml of water. The water phase was extracted with 3 × 100 ml of chloroform and the collected organic portions were washed with water and dried. Evaporation gave 12.1 g of crude methyl ester which was dissolved in benzene and chromatographed on an alumina column. Evaporation of the benzene yielded 9.1 g of a yellow liquid (65 %), which was stirred with 150 ml of 2 N NaOH solution at room temperature for 2 h. The reaction mixture was filtered and acidified with ice-cooled conc. HCl. The crystals were filtered off and washed with 10 ml of ice-water. Thus, 5.2 g acid was obtained. Another 0.8 g was obtained from the acidic filtrate through extraction with ether and evaporation: yield 6.0 g, m.p. ~95 °C (45 % based on VI).

The pure acid was obtained through recrystallization from hexane, m.p. 98–100 °C. NMR (DMSO-d_6): δ 6.78 (4-H, broad s, 1 H), 3.73 (5-CH_2, broad s, 2 H), 2.30 (2-CH_3, (s, 3 H). [Found: C 35.5; H 3.02; S 13.8. Calc. for $C_7H_7BrO_2S$ (M.wt. 235.10): C 35.76; H 3.00; S 13.64].

An attempt to prepare 3-carboxy-2-methyl-5-thienylacetic acid (III) from 3-bromo-2-methyl-5-thienylacetic acid (VII). In 100 ml of THF, 2.1 g (8.9 mmol) of 3-bromo-2-methyl-5-thienylacetic acid (VII) was dissolved. The solution was cooled to −70 °C and 13.6 ml (19.0 mmol) of 1.40 M butyllithium in hexane was added. The reaction mixture was left for 1 h at −70 °C with stirring, and was then poured onto solid carbon dioxide in ether. The ethereal phase was extracted several times with 2 N NaOH. The combined aqueous phases were acidified with conc. HCl (ice-cooling) and the acidic crude product was taken up in ether, which was evaporated, leaving 2.0 g of a brown oil. Upon treatment with CCl_4 the oil crystallized. The crystals melted between 100 and 120 °C with gas evolution. A sample of the crystals was dissolved in DMSO-d_6 and a NMR spectrum recorded, which showed the presence of aromatic protons around δ 6.67, methine protons at δ 4.83, methylene protons at δ 3.68, and methyl protons at δ 2.37 and 2.25. When the sample was heated slightly (~45 °C), the signal at δ 4.38 had disappeared and that at δ 3.68 had increased in height. The methyl signal at δ 2.25 had also disappeared. These results indicated that a thienyl malonic acid was present, among other compounds.

When the experiment was repeated with 3 equiv. of butyllithium, it did not give the desired thienylacetic acid. Instead a great deal of decomposition took place, which did not lead to significant amounts of acidic products.

Tetrachlorothiophene.[13] Hexachlorobutadiene (587 g, 2.25 mol) was heated with 192 g (6.0 mol) of sulfur. The sulfur dichloride was distilled off at 135–136 °C/760 mmHg, and the tetrachlorothiophene at 90–95 °C/12 mmHg. The crude product was recrystallized from methanol in the cold (−25 °C), giving 180 g of the title compound (36 %), m.p. 29–30 °C. [Lit.[13] m.p. 29.5–29.7 °C, 90 %].

3,4-Dichloro-2,5-dimethylthiophene (X). Tetrachlorothiophene (22.2 g, 0.100 mol) was dissolved in 200 ml of ether. To this solution 186 ml (0.210 mol) of 1.13 M butyllithium in hexane was added at 5–10 °C. After completion of the addition, the reaction mixture was allowed to reach room temperature. A white precipitate was formed. The suspension was stirred for another 1/2 h and 26.5 g (0.210 mol) of dimethylsulfate in 100 ml of ether was added dropwise (ice-cooling). After this addition (2.5 h) the reaction mixture was hydrolyzed with 20 ml of conc. ammonia solution and worked up by washing the ethereal layer with water to neutral reaction. After drying and evaporation of the solvent, the residue was distilled giving 13.1 g (72 %) of the title compound, b.p. 91–93 °C. NMR (CCl_4): δ 2.33 (CH_3, s). [Found: C 40.0; H 3.43. Calc. for $C_6H_6Cl_2S$ (M.wt. 181.08): C 39.80; H 3.34].

Metalation of 3,4-dichloro-2,5-dimethylthiophene with TMEDA-ethyllithium (1:1). The 1:1 complex between TMEDA and ethyllithium was prepared from 3.24g (0.028 mol) of TMEDA and 56 ml (0.028 mol) of 0.50 M ethereal ethyllithium. To this mixture was added 5.00 g (0.0276 mol) of 3,4-dichloro-2,5-dimethylthiophene together with 50 ml of ether. The solution became yellow and turned brown at reflux for 1/2 h. The reaction mixture was then poured onto solid carbon dioxide in ether. The ethereal phase was extracted with 2 N NaOH solution and the basic portions were acidified. Thus, 1.0 g of an acid mixture was isolated (m.p. 117–150 °C). A silylated sample (BSA) was analyzed by combined GLC-MS (OV 17, 2.0 m, 150–240 °C, 15 °C/min). Two components appeared in the proportions 3:4 (retention times 8 and 9 min, respectively). The mass numbers were 262 and 290, respectively. The ion distribution of the molecular fragments indicated that only *one* chlorine atom was present in each component. It was shown that the components were the trimethylsilyl esters of 4-chloro-2,5-dimethyl-3-thiophenecarboxylic acid (XI) and 4-chloro-5-methyl-2-propyl-3-thiophenecarboxylic acid (XII) after comparison with the retention times of the authentic silylated acids (BSA). NMR ($CDCl_3$) of the acid mixture ($-\overset{a}{C}H_2-\overset{b}{C}H_2\overset{c}{C}H_3$): δ 3.23 (a, t, 2 H); 1.3–1.9 (b, 5 lines, 2 H); 1.02 (c, t, 3 H) and two singlets at δ 2.70 and 2.38 which were assigned to methyl protons. From

the neutral ether phase 3.25 g of the starting material (65 %) was recovered.

4-Chloro-2,5-dimethyl-3-thiophenecarboxylic acid (XI). 2,5-Dimethyl-3-thiophenecarboxylic acid (1.0 g, 6.4 mmol) was dissolved in 25 ml of acetic acid, and 0.86 g (6.4 mmol) of *N*-chlorosuccinimide was added. The mixture was stirred overnight and then refluxed for 1 h. Most of the acetic acid was then evaporated and 20 ml of water was added to the residue. This mixture was extracted with ether and the collected ether portions were extracted with 3 × 10 ml of 2 N NaOH. Acidification of the basic water phase gave 0.65 g of an acid mixture. A silylated (BSA) sample of this mixture was analyzed by GLC (OV 17, 100 – 220 °C, 12 °C/min) and found to contain 12 % of the starting material, 71 % of the title compound and 17 % of 4-chloro-5-hydroxymethyl-2-methyl-3-thiophenecarboxylic acid or 4-chloro-2-hydroxymethyl-5-methyl-3-thiophenecarboxylic acid. The title acid was obtained pure after three recrystallizations from methanol: water, m.p. 192 – 194 °C. NMR (DMSO-d_6): δ 2.53 (CH_3, s, 3 H) and 2.30 (CH_3, s, 3 H). [Found: C 44.1; H 3.76. Calc. for $C_7H_7ClO_2S$ (M.wt. 190.65): C 44.10; H 3.70].

4-Chloro-2-hydroxymethyl-5-methyl-3-thiophenecarboxylic acid (XIV) or 4-chloro-5-hydroxymethyl-2-methyl-3-thiophenecarboxylic acid (XIII). To 1.56 g (0.0100 mol) of 2,5-dimethyl-3-thiophenecarboxylic acid in 50 ml of acetic acid, 3.18 g (0.0238 mol) of *N*-chlorosuccinimide was added. The reaction mixture was stirred at room temperature for 4 h and refluxed for 1 h. The acetic acid was evaporated and the residue treated as in the experiment above. Thus, 1.5 g (73 %) of the acid was obtained (m.p. ~170 °C). Recrystallization from methanol:water gave the pure acid (m.p. 177 – 179 °C). NMR (DMSO-d_6): δ 2.35 (CH_3, s, 3 H), 4.85 ($-CH_2-$, s, 2 H). Found *m/e* 206 (1 Cl), calc. for $C_7H_7{}^{35}ClO_3S$ = 206. [Found: C 40.80; H 3.50; S 15.38. Calc. for $C_7H_7ClO_3S$ (M.wt. 206.64): C 40.69; H 3.41; S 15.52].

3-Chloro-2-methyl-5-propylthiophene (XVb). 3-Bromo-2-methyl-5-propylthiophene (XVa) (9.1 g, 0.042 mol) was dissolved in 50 ml of dry ether and the solution was cooled to −70 °C, whereupon 75 ml (0.045 mol) of 0.6 M ethereal ethyllithium was added dropwise. After 15 min, 12 g (0.051 mol) of hexachloroethane in 150 ml of ether was added in 10 min. The reaction mixture was kept at −70 °C for another 4 h. The cooling bath was removed, the mixture was allowed to reach room temperature, and was then poured into water. The organic layer was separated and the water phase extracted with ether. After drying and evaporation of the solvent, the residue was distilled. Thus, 1.5 g (21 %) of the pure title compound was obtained, b.p.$_{13}$ 97 – 99 °C. Also a forerun, 4 g, b.p.$_{13}$ 93 – 97 °C, containing some hexachloroethane, was collected. NMR (CCl_4): δ 6.45 (4-H, broad s, 1 H), 2.32 (2-CH_3, s, 3 H); ($\underset{a}{CH_2}\underset{b}{CH_2}\underset{c}{CH_3}$) δ 2.65 (a, t, 2 H); ~1.63 (b, 6 lines, 2 H), 0.97 (c, t, 3 H). J_{ab} 7.4 Hz; J_{bc} 7.0 Hz. [Found: C 53.9; H. 6.24. Calc. for $C_8H_{11}ClS$ (M.wt. 174.69): C 55.00; H 6.35].

4-Chloro-5-methyl-2-propyl-3-thiophenecarboxylic acid (XII). To 1.0 g (5.7 mmol) of 3-chloro-2-methyl-5-propylthiophene in 25 ml of acetic acid, 1.0 g (6.3 mmol) of bromine dissolved in 10 ml of acetic acid was added. After 4 h at room temperature, the reaction mixture was heated to reflux, cooled and poured into water. After extraction with ether, drying and evaporation the residue was distilled, giving 0.8 g (55 %) of almost pure 3-bromo-4-chloro-5-methyl-2-propylthiophene (XVI), b.p.$_{0.2}$ 77 – 80 °C. NMR (CCl_4): δ 2.38 (5-CH_3, s, 3 H); ($\underset{a}{CH_2}\underset{b}{CH_2}\underset{c}{CH_3}$) δ 2.70 (a, t, 2 H), 1.63 (b, 6 lines, 2 H), 0.98 (c, t, 3 H). This compound (0.61 g, 2.4 mmol) was dissolved in 10 ml of dry ether in a septum bottle and cooled to −70 °C, whereupon 1.8 ml (2.7 mmol) of 1.48 M butyllithium in hexane was injected with a syringe. After 15 min, the reaction mixture was poured onto solid carbon dioxide in ether. The usual work up yielded 0.35 g (67 %) of the title compound, m.p. 130 – 133 °C. Recrystallization from methanol:water gave the pure acid, m.p. 135 – 136 °C. NMR (DMSO-d_6): δ 2.32 (5-CH_3, s, 3 H); ($\underset{a}{CH_2}\underset{b}{CH_2}\underset{c}{CH_3}$) δ 2.93 (a, t, 2 H), 1.58 (b, 5 lines, 2 H); 0.90 (c, t, 3 H). $J_{ab} = J_{bc} = 7.5$ Hz. [Found: C 49.39; H 5.04; S 14.56. Calc. for $C_9H_{11}ClO_2S$ (M.wt. 218.70): C 49.42; H 5.07; S 14.66].

Tetramethylthiophene.[17] From 30.0 g (0.268 mol) of 2,5-dimethylthiophene, 72.4 g (0.804 mol) of 1,3,5-trioxane and HCl gas, 40.2 g (72 %) of 3,4-bis(chloromethyl)-2,5-dimethylthiophene was obtained, m.p. 68 – 69 °C (lit.[17] m.p. 73 °C). The bis(chloromethyl) compound, 40.0 g (0.191 mol), was subsequently reduced with 16.8 g (0.442 mol) of lithium aluminium hydride in ether to give the title compound; yield 17.3 g (65 %), b.p.$_{12}$ 76 – 77 °C (lit.[17] 67 %, b.p.$_{12}$ 74 – 79 °C).

3,4,5-Trimethyl-2-thienylacetic acid (XVII). A solution of 5.8 g (0.050 mol) of TMEDA in 25 ml of ether was added to 62.5 ml (0.050 mol) of 0.80 M ethyllithium in ether. After 10 min, 7.0 g (0.050 mol) of tetramethylthiophene [17] in 50 ml of ether was added all at once. Additional ether was added to a total volume of 150 ml, and the mixture was refluxed for 2 h (ether was added to maintain 150 ml). At this point 50 ml of the mixture was withdrawn with a pipette and poured onto solid carbon dioxide in ether. To the rest 11 g (0.10 mol) of ethyl bromide was added and reflux was continued for another hour. The ether phase was washed with water, dil. HCl and water to neutral reaction and dried. Evaporation left 4.1 g of an oil. IR: 3300, (≡C–H) 2100 (C≡C) and 2080 cm^{-1} C≡C. GLC analysis showed about 15 components, of

which the most abundant was the starting material (60 %). A component with $m/e = 168$ amounted to 20 % and could have the structure XXII suggested in the discussion. NMR (CCl_4) showed mainly the starting material; δ 2.20 and 1.90. Other signals were present in the aliphatic region, but could not be assigned to any specific structure. Attempted distillation of the crude product at reduced pressure was unsuccessful due to tar formation, and preparative TLC (silica gel, hexane:ether 9:1) gave no significant separation.

The carbonated ethereal portion was extracted with dil. NaOH solution and the collected basic water portions were acidified, giving 0.15 g of the title compound; yield 5 %, m.p.~ 110 °C. Recrystallization from hexane gave the pure acid, m.p. 116–118 °C. IR: C=O 1710 cm^{-1}. NMR ($CDCl_3$): δ 3.67 (2-CH_2-, s, 2 H); 2.30 (CH_3, s, 3 H), 2.00 (CH_3, m, 6 H), 11.0 (CO_2H, s, 1 H). [Found: C 58.6; H 6.42; S 17.4. Calc. for $C_9H_{12}O_2S$ (M.wt. 184.26): C 58.67; H 6.56; S 17.40].

3-Acetyl-2,4,5-trimethylthiophene (XVIII). To a mixture of 1.00 g (7.94 mmol) of 2,3,5-trimethylthiophene and 1.30 g (12.7 mmol) of acetic anhydride, 1 drop of 70 % perchloric acid was added. Heat was evolved and the reaction mixture was stirred at room temperature overnight. It was then poured into water, extracted with ether and the ether phases washed with water to neutral reaction. After drying and evaporation of the solvent, 1.1 g of crude product remained, which was distilled, $b.p._{0.5}$ 74–75 °C, 0.75 g (56 %). NMR (CCl_4): δ 2.45, 2.22, and 2.10 (aromatic CH_3, 4 broad s with fine structure); 2.32 ($COCH_3$, s). [Found: C 64.0; H 7.19; S 19.0. Calc. for $C_9H_{12}OS$ (M.wt. 168.26): C 64.25; H 7.19; S 19.06].

2,4,5-Trimethyl-3-thienylacetic acid (XIX). A mixture of 0.50 g (3.0 mmol) of 3-acetyl-2,4,5-trimethylthiophene, 1.6 g (3.6 mmol) of thallium trinitrate trihydrate and 1.0 ml of 70 % perchloric acid in 5 ml of methanol was stirred at room temperature for 18 h. The precipitated thallium mononitrate was filtered off, and 50 ml of water was added to the filtrate. After extraction with $CHCl_3$, drying and evaporation of the solvent, 0.55 g of a brown oil remained. This oil (the methyl ester of the title compound according to NMR) was refluxed in a mixture of 2 g of potassium hydroxide, 10 ml of water and 10 ml of ethanol for 4 h. The ethanol was evaporated and the residual solution acidified with dilute HCl. Thus, 0.35 g (63 %) of the title compound was isolated, m.p. 100–102 °C. Recrystallization from hexane in the cold (–25 °C) gave the pure acid, m.p. 104–106 °C. NMR (DMSO-d_6): δ 1.95 (CH_3, s, 3 H), 2.23 (CH_3, broad s, 6 H); 3.37 (3-CH_2-, s, 2 H). IR: C=O 1705 cm^{-1}. [Found: C 58.4; H 6.63; S 17.1. Calc. for $C_9H_{12}O_2S$ (M.wt. 184.26): C 58.67; H 6.56; S 17.40].

1,5-Dimethyl-2-pyrrylacetic acid. A solution of 5.8 g (0.050 mol) of TMEDA in 40 ml of hexane was added to 20 ml (0.050 mol) of 2.5 M butyllithium in hexane. During 40 min, 5.45 g (0.0500 mol) of trimethylpyrrole in 30 ml of hexane was added. The reaction mixture was stirred for 1 h at room temperature and then refluxed for 1/2 h. A brown precipitate was formed. The mixture was poured onto solid carbon dioxide and ether. The acid was extracted with aqueous sodium hydroxide solution. Acidification gave 2.42 g (32 %) of the title compound. M.p. ~114 °C. Recrystallization from ligroin yielded the pure acid, m.p. 119–120 °C (lit.[20] m.p. 119.5 °C). IR: C=O 1700 cm^{-1}. NMR (DMSO-d_6): δ 3.33 (1-CH_3, s, 3 H); 2.14 (5-CH_3, broad s, 3 H); 3.52 (2-CH_2-, s, 2 H); 5.73 (3-H, d, q); 5.65 (4-H, d). $J_{3,4}$ 4.9 Hz; $J_{4\text{-H},5\text{-CH}_3}$ 0.8 Hz.

GLC analyses were performed on a Varian 1400 Gas chromatograph. IR spectra were recorded on a Perkin Elmer 257 Grating Infrared Spectrophotometer, NMR spectra on a Varian A 60 spectrometer and mass spectra on an LKB 9000 mass spectrometer. Commercial BSA (*N,O*-bis(trimethylsilyl) acetamide, Pierce Chemical Company) was used as silylating agent. GLC analyses for identification were performed by mixing the sample to be analyzed with authentic samples of known substances ("mixing analyses"). $MgSO_4$ was used as drying agent. All reactions with metal organics were performed in a dry oxygen-free nitrogen atmosphere.

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Reactions of Conjugated Diallenes with Sulfur Dioxide. Formation of 2,5-Bis(alkylidene)-2,5-dihydrothiophene-1,1-dioxide Derivatives

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Reactions of sulfur dioxide with conjugated diallenes (*1*) take place at room temperature with formation of [4+2] cycloaddition products *viz.* 2,5-bis(alkylidene)-2,5-dihydrothiophene-1,1-dioxides (*2*) in good yields. The stereochemistry of the reaction shows that it is disrotatory with the two most bulky groups moving away from each other.

In compounds containing two allenic linkages separated by one single bond the two central double bonds are conjugated (Fig. 1). Such compounds represent a unique diene system and thus are prone to [4+2] cycloadditions; at the same time they also maintain the structural characteristics of an allene which means that they should be capable of undergoing [2+2] cycloadditions. This potentially ambident character of conjugated diallenes has been tested with some olefinic reagents,[1] but only [4+2] type cycloadditions have been observed. In the present work the addition of sulfur dioxide to various conjugated diallenes (*1*) is reported to yield exclusively the [4+2] cycloaddition products of the general structure *2*.

Several methods for the preparation of conjugated diallenes are available, but few have any general application. Recently a two-step procedure leading to diallenes has been developed, which involves reaction of conjugated dienes with dibromocarbene and treatment of the resulting bis-adducts with methyllithium.[2,3] *1a*, *1b*, *meso-1c*, and *meso-1d* were prepared by this method, while the remaining diallenes were prepared by other methods reported in the literature (see Experimental). The diallenes were dissolved in as little as possible of an alkane or benzene or a mixture of the two at room temperature, and a gentle stream of gaseous sulfur dioxide was passed into the stirred solution. The reactions were followed by thin layer chroma-

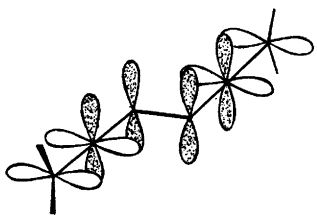
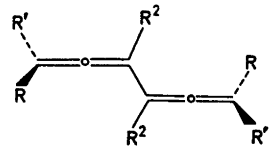
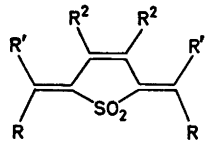


Fig. 1.



1



2

a. R=Me; R′=Me; R²=H
b. R=Me; R′=Et; R²=H
c. R=Me; R′=Ph; R²=H
d. R=Me; R′=*t*-Bu; R²=H
e. R=Ph; R′=Ph; R²=H
f. R=Ph; R′=Ph; R²=Br
g. R=Ph; R′=*t*-Bu; R²=Br

Table 1. Spectral properties of 2,5-bis(alkylidene)-2,5-dihydrothiophene-1,1-dioxides (*2*).

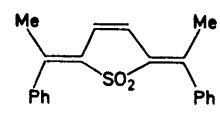
Compound	Yield %	NMR δ ($CDCl_3$)	IR (cm^{-1},KBr)	UV λ max (nm, ethanol)	ε
2a	85	1.95 (s) 6H	1710 w	203	7 400
		2.27 (s) 6H	1630 str		
		6.47 (s) 2H	1270 str	290	23 400
			1115 str		
2b	80	[a]	1625 str	200	15 500
			1275 str		
			1120 str	293	22 100
2c	78	2.64 (s) 6H	1750 w	200	29 800
		6.40 (s) 2H	1605 str		
		7.40 (m)10H	1280 str	317	29 300
			1120 str		
2d	74	1.29 (s) 18H	1600 str	199	15 900
		2.38 (s) 6H	1270 str		
		7.05 (s) 2H	1115 str	298	25 600
2e	73	6.65 (s) 2H	1580 (m)	215	57 500
		7.4 (m)20H	1560 (m)		
			1300 (str)	245	25 600
			1125 (str)	353	32 000
2f	73		1575 (str)	200	48 500
			1555 (str)	247	23 000
			1320 (str)	284	21 300
			1140 (str)	308	19 000
				362	25 000

[a] Mixture of isomers; two overlapping triplets at 1.1, 6 H; two quartets at 2.3 and 2.7, 4 H; two singlets at 1.94 and 2.25, 6 H; singlet at 6.47, 2 H.

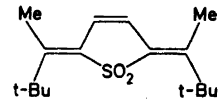
tography (TLC), and reaction times from 30 min to 7 h were needed for completion. The only product obtained in each case was the sulfolene derivative *2*.

The yields recorded in Table 1 refer to recrystallized analytically pure material. The structures were determined on the basis of spectroscopic evidence. Furthermore, a compound assigned structure *2a*, obtained by the base-catalyzed condensation of acetone with 2,5-dihydrothiophene-1,1-dioxide,[4] was indentical with our product. The structures of the sulfolene derivatives *2c* and *2d* were particularly interesting since both starting diallenes *1c* and *1d* had the *meso* configuration. A [4+2] cycloaddition of sulfur dioxide could theoretically yield three isomeric compounds, *viz.* *2c*, *3* and *5*, or *2d*, *4* and *6*, respectively.

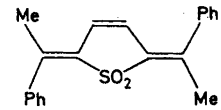
The products were homogenous as shown by TLC. The NMR spectra (Table 1) consisted in both cases of three singlets, excluding unsymmetrical structures *5* and *6*. The chemical shift of a methyl group attached to a double



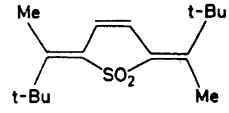
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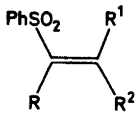


5



6

Table 2. NMR spectra of vinylsulfones.



R	R^1	R^2	$\delta(R^1)$	$\delta(R^2)$
Solvent: CCl_4; Ref. 5.				
H	CH_3	H	2.14	
H	H	CH_3		1.90
CH_3	CH_3	H	2.13	
CH_3	H	CH_3		1.72
Solvent: $CDCl_3$; Ref. 6.				
H	CH_3	$NHCH_2Ph$	2.16	
H	$NHCH_2Ph$	CH_3		1.86
H	CH_3	SPh	2.44	
H	SPh	CH_3		1.75

bond depends strongly on its *E* or *Z* relationship to a vicinal group and in vinyl sulfones methyl-groups in *E* position are shifted upfield (Table 2). In compound *2a* the methyl groups appear at δ 1.95 and 2.27, and are therefore assigned to *E*- and *Z*-methyl groups, respectively. The methyl groups of the products from the diallenes *1c* and *1d* absorb at 2.64 and 2.38 compatible with structures *2c* and *2d* which have a *Z* configuration of the methyl groups. Additional evidence for structure *2d* is also provided by the strong deshielding effect of the *t*-butyl group on the vinyl protons; compared with compound *2a*; a down-field shift of 0.58 ppm was observed.

It would be interesting to compare the UV spectrum of compound *2c* with that of 1,6-diphenyl-*1Z,3Z,5Z*-hexatriene, but the compound is unknown; however, Lunde and Zechmeister [7] who measured the UV spectra of the *1Z,3E,5Z*- and *1Z,3Z,5E*-isomers conclude that the all-*Z* isomer should absorb well below 334 nm. The absorption maximum at 317 nm as well as the lack of fine structure in the UV spectrum of *2c* is fully in accordance with this prediction.

Even without kinetic measurements it is clear that the reactivity of the diallene diminishes from *1a* to *1f*; the diallene *1g* was extremely unreactive as it was recovered unchanged after one hour in neat sulfur dioxide at 100 °C.

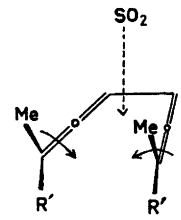


Fig. 2.

According to Woodward and Hoffmann [8] the concerted addition of sulfur dioxide to conjugated dienes is thermally allowed as a linear cheletropic reaction; the required disrotatory process has been observed.[9] The formation of sulfolene derivatives *2* from the diallenes *1* and sulfur dioxide show that the reaction is formally a [4+2] cycloaddition, and the stereochemical results strongly indicate that it is concerted. In such a process sulfur dioxide will most probably approach a cisoid diallene from the least hindered side, followed by a disrotatory move in which the two most bulky groups rotate away from each other as depicted in Fig. 2.

One can certainly imagine both radical and ionic mechanisms since unusual allylic stabilization of the intermediates is predictable; however, it would be very much a coincidence, if such reaction paths should exhibit the clean stereochemistry observed in the above cycloaddition reaction.

EXPERIMENTAL

NMR spectra were recorded on a Varian A60 Spectrometer. A Perkin-Elmer model 457 spectrophotometer was used for obtaining IR spectra, and UV spectra were recorded on a Cary 14 spectrophotometer. Elemental analyses were carried out by Ilse Beetz Microanalytical Laboratory, 8640 Kronach, Germany. All melting points are uncorrected.

2,5-Bis(2-propylidene)-2,5-dihydrothiophene-1,1-dioxide (*2a*). Sulfur dioxide was passed during 2 h into a stirred solution of 3.4 g (26 mmol) of 2,7-dimethyl-2,3,4,5-octatetraene (*1a*) in 50 ml dry pentane. Filtration gave 4.5 g (86 %) of *2a*, m.p. 261 – 263 °C (decomp) from acetone (lit.[4] m.p. 261 – 263 °C).

2,5-Bis(2-butylidene)-2,5-dihydrothiophene-1,1-dioxide (*2b*). Sulfur dioxide was passed during 2 h into a stirred solution of 1.0 g (6.1 mmol) of 3,7-dimethyl-3,4,6,7-decatetraene (*1b*) [3] in 20 ml of dry pentane. Filtration yielded 1.1 g (80 %) of *2b*, m.p. 142 – 143 °C

(decomp) from acetone-water, (Found: C 63.71; H 7.78. Calc. for $C_{12}H_{18}SO_2$: C 63.68; H 8.02).

anti,anti-2,5-Bis(1-phenylethylidene)-2,5-dihydrothiophene-1,1-dioxide (*2c*). Sulfur dioxide was passed during 1 h into a stirred solution of 0.15 g (0.58 mmol) of *meso*-3,7-diphenyl-2,3,5,6-octatetraene (*1c*) [3] in 10 ml of heptane-benzene 1:1. TLC showed that the diallene had been consumed after 30 min. Evaporation of the solvents and recrystallization of the residue from acetone-water gave 0.14 g (78 %) of *2c*, m.p. 155 °C. (Found: C 74.62; H 5.61. Calc. for $C_{20}H_{18}SO_2$: C 74.52; H 5.63).

anti,anti-2,5-Bis-(2,2-dimethyl-3-butylidene)-2,5-dihydrothiophene-1,1-dioxide (*2d*). Sulfur dioxide was passed during 2 h into a stirred solution of 0.25 g (1.1mmol) of *meso*-2,2,3,8,9,9-hexamethyl-3,4,6,7-decatetraene (*1d*) [3] in 10 ml of dry hexane. The product was filtered and recrystallized from hexane to give 0.23 g (74 %) of *2d*, m.p. 173 °C. (Found: C 68.94; H 9.27. Calc. for $C_{14}H_{26}SO_2$: C 68.05; H 9.28).

2,5-Bis(diphenylmethylidine)-2,5-dihydrothiophene-1,1-dioxide (*2e*). Sulfur dioxide was passed during 3 h into a stirred solution of 0.30 g (0.78 mmol) of 1,1,6,6-tetraphenyl-1,2,4,5-hexatetraene (*1e*) [10] in 30 ml benzene. The solvent was evaporated and the residue recrystallized from petroleum ether (60–80 °C–benzene to give 0.25 g (73 %) of *2e*, m.p. 229 °C (decomp). (Found: C 80.86; H 5.24. Calc. for $C_{30}H_{22}SO_2$: C 80.69; H 4.97). In cyclohexane *2e* crystallized with two molecules of solvent, m.p. 224 °C (decomp). (Found: C 82.15; H 7.32. Calc. for $C_{42}H_{46}SO_2$; C 82.04; H 7.54).

2,5-Bis(diphenylmethylidene)-3,4-dibromo-2,5-dihydrothiophene-1,1-dioxide (*2f*). Sulfur dioxide was passed during 7 h into a stirred solution of 0.27 g (0.50 mmol) of 3,4-dibromo-1,1,6,6-tetraphenyl-1,2,4,5-hexatetraene (*1f*) [11] in 10 ml of benzene and 5 ml of hexane. The product was precipitated by addition of hexane. Filtration and recrystallization from diethyl ketone gave 0.22 g (73 %) of *2f*, m.p. 260 °C (decomp.). (Found: C 59.67; H 3.48. Calc for $C_{30}H_{20}SO_2Br_2$: C 59.61; H 3.34).

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Photochemistry of Sulfur Compounds Related to the 1,2-Dithiole System. V. Formation of 1,2-Dithiolyl Radicals and Dithioketonate Anions in the Photolysis of 3,5-Disubstituted 1,2-Dithiolylium Salts

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It has been shown in a series of eleven 3,5-disubstituted 1,2-dithiolylium salts and unsubstituted 1,2-dithiolylium salts that irradiation of these salts in ethanolic solutions gives rise to 1,2-dithiolyl radicals and dithioketonate anions. Rate constants for the first order decay of these radicals and anions are given in absolute and 96 % ethanol respectively.

DITHIOLYL RADICALS

One of the conditions necessary for a radical to be observable is a favourable position of the equilibrium (1) to avoid disproportionation of the radical.

$$Ox + Red \rightleftharpoons 2\ Rad \qquad (1)$$

Further it is necessary, that the equilibrium (2) must be such, that measurable amounts of the radical exist.

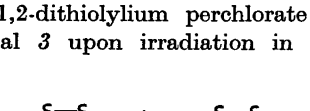
$$2\ Rad \rightleftharpoons Dimer \qquad (2)$$

It has been shown by theoretical studies both by PPP methods [1] and by CNDO/2 calculations [2] that both these prerequisites are fullfilled by the dithiolyl radical *1*.



1

It has previously been shown that 3,5-diphenyl-1,2-dithiolylium perchlorate *2* gives the radical *3* upon irradiation in ethanolic solution.[3]



2 3

It has now been demonstrated that this is a general reaction for 3,5-disubstituted 1,2-dithiolylium salts in ethanolic solution.

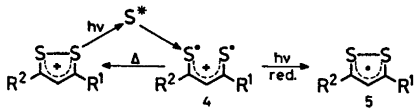
The quantum yield for the reaction was found to be unaffected by the presence of oxygen, which led us to the assumption, that the reaction proceeds *via* an excited singlet state.

The formation of the radical *5* is a biphotonic process since the quantum yield of *5* was found to be proportional to the square of the light intensities. This means as shown by Porter an coworkers,[4] that a photochemically formed intermediate undergoes a further photochemical reaction in competition with a thermal reaction.

The decay of *3* was found to obey first order kinetics.

The visible spectrum of the photoproduct [3] was identical with the visible spectrum of dithiolyl radicals generated by one electron cathodic reduction [5] of 1,2-dithiolylium salts ($\lambda_{max} = 650$ and 450 nm, it was not possible to obtain the absorption spectrum at wavelengths lower than 450 nm due to the strong absorption band of the starting dithiolylium salt in this region).

On the basis of these observations it was proposed that dithiolyl radicals were formed *via* the following pathway with the solvent acting as reductant.

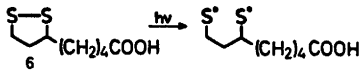


4 5

The dithiyl radical *4* was proposed as an intermediate since it is a well established fact that disulfides give thiyl radicals upon irradiation.[6]

$$RS\text{-}SR^1 \xrightarrow{h\nu} RS^{\bullet} + {}^{\bullet}SR^1$$

It has further been reported[7] that lipoic acid *6* as well as other 6 and 7 membered cyclic disulfides gives dithiyl radicals upon irradiation at low temperatures. The structure of the diradicals has been established by ESR spectroscopy.[7]



6

In Table 1 the rate constants are given for the first order decay of the investigated series of 1,2-dithiolyl radicals in absolute ethanol.

The mechanism of the decay was not further studied, but they probably decay by reaction with the solvent under destruction of the dithiol system.[8]

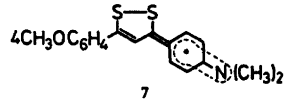
It was observed that the radicals decay faster if water is present. The 3,5-diphenyl-1,2-dithiolyl radical has, *e.g.*, $k_{96\ \%\ \text{ethanol}} = 1500\ s^{-1}$.

If the variations in rate constants in Table 1 are compared to the variation of the substituent pattern it is observed that the introduction of electron donating groups such as a *p*-methoxyphenyl group augments the reactivity of the radical whereas an electron attracting group such as *p*-bromophenyl diminishes the reactivity.

Table 1. Rate constants for the 1st order decay of 1,2-dithiolyl radicals in absolute ethanol at 25 °C.

R^1	R^2	$k\ s^{-1}$
C_6H_5	C_6H_5	903
C_6H_5	4-$CH_3OC_6H_4$	2020
C_6H_5	4-$CH_3C_6H_4$	895
C_6H_5	4-BrC_6H_4	735
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	880
4-$CH_3OC_6H_4$	4-$CH_3OC_6H_4$	2300
4-$CH_3OC_6H_4$	4-$(CH_3)_2NC_6H_4$	49
C_6H_5	CH_3	2040
C_6H_5	SCH_3	2480
t-C_4H_9	t-C_4H_9	252
CH_3	CH_3	4270
H	H	754

The extremely low reactivity of the product derived from 3-(*p*-methoxyphenyl)-5-(*p*-dimethylaminophenyl)-1,2-dithiolylium salts is probably due to the fact that the photoproduct is not a dithiolyl radical but an aminyl radical *7* since it is known that nitrogen containing radicals often show high stability.[9]

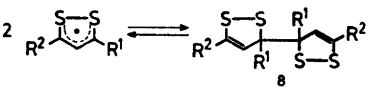


7

If one phenyl group in *2* is substituted by a methyl group the reactivity augments due to the lower steric hindrance of the methyl group. The di-*tert*-butyl radical is only slightly reactive due to strong steric hindrance.

The unsubstituted 1,2-dithiolyl radical proved to be unusually stable. This has also been observed by thermolytical formation of the dithiolyl radical in the ion source of the mass spectrometer.[10] No satisfactory explanation can be given for this observation.

If the rate constants in Table 1 are compared to the ratio monomer:dimer given in Ref. 5 the same general trend is found concerning the influence of substitution on the reactivity of the radical. If the radical is generated in acetonitrile it can only decay by formation of dimers *8*, *i.e.* the substrate with which it reacts is rather sterically hindered, and different for different dithiolyl radicals. The radicals generated in this study all decay by reaction with the same substrate, ethanol, the rate constants in Table 1 therefore give a more true picture of the reactivity of the radicals.



8

We have in several cases been able to measure the decay rates of radicals where the couple (dithiolylium salt/dithiolyl radical) showed electrochemical irreversibility on a platinum electrode, *i.e.* $E_{pc} - E_{pa} > 60$ mV.[5] This is due to the fact that it is possible to detect radicals with much shorter life time with the method used in this study. 3-Methyl-5-phenyl-1,2-

dithiolylium perchlorate, *e.g.*, displays irreversible electrochemistry at room temperature on a platinum electrode in acetonitrile solution, but the electrochemistry became reversible at −70 °C.[11] In the photolysis experiment it was possible to determine the decay rate of the corresponding radical at room temperature.

DITHIOKETONATE ANIONS

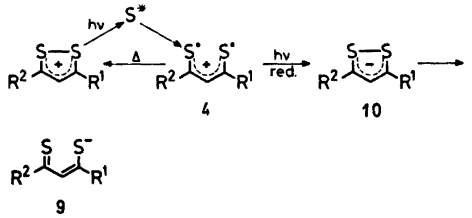
During the photolysis of 1,2-dithiolylium salts another species with a longer life time than dithiolyl radicals was observed. We have ascribed the dithioketonate anion structure *9* to these compounds.



The absorption spectrum of the compound derived from 3,5-diphenyl-1,2-dithiolylium salts [3] was found to be indentical to the absorption spectrum of the dithioketonate anion prepared electrochemically by two electron reduction of the same salt; [5] $\lambda_{max} = 490$ nm. It has been reported that dithioketonate anions in, *e.g.*, Ni chelates have an absorption band near 480 nm.[12]

The first product formed is probably the anion *10*, which on the basis of CNDO/2 calculations [2] and the irreversibility of the electrochemical reduction of dithiolyl radicals [5] has been shown to be less stable than its valence tautomer the dithioketonate anion.

The formation of *9* is found to be a biphotonic process (*cf.* p. 831). As the decay of the dithiolyl radical is thermal we assume that the anion *10* and the dithioketonate anion is formed directly from the dithiyl radical *4*.

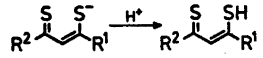


Thermally the dithioketonate anions decay in a first order reaction. The rate constants for the decay are given in Table 2.

Table 2. Rate constants for the 1st order decay of dithioketonate anions in 96 % ethanol at 25 °C.

R^1	R^3	k s^{-1}
C_6H_5	C_6H_5	0.774
C_6H_5	4-$CH_3OC_6H_4$	1.56
C_6H_5	4-$CH_3C_6H_4$	0.941
C_6H_5	4-BrC_6H_4	0.781
4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	1.17
4-$CH_3OC_6H_4$	4-$CH_3OC_6H_4$	7.35
4-$CH_3OC_6H_4$	4-$(CH_3)_2NC_6H_4$	not observable
C_6H_5	CH_3	41.4
C_6H_5	SCH_3	4.81
t-C_4H_9	t-C_4H_9	282
CH_3	CH_3	40.4

It was observed that the amount of dithioketonate anion formed as well as the decay rate of the dithioketonate anions were augmented by the presence of water in the alcohol. It was further noticed that both these factors were strongly influenced by the presence of acid. In 10^{-4} M ethanolic hydrogen chloride it was not possible to detect any dithioketonate anion after the photolysis, this may be due to a neutralization of the fairly basic anion.



As the formation and decay of dithiolyl radicals are only slightly dependent on the presence of acid, it is probably not the formation of the dithiyl radical which is hindered by acid nor is this biradical destroyed by acid.

It can be noticed from Table 2, that the decay of the dithioketonate anions is only slightly dependent on the nature of the substituents present, the aliphatic ones being a little less stable than the aromatic ones.

Both the formation of dithiolyl radicals as well as dithioketonate anions were independent of the nature of the anion in the starting dithiolylium salt.

EXPERIMENTAL

1,2-Dithiolylium salts. For the preparations of these salts, *cf.* Ref. 5 and references given there.

Equipment. Conventional flash kinetic equipment was used.[13] The flash lamps were filled with air, and flash energies were reproducible to ±2 %. The decay rates were constant to ±5 %. The concentration of dithiolylium salts was 10^{-4} M. The measurements were always carried out on freshly prepared solutions which were only used for one flash.

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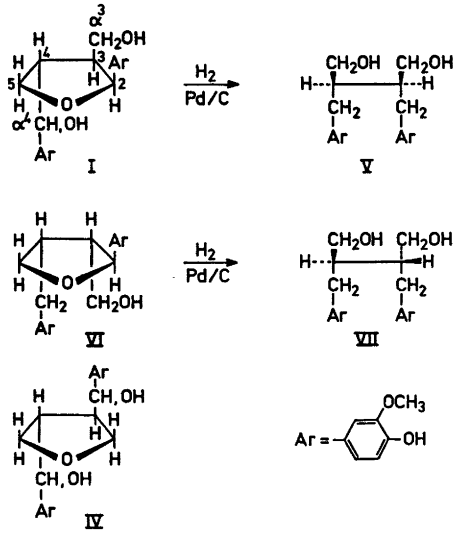
A New Lignan from Norway Spruce

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A new lignan tetrahydro-α^4, *r*-2-bis(4-hydroxy-3-methoxyphenyl)-*c*-3, *t*-4-furandimethanol (I), has been isolated from sound root sapwood as well as from the reaction zone induced by attack of *Fomes annosus*. The identification is based on NMR, MS, and hydrogenation studies. Two other nonidentified lignans were isolated and characterized by MS. HPLC and TLC data are given for a series of lignans.

In connexion with studies on changes in root sapwood from Norway spruce [*Picea abies* (L.) Karst.] attacked by *Fomes annosus* (Fr.) Cke, small amounts of a crystalline, apparently new lignan (I) (m.p. 148 – 152 °C: $[\alpha]_D$ – 10.4°) were isolated, together with crude fractions containing two other nonidentified compounds (II and III). The colour reactions and mobilities of the three compounds in thin layer chromatography (TLC) were similar to those of the lignan liovil (IV) (Table 1). Using high-pressure liquid chromatography (HPLC), small amounts of II and III have now been obtained in a pure state. The mass spectra of compounds I – III showed very similar fragmentation patterns (the more important peaks are given in Table 2) and the same molecular ion (*m/e* 376) as found for liovil (IV). The available amounts of compounds II and III, apparently also lignans, were too small for further identification, but the crystalline compound I was identified as described below.



I V VI VII IV

The elemental analysis and the mass spectrum of I were in agreement with the formula $C_{20}H_{24}O_7$. Catalytic hydrogenation yielded a product (V), chromatographically indistinguishable from (–)-*seco*-isolariciresinol (VII), the known hydrogenation product from (+)-lariciresinol (VI).[2] The available amount of compound V did not permit an accurate optical rotation determination. The mass spectra of V and VII were very similar (see EXPERIMENTAL), but NMR revealed slight but significant differences between the two com-

Table 1. Chromatographic properties of compounds I – VII.

Compound	LC retention time (min)	TLC: R_F value relative to VI		Colour with spray
		Solvent *a*	Solvent *b*	
I	10.0	0.45	0.72	red orange
II	8.0	0.35	0.86	red orange
III	19.2	0.52	0.67	red orange
IV	4.8	0.45	1.00	red orange
V	5.4	0.45	0.72	green
VI	6.2	1.00	1.00	brown
VII	5.4	0.45	0.72	green

Table 2. Mass spectra of compounds I – IV.

m/e	Intensities, % I	II	III	IV
65	17	15	20	10
69	44	26	17	27
77	9	7	12	7
81	10	7	13	9
91	7	5	12	5
93	22	20	23	9
94	12	8	11	8
122	14	9	11	8
124	9	5	12	6
131	10	5	13	6
137	100	100	100	100
138	65	42	26	29
151	17	11	22	6
153	88	53	53	32
175	26	7	24	13
180	10	7	10	5
206	27	11	16	12
340	5	2	3	8
358	25	7	11	11
376, M^+	24	11	11	3

pounds (Table 3). Most important, only compound VII showed separate doublets for the primary methylene protons H_A, H_B and a doublet for the methylene protons H_C,H_D. This indicated that compound V was the diastereomer of compound VII, since the latter compound is optically active, V must be the *meso*-isomer.

The NMR spectrum of I was very similar to those of lignans containing a 2,3,4-substituted tetrahydrofuran ring, [3–10] and the fact that the compound yielded *meso-seco*-isolariciresinol (V) on hydrogenation indicated a lariciresinol structure with an extra benzylic hydroxyl and with *trans*-oriented 3- and 4-protons.

The structure determination of I is supported by successive spin decoupling of the H-3 and H-4 protons in the NMR spectrum of I and its tetraacetate, respectively. The sharp doublets at δ 4.47 and 4.50 in the NMR spectrum of I must arise from two single benzylic hydrogens, the doublet at δ 4.47 being due to the hydrogen in the α^4-position.

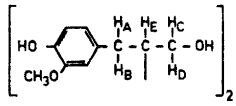
The upfield shift (δ 4.50) and the large coupling constant (J 8.4 Hz) for proton H-2 in the spectrum of I compared with (+)-lariciresinol, VI, (δ 4.75, J 6.5 Hz) indicates *cis*-oriented 2- and 3- protons. The *trans*-orientation of the corresponding protons in (+)-lariciresinol (VI) is based on its formation by hydrogenation of (+)-pinoresinol,[11] the absolute configuration of which is well established.[12–14] NMR spectra of the related tetrahydrofuro[3,4-*c*]furan lignans (*e.g.* pinoresinol) seems to offer the closest parallel to those of compounds I and VI. In their spectra the benzylic hydrogen is reported at δ 4.40 – 4.45 (J 6.0 – 7.0 Hz) and 4.80 – 4.85 (J 4.0 – 5.5 Hz) for *cis*- and *trans*-oriented 2- and 3-protons, respectively.[3,4,9,10]

These results verify the suggested structure of compound I or its enantiomer.

EXPERIMENTAL

TCL was performed on Silica Gel HF_{254} (Merk) plates with (*a*) 1:1 dichloromethane-acetonitrile and (b) 7:3 hexane-ethanol as solvents. The plates were sprayed with diazotized sulfanilic acid in 10 % aqueous sodium carbonate, followed by light spraying with 50 % sulfuric acid.

Table 3. NMR data of compounds V and VII.



	2-H	5-H	6-H	H_A	H_B	H_C	H_D	H_E	OMe
V	6.55 – 6.70 m			2.60 d		3.45 – 3.80 m		1.85 – 2.15 m	3.80 s
				$J_{AE}=J_{BE}=7.5$ Hz					
VII	6.59 d	6.66 d	6.53 q	2.60 d	2.62 d	3.59 d		1.75 – 2.10 m	3.73 s
$J_{26}=1.6$ Hz $J_{56}=7.8$ Hz $J_{AE}=6.8$ Hz $J_{BE}=7.4$ Hz $J_{CE}=J_{DE}=5.0$ Hz									

High-pressure liquid chromatography (HPLC) was performed on a Waters ALC-202 instrument with a 180 cm × 3 mm column containing Silica Gel (Corasil II, Waters) and 9:1 petroleum ether (b.p. 40 – 60 °C) – ethanol as solvent. The flow rate was 5 ml/min, and the detector a UV-monitor operating at 280 nm.

The NMR spectra were recorded on a Varian HA-100D instrument, in methanol-d_4 (if not otherwise stated) using tetramethylsilane as an internal standard. The singlet, doublet, quartet and multiplet patterns are designed s, d, q, and m, respectively. The mass spectra were recorded on a Varian CH-7 instrument.

A previous paper [1] described the isolation of compound I (20 mg) in the crystalline state from the mother liquor after crystallization of liovil. This was obtained from a subfraction after chromatography on silicic acid of the 2-butanone extract of the reaction zone from roots of Norway spruce attacked by *Fomes annosus* (fraction 8 in experiment C in that report). The mother liquor thus obtained, combined with the next subfraction (fraction 9), was fractionated in the present investigation on a silicic acid column (90 × 2 cm) using solvent *a*. From a subfraction containing compounds II, III, liovil and some I, small amounts of the unidentified compounds II and III (*ca.* 1 mg of each) were obtained in a chromatographically pure state by repeated HPLC. The retention times, using the conditions given above, and the mobilities and colour reaction in TLC are given in Table 1, together with similar information for compounds IV – VII for comparison.

In a similar way, using a combination of chromatography on Sephadex LH-20, silicic acid and HPLC, compounds I – III were also detected in a 2-butanone extract of sound sapwood from the previous investigation. Compound I was thereby also isolated in sufficient amount to yield an NMR spectrum, identical with that of compound I isolated from the reaction zone.

Compound I. Crystallized from ethanol, m.p. 148 – 152 °C, $[\alpha]_D^{20}$ −10.4° (*c* 1.8, methanol). (Found: C 63.8; H 6.4 $C_{20}H_{24}O_7$ requires C 63.8; H 6.4.) IR; ν_{max} (KBr): strong bands at 1035, 1060, 1130, 1160, 1275, 1435, 1520, 1600, and 1610 cm^{-1}. The MS data are given in Table 2. NMR: δ 2.26 (m, 1 H, H-3), 2.62 (m, 1 H, H-4), 3.66 (m, 4 H, H-5, H-5′, H-α^3, H-$\alpha^{3'}$), 3,84 (s, 3 H, OMe), 3.86 (s, 3 H, OMe), 4.47 (d, 1 H, *J* 9.2 Hz, H-α^4), 4.50 (d, 1 H, *J* 8.4 Hz, H-2), 6.7 – 7.0 (m, 6 H).

I-tetraacetate (Ac_2O/pyridine) amorphous. NMR ($CDCl_3$): δ 1.96 (s, 3 H, aliphatic acetyl), 2.05 (s, 3 H, aliphatic acetyl), 2.30 (s, 6 H, 2 aromatic acetyls), 2.32 (m, 1 H, H-3), 2.67 (m, 1 H, H-4), 3.78 (m, 2 H, H-5 and H-5′), 3.85 (s, 6 H, 2 methoxyls), 4.11 (q, 1 H, *J* 6.6 and 11.7 Hz, H-α^3), 4.37 (q, 1 H, *J* 5.0 and 11.7 Hz, H-$\alpha^{3'}$), 4.57 (d, 1 H, *J* 7.6 Hz, H-2), 5.79 (d, 1 H, *J* 9.6 Hz, H-α^4), 6.8 – 7.1 (m, 6 H).

Compound I (*ca.* 5 mg), in dry tetrahydrofuran (10 ml), was hydrogenated in the presence of 5 % palladium on carbon for 3 h under ambient conditions, and the catalyst was then filtered off and carefully washed with methanol. The product obtained (compound V) after evaporation, was purified from small amounts of impurities by TLC, using solvent *b*. MS: *m/e* 77 (6 % of base peak), 91(4), 94(8), 122(10), 137(*100*), 138(21), 163(4), 189(5), 194(4), 362(12, M+). Compare the MS of (−)-*seco*-isolariciresinol (VII) run under similar conditions (direct inlet, 140 °C): *m/e* 77 (9 % of base peak), 91(6), 94(12), 122(16), 137(*100*), 138 (38), 163(7), 189(10), 194(6), 362(22), M+).

The NMR data of compounds V and VII are given in Table 3.

Compounds II and III. The MS data are given in Table 2 and the chromatographic data in Table 1. The red-orange colour developed on spraying is notable, indicating an α-hydroxyvanillyl structure.

(+)-*Lariciresinol* (VI). NMR: δ 2.2 – 3.0 (m, 4 H), 3.81 (s, 3 H, OMe), 3.83 (s, 3 H, OMe), 3.5 – 4.1 (m, 4 H, $-CH_2-$), 4.75 (d, 1 H, *J* 6.5 Hz, H-2), 6.45 – 6.95 (m, 6 H).

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A Study of DNA from Chloroplasts Separated by Counter-current Distribution

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Three types of chloroplasts (Peak I, Peak II, and Peak III chloroplasts) isolated by counter-current distribution in aqueous polymer two-phase systems have been studied with respect to DNA content. (The characterization was performed by studying the buoyant density, the melting profile and the amount of DNA of each type). The DNA was found to differ between the three types of chloroplasts.

The counter-current distribution (CCD) of chloroplasts using aqueous polymer two-phase systems has shown that a chloroplast preparation is composed of three types of chloroplasts.[1] These differ with respect to the outer most membrane surrounding the chloroplast. One class has an intact envelope (Peak I), another type has a broken envelope (Peak II) and in the third type the chloroplast envelope is surrounded by a layer of cytoplasm enclosed within a membrane (Peak III). This cytoplasmic layer also contains mitochondria and peroxisomes. It is thus obvious that it is necessary to achieve a good separation of the different types of chloroplasts before a study of the chloroplasts can be carried out.

Chloroplasts have, by many authors,[2–5] been shown to contain DNA. The study was from the beginning concentrated on the characterization of the DNA with respect to buoyant density, melting characteristics, homogeneity, and content of DNA in the chloroplast. The main problem was to obtain clean preparations not contaminated with nuclear DNA, mitochondrial DNA, or DNA from other organisms, such as algae or bacteria.

The aim of this work was to investigate whether the three types of chloroplasts described above differed with respect to content, buoyant density and melting profiles of the DNA contained in them.

MATERIALS AND METHODS

Polymers used were Dextran 500, from Pharmacia Fine Chemicals, Uppsala, Sweden, and poly(ethylene glycol), Carbowax 6000 and Carbowax 4000, from Union Carbide, New York, USA. Cesium chloride was Suprapur, Merck, Darmstadt, Germany. All other chemicals used were of analytical reagent grade. The water used was distilled twice in a quartz apparatus.

Spinach (Viking II, Weibulls, Landskrona, Sweden) was grown in artificial light, with a light intensity of 5000 Lux, alternating with dark periods of 12 h. The temperature during the growth was 18 °C and the relative humidity 70 %.

Preparation of chloroplasts. 500 – 700 g spinach leaves were harvested and washed 3 times with 5 l of newly distilled water at a temperature of 4 °C. The leaves were homogenized 3 × 2 s in 2 l preparation medium (0.05 M potassium phosphate buffer pH 7.8 and 0.4 M sucrose) in a Turmix blendor. The homogenate was filtered through four layers of perlon net (Monodur 31 μ Vereignigte Seidenwebereien AG, Krefeldt, W. Germany). The filtrate was then centrifuged in a Sorvall RC 2B high speed centrifuge for 10 min at 400 *g*. The pellet was resuspended in the preparation medium and centrifuged for 15 min at 400 *g*. This pellet was used in the sample phase system.

Two-phase system. The phase system was composed of 630 g 20 % w/w dextran, 305 g 40 % w/w poly(ethylene glycol) 4000, 500 ml 30 % w/v sucrose, 50 ml 0.2 M potassium phosphate buffer pH 7.8 and water to 2012 g. This system was scaled up by a factor of ten as compared to Ref. 1.

Sample phase system. The chloroplast pellet in a typical experiment weighed about 13 g. It was suspended in 11 g of upper phase of the phase system mentioned above. To this suspension were added 7.31 g 40 % w/v poly(ethylene glycol) 4000, 0.6 g 20 % w/w dextran, 8.5 ml 30 % w/v sucrose and water to 45 g.

Counter-current distribution. The CCD was carried out in an all glass machine (Gallenkamp, England). The capacity of each tube for stationary phase was 40 ml. To get a stationary interphase each chamber except chamber 2 was filled with 35 ml of the lower phase and 45 ml of the upper phase. Chamber 2 was filled with 35 ml of the lower phase and the sample system. Number of transfers were 19, the shaking time 45 s and the settling time 20 min. The temperature during the run was +2 to +4 °C.

Extraction of DNA. The DNA was extracted from chloroplasts using the method described by Rudin and Albertsson.[6] In the present case the volumes of that procedure were scaled down by a factor of six.

Removal of polymers. Poly(ethylene glycol) and lithium phosphate buffer pH 7.8 were added to the upper phase from the extraction procedure to obtain a final concentration of 10 % w/w and 10 mM, respectively. The phase system thus formed separates into an upper phase which is free from dextran and containing all the DNA and a small lower phase. The upper phase was removed and CsCl was added to it to a density of 1.7 g cm^{-3}. In the resulting two-phase system the poly(ethylene glycol) distributed exclusively to the upper phase. The DNA was found in the polymer free CsCl phase. The CsCl was then removed by dialysis.

Ultracentrifugation. The density equilibrium centrifugation experiments were performed in a Spinco analytical ultracentrifuge Model E fitted with a Kel F centerpiece and MSE Centriscan 75 ultracentrifuge using the technique described by Schildkraut, Marmur and Doty.[7] DNA from *Micrococcus lysodeikticus* was used as reference DNA. The calculations were based upon a banding at the density 1.731 g cm^{-3} for the reference DNA. The centrifugations were carried out at 44 000 rpm and 20 °C. After at least 20 h the cell contents were analysed and in the case of Spinco the photographs were analysed in a Beckman Analytrol.

Melting curves. The melting curves were obtained using a Zeiss PMQ II spectrophotometer with termostated cuvette holder. The temperature was raised at the rate of 1 °C/min.

Chlorophyll measurements. Chlorophyll was determined by the method of Arnon.[8]

Calculation of DNA content. The calculation of DNA per chloroplast was referred to the chlorophyll content, one chloroplast containing 1×10^{-6} μg chlorophyll.[9]

RESULTS

The three types of chloroplasts obtained by CCD are classified according to their position in the CCD-diagram: Peak I chloroplasts partition to the left, Peak II chloroplasts partition in the middle and Peak III chloroplasts partition to the right.[1] Fig. 1 shows a CCD-experiment of 19 transfers with chloroplasts as sample. Peak I chloroplasts were collected from tube number 1 to 5, Peak II chloroplasts from tubes number 8 to 10 and Peak III chloroplasts from tubes number 13 to 18. Karlstam and Albertsson showed that the absorbance ratio (A_{550}/A_{680}) could be used to determine percentage of intact chloroplasts.[10] Intact chloroplasts have an absorbance ratio of close to 1. Peak I contains mainly intact chloroplasts. The broken chloroplasts found in Peak I are probably caused by destruction of intact chloroplasts during the end of the CCD.

Each type of chloroplasts collected as above were washed with preparation medium. The

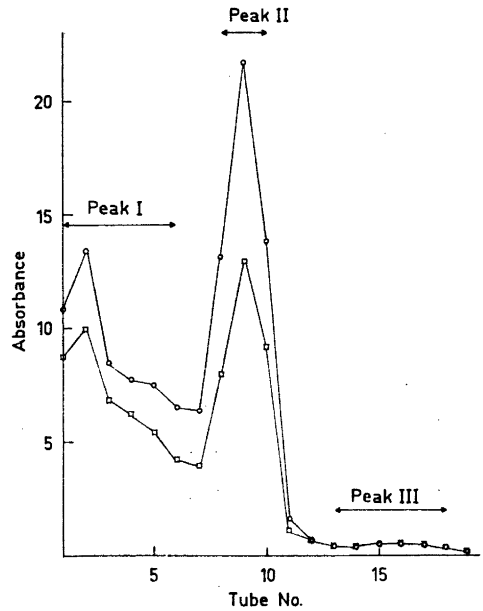


Fig. 1. Chloroplasts from 700 g spinach leaves were distributed in a glass Craig apparatus. Each tube had a capacity of bottom phase of 40 ml. The number of transfers was 19 and the temperature during the experiment 2 °C. Absorbance at 680 nm (○) and absorbance at 550 nm (□).

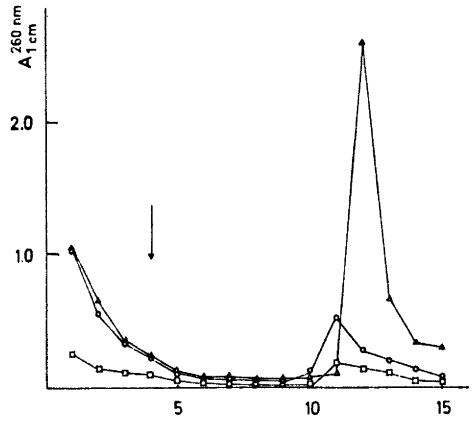


Fig. 2. Extraction of DNA from the three peaks shown in Fig. 1. The extraction profile for Peak I (○), for Peak II (△), and for Peak III (□), respectively. Vertical arrow indicates a shift in phase system to the one in which the DNA is transferred to the upper phase (tube numbers 11 and 12).

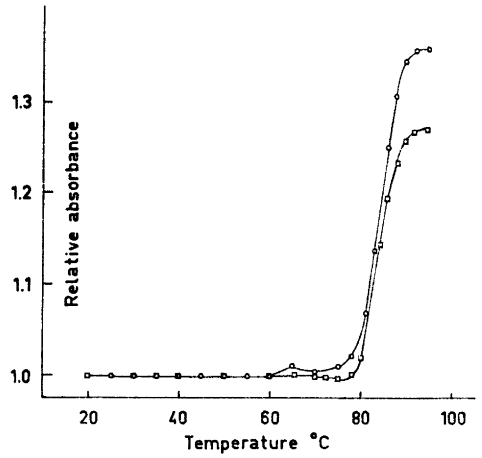


Fig. 3. Melting curves for Peak I DNA (□) and Peak II DNA (○). The concentration of salts was 0.15 M NaCl and 0.015 M sodium citrate.

DNA was then extracted from the chloroplasts in an aqueous polymer two-phase system and the results are shown in Fig. 2. The arrow in the figure indicates a shift of phase system to the one in which the DNA is extracted to the upper phase as the salt concentration diminishes. This usually occurs in tubes number 11 or 12. The DNA used in the subsequent experiments was taken from tube number 11 for Peak I, tube number 12 for Peak II and tube number 11 for Peak III. The number of chloroplasts used in these experiments was based on the chlorophyll content in the three chloroplast pellets obtained after the CCD. The DNA content of the three types of chloroplasts is recorded in Table 2. The value, 9×10^{-14} g DNA per chloroplast for Peak III DNA is somewhat uncertain due to the low amount of DNA obtained in the extraction procedure. It is nevertheless significantly higher than the

Table 1. Buoyant densities of chloroplast DNA from Peak I, Peak II and Peak III chloroplasts.

Preparation	Native DNA	Heat denatured DNA	Alk. denatured DNA
Peak I			
1	1.697	1.714	1.713
2	1.696	1.713	1.713
3	1.696	1.714	1.713
4	1.696		
5	1.696		
Peak II			
1	1.694	1.710	1.710
2	1.695	1.711	1.712
3	1.694	1.710	1.710
4	1.694		
5	1.694		
Peak III			
1	1.697		
2	1.696		
3	1.696		

Table 2.

	DNA per chloroplast ($\times 10^{14}$ g).	Mol per cent (GC) calculated from T_m according to Marmur and Doty[11].	Mol per cent (GC) calculated from buoyant density according to Schildkraut *et al.*[7]
Peak I Chloroplast DNA	3.3	36	37
Peak II Chloroplast DNA	7.2	35	35
Peak III Chloroplast DNA	9	36	37

DNA content in the other fractions for which the values were 3.3×10^{-14} g DNA per chloroplast for Peak I and 7.2×10^{-14} g DNA per chloroplast for Peak II.

Heat denaturation of Peak I and Peak II DNA was performed in a solution containing 0.15 M NaCl and 0.015 M Na-citrate. The results are shown in Fig. 3. A melting point (T_m) of 84 °C and a hyperchromicity of 28 % was obtained for Peak I DNA. For Peak II DNA T_m was the same but the hyperchromicity was 35 %. The hyperchromicity varied between 28 % and 33 % for Peak I DNA and between 30 % and 35 % for Peak II DNA in the melting experiments performed.

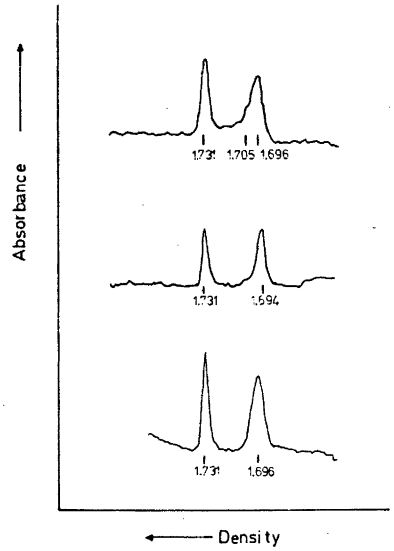


Fig. 4. Densitometer tracings of samples equilibrated in a CsCl density gradient formed by centrifugation at 44 000 rpm. The peak to the left is the standard DNA (*Micrococcus lysodeikticus* DNA). The curve at the top represents Peak III DNA, the curve in the middle Peak II DNA and the curve at the bottom of the diagram Peak I DNA.

The buoyant density analytical patterns for Peak I, Peak II and Peak III DNA are shown in Fig. 4. The three experiments were carried out on the DNA prepared from the three chloroplast types obtained from the same initial chloroplast preparation. In each experiment DNA from *Micrococcus lysodeikticus*, prepared according to Rudin and Albertsson [6] was used as marker. The shoulder at 1.705 for Peak III DNA is probably caused by mitochondrial DNA from the cytoplasm layer surrounding Peak III chloroplasts. Peak I DNA and Peak III DNA:s banded at a density of 1.696 g cm^{-3} while Peak II DNA banded at 1.694 g cm^{-3}. The small peaks between the shoulder and the marker DNA were identified as irregularities in the photograph, due to dust in the lens system. As shown in Table 1 the density values are reproducible, which indicates that there are differences between Peak I DNA and Peak II DNA. Heat denatured and alkaline denatured DNA was run in a CsCl-gradient. Peak I DNA was found to band at 1.713 g cm^{-3} while Peak II DNA banded at 1.711 g cm^{-3}. This is in agreement with the difference found between native Peak I and Peak II DNA.

Table 2 shows the mol per cent Guanosine-Cytosine (G−C) in each type of chloroplast DNA determined from the buoyant density and the melting point, respectively, of the corresponding DNA. The agreement between the two methods is good and shows that the mol per cent (G−C) is 35−37 in the DNA from the three chloroplast fractions.

DISCUSSION

The buoyant density values for spinach nuclear DNA of 1.694 and chloroplast DNA of 1.696 reported by Whitfield and Spencer [5] agree with the values above for Peak II and Peak I DNA, respectively. We therefore conclude that Peak I DNA is chloroplast DNA, Peak II DNA is composed mainly of nuclear DNA, and finally Peak III DNA consists of chloroplast DNA and mitochondrial DNA.

Suyama and Bonner [12] have reported a banding of different mitochondrial DNA from plants at 1.706 g cm^{-3}. Tewari [13] has reported the same buoyant density for mitochondrial DNA. It is therefore reasonable to assume that the shoulder in the buoyant density profile at 1.705 for Peak III DNA in Fig. 4 represents mitochondrial DNA.

Kolodner and Tewari [14] have reported a value of $(0.5-1.5)\times10^{-14}$ g DNA per chloroplast while Chiba and Sugahara [15] have reported a higher value, $(3.8-6.9)\times10^{-14}$ g DNA per chloroplast. The lower value may be due to losses of DNA in the extraction procedure. Our value 3.3×10^{-14} g DNA per Peak I chloroplast seems high compared to Tewari's result but agrees fairly well with the results of Chiba and Sugahara. The following reasons can be suggested for the high value 9×10^{-14} g DNA per Peak III chloroplast.

1. Peak III chloroplasts contain less chlorophyll than Peak I chloroplasts.

2. It has been suggested that chloroplasts undergo division.[16] In the mitotic state the chloroplasts contain double amount of DNA. Peak III chloroplasts might be chloroplasts in the mitotic state; this would explain the high amount of DNA.

3. DNA from the mitochondria in Peak III contributes to the DNA content.

A combination of point 3 and one or both of the other is a probable explanation for the higher value of the DNA content in Peak III chloroplasts. The DNA in Peak II chloroplasts is as mentioned earlier probably nuclear DNA. The DNA cannot be losely bound to the membranes because in that case it would have been washed away when the chloroplasts were collected after the CCD. Further the DNA has a high partition coefficient and would have been found to the far right in the CCD-diagram. It is thus probable that the nuclear DNA is rather tightly bound to the membrane system in Peak II chloroplasts. This binding might occur during the cell breakage and the subsequent handling of the leaf extract. DNA obtained from chloroplast preparations containing both intact and broken chloroplasts, would therefore be a mixture of chloroplast and nuclear DNA. It is therefore essential first to isolate intact chloroplasts surrounded by their envelope, before DNA extraction if one wants to claim that the DNA is of chloroplast origin.

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Structural Studies of Glycoproteins from *Phaseolus vulgaris*

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Sugar analysis, methylation analysis, enzymic hydrolysis and hydrazinolysis were used to study glycoproteins isolated from the primary leaves of *Phaseolus vulgaris* var. Pinto 36 h after sham-inoculation. The glycoproteins contained β-(1→4)-linked D-xylopyranose residues as well as (1→2)-, (1→3)-, and (1→5)-linked L-arabinofuranose residues. Smith degradation of the products of hydrazinolysis indicated that the majority of terminal arabinofuranose residues is linked to O-2 of adjacent residues. Glycoproteins isolated after inoculation with tobacco necrosis virus had a more variable composition and generally contained (1→4)-linked glucose in addition to the above structural elements. Glycoproteins isolated from *P. vulgaris* var. Black Valentine after sham-inoculation were similar to those isolated from the Pinto variety.

Carbohydrate moieties of the glycoproteins appear to be linked *via* hydroxyproline to protein.

The structure of plant glycoproteins has not been widely studied although they are important cell wall constituents,[1] occur in endosperm [2] and cytoplasm,[1] possess lectin activity [3] and may be important for plant virus localization.[4,5] Generally, the carbohydrate moiety is linked to hydroxyproline through α-L-arabinofuranosyl residues [3,6,7] but recently D-galactose has been found to be linked to hydroxyproline [2] and galactose-serine linkages also occur in plant cell wall glycoproteins.[8] L-Arabinose and D-galactose are the most commonly found carbohydrate constituents of plant glycoproteins [2,3,6,7] although, in addition, D-xylose and D-glucose have been found [9,10] as well as D-glucosamine.[11]

Glycoprotein having structural features similar to cell wall glycoproteins but lower hydroxyproline content are produced as a result of mechanical introduction of plant viruses.[4,5] The low hydroxyproline content possibly pointed to an anomalous linkage between carbohydrate and protein moieties and the present study sought to investigate this possibility.

EXPERIMENTAL

Plant and virus. Phaseolus vulgaris L. var. Pinto and Black Valentine received tobacco necrosis virus (Pinto) or were sham-inoculated (Pinto and Black Valentine) as outlined in previous publications.[4,5]

Extraction and purification. Primary leaves harvested 36 h after inoculation were extracted as previously described [4] at 2 °C, unless otherwise stated, yielding non-dialyzable precipitates which were dissolved in 10 % pyridine, insoluble material removed by centrifugation and freeze dried. Lipids were extracted [4] from the dried preparations and the extracted material (in approximately 50 mg batches) dissolved in phosphate buffer (0.02 M, pH 9.0) then chromatographed using a column (2.5 × 50 cm) of Sephadex G-150 developed with the buffer at a flow rate of 3 ml/h. In some cases, the glycoproteins did not dissolve completely in phosphate buffer prior to gel filtration; the insoluble material (up to 30 %) was removed by centrifugation. Fractions (3 ml) were collected; an aliquot of each fraction was diluted and its absorbance at 280 nm measured. Fractions containing the first and second peak of material absorbing at this wavelength were pooled, dialyzed and lyophilized. To measure reproducibility, two preparations from sham-inoculated plants (hereafter referred to as glycoproteins I) and three preparations from TNV-inoculated plants (hereafter referred to as glycoproteins II) were extracted and chromatographed as described.

Hydroxyproline-polysaccharide complexes are extracted from *Lupinus* hypocotyls by alkali at an extraction temperature of 20 °C but not at 2 °C.[9] To test the effect of extraction

temperature on glycoprotein recovery, primary leaves were extracted at 2 °C to yield glycoprotein II (preparation 3) then the leaves were reextracted with 1 M sodium hydroxide at 24 °C. The material extracted at the higher temperature was purified by gel filtration to yield preparation 4, peaks 1 and 2.

To produce enough material for degradation using pronase and hydrazinolysis, batches (approx. 200 mg) of glycoprotein I were purified as described above except the column effluent was monitored at 254 nm with an LKB Uvicord and the first broad peak absorbing at 254 nm was collected, dialyzed and lyophilized.

Acrylamide gel electrophoresis. Electrophoretic separation used a modification of the methods of Ornstein [12] and Davis [13] described previously.[4] The gels were cleaved longitudinally and the halves stained for carbohydrate or protein. Ovalbumin and β-lactoglobulin were used as controls.

Chemical analysis. Protein content was measured by nitrogen analysis. Carbohydrate content was estimated with anthrone. [14] Sugars were identified by gas liquid chromatography (GLC) of the alditol acetate derivatives. [15]

The products of pronase treatment and hydrazinolysis, that is amino acids and peptides or amino acid hydrazides, respectively, were measured quantitatively with ninhydrin. [16] Tests for uronic acids and amino sugars used the methods of Dische [17] and Tracey,[18] respectively.

For amino acid analysis, samples were hydrolyzed with 6 M HCl in sealed tubes *in vacuo* at 110 °C for 36 h. The amino acid composition of the hydrolyzate was determined by the method of Moore and Stein [19] using a JEOL Model 5 AH amino acid analyzer.

Methylation analysis. Samples were methylated twice using the Hakomori [20] procedure, hydrolyzed and converted to alditol acetate derivatives which were analyzed by GLC using the following columns: (1) 3 % ECNSS-M on Gas Chrom Q (100–200 mesh) at 165 °C, (2) 3 % OV-225 at 175 °C, (3) SP 1000 capillary column (0.25 mm × 25 m) at 240 °C. For quantitative evaluation of analyses obtained by GLC, a Hewlett Packard 3370 B integrator was used. Mass spectrometry used a Perkin-Elmer 270 combination gas chromatograph-mass spectrometer or, for compounds containing nitrogen, a Varian Mat 311B instrument. A S.C.O.T. OV-225 column was used to separate the compounds by GLC prior to recording mass spectra (MS) in the Perkin-Elmer instrument and an OV-1 column was used in the Varian instrument.

Pronase treatment. As a preliminary experiment, glycoprotein I (peak 1, 35 mg) was treated with pronase (Sigma, protease type VI, 3.4 units/mg) as described by Brown and Kimmins.[4] The products were separated by gel filtration on Sephadex G-150 as described above and the amino acid composition of the two carbohydrate containing fractions having the highest molecular weight determined. Based on the results of these amino acid analyses, the glycoprotein (253 mg) prepared by chromatography of 200 mg batches was treated with appropriate quantities of reagents and the products of enzymic hydrolysis isolated by gel filtration.

Hydrazinolysis. The glycopeptide fraction (30 mg) obtained by pronase digestion was treated with hydrazine (1.5 ml) and hydrazine sulfate (23 mg) as described by Yosizama *et al.*[21] for 10 h at 100 °C. The hydrazinolyzate was dried *in vacuo* over conc. sulfuric acid, then dissolved in water (2 ml) and chromatographed using a column of Sephadex G-25 (1.5 × 50 cm) developed with water (1.2 ml/h). Fractions (1.8 ml) were collected and tested for carbohydrate and amino acid hydrazides.

Two carbohydrate containing fractions were obtained by the above chromatographic procedure. These were dried by lyophilization and weighed. Fraction I (13 mg) was analyzed as follows: (1) part (2 mg) was hydrolyzed using mild conditions (0.01 N HCl, 100 °C, 3 h). The hydrolyzate thus obtained was divided into two parts. One part (2/3) was analyzed by paper chromatography (ethyl acetate, pyridine, water; 8:2:1) using arabinose and hydroxyproline hydrazide as standards and alkaline silver nitrate [22] for development. The other part (1/3) was converted to trimethylsilyl (TMS) derivatives [23] then analyzed by GLC using a 3 % OV-225 column at 110 °C and a SP 1000 column at 130 °C and by GLC-MS using a 3 % OV-1 column at 130 °C. In addition to these GLC procedures, the TMS derivatives were analyzed by GLC-MS as described for cylic (4-*trans*-hydroxy-L-prolyl) -4-*trans*-hydroxy-L-proline. (2) Part (2.0 mg) of fraction I was hydrolyzed using strong conditions (1.0 M HCl, 100 °C, 6 h) and the products analyzed by GLC-MS as TMS derivatives using the same procedures as described above for the products of mild hydrolysis. (3) Part (1.5 mg) was used for molecular weight determination using a column of Sephadex G-50, G-75 calibrated with oligosaccharides obtained from dextran.[24] (4) Part (1.5 mg) was hydrolyzed (0.01 M HCl, 100 °C, 3 h) and the molecular weight distribution of the hydrolyzate determined as described above. (5) Part (2 mg) was methylated and analyzed as described under methylation analysis.

Fraction II (8 mg) obtained by column chromatography of the hydrazinolysate was investigated by methylation analysis which used 2 mg of this material.

The paper chromatography and GLC described above for fraction I required hydroxyproline hydrazide and cyclic (4-*trans*-hydroxy-L-prolyl)-4-*trans*-hydroxy-L-proline as standards. The first compound was synthesized by hydrazinolysis of L-hydroxyproline methyl ester; the structure of the product was verified

by nuclear magnetic resonance and MS. The cyclic compound was synthesized from L-hydroxyproline methyl ester as described by D'Alagni and Pispisa [25] and the product had a melting point of 242–247 °C (decomp.). A 3 % OV-1 column at a starting temperature of 160 °C increasing to 240 °C at 10 °C/min was used for GLC-MS of the silyl derivative of this compound.

Smith degradation. The remaining parts of fractions I and II (4 and 6 mg, respectively) were degraded using the Smith procedure as described by Lindberg *et al.*[26] with the following modifications. After periodate oxidation, the product was purified by gel filtration using Sephadex G-25 (1.5 × 50 cm) and copper reduction [27] for monitoring fractions. The oxidized product was reduced with sodium borohydride and excess reagent removed by ion exchanging sodium with Dowex 50 and codistilling the boric acid thus generated with methanol. Before addition of Dowex 50, the solution was cooled to 2 °C and maintained at this temperature until the methanol codistillation step which was done at 30 °C under reduced pressure. After methylation, part of fractions I and II (1.5 and 2 mg, respectively) was hydrolyzed, converted to alditol acetate derivatives and analyzed by GLC while the remainder of each fraction was treated with 50 % aqueous acetic acid (100 °C, 1 h), reduced ($NaBH_4$ in dioxane-ethanol, 3:1), remethylated (CD_3I) then analyzed by GLC.

Xylanase treatment. Glycoprotein I extracted at 2 °C from *P. vulgaris* var. Black Valentine (24 mg) was suspended in 0.1 M acetate buffer (pH 5.5, 2 ml) and xylanase (14 μg in 0.1 ml water) was added. The mixture was incubated at 37 °C while the production of reducing groups was measured every 30 min for 4 h. Incubation was continued for 12 h to insure complete hydrolysis; then the hydrolyzate was converted to alditol acetate derivatives and analyzed by GLC. The xylanase was produced by *Trichoderma viride* (All Japan Biochemicals Co., Nishinomiya, type Onozuka SS) and isolated as described by Sinner. [28] The preparation had the following specific activities: xylanase 101.5; CM cellulase 0.22; mannanase 0.00; β-xylosidase < 0.02; β-glucosidase; < 0.03 and β-galactosidase; 0.00.

RESULTS

Extraction and purification. Separation of glycoproteins by gel filtration produced two

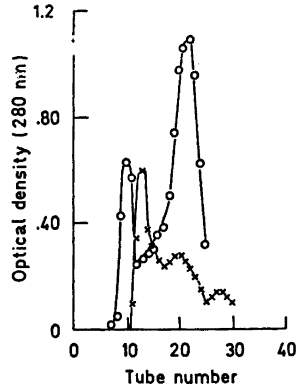


Fig. 1. Absorption (280 nm) of diluted fractions obtained by column chromatography using Sephadex G-150 of glycoproteins I (preparations 1) (○) or glycoproteins II (×). Glycoproteins I: peak 1 (tubes 8–12 incl.); peak 2 (tubes 18–25 incl.). Glycoprotein II: peak 1 (tubes 11–16 incl.); peak 2 (tubes 18–23 incl.). Yields: glycoprotein I; 66 mg placed on column; peak 1, 26 mg; peak 2, 21 mg. Glycoprotein II; 46 mg placed on column; peak 1, 22 mg; peak 2, 16 mg.

Table 1. Carbohydrate and protein content of glycoproteins.

Sample	Preparation No.	Glycoproteins I, % Carbohydrate	Protein[a]	Glycoproteins II, % Carbohydrate	Protein[a]
Peak 1	1	12	–	14	–
	2	17	74	16	77
	3	–	–	12	74
	4[b]	–	–	4	–
Peak 2	1	6	–	11	–
	2	7	76	13	77
	3[b]	–	–	4	–

[a] Protein was measured by nitrogen analysis and assuming a nitrogen content of 13.65 % based on the amino acid analysis. [b] Extracted at 24 °C.

Table 2. Sugar composition of glycoproteins.

Sample	Preparation No.	Glycoproteins I, % Ara[a]	Xyl	Man	Gal	Glc	Glycoproteins II, % Ara	Xyl	Man	Gal	Glc
Peak 1	1	39	47	2	6	6	18	2	2	4	73
	2	14	61	18	4	3	15	59	12	6	8
	3	–	–	–	–	–	31	–	13	16	40
	4[b]	–	–	–	–	–	33	7	7	17	36
Peak 2	1	14	43	2	14	24	6	11	3	2	78
	2	24	47	5	6	17	5	21	8	5	61
	3[b]	–	–	–	–	–	52	7	9	15	17

[a] Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose and Glc, glucose. [b] Extracted at 24 °C.

fractions (Fig. 1). Each was homogeneous by disc gel electrophoresis and showed coincident protein and carbohydrate bands. Although there was variation in the degree of separation and relative amounts of peak 1 and 2 in subsequent preparations, all showed a similar distribution of components during gel filtration.

Chemical analysis. Carbohydrate and protein accounted for most of the weight of the glycoprotein preparations (Table 1). The sugar composition of the glycoproteins indicated that more variation occurred in glycoproteins II than glycoproteins I (Table 2). The most abundant sugars were arabinose and xylose although in some cases glucose accounted for a considerable portion of the carbohydrate content, particularly in glycoproteins II. Colourimetric tests for uronic acids using native glycoproteins I and amino sugars after acid hydrolysis indicated that the content of these sugars was less than 0.5 % for uronic acids and 0.1 % for amino sugars. In addition, the

Table 3. Amino acid composition of glycoproteins and glycopeptides (Preparation 1).

Amino acid	Molar ratio Glycoprotein I Peak 1	Glycoprotein II Peak 1	Glycoprotein II Peak 2	Glycopeptides[a] Peak 1	Glycopeptides[a] Peak 2
Lysine	8	10	7	9	9
Histidine	2	1	1	7	6
Ammonia	13	90	84	15	20
Arginine	4	5	4	3	3
Unknown[b]	trace	trace	trace	10	8
Hydroxyproline	trace	trace	trace	18	9
Aspartic acid	8	11	11	7	12
Threonine	4	5	7	3	6
Serine	5	10	7	9	9
Glutamic acid	9	14	13	4	8
Proline	4	6	6	4	4
Glycine	7	14	20	9	13
Alanine	8	6	6	1	11
Valine	6	9	8	5	7
Methionine	1	1	1	–	–
Isoleucine	5	5	4	2	3
Leucine	10	9	8	3	5
Tyrosine	3	3	3	3	2
Phenylalanine	4	4	4	2	2

[a] Glycopeptides produced by pronase treatment of glycoprotein I (peak 1) and separation of the carbohydrate containing fractions by gel filtration. [b] The molecular weight and colour coefficient for ninhydrin were assumed to be the same as hydroxyproline for calculating molar ratios.

Table 4. Methylation analysis of glycoproteins (preparation 2).

Sugar[a]	Tg[b] SP 1000	ECNSS-M	OV-225	Molar proportions (%) Glycoprotein I Peak 1	Glycoprotein I Peak 2	Glycoprotein II Peak 1	Glycoprotein II Peak 2
2,3,5-Tri-*O*-Me-Ara	0.55	0.48	0.45	2	—	—	1
2,3,4-Tri-*O*-Me-Xyl	0.74	0.66	0.55	2	2	3	5
3,5-Di-*O*-Me-Ara	0.92	0.91	0.80	—	8	2	3
2,5-Di-*O*-Me-Ara	0.96	1.06	0.89	—	16	1	1
2,3,4,6-Tetra-*O*-Me-Glc or -Man	1.00	1.00	1.00	2	—	1	7
2,3-Di-*O*-Me-Xyl	1.20	1.54	1.19	38	33	57	26
2,3,6-Tri-*O*-Me-Glc	1.84	2.48	2.29	3	1	3	43
Ara	—	—	—	3	4	5	—
Xyl	—	—	—	9	25	—	—
Man	—	—	—	17	—	22	—
Gal	—	—	—	3	—	2	—
Glu	—	—	—	19	—	5	—

[a] See Table 2 for abbreviations. [b] Tg is the retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

sugar composition of glycoprotein I (peak 1) remained unchanged following reduction,[29] further indicating the absence of uronic acids.

The glycoproteins had similar amino acid compositions (Table 3). The ammonium content was high in glycoproteins II following hydrolysis, otherwise the analyses showed only minor variation. All analyses indicated the presence of only traces of hydroxyproline and a more acidic unknown component which reacted with ninhydrin to produce a product absorbing more strongly at 440 nm than at 520 nm.

Methylation analysis. All preparations of glycoproteins were subjected to methylation analysis.The results presented for glycoproteins I had the best agreement between the sugar composition before methylation and recovery of *O*-methyl derivatives; however, methylation of preparation 1 yielded the same methylated sugars. Unmethylated sugars were present in glycoproteins I after two treatments using the Hakomori procedure (Table 4). Although unmethylated sugars were detected, no peaks were found during GLC–MS which would indicate mono-*O*-methylated pentoses or di-*O*-methylhexose; that is, the residues appeared to be methylated completely or not at all. Attempts to improve efficiency by pronase digestion and mild acid hydrolysis (0.01 N HCl, 100 °C, 3 h) before methylation were unsuccessful; that is, the methylation analyses after these treatments were very similar to the analyses obtained using native glycoproteins. Glycoprotein II (preparation 2, peak 1) had about the same composition of methylated sugars as glycoprotein I. Glycoproteins II having glucose as the most abundant sugar contained more 2,3,4,6-tetra-*O*-methylhexose and 2,3,6-tri-*O*-methylglucose; the ratio of tetra to tri was in the range 1 to 3 – 7.

No unmethylated sugars were detected following methylation of the glucose-rich preparation (glycoprotein II, peak 2) indicating that methylation was complete.

2,3,5-Tri-*O*-methylarabinose indicated terminal arabinofuranose units whereas 3,5- and 2,5-di-*O*-methylarabinose demonstrated (1→2)- and (1→3)-linked arabinofuranose residues, respectively. 2,3,4-Tri-*O*-methylxylose indicated terminal xylopyranose units and 2,3-di-*O*-methylxylose could have arisen from (1→5)- or (1→4)-linked residues. 2,3,4,6-Tetra-*O*-methylhexose demonstrated terminal glucose or mannose residues and 2,3,6-tri-*O*-methylglucose revealed the presence of (1→4)- or (1→5)-linked glucose residues. Since mild acid hydrolysis of glycoproteins I and II released only arabinose, xylofuranose and glucofuranose residues are not present.

Pronase treatment. Glycoprotein I (peak 1) was hydrolyzed with pronase and two glycopeptide fractions isolated. Amino acid analysis (Table 3) indicated that the glycopeptide fractions were enriched in hydroxyproline and

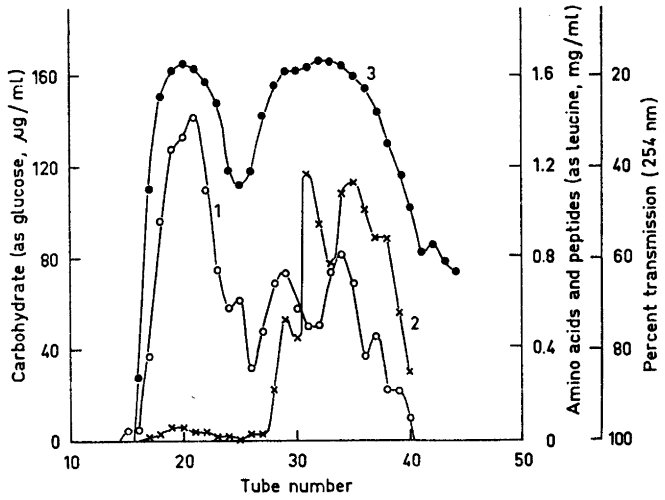


Fig. 2. Gel filtration (Sephadex G-150) of pronase digest of glycoprotein I. Yield: 32 mg (tubes 17 to 25 incl.) of 253 mg placed on column. Curve 1: carbohydrate. Curve 2: peptides and amino acids. Curve 3: absorbance at 254 nm.

an unknown compound. This suggested that hydroxyproline was important for linkage of carbohydrate and protein moieties. This procedure removed 80 – 90 % of the protein, therefore, a larger quantity of glycoprotein I was hydrolyzed with pronase and the products of hydrolysis fractionated by gel filtration (Fig. 2). The glycopeptide fraction was treated with hydrazine without analysis.

Hydrazinolysis. Gel filtration of the products of hydrazinolysis resulted in the isolation of two fractions rich in carbohydrate (Fig. 3). Paper chromatography of fraction I, $[\alpha]_{578}$ + 8 °, after mild acid hydrolysis resulted in the detection of arabinose (R_F 0.36) but no hydroxyproline hydrazide (R_F 0.54). In addition, arabinose was the only sugar detected by GLC after mild hydrolysis (Table 5). Acid hydrolysis with 1 M HCl released xylose and glucose in addition to arabinose. GLC indicated that cyclic (4-*trans*-hydroxy-L-prolyl)-4-*trans*-hydroxy-L-proline was not present in either hydrolyzate although more volatile unidentified components were detected. The unidentified compounds gave mass spectra which were similar to each other and indicated that they were not carbohydrate derivatives.

Molecular weight determination of fraction I before and after mild acid hydrolysis indicated that before hydrolysis, $\overline{M}_w$ was 27 800 and $\overline{M}_n$ was 15 900. The ratio $\overline{M}_w/\overline{M}_n$ was 1.75 indicating a heterogeneous distribution of chain lengths. The highest fragment had a molecular weight of 48 800, whereas the smallest fragment had a molecular weight of 3 360.

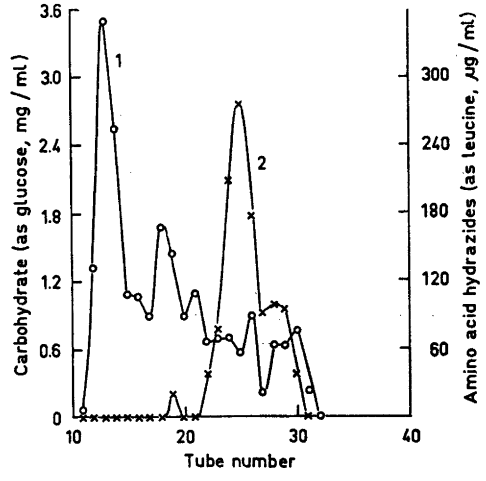


Fig. 3. Fraction of hydrazinolysate by gel filtration (Sephadex G-25). Yields: Fraction I; 13 mg (tubes 11 – 16 incl.) and fraction II; 8 mg (tubes 17 – 20 incl.). Curve 1: carbohydrate. Curve 2: amino acid hydrazides.

Table 5. Analysis of trimethylsilylated acid hydrolyzates of fraction I obtained by hydrazinolysis.

Compound	Ta[c]		Ta (standards)		% [d]	
	OV-225	SP 1000	OV-225	SP 1000	Mild acid	Strong acid
Arabinose[a]	1.00	1.00 (1.08)	1.00	1.00 (1.10)	69	31
Arabinose[a]	1.40	1.50 (1.73)	1.40	1.54 (1.72)		27
Xylose	1.54	1.80	1.53	1.75	–	14
Xylose	1.96	2.82	1.95	2.79	–	4
Mannose	–	–	1.53	–	–	–
Mannose	–	–	2.76	–	–	–
Glucose[b]	2.48	–	2.57	–	–	–
Glucose[b]	4.50	–	4.61	–	–	–
Unknown 1	1.17	0.96	–	–	12	12
Unknown 2	–	1.29	–	–	6	6
Unknown 3	–	2.15	–	–	13	–

[a] The figures in brackets indicate additional peaks found after mild hydrolysis of fraction I and, in the case of the standards, phenyl-α-L-arabinofuranoside. [b] Glucose was identified separately using a column of OV-225 at 130 °C and the quantity of this sugar is not included in the calculation of percent. [c] Retention time relative to silylated β-arabinose. [d] Percent obtained by integration and uncorrected for detector response.

After mild hydrolysis $\overline{M}_w$ was 8 990, $\overline{M}_n$ was 1 600 and the ratio $\overline{M}_w/\overline{M}_n$ was 5.6 indicating very large differences in chain length. The highest molecular weight was 34 490 whereas the lowest was 170.

Methylation analysis of fraction I (Table 6, column a) indicated the presence of terminal arabinofuranose and glucopyranose units. In addition (1→2)- and (1→3)-linked arabinofuranose units were demonstrated. 2,3-Di-*O*-methylarabinose could have arisen from either (1→4)- or (1→5)-linked arabinose; however, since arabinose occurs in the furanose form when (1→2)- and (1→3)-linked, the latter possibility is considered to be more likely. The presence of 2,3-di-*O*-methylxylose also indicated (1→4)- or (1→5)-linkages; however, the result of mild acid hydrolysis eliminates the latter possibility.

Methylation of fraction II (Table 6, column d) demonstrated terminal arabinofuranose in addition to (1→2)- and (1→3)-linked arabinofuranose units.

Smith degradation. Analysis of the products of the modified Smith degradation prior to treatment with 50 % acetic acid indicated that all terminal glucose, (1→4)-linked xylose, and (1→5)-linked arabinose residues of fraction I had been oxidized (Table 6, column b). The presence of 2,3,5-tri-*O*-methylarabinose in both

Table 6. Methylation analysis of fractions I and II obtained by hydrazinolysis.

Sugar[a]	Tg[b]			Molar proportions (%)					
	SP 1000	ECNSS-M	OV-225	Fraction I			Fraction II		
				a	b	c	d	e	f
2,3,5-Tri-*O*-Me-Ara	0.55	0.48	0.45	21	22	55	20	16	55
3,5-Di-*O*-Me-Ara	0.92	0.91	0.80	13	28	–	30	34	7
2,5-Di-*O*-Me-Ara	0.96	1.05	0.89	24	50	45	50	50	37
2,3,4,6-Tetra-*O*-Me-Glc	1.00	1.00	1.00	8	–	–	–	–	–
2,3-Di-*O*-Me-Ara	1.12	1.35	1.07	12	–	–	–	–	–
2,3-Di-*O*-Me-Xyl	1.20	1.54	1.19	22	–	–	–	–	–

[a+b] See Table 4.

fractions (columns b and e) indicated that some of the modified sugar residues had been hydrolyzed probably by acid generated when Dowex 50 was used to remove excess sodium borohydride. Following hydrolysis with 50 % acetic acid and remethylation, an increased content of 2,3,5-tri-*O*-methylarabinose was observed with a corresponding decrease of 3,5-di-*O*-methylarabinose (columns c and f). These results indicate that most terminal arabinose units were linked to O-2 of adjacent arabinose residues.

Effect of variety variation. Phaseolus vulgaris var. Black Valentine was sham-inoculated, extracted at 2 °C then subsequently at 24 °C and glycoproteins I (peak 1) isolated by gel filtration. Glycoprotein I extracted at 2 °C contained 5 % carbohydrate and 70 % protein, whereas the material extracted at 24 °C contained 7 % carbohydrate and 51 % protein. The sugar composition (Table 7) was similar to other preparations of glycoprotein I. Methylation analysis (Table VIII) indicated the same glycosidic linkages as found in glycoproteins isolated from *P. vulgaris* var. Pinto.

Treatment of glycoprotein I extracted at 2 °C with xylanase produced reducing groups equivalent to 416 μg xylose (theoretical yield, 400 μg). GLC indicated that the hydrolyzate contained xylose and a smaller quantity of arabinose.

Table 7. Sugar composition (%) of glycoproteins I from *P. vulgaris* var. Black Valentine.

Sugars	2 °C[a]	24 °C[a]
Arabinose	41	26
Xylose	31	17
Mannose	13	22
Galactose	6	7
Glucose	9	28

[a] Temperature of extraction.

Table 8. Methylation analysis[a] of glycoproteins I from *P. vulgaris* var. Black Valentine.

Sugars	Molar proportions (%) 2 °C	24 °C
2,3,5-Tri-*O*-Me-Ara	11	7
2,3,4-Tri-*O*-Me-Xyl	5	14
3,5-Di-*O*-Me-Ara	6	–
2,5-Di-*O*-Me-Ara	11	13
2,3-Di-*O*-Me-Ara	8	13
2,3-Di-*O*-Me-Xyl	53	16
2,3,6-Tri-*O*-Me-Glc	–	13

[a] See Tables 4 and 6 for abbreviations and Tg values.

DISCUSSION

Following mechanical introduction of plant viruses, glycoproteins have been isolated and characterized [4] and their production studied.[5] The aim of the present work was to characterize glycoproteins produced when virus was omitted but leaves were brushed with Celite in the normal manner (sham-inoculation). During this investigation it became apparent that differences in glycoprotein composition occurred. To determine the cause of this variability, the effect of extraction temperature was investigated as well as duplicate samples from sham-inoculated plants and triplicate samples from TNV-inoculated plants. In addition, two varieties, Pinto and Black Valentine, were used for the sham-inoculation experiments.

Glycoproteins extracted at 2 °C from *P. vulgaris* var. Pinto after sham- or TNV-inoculation had about 14 % carbohydrate whereas material extracted at 24 °C contained less carbohydrate and consequently was not investigated further. Extraction at 2 °C and 24 °C of *P. vulgaris* var. Black Valentine after sham-inoculation, produced glycoprotein preparations having lower carbohydrate content than similar preparations from the Pinto variety. The low protein content of material extracted from Black Valentine at 24 °C indicated that more impurities were extracted at the higher temperature. The sugar composition of glycoproteins extracted after sham-inoculation was approximately the same regardless of which variety was used. Glycoproteins extracted after TNV inoculation had a more variable sugar composition which may be caused by the disruption of cellular processes by the virus following mechanical inoculation. Generally, the presence of virus caused a decreased content of xylose with a corresponding increase in

glucose. The amino acid composition of glycoproteins from *P. vulgaris* var. Pinto were similar to each other, to other analyses,[4] to glycoproteins from *P. vulgaris* var. Black Valentine,[30] and except for the low content of hydroxyproline to plant cell wall glycoproteins.[11] Methylation analysis of glycoproteins from Pinto after TNV-inoculation and both varieties after sham-inoculation indicated that the same glycosidic linkages were present in all preparations. Investigation of glycoproteins rich in glucose indicated that this sugar occurred as either terminal or (1→4)-linked residues. The ratio of tetra to tri-*O*-methylglucose indicated that glucose probably occurred as low molecular weight oligosaccharides. Perhaps this low degree of polymerization accounts for the relative ease with which these glycoproteins are fully methylated in contrast to preparations rich in xylose. Xylose was invariably (1→4)-linked whereas arabinose occurred as (1→2)- and (1→3)-linked arabinofuranose units.

Methylation analysis of glycoproteins extracted at 2 °C from *P. vulgaris* var. Black Valentine indicated the same glycosidic linkages as found in Pinto except for the absence of terminal glucose units and the presence of terminal xylose units. Extraction at 24 °C provided material having similar linkages except for the absence of (1→2)-linked arabinofuranose units and the presence of (1→4)-linked glucose. No evidence of incomplete methylation was found in either preparation. Xylose was produced when material extracted at 2 °C was treated with xylanase indicating that β-(1→4)-D-xylopyranose linkages were present.

Treatment of the glycoproteins with pronase hydrolyzed sufficient peptide bonds so that subsequent gel filtration removed about 90 % of the amino acid residues. Amino acid analysis revealed substantial enrichment of hydroxyproline in the carbohydrate containing fractions and implicated this amino acid in carbohydrate-protein linkage. Enrichment of an unidentified acidic component also occurred and the structural significance of this compound remains to be determined.

Hydrazinolysis allowed removal by gel filtration of more of the amino acid residues not attached to carbohydrate and there is considerable evidence[7] which indicates that few, if any, glycosidic linkages are cleaved by this procedure. Nevertheless, some low molecular weight carbohydrate components were lost during gel filtration. Although only results of hydrazinolysis of glycoprotein rich in xylose are presented, the same procedure was applied to glucose-rich glycoproteins; however, the low recovery caused by incomplete separation of carbohydrate from amino acid hydrazides during gel chromatography did not permit further structural studies.

Methylation analysis of the products of hydrazinolysis indicated the presence of the same linkages as found in the original glycoprotein, but in addition, (1→5)-linked arabinofuranose units were found. During methylation analysis of native glycoproteins, GLC revealed many peaks which mass spectrometry demonstrated were not sugars. GLC of the methylated product of hydrazinolysis detected only sugars and all peaks were identified. In addition, there was no evidence of incomplete methylation as occurred using native glycoproteins from Pinto.

The two fractions isolated after hydrazinolysis of Pinto glycoproteins had an average degree of polymerization based on the results of methylation analysis of about 2 for the fraction eluted first and 4 for the fraction eluted second. Furthermore, molecular weight determination by gel filtration indicated a very much higher degree of polymerization than did the methylation analysis. This result suggests that the apparent degree of polymerization is an artifact of hydrazinolysis. Indeed, Heath and Northcote[7] have shown that adjacent hydroxyproline residues in protein will dimerize during hydrazinolysis. However, the cyclic dimerization product was not detected in the hydrolyzates of the products of hydrazinolysis nor was hydroxyproline hydrazide. This discrepancy must involve arabinofuranose linkages since mild acid hydrolysis caused a ten-fold decrease in degree of polymerization as indicated by $\overline{M}_n$.

Smith degradation of the products of hydrazinolysis indicated that terminal arabinose units are linked to O-2 of adjacent residues whereas arabinose units closer to the reducing end are (1→3)-linked. Although the structural significance of the (1→5)-linked arabinose

units is difficult to evaluate, it is probably that (1→4)-linked xylose units are on the perifery and that these chains can be quite long. The low optical rotation of the products before Smith degradation is consistent with β-(1→4)-linked D-xylose units and α-linked L arabinofuranose units. Other studies have demonstrated conclusively the presence of L-arabinofuranose [2,8] in plant derived material which is α-linked. [2,8,31]

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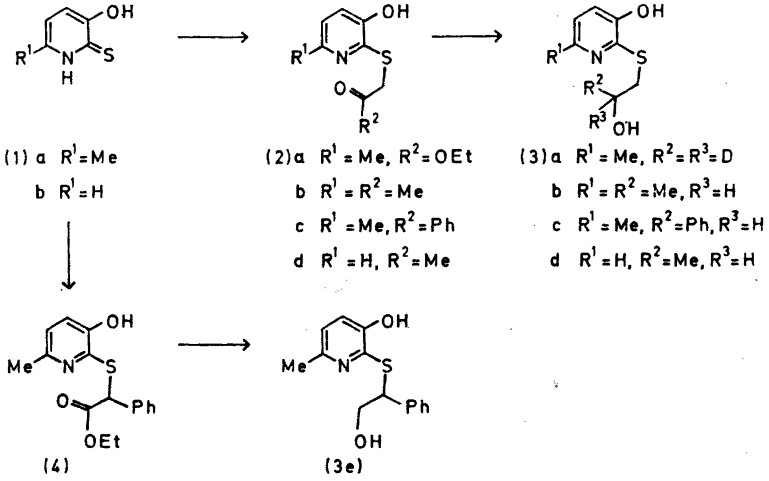
N-Quaternary Compounds. Part XLII.[1] Competitive Neighbouring Group Participation in Cyclisation Reactions of 2-Pyridylthioethers

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2-β-Hydroxyalkylthiopyridines in cold thionyl chloride react to yield the corresponding cyclised dihydrothiazolo[3,2-*a*]pyridinium derivative and a mixture of two 2-β-chloroalkylthiopyridine isomers. Further rearrangements may occur on cyclisation of the latter. The product compositions depend on the nature of the substituents and is in part rationalised by an episulfonium intermediate. Acid catalysed cyclisation of a 2-vinylthiopyridine, a 2-allylthiopyridine, and of 2-β-hydroxyalkylthiopyridines, however, proceeded without rearrangement.

Sulfur neighbouring group participation [2–5] has been used to explain the formation of a mixture of 2- and 3-carboxydihydrothiazolo[3,2-*a*]-pyridinium derivatives in the cyclisation of a 2-bromo-3-(2-pyridylthio)propionic acid.[6] Analogous reactions have been reported in that α-chloro-β-alkylthio- or β-arylthiopropionitrile or corresponding propionates react with nucleophiles to preferentially yield the product with the thioether group in the α-position.[2] The reaction path is more complicated on substitution of the side-chain β-carbon in 2-ethylthiopyridines since neighbouring group participation is possible from either the thioether sulfur atom or the pyridine nitrogen atom. Some ambiguity may also be attached to the role of the carboxy group in the cyclisation reaction described [6] although the carboxy group is largely un-ionised in acid solution.[3] Nevertheless, we have in the present work excluded the carboxy function from the sulfur side-chain. In the simplest example the side-chain carries only the leaving group on the β-carbon atom (Scheme 1). In order to distinguish



Scheme 1.

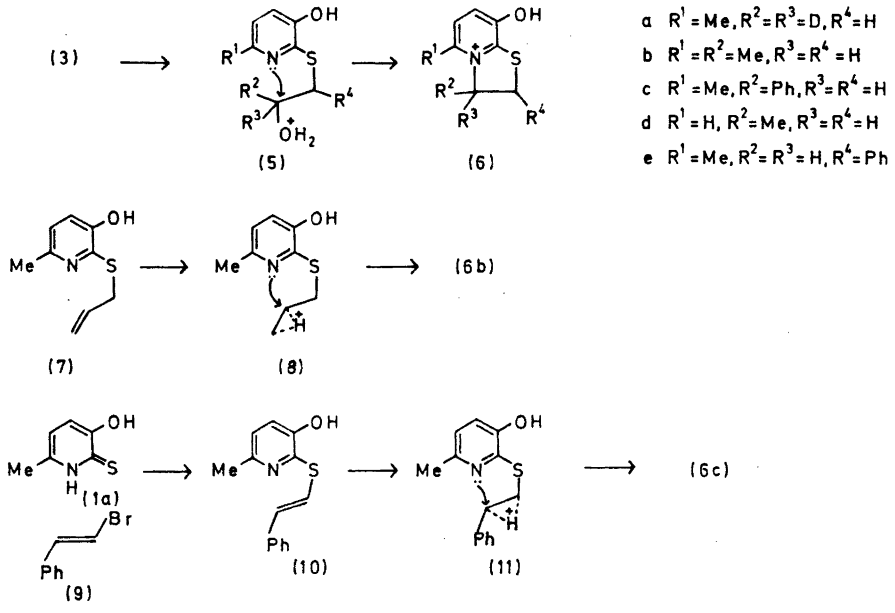
between the two methylene groups in the reaction product the terminal methylene group was deuteriated *3a*. The influences of alkyl and aryl substituents in the side-chain on the course of the cyclisation reaction of β-hydroxy derivatives have been studied.

The β-hydroxy derivatives were prepared as shown in Scheme 1. The thiolactams *1* are selectively alkylated on the sulfur atom.[7] Sodium borohydride reduction of the ketones *2b – d* thus prepared furnished the α-substituted derivatives *3b – d*; lithium aluminium hydride reduction of the ester *4* yielded the α-substituted derivative *3e*. The ester *2a* was reduced by lithium aluminium deuteride to the β,β-dideuterio derivative *3a*.

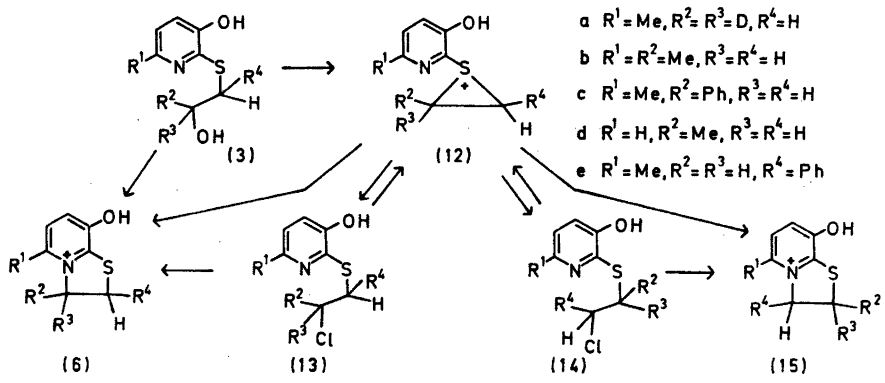
It has been found that direct substitution of the hydroxy group in *3* can be effected by heating in acetic acid or formic acid. In strong hydrochloric acid, however, little cyclisation took place presumably because the pyridine is protonated in strong acid solution. The product formed corresponds to a direct cyclisation reaction of 3; in no case was the rearranged isomer *15* (Scheme 3) seen (NMR). It is conceivable,[2–4] however, that some of the cyclic product may have been formed by acid catalysed cyclisation of a vinyl intermediate generated initially by water elimination. Therefore the synthesis of a suitable *S*-vinyl compound was developed by heating the thiolactam *1a* and β-bromostyrene together in acetic acid at 80 °C. The *S*-vinyl product *10* was indeed cyclised to *6c* on heating in acetic acid at elevated temperatures which shows that *S*-vinyl intermediates are possible. The vinylation reaction (*9→10*) corresponds to an overall nucleophilic displacement of the bromine atom; the substitution pattern is governed by the electron accepting properties of the phenyl ring.

In a related acid catalysed reaction the allyl derivative *7* was cyclised to *6b* without rearrangement (NMR) by heating in acetic acid. This reaction is analogous to our previously reported preferential five-ring cyclisation of *7* by bromination.[7] The generally more rapid formation of five- than six-membered rings [3] may be ascribed to the more negative activation entropy for the latter process.[8]

In contrast to the acid catalysed cyclisation reactions which proceeded without isomerisations, the reactions in thionyl chloride have yielded mixtures of isomeric products (Scheme 3). The compositions of the crude reaction products were determined by NMR. The



Scheme 2.



Scheme 3.

magnetic non-equivalence of the methylene protons on the α-carbon atom in the *S*-side-chain in the substituted chloro derivatives *13* and *14* and in the corresponding dihydro-thiazolo[3,2-*a*]pyridinium derivatives often complicated the spectral analyses due to overlap of the proton signals. Fortunately the chemical shifts of the protons in the methyl and phenyl substituents differ slightly before and after cyclisation and have also been used in the composition analyses. The structural assignments of the isomeric quaternary products are ascertained by comparison with authentic 2-substituted isomers previously prepared by non-ambiguous methods.[9,10] In the case of the deuteriated derivative *3a* identification of the *N*-quaternary product formed was evident from the relative chemical shifts of the methylene protons.[11]

Analogues of the β-halides *13* and *14* are readily cyclised.[6] The reactions in thionyl chloride were therefore run in the cold; the reaction time was 12–14 h. The deuteriated hydroxy derivative *3a* yielded a mixture of the *N*-quaternary compound *6a* and the chlorinated derivative *13a* in the ratio 6:1; no rearranged isomer (*14* or *15*) was seen (NMR). Heating the crude product mixture in ethyl acetate for 5 h, however, gave the quaternary isomers *6* and *15* in the ratio 20:1. Consequently some of the chloro compound *13a* has been cyclised *via* an episulfonium intermediate *12*. From the β-methyl derivative *3b* the amount of cyclised *N*-quaternary product was almost negligible while the 6-desmethyl analogue *3d* yielded about 10 % of the cyclised product *6d*. The major products in both these cases were the chloro isomers *13* and *14* which were formed in the approximate ratio 3:2. It seems likely that steric retardation of the cyclisation reaction is a major reason for the formation of the chloro isomers. The β-phenyl derivative reacted similarly with 20 % cyclisation to the 3-isomer *6c*; the major products were the chloro isomers *13c* and *14c* in the ratio 2:1. The α-phenyl derivative (*3e*) yielded about 20 % of the cyclised isomer *6e*. The reaction in this case yielded several products but the major components were the chloro isomers *13e* and *14e* in the ratio 1:2; on heating the mixture of the chloro compounds in ethyl acetate cyclisation occurred almost exclusively to the 3-phenyl isomer *15e*. The same product *6c* was obtained from the chloro isomer mixture derived from the β-phenyl derivative *3c*. The isomer ratios in the β-methyl series (*b* and *d*) were little affected on cyclisation of the chloro isomer mixture (*13* and *14*).

The product compositions are interpreted in terms of reaction paths which may not, or may go partially or almost completely through an episulfonium intermediate *12*. The formation of an episulfonium intermediate shows that sulfur neighbouring group participation is involved in the reaction. The possibility also exists for neighbouring group participation from the pyridine nitrogen atom. Participation from either heteroatom in its initial stage leads to partial bonding to the reaction centre with the formation of an internally solvated ion pair. Collapse of such a five-membered ring structure leads directly to the stable dihydrothiazolo-

pyridinium system in which case no rearranged isomer is formed. Collapse of the ion pair bonding with the thioether sulfur atom, however, leads to the highly reactive episulfonium intermediate which may suffer addition of the pyridine nitrogen atom to either episulfonium carbon atom and thus give rise to two structural isomers. Chloride ion addition to the reactive intermediates, however, will compete with the cyclisation reactions. The chlorine atom in the cyclisation of *13* and *14* may be displaced by the same type of mechanisms. The episulfonium intermediate *12* formed in the cyclisation of the deuteriated chloro isomer *13a* is symmetrical neglecting the deuterium isotope effect. The intermediate *12* is therefore expected to react with almost the same probability at either carbon atom resulting in an 1:1 product isomer ratio. The small fraction of rearranged product *15a* observed points to effective competitive neighbouring group participation from the pyridine nitrogen atom. In the reaction of the episulfonium carbon atom the nitrogen nucleophile must add *cis* to the sulfur which appears possible if the carbon-sulfur bond is stretched or almost broken so that the sulfur holds the partially charged carbon atom from some distance.

It will be recalled that all the direct cyclisation reactions have occurred without isomerisation. In thionyl chloride initial esterification is expected and the chlorosulfite ester group is then intramolecularly displaced or substituted by a chloride ion in an intra- or intermolecular process. The amount of chloro compound formed seems to increase with β-substitution (*3b – d*) possibly because the rate of cyclisation is decreased due to increase in non-bonded interaction although chain-branching often promotes cyclisation.

Ring opening of the substituted episulfonium intermediate either by the pyridine nitrogen atom or by the chloride ion is expected to occur in such a way that the nucleophile adds to the more stabilised incipient carbonium ion; the product ratio for the β-methyl chloro isomers *13* and *14* were about 3:2. Phenyl group stabilisation (*3c*) increased this ratio to about 2:1 while the ratio for the α-phenyl analogue (*3e*) was about 1:2. Cyclisation of the mixture of chloro derivatives from the phenyl isomers *3c* and *3e* gave only the 3-phenyl substituted cyclised product in accordance with an episulfonium intermediate. The isomer ratios in the chloro compounds, however, indicate that chlorine substitution occurs both by direct substitution and *via* an episulfonium intermediate.

It has been the purpose of this work to show that neighbouring group participation from the thioether sulfur atom is to be expected in the cyclisation of 2-ethylthiopyridines with a suitable leaving group on the β-carbon of the S-side-chain. The extent to which the sulfur participation in the reaction leads to an episulfonium intermediate or to direct cyclisation appears to depend on the nature of the leaving group besides on substituent and solvent stabilisation of the incipient carbonium ion.

EXPERIMENTAL

The NMR spectra were recorded with a Varian A-60A or a Varian A-100 instrument.

Ethyl (3-hydroxy-6-methyl-2-pyridylthio)acetate (2a).*HCl*. Thionyl chloride (7 ml) was added slowly with stirring to ethanol (70 ml) at −70 °C before 3-hydroxy-6-methyl-2-pyridylthioacetic acid (1.0 g, 0.005 mol) was added. The reaction mixture was allowed to reach room temperature overnight. The ethanol was evaporated, the residue triturated with ether and the residue crystallised from ethanol/acetone; yield 0.92 g (70 %), m.p. 155 °C. (Found: C 45.49; H 5.72. Calc. for $C_{10}H_{13}NO_3S.HCl$: C 45.60; H 5.32); δ(TFA) 2.8(Me), 1.3 and 4.5 (OEt), 4.1(*S*-CH_2), 7.6 and 8.0 (AB, 2 H-Py).

The free base, required for the synthesis of *3a*, was isolated by extraction of a neutralised aqueous solution with ether.

Ethyl 2-(3-hydroxy-6-methyl-2-pyridylthio)-phenylacetate (4) was prepared as above from 2-(3-hydroxy-6-methyl-2-pyridylthio)-phenylacetic acid.[12] The isolated free base was crystallised from toluene; yield 88 %, m.p. 131 °C. (Found: C 63.12; H 5.94. Calc. for $C_{16}H_{17}NO_3S$: C 63.36; H 5.65); δ($CDCl_3$) 2.4(Me), 1.2 and 4.1(OEt), 5.4(CH), 6.8 and 7.0(AB, 2 H-Py).

2-Phenacylthio-3-hydroxy-6-methylpyridine (2c). Potassium carbonate (20.0 g, 0.145 mol) was added to a solution of 3-hydroxy-6-methylpyrid-2-thione (16.4 g, 0.116 mol) in dimethylformamide (150 ml) followed by slow addition of ω-bromoacetophenone (26.4 g, 0.133 mol). The reaction mixture was stirred at room temperature overnight before filtration. The title compound was precipitated on dilution of the filtrate with water and pH adjustment to *ca.* 5; yield 29.1 g (95 %), m.p. 145 °C after recrystallisation from toluene. (Found: C 64.75;

H 5.19. Calc. for $C_{14}H_{13}NO_2S$: C 64.86; H 5.05); δ(TFA) 2.9(Me), 4.8(CH_2), 7.5–8.2(5 H-Ph, 2 H-Py).

2-β-Hydroxy-α-propylthio-3-hydroxy-6-methylpyridine (3b). Sodium borohydride (10.0 g, 0.26 mol) was added to a solution of 2-acetonylthio-3-hydroxy-6-methylpyridine [7] (10.0 g, 0.05 mol) in methanol (200 ml). The solution was left at room temperature for 1 h before evaporation of most of the methanol. Water was then added and the pH adjusted to *ca.* 5 before chloroform extraction. Evaporation of the washed and dried chloroform extracts left the title compound (9.1 g, 90 %); m.p. 117 °C after recrystallisation from toluene (Found: 53.88; H 6.49. Calc. for $C_9H_{13}NO_2S$: C 54.26; H 6.58); δ(TFA) 1.5(d, *J* 6.5 Hz, *Me*–CH), 2.7(Me–6), 3.4(S-CH_2), 4.5(m, CH), 7.5 and 7.8(AB, 2 H-Py).

2-β-Hydroxy-β-phenylethylthio-3-hydroxy-6-methylpyridine (3c).*HCl* was prepared from 2-phenacylthio-3-hydroxy-6-methylpyridine as above. The product was crystallised as the HCl-salt from a small volume of ethanol at −20 °C; yield 80 %, m.p. 183 °C. (Found: C 57.18; H 5.41. Calc. for $C_{14}H_{15}NO_2S$.HCl: C 57.48; H 5.37); δ(TFA) 2.7(Me), 3.6(d, CH_2), 5.3(t, CH), 7.4(5 H-Ph), 7.8 and 7.4(AB, 2 H-Py).

2-β-Hydroxy-α-propylthio-3-hydroxypyridine (3d) was prepared from 2-acetonylthio-3-hydroxypyridine [7] in 91 % yield, m.p. 101 °C (diisopropylether). (Found: C 52.27; H 6.11. Calc. for $C_8H_{11}NO_2S$: C 51.88; H 5.99); δ(TFA) 1.5(d, Me), 4.6(m, CH), 3.2–3.5(S-CH_2), 7.5–8.2(2 H-Py).

2-β-Hydroxy-α-phenylethylthio-3-hydroxy-6-methylpyridine (3e). Lithium aluminium hydride (0.75 g, 0.02 mol) was added gradually to a solution of ethyl 2-(3-hydroxy-6-methyl-2-pyridylthio)phenylacetate (1.52 g, 0.005 mol) in anhydrous ether (100 ml). The reaction was stopped by ethyl acetate addition after 1 h. After addition of water and neutralisation the title compound was extracted into ethyl acetate which was washed and dried before evaporation. The residual crystalline material weighed 1.10 g (77 %) and had m.p. 135 °C. (Found: C 64.60; H 5.89. Calc. for $C_{14}H_{15}NO_2S$: C 64.36; H 5.79); δ($CDCl_3$) 2.4(Me), 4.1(d, *J* 6.5 Hz, CH_2), 4.6(t, CH), 7.2(5 H, Ph) 6.8 and 7.0(AB, 2 H-Py).

2-β,β-Dideuterio-β-hydroxyethylthio-3-hydroxy-6-methylpyridine (3a) was prepared as above from ethyl (3-hydroxy-6-methyl-2-pyridylthio)-acetate using lithium aluminium hydride-d_4; the non-deuteriated material has previously been described.[13]

3,5-Dimethyldihydrothiazolo[3,2-a]pyridinium-8-oxide (6b).*HCl by acid cyclisation of* 7. 2-Allylthio-3-hydroxy-6-methylpyridine [7] (1.82 g, 0.01 mol) was dissolved in acetic acid (150 ml) and the solution heated in a pressure vessel at 145 °C overnight. The solution was then evaporated, the residue triturated with ether and the remaining solid heated in 6 N HCl for 30 min before evaporation. Recrystallisation of the solid residue furnished the title compound; yield 1.17 g (54 %), m.p. 277 °C (decomp.). (Found: C 49.87; H 5.59. Calc. for $C_9H_{11}NOS$.HCl: C 49.65; H 5.56); δ(TFA) 1.7(d, *J* 6.5 Hz, Me-3), 2.8(Me-5), 3.5 and 4.1(AB of ABX, *J* 12,7, *J* 1 Hz, CH_2), 5.7(X of ABX, –CH), 7.3 and 7.6(AB, 2H-Py).

3-Phenyl-5-methyldihydrothiazolo[3,2-a]pyridinium-8-oxide (6c).*HBr by acid catalysed cyclisation of* 10. A solution of 2-β-styrylthio-3-hydroxy-6-methylpyridine.HBr (13.0 g, 0.04 mol) in acetic acid (400 ml) was heated at 150 °C in a pressure bottle overnight. The acetic acid was then distilled off at reduced pressure and the solid residue triturated with acetone before recrystallisation from isopropanol; yield 9.1 g (70 %), m.p. 264 °C (decomp.). (Found: C 52.04; H 4.41. Calc. for $C_{14}H_{13}NOS$.HBr: C 51.86; H 4.35); δ(TFA) 2.5(Me), 3.6 and 4.5(AB of ABX, *J* 12,8 < 1 Hz), 6.6(X of ABX, CH), 7.0–7.5(5 H-Ph, 1 H-Py), 7.8(H-Py).

3-Methyldihydrothiazolo[3,2-a]pyridinium-8-oxide (6d).*HCl. Cyclisation of β-hydroxy derivatives in acetic acid.* A solution of 2-β-hydroxy-α-propylthio-3-hydroxypyridine (1.85 g, 0.01 mol) in acetic acid (30 ml) was heated under reflux overnight. The acetic acid was then evaporated and the residue refluxed for 1 h in 6 N HCl to hydrolyse any acetate formed in the cyclisation reaction. The HCl solution was next evaporated and the solid residue recrystallised from isopropanol; yield 1.10 g (54 %), m.p. 255 °C (decomp.). (Found: C 47.24; H 4.83. Calc. for C_8H_9NOS.HCl: C 47.17; H 4.95); δ(TFA) 1.9(d, *J* 6.5 Hz, Me), 3.5 and 4.1(AB of ABX, *J* 12,7, 6 Hz), 5.5(X of ABX,CH), 7.3–8.3(3 H-Py).

All the 2-β-hydroxyethylthiopyridines (*3*) were found to react in the same way and the products were purified by recrystallisations from isopropanol; yields 50–70 %. NMR analyses of the crude cyclised products showed the rearranged isomer *15* to be absent.

Reactions of 2-β-hydroxyethylthiopyridines (3) *in thionyl chloride solution; formation of* 6/15 *and* 13/14. The 2-β-hydroxyethylthiopyridine (0.05 mol) was dissolved in cold thionyl chloride (30 ml) and the solution left at room temperature for 12–14 h. The reaction mixture was then evaporated at reduced pressure to avoid heating. The residual solid was analysed by NMR in TFA solution.

The mixture of the cyclised product *6* and the chlorinated products *13* and *14* were then heated in ethyl acetate under reflux for 5 h and the solution evaporated before NMR analysis as above.

trans-*2-β-Styrylthio-3-hydroxy-6-methylpyridine* (10).*HBr*. A solution of β-bromostyrene (25.0 g, 0.14 mol) and 3-hydroxy-6-methylpyrid-2-thione (10.0 g, 0.07 mol) in acetic acid (150 ml) was heated at 80 °C for 10 h. The product crystallised from the cold solution and

was recrystallised from acetic acid; yield 15.0 g (66 %), m.p. 200 °C. (Found: C 51.84; H 4.54. Calc. for $C_{14}H_{13}NOS.HBr$: C 51.86; H 4.35); δ(DMSO-d_6) 2.6(Me), 6.9(d, J 16 Hz, $-CH=$), 7.2 – 7.8 ($-CH=$, 5 H-Ph, 3 H-Py).

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Phloroglucinol Derivatives of *Dryopteris crassirhizoma* from Japan

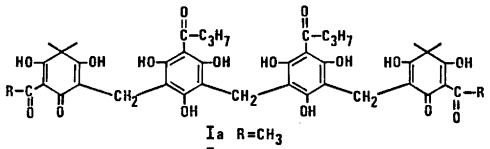
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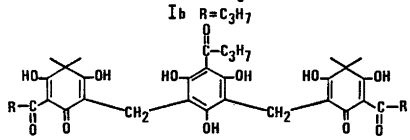
The phloroglucinol composition in *Dryopteris crassirhizoma* Nakai, a Japanese member of the *D. filix-mas* complex, has been investigated. The results are compared with those of European *D. filix-mas* s. str.. *D. crassirhizoma* contains large amounts of dryocrassins of which the homologue ABBA (Ia) predominates and some flavaspidic acid. Filixic acid, one of the main compounds in *D. filix-mas* s. str., has not been detected in *D. crassirhizoma*.

Dryopteris crassirhizoma Nakai is a diploid sexual taxon, which occurs in Japan, Sakhalin, southern Kuriles, Korea and northern China.[1] It is a member of the *Dryopteris filix-mas* complex. The oleoresin of *D. crassirhizoma* has been listed in the Japanese Pharmacopoeia as an anthelminticum.[2] According to chemical investigations of Hisada and Noro,[3] *D. crassirhizoma* contains flavaspidic acid, albaspidin (not in every specimen), filixic acid, and a substance similar to filixic acid (substance F).* This has later been identified as dryocrassin (Ia) (dryocrassin-ABBA) (methylene-bis-norflavaspidic acid-ABBA), a tetracyclic phloroglucinol derivative.[4] It also occurs in *D. polylepis* (Fr. & Sav.) C. Chr.[5,6] The corresponding tricyclic compound, filixic acid-ABA (IIa), has previously been isolated from *D. dickinsii* (Fr. & Sav.) C.Chr.[7,8] and *D. parallelogramma* (Kunze) Alston.[9] Another tetracyclic phloroglucinol, methylene-bis-norflavaspidic acid-BBBB (Ib), has been isolated from *D. austriaca* s. lat. by Penttilä and Sundman.[10]** The corresponding tricyclic compound filixic acid-BBB (IIb), is widely distributed in taxa of the *D. villarii* and *D. filix-mas* complex.[11–12] It has also been isolated in trace amounts from *D. austriaca* s.l. lat.,[13] and from *D. dickinsii*.[7,8]

Material. Rhizomes of *D. crassirhizoma* for chemical analysis were kindly collected by professor K. Iwatsuki, Kyoto University, and Dr. T. Seki, Hiroshima University, Japan. The collection data follow: Japan: Mt. Hiba, alt. *ca.* 1250 m, Hiba-gun, Hiroshima Pref., Aug. 28, 1973, leg. T. Seki (2 rhizomes), Seryntoye, north of Kyoto, alt. *ca.* 400 m, Honshu, Kyoto Pref., July 6, 1973, leg. K. Iwatsuki (*ca.* 10 rhizomes).



Ia R=CH_3
Ib R=C_3H_7



IIa R=CH_3
IIb R=C_3H_7

RESULTS AND DISCUSSION

As in previous investigations [11–12] the phloroglucinol mixture (crude filicin) from *D.*

* For the chemical formulae not presented here, see Refs. 11 and 14.

** The correct botanical source is most probably *D. assimilis* S. Walker or *D. spinulosa* Watt.

Table 1. Composition of the phloroglucinol derivatives in *D. crassirhizoma* and *D. filix-mas*.

Taxon and ploidy	*D. crassirhizoma* 2×	*D. filix-mas* 4×
Source	Japan	Finland
Dryocrassin	+++[a]	−
Filixic acid	−	+++[b]
Flavaspidic acid	+[a]	+++[b]
Albaspidin	−	−
Para-aspidin	−	+[b]
Desaspidin	−	+[b]
Trisdesaspidin	−	+[b]

[a] Mainly consisting of acetylhomologues (see text). [b] Mainly consisting of butyryl- and propionyl-homologues.[11,15]

crassirhizoma was separated by column chromatography on silica gel. Several samples of dryocrassin (I) with slightly different melting points were isolated by us. In addition, flavaspidic acid was detected by thin-layer chromatography. The albaspidin and the filixic acid, detected by Hisada and Noro,[3] were not found. It is possible that these compounds are not natural substances in *D. crassirhizoma*, but have been formed by the so-called rottlerone change during the MgO-treatment in the isolation of crude filicin (*cf.* Ref. 14). The semiquantitative composition of the phloroglucinol derivatives from *D. crassirhizoma* is given in Table 1. The corresponding results for European *D. filix-mas* taken from previous investigations [15] are also included in the table. This taxon is related to *D. crassirhizoma* morphologically, but is known to be tetraploid. Dryocrassin-ABBA (Ia) is the main compound in *D. crassirhizoma*. There are also small amounts of flavaspidic acid present. The phloroglucinol composition of *D. filix-mas* is quite different; flavaspidic acid predominates and much filixic acid is present also. In addition, there are small amounts of para-aspidin, desaspidin and traces of trisdesaspidin. No dryocrassin has been found in *D. filix-mas*. There was no detectable variability in the different rhizomes of *D. crassirhizoma* investigated. The reductive alkaline cleavage of the crude filicin of *D. crassirhizoma* and the investigation of the acylfilicinic acids formed, indicated that this taxon contains virtually only acetyl (A) homologues (95 %) with traces of propionyl (P) homologues (5 %). In distinct contrast to *D. filix-mas* and all other *Dryopteris* species so far investigated,[11] no butyryl (B) homologues were detected.

The mass spectrum of dryocrassin (m.p. 210–215 °C) (Fig. 1) shows a molecular peak at *m/e* 820 corresponding to $C_{48}H_{68}O_{16}$ (dryocrassin-ABBA (Ia)). Weak peaks at *m/e* 848, 834, and 806, combined with the results of chromatography (*cf.* below), suggest the presence of PBBP, PBBA and/or PPBP, and APBA homologues in trace quantities. The fragmentation pattern is very similar to that found earlier for filixic acid-BBB (IIb) and -ABA (IIa).[9,16,17] On analogy with the earlier cases [16,17] the thermic rottlerone change has to be taken into consideration in the present case, too.

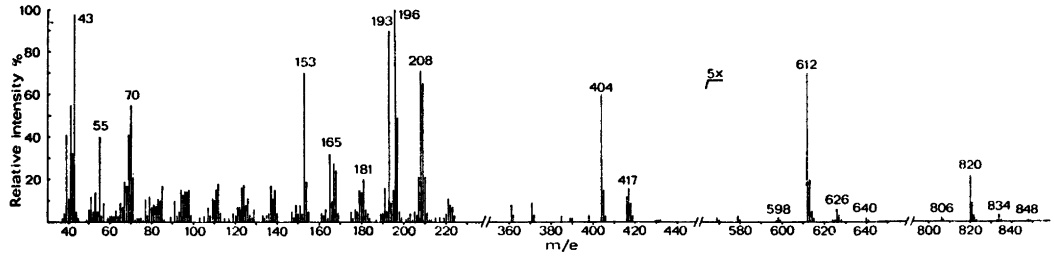


Fig. 1. Mass spectrum (70 eV, *T* 220 °C) of dryocrassin (m.p. 210–215 °C).

Thus, it can be expected *inter alia* that the peaks at *m/e* 612 and 404 are partly due to filixic acid-ABA (IIa) and albaspidin-AA, respectively, thermally formed in the ionization chamber of the mass spectrometer before ionization.

The NMR spectrum ($CDCl_3$) of dryocrassin (m.p. 210–215 °C), showing the following signals, is in full agreement with the proposed structure (Ia). δ 0.98 (6 H, t, $J=7$ Hz, two $-CO-CH_2-CH_2-CH_3$ groups), 1.42 and 1.52 (12 H, each s, two *gem.* dimethyl groups), 1.72 (4 H, m, two $-CO-CH_2-CH_2-CH_3$ groups), 2.74 (6 H, s, two $-CO-CH_3$ groups), 3.18 (4 H, m, two $-CO-CH_2-CH_2-CH_3$ groups), 3.54 (4 H, s, two $C-CH_2-C$ groups), and 3.84 (2 H, s, one $C-CH_2-C$ group). The OH signals are omitted (*cf.* also ref. 4).

Reductive alkaline cleavage of dryocrassin. (*cf.* Ref. 18, Cleavage A). Two main products, namely acetylfilicinic acid and butyrylphloroglucinol were identified by paper and thin-layer chromatography. These were the same monocyclic compounds which were found by Noro *et. al.*[4] However, in agreement with the results from mass spectrometry (see above) and thin-layer chromatography (see below) trace amounts of propionylfilicinic acid were detected too. On the other hand, due to the very similar thin-layer chromatographic behavior of butyrylphloroglucinol and propionylphloroglucinol,[14] the latter could not be detected with certainty.

Thin-layer chromatography. All samples of dryocrassin gave one large spot, R_F 0.30, dryocrassin-ABBA (Ia), when chromatographed on thin-layers buffered to pH 6.0 in hexane-chloroform 1:1.[14,15] However, just visible or small spots due to homologous dryocrassins were present, too. These were tentatively identified as the dryocrassins-PBBP (R_F 0.40), -PBBA and/or -PPBP (R_F 0.38), and -APBA (R_F 0.27) according to the results from mass spectrometry and reductive alkaline cleavage.

The thin-layer chromatographic behaviours of dryocrassin-ABBA (Ia) and -PBBA and/or -PPBP and the corresponding tricyclic and bicyclic compounds, filixic acid-ABA (IIa) and albaspidin-AA, respectively, were also studied on thin-layers buffered to pH 4–8 using gradient technique.[19] As seen from Fig. 2. dryocrassin-ABBA (Ia)* is running slower than filixic acid-ABA (IIa) at every pH tested. Both substances show distinct pH-dependence. However, it appeared that the R_F-value of dryocrassin-PBBA and/or -PPBP* was identical with that of filixic acid-ABA (IIa). Therefore, an accurate identification of these substances and their higher homologues cannot be achieved without isolating crystalline compounds and submitting these to mass spectrometry and reductive alkaline cleavage.

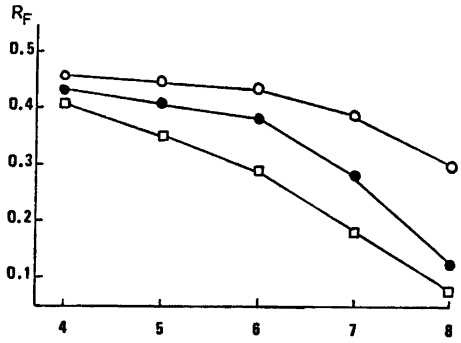


Fig. 2. Gradient TLC of dryocrassin-ABBA (□, brown), dryocrassin-PBBA and/or -PPBP and filixic acid-ABA (●, brown), and albaspidin-AA (○, red). Solvent: hexane-$CHCl_3$, 50:50. Colour with Fast Blue Salt B in parenthesis. Chromatographed 3 × 15 cm.

As apparent from Table 1. *D. crassirhizoma* contains large amounts of dryocrassin-ABBA (Ia) and traces of its homologues. There is most probably no filixic acid-ABA (IIa) present. These facts suggest interesting differences in the biosynthetic pathways of these two substances. So far *D. crassirhizoma* is unique among the representatives of the *D. filix-mas* complex in lacking filixic acid.

The morphology of *D. crassirhizoma* and *D. filix-mas* indicate that these two taxa are closely related. However, our chromatographic results do not suggest that these taxa are conspecific. Their ploidy levels are also different as already pointed out.

* Noteworthy, a slight decomposition of the dryocrassins were observed on the alkaline side of the plates.

EXPERIMENTAL

General. The mass spectrum was recorded on an A.E.I. MS-9 mass spectrometer at the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, through the courtesy of Dr. B. C. Das. The NMR spectrum was recorded on an I.E.F. 240 B spectrometer (240 MHz) at the Institut d'Électronique Fondamentale, Faculté des Sciences d'Orsay, France, through the courtesy of Drs. S. K. Kan and G. Massiot. The melting points have been determined on a Kofler micro hot stage and are uncorrected. The chromatographic methods were those previously reported.[11,13,19]

Preparation of ether extract and crude filicin of D. crassirhizoma. These were prepared from air-dried rhizomes (275 g) according to previously reported methods,[14,15] yielding 21.1 g (7.7 %) of ether extract (oleoresin) from which 5.224 g (1.9 %) of crude Mg-filicin and 6.620 g (2.4 %) of crude Ba-filicin were obtained.

Column chromatography of crude Mg-filicin. The crude Mg-filicin (5.124 g) was suspended in benzene and chromatographed on a column containing 265.5 g SiO_2 as previously described.[15] The fractions eluted with benzene or benzene/chloroform 1:1 (10 ml each), all contained dryocrassin as found by TLC. The fractions 1 – 35 (benzene) were combined, evaporated *in vacuo*, and the residue was crystallized from ether, yielding several subsequent fractions of dryocrassin with slightly different melting points. Those with the melting points 205 – 207 °C (94.5 mg) and 210 – 215 °C (33.1 mg) consisted of almost pure dryocrassin-ABBA (Ia) as found by TLC, alkaline cleavage, MS, and NMR spectra (see theoretical section). The last fractions eluted with chloroform/ethanol (95/5) consisted of impure flavaspidic acid (TLC). No crystals were obtained from methanol.

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Reduction of Phosphinoylacetonitriles to Phosphinoacetonitriles

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Four phosphinoacetonitriles, R_2PCH_2CN, have been prepared from the corresponding phosphine oxides by reduction with diphenylsilane. Their reactivities have been investigated. The results show that a cyanomethyl substituent lowers both the nucleophilic reactivity and the rate of oxidation of a tertiary phosphine.

Cyano groups are strong electron withdrawing substituents which generally lower the nucleophilic reactivity of nearby nucleophilic centers. Thus $P(CH_2CH_2CN)_3$ is a much weaker nucleophile than Et_3P and even Ph_3P.[1] A cyanomethyl substituent is expected to lower the nucleophilic reactivity more than a 2-cyanoethyl substituent, but not so strongly as a directly bonded cyano group. For example, the phosphorus atom in $P(CN)_3$ [2] has lost nearly all nucleophilic properties and becomes electrophilic. Cyanomethylphosphines, $R_nP(CH_2CN)_{3-n}$, are virtually unknown in the literature. Two compounds, $P(CH_2CN)_3$ [3] and Ph_2PCH_2CN,[4] have been mentioned in patents, but neither physical data nor preparative directions have been given. As a part of a study of the preparation and properties of cyanomethylphosphines we now describe some phosphinoacetonitriles, II, prepared by the following sequence:

$$R_2POEt \xrightarrow{ClCH_2CN} \underset{\text{Ia–d}}{R_2P(O)CH_2CN} \xrightarrow{Ph_2SiH_2} \underset{\text{IIa–d}}{R_2PCH_2CN}$$

R = a: Et; b: Pr^i; c: Bu^t; d: Ph.

The first step is the well-known Arbuzov reaction which has been shown to give high yields of Ia[5] and Id.[6] When applied to Pr^i_2-POEt the expected compound, Ib, was isolated in 80 % yield, together with a small amount of $Pr^i_2P(O)Cl$. However, Bu^t_2POEt gives equal amounts of $Bu^t_2P(OEt)CHCN$ and Bu^t_2-$P(O)Cl$. Phosphine oxide Ic is obtained only by subsequent addition of HCl to this mixture. This deviation from the normal Arbuzov reaction is ascribed to competitive attack by R_2POEt on the chlorine atom of $ClCH_2CN$ when R is sterically demanding. The reaction will be described in detail in a forthcoming report.[7]

The last step is the reduction of the phosphinoylacetonitriles I to the phosphinoacetonitriles II. Whereas lithium aluminium hydride could not be used in presence of cyano groups, diphenylsilane was found to reduce I to II in high yields at 150 – 240 °C.

Silanes have been used previously for the reduction of various phosphoryl compounds to primary, secondary, or tertiary phosphines.[8,9] We attempted similar reductions of $Ph(EtO)$-$P(O)CH_2CN$ (to $PhPHCH_2CN$) and $(EtO)_2$-$P(O)CH_2CN$ (to PH_2CH_2CN) with diphenylsilane. However, after 4 h at 160 – 190 °C only CH_3CN (*ca.* 40 and 60 %, resp.) could be isolated by distillation.

The phosphines II were characterized by ^{31}P NMR, ^1H NMR and IR spectroscopy (Table 1). The phosphorus chemical shifts are displaced high-field relative to the phosphine oxides I, and show the expected [10] variation with the substituents R. The coupling constants $^2J_{PH}$ of II are smaller than those of I, as generally found for $^2J_{PH}$ of methylphosphines relative to methylphosphine oxides. The small

Table 1. ^{31}P NMR, ^{1}H NMR, and IR data[a] for phosphinoylacetonitriles and phosphinoacetonitriles.

No.	Compound	δ_P	δ_H[b]	$^2J_{PH}$[b]	ν_{CN}
Ia	$Et_2P(O)CH_2CN$	47.3	2.96	14.2	2252
Ib	$Pr^i_2P(O)CH_2CN$	53.7	2.84	13.5	2249
Ic	$Bu^t_2P(O)CH_2CN$	58.2	2.82	12.6	2248
Id	$Ph_2P(O)CH_2CN$	25.0	3.39	15.1	2253
IIa	Et_2PCH_2CN	−24.4	2.42	5.1	2234
IIb	$Pr^i_2PCH_2CN$	0.7	2.36	4.5	2238
IIc	$Bu^t_2PCH_2CN$	27.0	2.36	2.5	2243
IId	Ph_2PCH_2CN	−17.8	2.89	4.4	2242

[a] NMR: *Ca.* 10 % solutions in $CDCl_3$ at *ca.* 30 °C, chemical shifts in ppm relative to internal TMS (δ_H) or external 85 % H_3PO_4 (δ_P), coupling constants (J) in Hz. Chemical shifts are positive for low-field shifts. IR: *Ca.* 10 % solutions in $CHCl_3$ at *ca.* 25 °C, ν in cm^{-1}. [b] For the CH_2CN group.

values of $^2J_{PH}$ found for $R_2P(O)CH_2X$ compounds where X is an electronegative substituent [11] are not observed in the case of I. The presence of nitrile groups are evident from the sharp band found in the IR spectra in the 2235 − 2255 cm^{-1} region.[12] IIa was further characterized by reaction with ethyl bromide to give $Et_3P^+CH_2CN\ Br^-$, which was indentical with the compound obtained from Et_3P and $BrCH_2CN$.

The nucleophilic reactivity of compounds II are low, as expected. Although IIa dissolves in CS_2 to give a red solution, no adduct could be isolated. Compounds IIb and IIc gave only faintly red solutions. Alkyl halides react slowly with IIa − d to give quaternary salts. Kinetic measurements on the reaction of IId with EtI in acetone at 35 °C, using the conductivity method of Henderson and Buckler,[1] showed, that IId reacts 8 times slower than Ph_3P and 22 times slower than Ph_2EtP. From the equation given by Henderson and Buckler[1] a Taft σ^* value of + 1.7 for the CH_2CN group is calculated. This is in agreement with the σ^* value of + 1.71 given by Stevenson and Williamson.[13]

The phosphines II are oxidized by exposure to air, although slowly. Thus only 30 % of IId was oxidized to Id when a few drops of IId was exposed to air on a watchglass for 72 h. The relative rates of oxidation of II in an oxygen atmosphere, initiated by azobisisobutyronitrile (AIBN), were estimated by competition experiments. IId was mixed with approximately equal amounts of IIa, IIb, IIc, or Ph_3P and the mixtures were oxidized at *ca.* 100 °C with a deficit of oxygen in the presence of AIBN. The ratios I/II were determined by NMR. Only small amounts of oxidation products other than I were observed. Assuming the same rate expressions (first order in R_3P) for these oxidation reactions, relative rate constants were calculated:

Compound	$k_{rel.}$
Et_2PCH_2CN	6.2
$Pr^i_2PCH_2CN$	2
$Bu^t_2PCH_2CN$	1.1
Ph_2PCH_2CN	1.0
Ph_3P	1.7

Although data for comparison are scarce in the literature,[14,15] these results indicate that (i) a cyanomethyl group reduces the rate of oxidation of a phosphine ($Ph_2PCH_2CN < Ph_3P$ and, presumably, $Ph_3P < Ph_2EtP$),(ii) there seems to be a steric effect on the rate ($Et_2PCH_2CN > Pr^i_2PCH_2CN > Bu^t_2PCH_2CN$).

EXPERIMENTAL

Analyses were carried out by the Microanalysis Department of this laboratory. IR spectra were obtained on a Perkin Elmer 337 Grating Infrared Spectrometer. NMR spectra were recorded on a Bruker HX-90 E Spectrometer. All NMR spectra were run in $CDCl_3$ at *ca.* 30 °C with internal deuterium lock on $CDCl_3$. Chemical shifts (ppm) are relative to internal TMS for ^1H spectra (δ_H) and external 85 % H_3PO_4 for ^{31}P spectra (δ_P), and are given as positive for lowfield shifts. The δ_P values are in most cases obtained from ^1H spectra by ^{31}P selective decoupling, and all assignments of coupling to phosphorus have been verifield by ^{31}P decouplings.

Phosphines and phosphinites were handled in a nitrogen atmosphere. The following compounds were prepared according to the literature: Pr^i_2POEt,[16] $Et_2P(O)CH_2CN$,[5] $Bu^t_2P(O)CH_2CN$,[7] $Ph_2P(O)CH_2CN$,[6] $Ph(EtO)P(O)CH_2CN$,[17] and $(EtO)_2P(O)CH_2CN$.[18]

P,P-*Diisopropylphosphinoylacetonitrile (Ib).* Pr^i_2POEt (16.2 g, 0.10 mol) was added in *ca.* 2 ml portions to slightly heated (*ca.* 50 °C) $ClCH_2CN$ (8.3 g, 0.11 mol) with stirring. The reaction was strongly exothermic and a reflux condenser was necessary. The reaction mixture was distilled *in vacuo* through a Claisen head. A forerun, b.p. 61 − 63 °C/0.5 mmHg (0.5 g)

consisted mainly of $Pr^i_2P(O)Cl$ (lit.[19] b.p. 50 °C/0.2 mmHg. NMR: CH_3: δ_H 1.15–1.50, multiplet, CH: δ_H 2.36, $^2J_{PH}$ *ca.* 7 Hz, $^3J_{HH}$ *ca.* 7 Hz, in good agreement with literature.[20] δ_P 86.8). $Pr^i_2P(O)CH_2CN$ distilled at 121.5–122.5 °C/0.30 mmHg (13.8 g, 80 %). The product crystallized upon standing, m.p. 58–61 °C. It is strongly hygrosopic and satisfactory elemental analysis could not be obtained. However, ^1H NMR showed no impurities other than water. NMR data: δ_P 53.7. CH_2CN: δ_H 2.84, $^2J_{PH}$ 13.5 Hz. CH_3: Nonequivalent CH_3 groups, $\delta_{H(A)}$ 1.33, $^3J_{PH(A)}$ 16.0 Hz, $\delta_{H(B)}$ 1.32, $^3J_{PH(B)}$ 16.6 Hz, Δ_{AB} 0.9 Hz, $^3J_{HH}$ 7.2 Hz. CH: δ_H 2.26, $^2J_{PH}$ 8.8 Hz (the assigment was assisted by data in $CDCl_3$–C_6D_6 mixtures where Δ_{AB} was larger).

P,P-*Diethylphosphinoacetonitrile (IIa).* A mixture of $Et_2P(O)CH_2CN$ (14.5 g, 0.10 mol) and Ph_2SiH_2 (18.4 g, 0.10 mol) was heated with stirring to 150 °C for 5 h. The reaction mixture was distilled *in vacuo* through a 15 cm Vigreux column to give Et_2PCH_2CN, b.p. 90–92 °C/13 mmHg (11.0 g, 85 %). No impurities were observable from its ^1H NMR spectrum. NMR data: δ_P −24.4. CH_2CN: δ_H 2.42, $^2J_{PH}$ 5.1 Hz. CH_3: δ_H *ca.* 1.11, $^3J_{PH}$ *ca.* 16 Hz, $^3J_{HH}$ *ca.* 7 Hz. CH_2CH_3: δ_H *ca.* 1.6, $^2J_{PH} < 2$Hz (second order spectrum).

P,P-*Diisopropylphosphinoacetonitrile (IIb).* A mixture of $Pr^i_2P(O)CH_2CN$ (17.3 g, 0.10 mol) and Ph_2SiH_2 (20.2 g, 0.11 mol) was heated with stirring to 200 °C for 3 h. Distillation *in vacuo* through a 15 cm Vigreux column gave $Pr^i_2PCH_2CN$, b.p. 92–92.5 °C/5.0 mmHg (11.8 g, 75 %). No impurities were observable from its ^1H NMR spectrum. NMR data: δ_P 0.7. CH_2CN: δ_H 2.36, $^2J_{PH}$ 4.5 Hz. CH_3: Nonequivalent CH_3 groups, $\delta_{H(A)}$ 1.17, $^3J_{PH(A)}$ 15.2 Hz, $\delta_{H(B)}$ 1.14, $^3J_{PH(B)}$ 11.7 Hz, $^3J_{HH}$ 7.0 Hz, Δ_{AB} 3.0 Hz. CH: δ_H 1.96, $^2J_{PH}$ 2.2Hz.

P,P-*Di-t-butylphosphinoacetonitrile* (*IIc*). A mixture of $Bu^t_2P(O)CH_2CN$ (4.02 g, 0.02 mol) and Ph_2SiH_2 (3.7 g, 0.02 mol) was heated with stirring to 240 °C for 15 h. Distillation *in vacuo* through a 10 cm Vigreux column gave $Bu^t_2PCH_2CN$, b.p.118–119 °C/5.8 mmHg, m.p. 6–8 °C (2.8 g, 75 %). No impurities were observable from its ^1H NMR spectrum. NMR data: δ_P 27.0. CH_2CN: δ_H 2.36, $^2J_{PH}$ 2.5 Hz. CH_3: δ_H 1.23, $^3J_{PH}$ 11.7 Hz.

P,P-*Diphenylphosphinoacetonitrile* (*IId*). A mixture of $Ph_2P(O)CH_2CN$ (24.1 g, 0.10 mol) and Ph_2SiH_2 (18.4 g, 0.10 mol) was heated with stirring to 190 °C for 6 h. Destillation *in vacuo* through a Claisen head gave Ph_2PCH_2CN, b.p. 146–147 °C/0.35 mmHg (18.0 g, 80 %). No impurities were observable from its ^1H NMR spectrum. NMR data: δ_P −17.8. CH_2CN: δ_H 2.89, $^2J_{PH}$ 4.4 Hz. Ph: δ_H 7.4 (m).

(Cyanomethyl)triethylphosphonium bromide. A mixture of Et_2PCH_2CN (0.26 g, 2×10^{-3} mol) and EtBr (0.6 g, 6×10^{-3} mol) in acetone (2 ml) was heated in an ampule to 100 °C for 24 h. The residue after evaporation was recrystallized from 2-propanol to give $Et_3P^+CH_2CN\ Br^-$ (0.38 g, 80 %), m.p. 228–230 °C. (Found: C 40.18; H 7.18; N 5.68; Br 33.25. Calc. for $C_8H_{17}BrNP$: C 40.35; H 7.20; N 5.88; Br 33.56). IR (KBr): ν_{CN} 2252 cm^{-1}. NMR ($(CD_3)_2SO$): δ_P 41.1 CH_2CN: δ_H 4.51. $^2J_{PH}$ 15.3 Hz. CH_3: δ_H 1.35, $^3J_{PH}$ 19.7 Hz, $^3J_{HH}$ 7.6 Hz. CH_2CH_3: δ_H 2.54, $^2J_{PH}$ 13.5 Hz.

The compound prepared from Et_3P and $BrCH_2CN$ had identical m.p., and NMR spectra.

Kinetic measurements. The conductivities were measured with a Radiometer type CDM 2 Conductivity Meter and a cell with platinized platinum electrodes. Acetone and EtI were purified through a column of Al_2O_3 (Woelm W 200 basic) and had conductivities of 0.3–2 μS. Solutions of Ph_2PCH_2CN (0.106 M) and EtI (0.192 M) in acetone were prepared, and 25 ml of each mixed in the conductivity cell at 35± 0.1 °C. The conductivity was measured until less than 0.4 % of Ph_2PCH_2CN had reacted. The concentration of $Ph_2EtP^+CH_2CN\ I^-$ was calculated from the conductivity values by comparison with the conductivities of standard solutions of the phosphonium salt. Similar experiments were made with Ph_3P. The rate constants were calculated from eqn. (4) of Henderson and Buckler.[1] Found for Ph_2PCH_2CN: $k = 7.2 \pm 0.2 \times 10^{-6}$ l mol^{-1} s^{-1}; for Ph_3P: $k = 5.8 \pm 0.2 \times 10^{-5}$ l mol^{-1} s^{-1}. The value for Ph_3P was higher than that (3.78×10^{-5}) given by Henderson and Buckler[1] probably because of residual water in our solvents.

(Cyanomethyl)diphenylethylphosphonium iodide. A mixture of Ph_2PCH_2CN (0.45 g, 2×10^{-3} mol) and EtI (1 ml, 12×10^{-3} mol) in acetone (5 ml) was refluxed for 4 h. After cooling, $Ph_2EtP^+CH_2CN\ I^-$ was filtered off and recrystallized from abs. ethanol. Yield 70 %, m.p. 179–179.5 °C. (Found: C 50.40; H 4.61; N 3.65; I 33.52. Calc. for $C_{16}H_{17}INP$: C 50.41; H 4.50; N 3.67; I 33.29).

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Structure Determination of Brominated 1,3,6-Triazacycl[3.3.3]-azines by Polyphosphoric Acid Decyanation of Their 4-Cyano Derivatives

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When the 7- and 9-bromo derivatives of 4-cyano-2-methyl-1,3,6-triazacycl[3.3.3]azine were treated with polyphosphoric acid, the corresponding decyanated derivatives were formed. These compounds were brominated in the 4-position with *N*-bromosuccinimide. We were then able to establish, by analogy, the structures of 4,7- and 4,9-dibromo-1,3,6-triazacycl[3.3.3]-azine.

Polyphosphoric acid (PPA) is a convenient reagent for substitution of a hydrogen atom for a cyano group.[1–3] When the bromocyanoazacyclazines *1*—*3* (*cf.* Chart 1) were treated separately with PPA at 145—185 °C for 15—20 min, low yields of the cyano-free compounds were obtained. Since the structures of *1*—*3* have

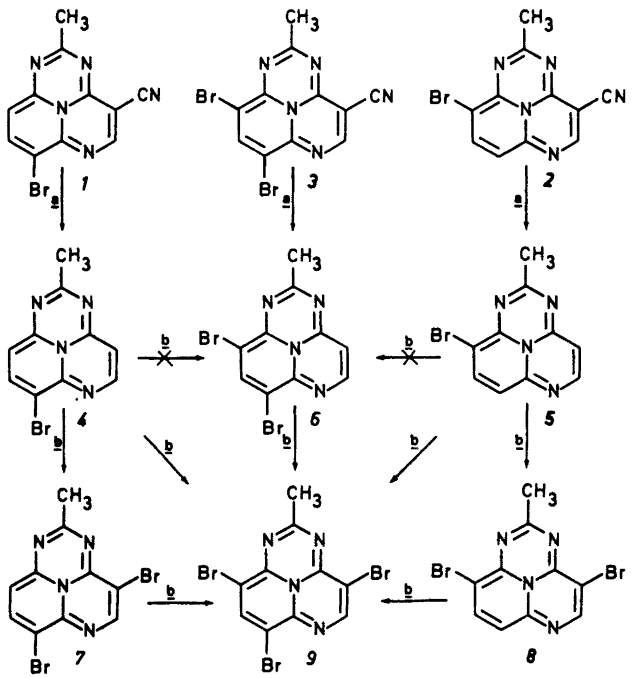
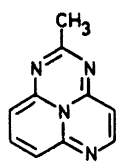
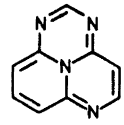


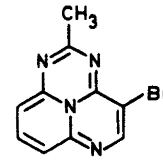
Chart 1. Reagents: *a* PPA; *b* NBS in $CHCl_3$.



10



11



12

Table 1. NMR spectral data for *7 – 9*, *12*, and *13 – 16* [5] (solvent: trifluoroacetic acid).

Compound	CH_3	H-2	H-5	H-7	H-8	H-9	Coupling constants (in Hz)
7	2.23	–	8.45	–	8.07	6.58	$J_{8-9}=8.5$
13	–	7.36	8.44	–	8.08	6.63	$J_{8-9}=8.5$
8	2.28	–	8.40	7.12	7.98	–	$J_{7-8}=9.1$
14	–	7.39	8.37	7.11	7.96	–	$J_{7-8}=9.4$
9	2.27	–	8.50	–	8.27	–	
15	–	7.38	8.48	–	8.26	–	
12	2.23	–	8.33	7.19	7.84	6.69	$J_{7-8}=8.6$ $J_{8-9}=7.9$ $J_{7-9}=1.0$
16	–	7.38	8.32	7.22	7.85	6.73	$J_{7-8}=8.5$ $J_{8-9}=8.1$ $J_{7-9}=1.0$

been established independently,[4] the structures for *4 – 6* follow. The PPA-method is so far the only way to obtain the dibromide *6*, since C-4 is the most reactive site in *10* (*vide infra*). Further bromination of *4 – 6* yielded *7 – 9* as indicated in Chart 1. Their structures were deduced from mass and NMR spectra (*cf.* Tables 1 and 2).

By the above sequence of reactions all expected * bromo derivatives of *10*, except *12*, have been obtained. Compound *12* was formed (77 %) along with *4* and *5* (18 % together) by mild bromination of *10*. Bromination of *12* with NBS gave two dibromo compounds, identical with those obtained from *4* and *5*; they therefore have structures *7* and *8*. Compounds *7* and *8* are more conveniently obtained in this way, or from *10*, than *via* the routes outlined in Chart 1. Further bromination of *7* and *8* gave the tribromide *9*.

In the decyanation procedure we always observed considerable amounts of 4-carboxamido derivatives having almost the same chromatographic mobilities as the corresponding decyanated compounds. These amides could be solvolysed in boiling ethanol in the presence of catalytic amounts of acid; the ethoxycarbonyl derivatives of the respective bromides then formed were easily separated from the decyanated compounds. Attempts to increase the yields of *4 – 6* were unsuccessful. Higher temperatures resulted in degradation of the cyclazine system, and treatment of *1 – 3* with PPA at lower temperatures gave only the respective carboxamides in good yields.

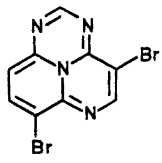
The chemical shifts for the aromatic protons in *7* and *8* have been used to establish, by analogy, the structures of two dibromo derivatives of *11*, reported earlier.[5] From theoretical considerations and from NMR data these compounds were predicted to be the 4,7- and 4,9-dibromides, but they could not be conveniently differentiated. One of them displayed chemical-shift values for the aromatic protons identical

Table 2. Mass spectral data for *7 – 9* and *12*.

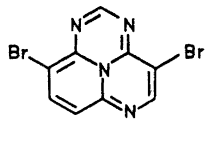
	M+	Rel. intensities
7	340, 342, 344	1:2:1
8	340, 342, 344	1:2:1
9	418, 420, 422, 424	1:3:3:1
12	262, 264	1:1

* Simple HMO-calculations, performed on *11*,[6] and arguments using resonance structures [5] seem to exclude electrophilic substitution at C-5 and C-8 in the 1,3,6-triazacycl[3.3.3]azine system.

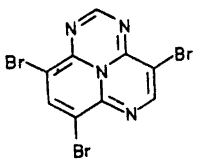
with those of *7* and the other one, values identical with those of *8* (Table 1); therefore they can now be assigned structures *13* and *14*, respectively.



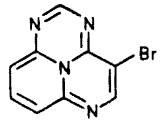
13



14



15



16

EXPERIMENTAL

General. NMR spectra were obtained with a Varian Model A-60 spectrometer and chemical shifts are reported in δ-values, using tetramethylsilane as internal reference. Mass spectra were recorded with a GEC-AEI 902 mass spectrometer at the Department of Medical Biochemistry, University of Göteborg. The IR spectra were determined in KBr with a Perkin-Elmer 337 infrared spectrophotometer. For TLC, Silica Gel GF_{254} (Merck) was used. Compounds *1*, *2*, *3*, and *10* were prepared as previously described.[2,4]

Decyanation of 1 *with PPA.* Freshly prepared PPA [7] (*ca.* 0.5 ml) was heated to 145°, 5 mg of *1* was added, the mixture was stirred at this temperature for 15 min, then cooled to 25°, and diluted with water. This solution was neutralized with 10 % aqueous sodium bicarbonate and extracted with chloroform. The organic layer was dried over magnesium sulfate and the solvent evaporated *in vacuo*. On the TLC-plate only one coloured band was observed ($R_F = 0.10$; EtOAc). This was shown to contain, in addition to *4*, the 4-carboxamido derivative of *4* (IR). The crude product was dissolved in 5 ml of ethanol, one drop of perchloric acid (70 %) was added, and the solution was heated under reflux for 1 h. The solvent was evaporated, the residue dissolved in chloroform, the organic layer washed with water, and then dried over magnesium sulfate. TLC showed that a new product ($R_F = 0.53$), which we believe is the ethoxycarbonyl derivative of *4*, had formed. Compound *4* was separated from this product and from small amounts of still unreacted amide by preparative TLC (EtOAc; the chromatogram was developed three times). M.S.: $M^+ = 262$, 264 (intensities 1:1). M.p.: decomposed above *ca.* 240 °C.

Bromination of 4 *to* 7 *and* 9. Five mg of *1*, treated with PPA, yielded a mixture of *4* and the 4-carboxamide of *4*. This mixture was dissolved in 5 ml of chloroform, 1 mg of NBS was added, and the solution was shaken for 5 min. The solvent was then evaporated and the residue was subjected to preparative TLC (EtOAc). *Ca.* 0.5 mg (7 %) of *7* + *9* (mainly *7*) and 1.5 mg (25 %) of the 4-carboxamido derivative* of *4* (IR, NMR) were isolated. TLC (EtOAc): $R_F = 0.37$ (*7*) and 0.57 (*9*).

Decyanation of 2 *with PPA.* Compound *2* (5 mg) was treated with 0.5 ml of PPA at 185 °C for 20 min and the reaction mixture was worked up and analysed as described for *1* above. Compound *5* was separated from the 4-carboxamido derivative of *5* on a TLC-plate (EtOAc; the chromatogram was developed five times). TLC: $R_F = 0.10$. M.S.: $M^+ = 262$, 264. M.p.: 193 – 195 °C.

Bromination of 5 *to* 8 *and* 9. A mixture of *5* and the 4-carboxamide of *5* (from 5 mg of *2*) was brominated as described for *4* above. Compounds *8* and *9* (mainly *8*; TLC) were isolated along with the 4-carboxamido derivative* (IR) of *5*. Yield of *8*: *ca.* 0.5 mg (7 %). TLC (EtOAc): $R_F = 0.45$ (*8*).

Decyanation of 3 *with PPA and bromination of* 6 *to* 9. A solution of 25 mg of *3* in 1 ml of PPA was stirred at 165 °C for 15 min. The reaction mixture was worked up as described for *1*. TLC (EtOAc): $R_F = 0.42$ (*6*) and 0.47 (4-carboxamide of *6*). The crude reaction product was dissolved in 5 ml of chloroform, 2 mg of NBS was added, and the solution was shaken for 10 min. Compound *9* and the 4-carboxamido derivative of *6* (IR, MS) were isolated by preparative TLC (EtOAc). Yield of *9*, *ca.* 1.5 mg (5 %) and of amide, 4 mg (15 %).

Bromination of 10 *to* 12 *with NBS.* A solution of 43 mg (0.23 mmol) of *10* and 41 mg (0.23 mmol) of NBS in 15 ml of chloroform was stirred at 25 °C for 20 min. The solution was filtered to remove succinimide and then evaporated. The residue was separated by preparative TLC (EtOAc, the chromatogram was developed twice). Yield of *12*: 43 mg (77 %); of *4* + *5*: 10 mg (18 %). Small amounts of *7* – *9* were also obtained. TLC: $R_F = 0.13$ (12). NMR and mass spectral data for *12* are presented in Tables 1 and 2, respectively.

Bromination of 10 *to* 7, 8, *and* 9 *with NBS.* A solution of 80 mg (0.44 mmol) of *10* and 150

* The possibility that the 4-carboxamido derivatives are converted to 4-brominated products can be eliminated, since the pure amides do not give *7* and *8* when treated with NBS under similar conditions.

mg (0.81 mmol) of NBS in 50 ml of chloroform was stirred for 15 min at −10 °C. The solution was filtered and the filtrate evaporated to dryness. From the residue, three products were isolated by preparative TLC (EtOAc): *7* (89 mg; 60 %), *8* (26 mg; 17 %), and *9* (17 mg; 9 %). NMR and mass spectral data for *7*–*9* are summarized in Tables 1 and 2, respectively.

Addendum: In Table 3, Ref. 5, two coupling constants have been exchanged. J_{8-9} (in *8*) should be 8.5 Hz and J_{7-8} (in *9*) should be 9.4 Hz.

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Structural Studies of Metabolic Products of Dopamine IV.* Crystal and Molecular Structure of (—)-Noradrenaline

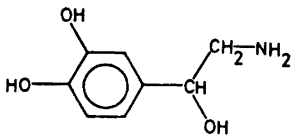
A. M. ANDERSEN

Department of Chemistry, University of Oslo, Oslo 3, Norway

The crystal structure of (−)-noradrenaline has been determined by X-ray methods, using 1000 observed reflections collected by counter diffractometer techniques. The crystals are orthorhombic, space group $P2_12_12_1$, with $a = 8.611(2)$, $b = 6.138(2)$, and $c = 14.912(4)$ Å. Least-squares refinements yielded a conventional R-factor of 0.036. Standard deviations in bond lengths are 0.003 Å and in bond angles 0.2°. The crystal structure of (−)-noradrenaline corresponds closely to that of (−)-adrenaline in molecular geometry, conformation, and arrangement in the crystals The results of the structure investigations are discussed in relation to the biological activity of these biogenic amines.

Noradrenaline (norepinephrine) is an adrenal medullary hormone as well as a neurotransmitter at most sympathetic postganglionic nerve endings and in certain synapses in the central nervous system. In the catecholamine biosynthesis noradrenaline is the immediate precursor of adrenaline, and differs from the latter by lacking a methyl substituent in the amino group. Both drugs act directly on α- and β-adrenergic receptors, but with noradrenaline the α-receptor stimulation generally dominates. Of each compound the naturally occurring $R(-)$-form is the more active isomer.

The crystal structure of (−)-noradrenaline hydrochloride and also the salts of several other sympathomimetic catecholamines have been determined by X-ray diffraction methods.[1] The present study forms part of a structure investigation of the free base form of these biogenic amines, as information



concerning their molecular characteristics is of fundamental importance for structure-activity relationship (SAR) studies.

EXPERIMENTAL

The crystallization procedure employed in the structure investigation of (−)-adrenaline [2] also yielded single crystals of (−)-noradrenaline. Three-dimensional intensity data were obtained from a crystal of dimensions $0.08 \times 0.12 \times 0.43$ mm³ on a SYNTEX $P\bar{1}$ automatic diffractometer with graphite crystal monochromated Mo$K\alpha$ radiation.

Oscillation and Weissenberg photographs indicated orthorhombic symmetry; systematically absent reflections determined the space group to be $P2_12_12_1$. Unit cell parameters were obtained from a least-squares treatment of diffractometer measurements on 15 general reflections.

Intensities of 1063 reflections were recorded using the $\omega - 2\theta$ scan technique with scan range 1.6° and scan speed 1° min^{-1} ($2\theta \leq 50°$), and 1 − 1.5 ° min^{-1} ($50° < 2\theta \leq 60°$). The ratio of total background couting time to scan time was 0.7. 1000 reflections having net intensities greater than $2.5\sigma(I)$ were considered to be observed, the remaining reflections were excluded from the further calculations. The intensity data were corrected for Lorentz and polarization effects and scaled to an absolute level by Wilson's statistical method.

Atomic form factors used were those of Doyle and Turner [3] for oxygen, nitrogen, and carbon atoms, and of Stewart *et al.* [4] for hydrogen atoms.

* The previous publication in this series: *Acta Chem. Scand. B 29* (1975) 239.

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations for non-hydrogen atoms ($\times 10^4$). The temperature factor is given by exp $-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)$.

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
O1	1627(2)	3005(3)	7898(1)	451(18)	1646(50)	342(8)	− 62(56)	−69(21)	−332(34)
O2	1188(2)	6486(3)	8997(1)	568(21)	1907(56)	440(10)	456(61)	−73(24)	−772(42)
O3	8388(2)	5068(3)	8380(1)	464(18)	1650(47)	392(9)	−301(56)	−93(24)	417(36)
N	9279(2)	537(4)	8580(2)	588(25)	1400(55)	344(11)	272(68)	−86(29)	151(45)
C1	5648(2)	4123(4)	8585(1)	495(24)	1263(59)	233(10)	7(71)	−37(26)	146(44)
C2	4383(2)	3104(4)	8188(2)	594(26)	1118(59)	286(11)	202(74)	27(29)	−226(46)
C3	2864(2)	3850(4)	8315(1)	494(24)	1205(60)	220(10)	− 86(68)	−36(27)	− 34(42)
C4	2661(2)	5675(4)	8871(1)	516(26)	1365(62)	231(10)	241(75)	45(26)	12(44)
C5	3908(2)	6683(4)	9278(1)	705(28)	1406(65)	239(10)	55(82)	−15(29)	−401(46)
C6	5407(3)	5904(4)	9138(2)	559(27)	1438(66)	245(11)	−243(71)	−99(28)	−214(46)
C7	7274(2)	3352(4)	8344(2)	485(24)	1402(61)	263(11)	− 31(71)	3(28)	54(49)
C8	7851(3)	1572(4)	8959(2)	697(31)	1576(69)	367(13)	245(83)	134(33)	348(55)

Table 2. Fractional atomic coordinates ($\times 10^3$) and isotropic thermal parameters with estimated standard deviations for hydrogen atoms.

Atom	x	y	z	B
HC2	455(3)	191(4)	782(1)	2.4(5)
HC5	371(3)	800(4)	965(1)	2.9(5)
HC6	622(3)	660(4)	941(1)	2.4(5)
HC7	719(3)	269(4)	768(2)	2.7(5)
H1C8	706(3)	44(5)	902(2)	3.5(6)
H2C8	813(3)	220(4)	955(2)	3.9(6)
H1N	975(3)	− 43(6)	897(2)	4.5(7)
H2N	1012(3)	148(5)	840(2)	4.1(7)
H3N	908(3)	− 21(5)	803(2)	3.5(6)
HO2	67(4)	573(6)	875(2)	5.9(11)
HO3	819(3)	607(5)	790(2)	4.8(7)

CRYSTAL DATA

Noradrenaline, $C_8H_{11}O_3N$
$a=8.611(2)$ Å; $b=6.138(2)$ Å; $c=14.912(4)$ Å. $V=788.1$ Å^3; $M=169.20$; $F_{(000)}=360$; $Z=4$; $D_{calc}=1.427$ g cm^{-3}.

STRUCTURE DETERMINATION

The structure was solved by direct methods [5] and refined by full-matrix least-squares technique. All computer programs employed are desribed in Refs. 5 and 6.

Calculated coordinates of hydrogen atoms bonded to carbon atoms were introduced, and a few cycles of anisotropic refinement of non-hydrogen atoms yielded an R-factor of 0.054. A difference Fourier map calculated at this stage revealed the positions of the remaining five hydrogen atoms at peaks ranging from 0.46 to 0.26 e Å^{-3}, compared with background at 0.20 e Å^{-3}. The hydrogen atoms were located in positions showing a zwitterionic structure of the noradrenaline molecule. Subsequent least-squares refinements including positional and isotropic thermal parameters of the hydrogen atoms yielded a conventional R-factor of 0.036 and a weighted R-factor of 0.034. A total difference Fourier map demonstrated irregular electron density with no peaks larger than 0.22 e Å^{-3}.

The final parameters for non-hydrogen atoms are given in Table 1 and for hydrogen atoms in Table 2. An analysis of the anisotropic vibration parameters indicated that the thermal motion in the catechol part of the molecule could be described in terms of a rigid body model. Bond lengths in this part of the molecule were accordingly corrected. Bond lengths and angles are listed in Table 3. The ellipsoids of thermal motion are illustrated in Fig. 1, which also presents corrected bond lengths.

The structure factor list may be obtained from this institute upon request.

DESCRIPTION OF THE STRUCTURE

The noradrenaline molecule is found to have a zwitterionic structure, formed by a proton transfer from the *meta* phenolic hydroxyl group to the nitrogen atom. This was also

Table 3. Bond lengths (Å) and bond angles (°) with estimated standard deviations in parantheses.

Bond length		Corrected	Bond angle	
C1 – C2	1.389(3)	1.391	C1 – C2 – C3	121.8(2)
C2 – C3	1.399(3)	1.401	C2 – C3 – C4	117.3(2)
C3 – C4	1.404(3)	1.409	C3 – C4 – C5	121.3(2)
C4 – C5	1.379(3)	1.381	C4 – C5 – C6	120.1(2)
C5 – C6	1.393(3)	1.395	C5 – C6 – C1	119.9(2)
C1 – C6	1.385(3)	1.390	C2 – C1 – C6	119.5(2)
C1 – C7	1.522(3)	1.524	C6 – C1 – C7	121.6(2)
C3 – O1	1.338(3)	1.340	C2 – C1 – C7	118.7(2)
C4 – O2	1.376(3)	1.378	C2 – C3 – O1	123.7(2)
C7 – C8	1.511(3)		O1 – C3 – C4	118.9(2)
C7 – O3	1.424(3)		C3 – C4 – O2	119.0(2)
C8 – N	1.496(3)		O2 – C4 – C5	119.7(2)
C2 – HC2	0.93(3)		C1 – C7 – C8	112.6(2)
C5 – HC5	0.99(3)		C1 – C7 – O3	112.4(2)
C6 – HC6	0.91(3)		O3 – C7 – C8	106.9(2)
C7 – HC7	1.08(3)		C7 – C8 – N	110.5(2)
C8 – H1C8	0.98(3)			
C8 – H2C8	0.99(3)			
N – H1N	0.93(3)			
N – H2N	0.96(3)			
N – H3N	0.96(3)			
O2 – HO2	0.74(3)			
O3 – HO3	0.96(3)			

observed for the adrenaline molecule in a previous structure investigation.[2] Both crystal structures are characterized by chains of zwitterions extending in the direction of a crystallographic axis. The ions are connected by hydrogen bonds to build double molecular layers, which are held together by van der Waals interactions. This arrangement of the noradrenaline molecules is illustrated in Fig. 2. Van der Waals contacts and hydrogen bond parameters are listed in Table 4. The charged oxygen atom is acting as an acceptor

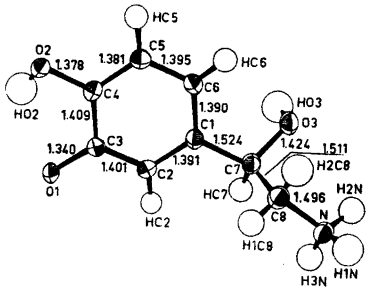


Fig. 1. Bond lengths (corrected) and 50 % probability ellipsoids. (The drawing was prepared using the computer program ORTEP[16]).

in three fairly short and approximately linear hydrogen bonds from two nitrogen atoms and one hydroxyl oxygen atom in neighbouring molecules. The hydrogen bonds of lengths 2.722 Å (N···O1), 2.808 Å (N···O1), and 2.624 Å (O3···O1) are directed nearly tetrahedrally about O1. The nitrogen atom is also acting as a hydrogen donor in a relatively weak hydrogen bond of length 3.046 Å to O2 in an adjacent molecule. The HO2 hydrogen atom is situated in the plane of the benzene ring at a distance of 2.26 Å from the O1 oxygen atom and 2.08 Å from O3 in a neighbouring molecule. The O2···O3 separation of 2.724 Å represents a normal hydrogen bond. The presence of an intramolecular hydrogen bond between O2 and O1 seems thus unlikely, particularly in view of the intermolecular hydrogen bond system already involving O1. In the crystals of adrenaline [2] the arrangement around the charged oxygen atom is almost indentical, only the phenolic hydrogen atom is situated out of the ring plane by −0.123 Å, and thus at larger distance from O1.

The C – O$^-$ bonds of noradrenaline and adrenaline [2] (1.340 and 1.348 Å) are significantly shorter than the phenolic C – O bonds.

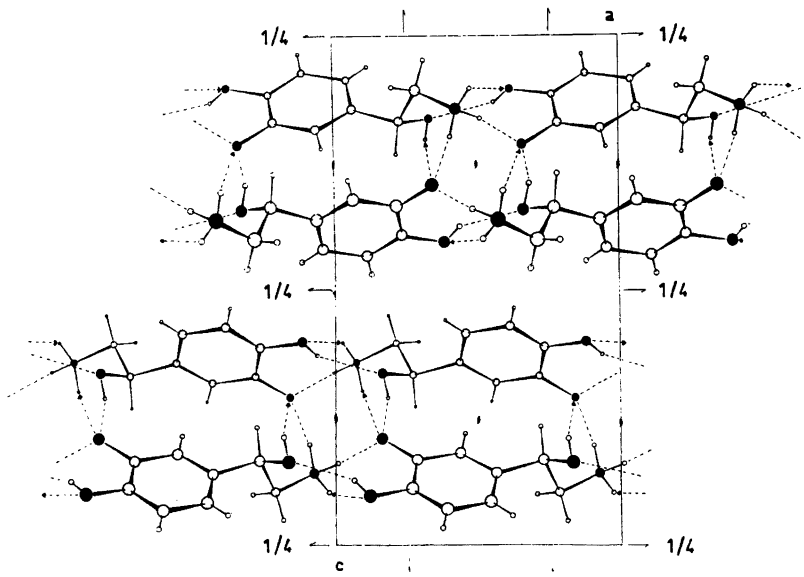


Fig. 2. The structure as seen down the *b*-axis. Hydrogen bonds are indicated by broken lines and the arrow-heads indicate atoms displaced by one unit cell up the *b*-axis.

Table 4.
a. Hydrogen bonded interactions X−H · · ·Y.

X	Y	X···Y (Å)	H···Y (Å)	X−H···Y (°)
N	O1(*a*)	2.722	1.77	170
N	O1(*b*)	2.808	1.87	168
N	O2(*c*)	3.046	2.26	142
O3(*b*)	O1	2.624	1.69	164
O2(*a*)	O3	2.724	2.08	145

b. Other short contacts (Å).

N	O3	2.902
O3	O1(*a*)	3.147
C2	C1(*b*)	3.600
C7	C4(*b*)	3.690
C5	C8(*d*)	3.425
C6	O2(*e*)	3.279

$a = x+1, y, z$
$b = -x+1, y-1/2, -z+3/2$
$c = x+1, y-1, z$
$d = x-1/2, -y+1/2, -z+2$
$e = x+1/2, -y+3/2, -z+2$

No structure determinations of comparable phenolates have hitherto been reported. However, the C−O⁻ distances observed in the two amines are larger than might be expected for this type of bond. This relative lengthening may be an effect of the extensive hydrogen bond system involving the charged oxygen atom in both crystal structures. The bond lengths in the phenolic hydroxyl group are normal; the values found in noradrenaline are 1.378 Å (C4−O2) and 0.74 Å (O2−HO2). The nitrogen atom is tetrahedrally bonded, and the conformation about the C−N bond is staggered. The bond distances C−N (1.496 Å) and N−H (0.93−0.96 Å) are all in the range commonly reported for a C−NH_3^+ group. Altogether, the molecular geometry of noradrenaline corresponds closely to that of adrenaline,[2] and except for the zwitterionic character, also to that reported for the salts of these biogenic amines.[7,8]

The catechol part of the molecule is nearly planar. The deviations from a least-squares plane through the ring carbon atoms are less than 0.007 Å for the atoms defining the plane, and for the exocyclic atoms 0.077 (O1), 0.037 (O2), and 0.137 Å (C7). The deviation of the HO2 hydrogen atom from the ring plane is 0.003 Å.

The ethylamine side chain is fully extended and approximately perpendicular to the ring

system. The dihedral angle C1 – C7 – C8 – N is 167.5° and C6 – C1 – C7 – C8 is 94.2°. This is the conformation usually encountered among the salts of the sympathomimetic amines in the crystals,[1] and is also the one observed in the crystal structure of (–)-adrenaline.[2] The dihedral angle C6 – C1 – C7 – O3 is – 26.6°. Thus, the hydroxyl group of the side chain is situated in a *trans* position relative to the *meta* oxygen atom of the benzene ring. An identical arrangement is found in the crystal structure of (–)-adrenaline,[2] but in the salts of the *R*(–)-form of these biogenic amines a *cis* position of the corresponding hydroxyl groups is observed.[1]

DISCUSSION WITH RELEVANCE TO BIOLOGICAL ACTIVITY

The conformational requirements of the sympathomimetic amines for adrenergic activity have been a central topic in SAR studies in recent years.[1,9–12] In a series of X-ray analyses [1] performed on the salts of these biogenic amines, the ethylamine side chain is with no exception found in an extended conformation. This is therefore regarded as the prefered conformation of the amines in the solid state.[1] The structure investigations of the free base form of adrenaline [2] and noradrenaline (present study) are consistent with this assumption, as both molecules are found with the extended conformation in the crystals. Whether this is also the more stable conformation in solution and of the isolated molecules has been extensively discussed.[1,9,10]

The crystal structures of noradrenaline and adrenaline [2] have revealed another interesting quality of these amines. Both amines have demonstrated the ability to form zwitterions with a proton transfered from the *meta* phenolic group to the amino group. This indicates that the former group is more acidic than the protonated amino group, which has been a subject of some discussion.[13] As the aromatic moiety is assumed to be particularly important for β-adrenergic activity,[9] the zwitterionic character might be involved in the receptor stimulation. In theories concerning drug-receptor interactions, the ability of the catechol moiety to engage in hydrogen bonding and complex formation have been discussed.[9,14,15] A negatively charged oxygen atom could also be engaged in other types of reactions; for example acting as a proton acceptor, or being involved in an ion-pair formation with a cationic receptor site corresponding to the type of interaction proposed for the protonated amino group.

The assumption that a zwitterionic form may be involved in β-receptor interactions is consistent with the increase in β-potency generally observed when going from primary to secondary catecholamines,[15] as the base strengths of the amines normally increase in this series, and hence the ability of zwitterion formation. It is furthermore reported that the adrenaline activity of the tertiary amine *N*-methyladrenaline is markedly reduced, and that the quaternary ammonium salt *N*,*N*-dimethyladrenaline is practically free from adrenergic effects.[15]

A catechol monoanion as participant in biological reactions has been mentioned in connection with the catalytic conversion of ATP to cyclic 3′5′-AMP, and also in the enzymatic *O*-methylation of the catecholamines.[14] The latter reaction is assumed to constitute the principal metabolic pathway of this class of amines, and is regarded as a nucleophilic reaction of a phenolic group with the electron-deficient carbon of *S*-adenosylmethionine.[13,14] This *O*-methylation occurs *in vivo* predominantly on the *meta* phenolic oxygen atom [13], the one found to be negatively charged in the crystals of both noradrenaline and adrenaline.[2]

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Organic Selenium Compounds. XIII.* Insertion Reactions of Methylenediamines with Carbon Diselenide

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The formation of dialkylaminomethyl dialkyldiselenocarbamates (III) from tetraalkylmethylenediamines and carbon diselenide is described. Compounds III are very rapidly hydrolyzed to give formaldehyde and dialkylammonium diselenocarbamates (IV), and in the presence of oxygen the latter undergo oxidation. The oxidation products, previously referred to as bis(selenocarbamoyl) triselenides, are tentatively assigned the structure of tetracoordinate bis(diselenocarbamato)selenium(II) compounds.

In the ^{1}H NMR spectra of III the *N*-alkyl groups become magnetically equivalent at unusually low temperature. The simultaneous disappearance of the ^{77}Se satellites in the Se–CH_2 signal indicates that the interchange of *N*-alkyl groups between diastereotopic positions in the dialkyldiselenocarbamate portion of compounds III occurs through an ion pair rather than by rotation around the R_2N–CSe_2 bond.

Kreutzkamp and Oei[1] have described the reaction of carbon disulfide with certain tetrasubstituted methylenediamines (I) which results in the formation of dialkylaminomethyl dialkyldithiocarbamates (II).

As a part of our studies of the reactivity of carbon diselenide we have investigated its reaction with some methylenediamines. We found that the reaction proceeded smoothly in dry hydrocarbon solvents, *e.g.* benzene or petroleum ether, to give dialkylaminomethyl dialkyldiselenocarbamates (III), the selenium analogues of II. However, under the reaction conditions used by Kreutzkamp and Oei

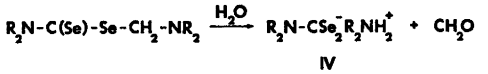
$R_2N-CH_2-NR_2 + CY_2 \rightarrow$
I

$R_2N-CY-Y-CH_2-NR_2$
II: Y = S; III: Y = Se

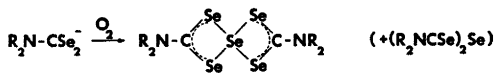
a: R = Me
b: R = Et
c: R,R = $-(CH_2)_5-$

(ethanolic solution) we instead obtained dialkylammonium dialkyldiselenocarbamates (IV).

Chemical properties of compounds III. An investigation of the chemical properties of the compounds III showed that these are inert towards dry alcohols but, in contrast to their sulfur analogues, are easily hydrolyzed to give IV and formaldehyde. On addition of $NiCl_2.6H_2O$ to an ethanolic solution of an ester III a precipitate of the corresponding nickel(II) diselenocarbamate immediately separates. The hydrolysis is very rapid and may even be effected by the traces of water adsorbed on the walls of glass vessels or moisture from the air. When in contact with air, solutions of compounds IIIa–c smell of formaldehyde, and diselenocarbamate salts (IV) or compounds of type V eventually separate.



IV



V

* Parts XII and XIV–XVII have been published: *Acta Chem. Scand.* *27* (1973) 3605; *24* (1970) 2055, 2061, 2065, 3213.

The latter, the conspicuous so-called bis(selenocarbamoyl)triselenides, are formed by an oxidation of IV, as has previously been reported.[2-4] It is remarkable that the diselenocarbamates differ from their thio analogues in that they on oxidation disproportionate into a mixture of mono- and triselenides whereas the latter exclusively give disulfides. We suggest that this difference can be ascribed to an extra stabilization of the triselenides by coordination of the central selenium atom to 4 atoms of selenium so that these compounds are actually bis(diselenocarbamato)selenium(II) compounds (formula V) rather than open-chain triselenides. This assumption has recently received support from an X-ray analysis of the oxidation product from pyrrolidinium 1-pyrrolidinecarbodiselenoate.[5]

It was attempted to introduce a second molecule of carbon diselenide into the esters III, which still possess a basic nitrogen atom. However, such a reaction did not take place. The expected products, methylene bis(selenocarbamates) (VI), which were readily prepared from IV and methylene bromide, were not observed. Instead the main products were compounds V together with higher molecular-weight substances with a high selenium content. Similar results were obtained when the tetraalkylmethylenediamines (I) were reacted with 2 mol of carbon diselenide.

$$2R_2N-CSe_2^- + CH_2Br_2 \rightarrow R_2N-\overset{\overset{Se}{\|}}{C}-Se-CH_2-Se-\overset{\overset{Se}{\|}}{C}-NR_2$$

VI

The formation of V in these reactions can be ascribed to hydrolysis of III to give diselenocarbamate salts followed by air oxidation. The higher molecular-weight compounds formed have not been studied in detail, but they presumably correspond to the so-called CSe_2-polymers, the possible composition of which has been discussed previously.[6]

Structural considerations. Infrared and electronic spectra suggest that the products from I and carbon diselenide are normal, covalent diselenocarbamate esters in accordance with formula III. A comparison of the infrared spectra of the selenium compounds (III) and the corresponding sulfur compounds (II) showed the expected shift in the 1000 cm^{-1} region towards lower wave numbers when sulfur was replaced by selenium.[7] The electronic spectra closely resemble those of other diselenocarbamates.[8] The weak absorption band in the visible part of the spectrum, assigned to the $n-\pi^*$ transition,[8] manifests itself by a light yellow colour of the selenium compounds III, the sulfur compounds II being colourless.

However, the ^1H NMR spectra of the reaction products, recorded at room temperature, were not immediately consistent with structure III. Unexpectedly, the amide *N*-alkyl groups of IIIa–c proved magnetically equivalent at room temperature, and the $Se-CH_2$ signal showed no ^{77}Se satellites (neither could ^{77}Se

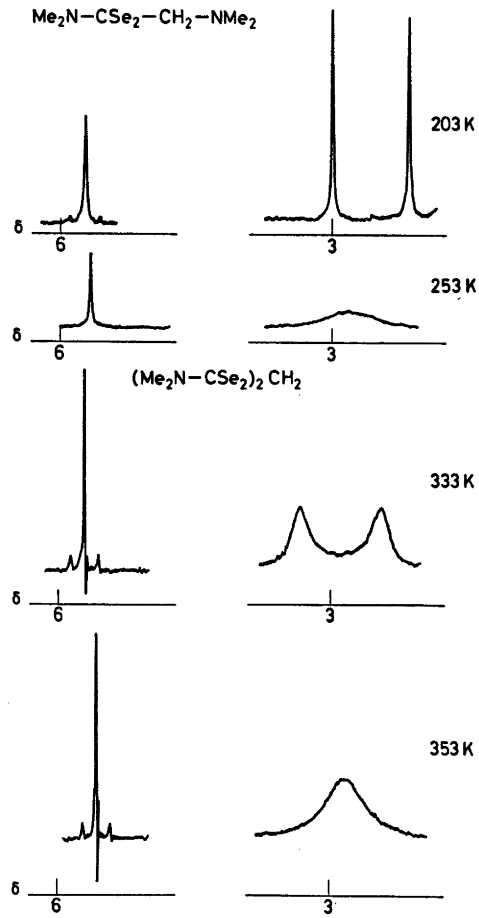


Fig. 1. Temperature dependent NMR spectra of compounds IIIa and VIa (solvent: toluene-d_8).

Table 1. Coalescence temperatures, T_C (K, ±2).

Compound	Solvent Toluene-d_8			C_6D_5Br	$CDCl_3$
	$\Delta\nu$ (Hz)	T_C (K)	$\Delta G^{\ddagger}$ (kcal/mol)	T_C (K)	T_C (K)
IIa	30	303	15.1	283	243
IIIa	52	253	12.4	233	< 218[a]
VIIa	45	318	15.7		313
VIa	61	348	17.0		> 333[b]

[a] In CD_2Cl_2 coalescence was observed at *ca.* 193 K. [b] The extent of exchange broadening suggests that coalescence will occur in the 343–348 K interval.

satellites be observed in the ^{13}C NMR spectrum of IIIa).

The latter fact implies that the CH_2 group cannot stay bonded to the same selenium atom all the time, and this observation prompted us to a closer investigation of the structure of compounds III.

The ^1H NMR spectra of IIIa and its thio analogue, IIa, were recorded as a function of temperature and solvent and compared to those of VIa and its thio analogue (VIIa), the latter two taken to represent the normal behaviour of diseleno- and dithiocarbamates.

Fig. 1 shows the temperature dependent spectra of IIIa and VIa (only amide–NCH_3 and Se–CH_2 signals shown) and demonstrates that the coalescence of the N–CH_3 signals of III is accompanied by the disappearance of the ^{77}Se satellites of the Se–CH_2 signal. In contrast to this behaviour these satellites in the spectrum of VIa are not affected by the interchange of the N–CH_3 groups.

The temperatures for coalescence of the amide–NCH_3 signals of IIa, IIIa, VIa, and VIIa in the solvents toluene-d_8, bromobenzene-d_5 and deuteriochloroform are recorded in Table 1.

An estimate of the barriers to exchange of methyl groups in toluene-d_8 was obtained by calculating $\Delta G^{\ddagger}$ at the coalescence temperature (T_C) using the relations $k_r = \pi/2^{\frac{1}{2}}\Delta\nu^{\circ}$ and $\Delta G^{\ddagger} = RT \ln (kT_C/hk_r)$, where k_r is the reorientation rate constant.[9,10]

Similar calculations were not carried out for the solvents bromobenzene and chloroform since in many cases either T_C or the peak separation in the absence of exchange ($\Delta\nu^{\circ}$) could not be determined. However, it seems a safe estimate that the $\Delta G^{\ddagger}$ values for IIa and IIIa both decrease by *ca.* 1 kcal/mol from toluene to bromobenzene and further by *ca.* 2 kcal/mol from bromobenzene to chloroform whereas VIa and VIIa show no significant difference between the barriers in toluene and chloroform.

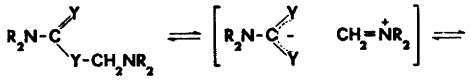
The spectra of VIa and VIIa are consistent with the expected behaviour of normal diseleno- and dithiocarbamates, respectively. The ^{77}Se satellites in the spectrum of VIa are retained above the coalescence temperature in accordance with an exchange of *N*-methyl groups through rotation around the C–N bond. The barrier for VIIa agrees very well with the value previously reported [11] for methyl *N*,*N*-dimethyldithiocarbamate (15.6 kcal/mol in *o*-dichlorobenzene). Likewise the lack of solvent sensitivity of both barriers is in accordance with previous reports on the rotational barriers for a number of thio- and selenoamide structures.[11–13]

Rotational barriers for diselenocarbamates have not previously been reported, but the value observed here for VIa suggests that this type of compound will normally conform to the general rule that a selenoamide structure displays a higher rotational barrier than its thio analogue.[13]

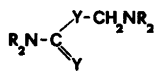
Apart from the disappearance of the ^{77}Se satellites simultaneosuly with the coalescence of the *N*-methyl signals in the spectrum of IIIa other anomalies are found in the spectra of IIa and IIIa. Firstly, IIIa displays a lower barrier to exchange of *N*-methyl groups than IIa, *i.e.* contrary to the usual trend. Secondly, both barriers are, contrary to those of VIa and VIIa, strongly solvent-dependent, the coalescence temperatures being lowered by an increase in solvent polarity. Thirdly, the

coalescence temperatures are lower than those of VIa and VIIa in all three solvents.

These results indicate that the energy barriers of compounds II and III are not barriers to rotation but rather barriers to transfer of the CH_2-NR_2 group from one Y atom (Y = S or Se) to the other. The solvent influence suggests an ion-pair mechanism for the exchange:



VIII



In the case of IIIa the disapperance of the ^{77}Se satellites furnishes direct evidence for the occurrence of a rearrangement reaction while for IIa such a process can only be inferred by analogy. However, we feel that in particular the fact that both exchange processes show the same solvent dependence, different from that found for VIa and VIIa, furnishes strong circumstantial evidence for an exchange by rearrangement also in the case of IIa. At least this appears to be the case in the more polar solvents, *e.g.* $CDCl_3$ ($\Delta T_{VIIa/IIa} = 70$ °C), whereas in toluene ($\Delta T = 15$ °C) the exchange might be effected by rotation as well.

In their tendency to undergo ionization II and III resemble the so-called *N*-(chloromethyl)-amines. *N*-(Chloromethyl)piperidine [14] is a solid, salt-like compound, insoluble in nonpolar solvents, and we found that its conductivity in acetonitrile or nitromethane solution amounts to *ca.* 20 % of that of a uni-univalent electrolyte. This would be in accordance with its formulation as *N*-methylenepiperidinium chloride, $C_5H_{10}N^+ = CH_2Cl^-$, the conductivity being diminished by strong ion-pair formation. It seemed possible that compounds II and III at higher temperatures might exist in a similar ionic structure, *i.e.* as methyleneiminium dithio- or diselenocarbamates, although they differ from the chloride in being readily soluble in non-polar solvents.

However, the conductivity of a freshly prepared solution of IIIc in acetonitrile was almost zero. The conductivity of the solution increased somewhat with time, but this increase is due to hydrolysis by traces of water which it is almost impossible to exclude. The acetonitrile solution finally smelled of formaldehyde, and crystals of compound Vc separated.

The conductivity measurements show that compounds III are not ionic in the ground state. This conclusion is supported by the finding that they do not react with benzyl bromide to form benzyl diselenocarbamates. It must therefore be concluded that the structure VIII represents either an activated complex or a very unstable intermediate in the rearrangement reaction.

The fact that the barriers for the selenium compounds III are lower than for their thio analogues gives an explanation of their higher reactivity towards water. That the $Se-CH_2NR_2$ bond is more polar than the $S-CH_2NR_2$ bond is indicated by the CH_2 signal being found at lower field for the selenium compounds. For methylenepiperidinium chloride the CH_2 proton signal is as low as δ 8.4 (in CD_3NO_2).

For the sulfur compound IIa both methyl signals of the Me_2NCS group are shifted upfield when we shift from chloroform to chlorobenzene as the solvent, but the high field one is shifted most strongly (Table 2). For thioamides it has been found that substituents *cis* to C=S exhibit smaller shifts than *trans* substituents on dilution with benzene.[15,16] If this rule holds true also for the compounds discussed here the high field one of the nonequivalent *N*-methyl signals is due to the methyl group trans to C=S or C=Se.

Mechanism of the formation of compounds III. In the investigation of the chemical properties of III it was found that these compounds do not react with carbon diselenide to give VI although the dialkylaminomethyl nitrogen

Table 2. Solvent shift (δ values) of nonequivalent *N*-methyl signals at 233 K.

Compound	$CDCl_3$	C_6H_5Cl
IIa	3.64	3.19
	3.55	2.94
IIIa	3.69	3.22
		2.88

should be sufficiently basic to form a bond to carbon diselenide. On the basis of the result that a Se–Se′ rearrangement proceeds readily in III but not in VI we suggest that the formation of III from carbon diselenide and I proceeds by a primary addition to give the dipolar ion IX followed by rearrangement *via* a methyleneiminium intermediate (VIII):

$$R_2N-CH_2-NR_2 + CSe_2 \rightarrow R_2N^+-CH_2-NR_2 \rightarrow \quad (CSe_2^-) \quad \text{IX}$$

$$R_2NCSe_2^-CH_2=NR_2^+ \rightarrow \text{III} \quad \text{VIII}$$

Even if the first step of the sequence could also take place from III and carbon diselenide, insertion of the latter would be blocked by the inability of the selenocarbamoylselenomethyl group to form a cation.

EXPERIMENTAL

Infrared spectra of KBr discs were recorded on a Perkin-Elmer model 337 grating spectrometer and visible spectra on a Unicam SP 800A instrument. The ^{1}H NMR spectra entered below were recorded at 40 °C on a Varian Model A-60A instrument with TMS as internal standard, and those used for the determination of coalescence temperatures (Table 1) on a Bruker WH-90 instrument.

Dimethylaminomethyl N,N-*dimethyldiselenocarbamate (IIIa).* When a solution of carbon diselenide (0.85 g) in petroleum ether was added to a solution of *N,N,N′,N′*-tetramethylmethylene diamine (0.51 g) in petroleum ether a small amount of an oil separated after a few minutes. The solution was decanted from the oil and evaporated in vacuum. The residue was dissolved in 20 ml of pentane and the solution filtered and cooled at −30 °C for 12 h. The compound separated as well-developed light yellow crystals. Yield 59 %. M.p. 48–49 °C. (Found: C 26.35; H 5.24; N 10.37. Calc. for $C_6H_{14}N_2Se_2$: C 26.44; H 5.20; N 10.36).

The corresponding sulfur compound (IIa) was prepared in a similar manner, using benzene as solvent and heating the solution at 40 °C for 15 min. The benzene was removed in vacuum and the residue dissolved in pentane. On cooling the compound separated as colourless crystals. Yield 56 %. M.p. 39–41 °C. (Found: C 40.44; H 7.92; N 15.72. Calc. for $C_6H_{14}N_2S_2$: C 40.68; H 7.80; N 16.07).

The main difference between the infrared spectra of II and IIa is found below 1000 cm^{-1}, where strong bands at 980 and 600 cm^{-1} in the spectrum of the sulfur compound are replaced by strong bands at 860, 530, and 495 cm^{-1} in the spectrum of the selenium compound. However, there is also a characteristic difference in the 1200 cm^{-1} region where a doublet at 1240 and 1260 cm^{-1} in the spectrum of IIa is shifted to 1222 and 1196 cm^{-1} in the spectrum of II. A pronounced difference in this region between corresponding sulfur and selenium compounds is only observed when the CS_2 and CSe_2 groups are bound to light groups, such as the methyl group in dithioacetates and diselenoacetates.[14]

^{1}H NMR spectra. IIIa ($CDCl_3$): δ 2.36 (s, 6 H); 3.58 (s, 6 H); 5.58 (s, 2 H); (C_6D_6): 2.34; 2.27; 5.70. IIa ($CDCl_3$): δ 2.34 (s, 6 H); 3.50 (s, 6 H); 5.15 (s, 2 H).

Diethylaminomethyl N,N-*diethyldiselenocarbamate (IIIb).* When a solution of carbon diselenide (0.85 g) in pentane (5 ml) was added to a solution of *N,N,N′,N′*-tetraethylmethylenediamine (0.80 g) in pentane (5 ml) an exothermic reaction took place. On cooling a yellowish precipitate separated. This was identified as diethylammonium diethyldiselenocarbamate. The filtrate left on evaporation a yellow oil which decomposed on attempts at purification. However, the crude product gave acceptable analyses and spectra for the expected compound. (Found: C 37.55; H 7.02; N 8.37. Calc. for $C_{10}H_{22}N_2Se_2$: C 36.85; H 6.74; N 8.51). ^{1}H NMR spectrum ($CDCl_3$): δ 1.12 (t, 6 H); 1.32 (t, 6 H); 2.67 (q, 4 H); 4.02 (q, 4 H); 5.55 (s, 2 H).

The compound is extremely sensitive to humidity. A pentane solution soon smells of formaldehyde and on standing deposits a reddish precipitate of the "triselenide" (Vb).

Se-Piperidinomethyl piperidine-1-carbodiselenoate (IIIc). Carbon diselenide (0.85 g) dissolved in petroleum ether (5 ml) was added to a solution of 1,1′-methylenediperidine (0.79 g) in petroleum ether (5 ml). On standing light yellow crystals separated. Yield 1.42 g (88 %). M.p. 93–94 °C (recryst. from pentane). (Found: C 40.92; H 6.33; N 7.96; Se 44.70. Calc. for $C_{12}H_{22}N_2Se_2$: C 40.92; H 6.29; N 7.95; Se 44.83).

The corresponding sulfur compound (IIc), prepared in ethanolic solution, formed colourless crystals with m.p. 60 °C in accordance with Ref. 1.

In contrast to the selenium compound (IIIc) which in ethanolic solution rapidly forms the "triselenide" Vc, the sulfur compound is quite stable in this solvent. However, on standing of an ethanolic solution of IIc for 6 months crystals with m.p. 130 °C separated. These were identified as bis(pentamethylene)thiuram disulfide.

The main differences between the infrared spectra of IIIc and IIc is the replacement of strong bands at 975, 890, 640, and 575 cm^{-1} in the spectrum of the sulfur compound by strong bands at 825, 782, 490, and 420 cm^{-1} in the spectrum of the selenium compound.

Electronic spectra. λ_{max} nm (log ε): IIIc, 274 (4.24), 327 (4.00), 394 (2.59); IIc, 216 (3.88), 262 (3.72), 290 (3.66). ^{1}H NMR spectra (in $CDCl_3$). IIIc: broad, unresolved signals at δ 1.5 – 1.7 (12 H), 2.5 – 2.7 (4 H), and 4.2 – 4.3 (4 H), sharp singlet at δ 5.62 (2 H). IIc: similar signals at δ 1.5 – 1.7 (12 H), 2.5 – 2.7 (4 H), 4.1 – 4.3 (4 H), and 5.25 (s, 2 H).

Bis(1-piperidinecarbodiselenoato)selenium(II) (compound Vc). Carbon diselenide (1 mmol) in benzene (10 ml) was added dropwise to a stirred solution of 1,1′-methylenedipiperidine (0.5 mmol) in benzene (10 ml). The solution was first yellow but the colour changed to red when half of the carbon diselenide had been added. On standing in a refrigerator for 12 h a yellow precipitate had formed. This was filtered off, and pentane was added, which again gave a precipitate and a solution from which 45 % of pure IIIc could be isolated. Treatment of the precipitates with methylene chloride yielded a slight amount of insoluble, apparently high-polymeric material, some less soluble fractions with *ca.* 14 % C, and some more soluble fractions with *ca.* 24 % C. The latter had almost identical infrared spectra and were purified by dissolution in methylene chloride, filtration through Al_2O_3, and precipitation with hexane. The resulting reddish powder with m.p. 190 – 191 °C had the composition $C_{12}H_{20}N_2Se_5$. (Found: C 24.76; H 3.42; N 4.90. Calc.: C 24.55; H 3.43; N 4.77). The infrared spectrum of this product proved to be identical with that of a product prepared earlier by oxidation of piperidinium 1-piperidinecarbodiselenoate.

The reaction between IIIc and carbon diselenide in benzene gave a similar result: along with unchanged IIIc the main product was compound Vc, a part of which separated from the benzene solution as well-developed red crystals.

Methylene bis(N,N-dimethyldiselenocarbamate) (VIa). Dimethylammonium dimethylselenocarbamate was prepared by leading gaseous dimethylamine into a solution of carbon diselenide (0.85 g) in petroleum ether (50 ml). The crystalline precipitate (1.2 g) was suspended in methylene chloride (150 ml) and 1 g of methylene bromide was added. The mixture was heated to boiling for 5 min, cooled, washed with water and dried. The residue obtained by evaporation of the solution was dissolved in 10 ml of boiling toluene and the hot solution was filtered through Al_2O_3 (W-200 neutral). On addition of petroleum ether to the filtrate a reddish precipitate was obtained. This was dissolved in the minimum amount of hot toluene and the solution was freed from a red insoluble substance by centrifugation. On addition of pentane and cooling in ice yellow crystals separated (113 mg). M.p. 165 – 167 °C. (Found: C 19.05; H 3.31; N 6.20. Calc. for $C_7H_{14}N_2Se_4$: C 19.06; H 3.19; N 6.34). ^{1}H NMR ($CDCl_3$): δ 3.37 (s, 6 H); 3.68 (s, 6 H); 5.42 (s, 2 H; satellites 2 Se, J (^{77}Se – H) = 18 Hz).

Methylene bis-(1-piperidinecarbodiselenoate) (VIc). Carbon diselenide (10 mmol) in petroleum ether (50 ml) was added to piperidine (20 mmol) in the same solvent at room temperature. The resulting piperidinium 1-piperidinecarbodiselenoate (3.4 g) was dissolved in methylene chloride and methylene bromide (2 g) was added. The mixture was heated until it just boiled and left at room temperature for 1 h. Then hexane (30 ml) was added and the precipitated product was filtered off, washed thoroughly with water to remove piperidinium bromide, dried, and recrystallized from toluene. Yield 2.08 g (80 %). M.p. 200 – 201 °C. (Found: C 29.78; H 4.16; N 5.29. Calc. for $C_{13}H_{22}N_2Se_4$: C 29.89; H 4.21; N 5.36).

^{1}H NMR spectrum ($CDCl_3$): broad, unresolved signals at δ 1.75 (12 H); 3.80 (4 H); and 4.44 (4 H); sharp singlet at 5.51 (2 H; satellites 2 Se; J (^{77}Se – H) = 19 Hz).

Methylene bis(N,N-dimethyldithiocarbamate) (VIIa). Dimethylammonium dimethyldithiocarbamate was prepared from dimethylamine (50 mmol) dissolved in petroleum ether (200 ml) and carbon disulfide (25 mmol). The precipitate was filtered off and immediately dissolved in methylene chloride (40 ml). Methylene iodide (25 mmol) was added and the solution was left at room temperature for 4 h. The reaction mixture was washed with water and evaporated to dryness. The residue was recrystallized from toluene-petroleum ether to give 2.7 g (68 %) of VIIa. (Found: C 33.25; H 5.75; N 11.07. Calc. for $C_7H_{14}N_2S_4$: C 33.07; H 5.51; N 11.02).

^{1}H NMR spectrum ($CDCl_3$; 33 °C): singlet at δ = 5.35 (2 H), broad signal with two peaks at δ 3.39 and 3.50 (12 H). Coalescence temperature 40 °C, see Table 1.

N-*Methylenepiperidinium chloride* was prepared from 1,1′-methylenedipiperidine and acetyl chloride.[14]

^{1}H NMR spectrum (CD_3NO_2): broad signals at δ 1.92 (6 H) and δ 4.18 (4 H), singlet at δ 8.40 (*ca.* 2 H).

Molar conductivity (25 °C; *c* 0.1 – 0.01 mol/l): 13.8 S cm^2 mol^{-1} in nitromethane and 34 S cm^2 mol^{-1} in acetonitrile. According to the values found for the conductivity of uni-univalent electrolytes in nitromethane[18] and acetonitrile,[19] the chloro compound is 15 – 20 % dissociated under these conditions. Its physical properties and the presence of only one CH_2 signal in the NMR spectrum suggest that the undissociated molecules are ion pairs rather than molecules with covalently bound chlorine.

Diethylmethyleneiminium chloride was prepared from *N,N,N′,N′*-tetraethylmethylenediamine and benzoyl chloride.[14]

^{1}H NMR spectrum ($CDCl_3$): δ 1.49 (t, 6 H), 3.95 (q, 4 H), and 8.55 (s, 2 H), unchanged after 24 h.

Molar conductivity in acetonitrile (25 °C; c 0.008 mol/l): 41.2 S cm^2 mol^{-1}. The conductivity diminished with time and after 24 h

reached the value 14.9 S cm^2 mol^{-1}, corresponding to complete hydrolysis to diethylammonium chloride, which was found to have this conductivity under the same conditions.

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Short Communications

Bacterial Carotenoids. XLVII. * C_{50}-Carotenoids. 15. ** Absolute Configuration of Decaprenoxanthin

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Hitherto the constitutions of 16 C_{50}-carotenoids have been reported.[1–5] They all exhibit extra five carbon substituents in 2,2′-positions of a traditional C_{40}-carotenoid, suggesting common biosynthetic pathways.[6,7]

Recently, we assigned the absolute configuration to *C.p.* 450 (*1*, Scheme 1)[8] from CD-correlation with (2R,2′*R*)-2,2′-dimethyl-β,β-carotene (*2*).[9]

We now present evidence for assignment of the absolute configuration *3* to decaprenoxanthin.[10,11]

Decaprenoxanthin (*3*) has two chiral centers in each ε-ring (C-2, C-6 and C-2′, C-6′). Since decaprenoxanthin is optically active,[12] it is not a *meso* compound, and the chirality of each end-group is from biosynthetic considerations likely to be the same. This is supported by the ^{1}H NMR spectrum,[10] demonstrating that decaprenoxanthin is centro-symmetric.

Four stereochemical alternatives, consisting of two enantiomeric pairs (A, ent. A (2,6-*trans*); B, ent B (2,6-*cis*)) for these end-groups exist. In principle, CD-spectra of two model carotenoids with end groups A or ent. A, B or ent. B should allow a differentiation between these four possibilities.

Two optically active 2,2′-dimethyl-carotenes of type A,A (*4*) and B,B (*5*) have been synthesized[13] from the irones *6* and *7* by routes known to retain the stereo-integrity of the optically active irones[14] *via* the C_{15}-alcohols *8* and *9*. Irone *7* is a natural constituent of Iris oil; *6* was isolated after base-catalyzed isomerization of natural irones according to Rautenstrauch and Ohloff.[15]

The similarities of the CD spectra (Fig. 1) of the 2,6-*trans* model dimethyl-carotene

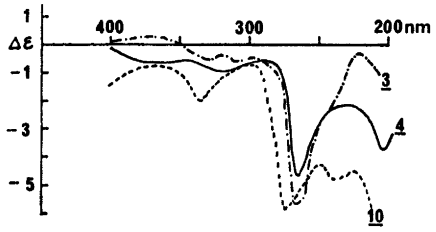
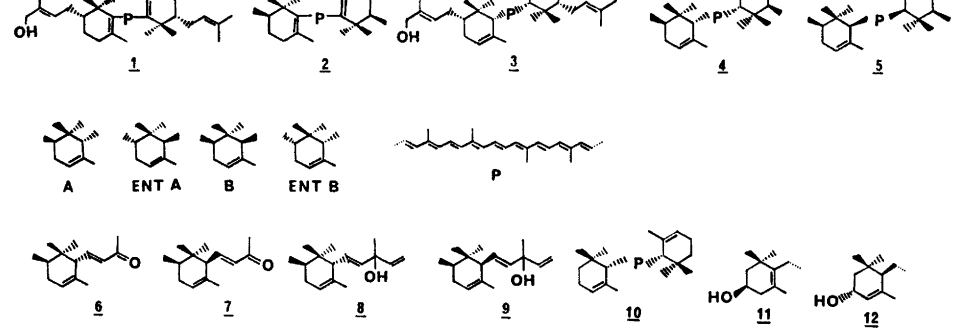


Fig. 1. CD-spectra of *4* (EPA = ether, isopentane, ethanol 5:5:2———; *10* (dioxane)[14] — — —, and decaprenoxanthin (*3*, EPA)[10] — · —.



Scheme 1.

* Part XLVI. *Acta Chem. Scand. B 29* (1975) 921.
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Table 1. δ-values ($CDCl_3$) of the *gem.* dimethyl signals of some model compounds and of decaprenoxanthin.

Compound		δ *gem.* dimethyl		Difference in chemical shift Δ
7		0.72	0.87	0.15
9	2,6-*cis*	0.63	0.85	0.22
5		0.68	0.87	0.19
6		0.83	0.87	0.04
8	2,6-*trans*	0.77	0.81	0.04
4		0.81	0.82	0.01
Decaprenoxanthin [10]		0.75	0.95	0.20

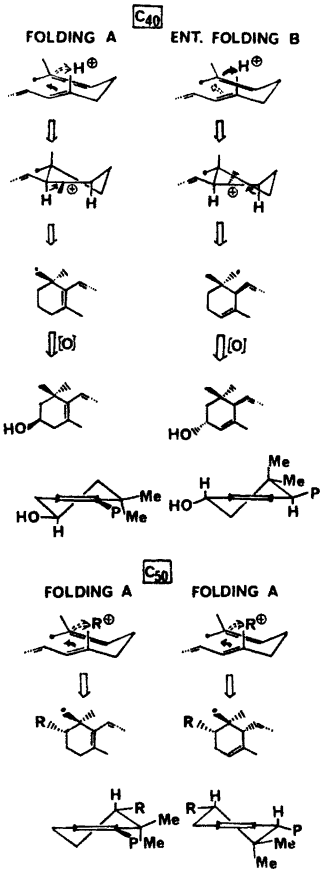
(*4*)[13] and of decaprenoxanthin is striking and could be taken to support the same (2*S*,6*R*,2′*S*,6′*R*) configuration for decaprenoxanthin.

However, the ^1H NMR signals of the *gem.* dimethyl groups of the model compounds *4*–*9* (Table 1) clearly demonstrate a smaller shielding of one of the *gem.* dimethyl groups in the 2,6-*trans* than the 2,6-*cis* derivatives. Assuming that the influence on the *gem.* dimethyl signals caused by a 2-methyl group is comparable with that of a 2-(4-hydroxy-3-methyl-2-butenyl) substituent, the situation (Table 1) for decaprenoxanthin[10] clearly favours 2,6-*cis*. The CD results therefore need further consideration. When comparing the CD spectrum (Fig. 1) of unnatural (6*S*,6′*S*)-ε,ε-carotene (*10*)[16] with that of *4* it becomes evident that the chiral center at C-2 does not contribute significantly to the *CD*. This is compatible with the concept of preferred helicity of the chiral cyclohexene half-chair[17,18] The conformation with the polyene chain quasiequatorial is expected to be dominant for *10*, *4* and particularly stabilized for *3* with the isopentenyl substituent equatorial. The CD spectrum of decaprenoxanthin (Fig. 1) is therefore compatible with structure *3* (2,6-*cis*, 2′,6′-*cis*; ent B, ent B).

That the CD of 2-substituted ε-rings is dictated nearly exlusively by the C-6 center has already been observed by Rautenstrauch and Ohloff for α-ionone and the corresponding epimeric irones.[15] The same appears to be true for a 3-substituted ε-ring.[19]

Attempts to confirm assignment *3* for decaprenoxanthin by base-catalyzed isomerization[20,21] to the β-analogue were made. Decaprenoxanthin was reisolated from *Flavobacterium dehydrogenans*.[10] Base-catalyzed isomerization of the ε- to β-end groups, successful with ε,ε-carotene and lutein, did not yield the expected products when applied to decaprenoxanthin. Chromatographic, spectroscopic and mass spectrometric evidence indicated that isomerization was accompanied by other fundamental changes in structure (probably involving the additional 5-carbon units at the 2-positions). As a result correlation of the configuration at C-2 of decaprenoxanthin with that of *C.p.* 450 failed.

Decaprenoxanthin (2*R*,6*R*,2′*R*,6′*R*)-2,2′-bis(4-hydroxy-3-methyl-2-butenyl)-ε,ε-carotene thus has the same absolute configuration at C-2,2′ as *C.p.* 450 (*1*) isolated from *Corynebacterium poinsettiae*[8] and opposite configuration at C-6 to all C_{40}-carotenoids with ε-end groups,[1] which leads to the following biosynthetic considerations depicted in Scheme 2.



Scheme 2.

From work on the trisporic acid biosynthesis[22,23] cyclization resulting in β- and ε-end groups must proceed through enantiomeric foldings of the chain forms if the cyclizations involve loss of axial H and are analogous with other terpenoids. Subsequent equatorial hydroxylation of half-chairs of the same helicity is consistent with the stereochemistry of the zeaxanthin (*11*) and lutein (*12*) end groups.

In the C_{50}-series addition of 2 five-carbon units (probably isopentenyl or dimethylallyl pyrophosphate) could precede or initiate cyclization. No cyclic C_{40}-carotenoids have as yet been found in organisms producing C_{50}-carotenoids. Folding A, followed by 5-carbon addition/cyclization and loss of the axial H at C-4 would give the observed stereochemistry for the *C.p.* 450 (*1*) end groups and the same folding is required to give the decaprenoxanthin (*3*) end group. In the ε-series the half-chairs are stabilized by extra equatorial substituents (OH in *12* and alkyl in *3*). Different enzymes must be responsible for cyclization to ε-rings in carotenoids in the C_{40}-series and in the C_{50}-series; the latter so far being restricted to non-photosynthetic, spherical or rod-shaped, aerobic bacteria.

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The Resistance of Glycoproteins to Proteolytic Inactivation

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The carbohydrate constituents of globular glycoproteins are probably located at the surface and may thus be expected to act as local shields and enhance the protection of the polypeptide towards degradation by proteolytic enzymes. Supporting evidence for this hypothesis has been obtained by different groups.[1-3] and the results were obtained from experiments using glycoproteins found in nature.

A novel approach to such studies is provided by the possibility of attaching, covalently, soluble carbohydrates to polypeptides by the cyanogen bromide method.[4] The evidence obtained so far indicates that a considerable increase in stability towards inactivation by heat and by proteolytic enzymes may be imparted to proteins in this way.[5,6]

In the present communication we describe extensions to our previous experiments [6] and have examined the proteolysis of different fractions of a lysozyme-dextran T10 preparation as well as the inactivation by autolysis of chymotrypsin, trypsin and their dextran conjugates.

Experiments. The enzymes used were egg white lysozyme, EC 3.2.1.17 (Nutritional Biochemicals Corporation, U.S.A), alpha-chymotrypsin, EC. 3.4.21.1 (Koch-Light Laboratories, England) and trypsin, EC. 3.2.21.4 (Sigma Chemical Co., U.S.A.). Dextran-T10 (Pharmacia, Sweden) with an average molecular weight of 10 000 daltons was used as the carbohydrate attached to the enzymes. Partial characterisation of the preparations is included in Table 1.

The conjugates were prepared from cyanogen bromide activated T10[4] (400 mg) which was attached to free amino- and carboxyl groups[7] in the proteins (100 mg). Subsequent to the coupling procedure, the lysozyme-T10 preparation was subjected to gel exclusion chromatography on Sephadex G-100 and the three fractions, A, B, and C were obtained from the early, middle, and late parts of the elution diagram, respectively.

Table 1. Protein-dextran T10 preparations.

Preparation	Relative specific enzyme activity (%)	Carbohydrate content (%, w/w)
Lysozyme-T10-A	16	40
Lysozyme-T10-B	39	29
Lysozyme-T0-C	24	17
Chymotrypsin-T10	55	80
Trypsin-T10	58	80

Lysozyme was assayed using the lysis of bacterial cell walls [8] and the proteases were assayed by the caseinolytic method.[9] Relative specific enzyme activities were obtained by comparison with the original enzymes and the calculations were based on the protein content of the conjugates. Percentage inactivation was determined by comparing the assay readings to standard curves of "amount of enzyme" *versus* "assay reading".

The carbohydrate content of the lysozyme-T10 fractions was determined by gas chromatography of the trimethyl silyl derivatives of the methyl glucosides obtained by methanolysis [10] but the compositions of the protease-conjugates were taken directly from the amounts used in the coupling procedure as no fractionation was carried out.

All proteolytic degradations were carried out in sealed tubes at 37 °C in the presence of traces of toluene in order to prevent bacterial growth. Lysozyme and the lysozyme-conjugates were incubated in 25 mM citrate-phosphate buffer (pH 6.2) containing 5 mM calcium chloride in order to stabilise chymotrypsin. The concentrations of lysozyme and chymotrypsin in these mixtures (Nos. 1 – 4) were 0.05 mg/ml and 0.2 mg/ml, respectively.

The autolytic inactivation of the protease conjugates and the free proteases were performed in 100 mM phosphate buffer (pH 7.5). The chymotrypsin concentrations in incubation mixtures 5 and 6 were 0.5 mg/ml and 0.7 mg/ml respectively, and the trypsin concentrations in mixtures 7 and 8 were 1.0 mg/ml and 1.7 mg/ml, respectively.

For control purposes the free enzymes were incubated in the presence of concentrations of dextran T10 equivalent to the amount of T10 present in the corresponding conjugate-incubations. This was done in case the dextran might have a modifying influence on the proteolytic enzymes.

Results and discussions. The results obtained by Marshall and Rabinowitz [5] and by us [6] (Table 2) show that the covalent attachment of carbohydrate to lysozyme, trypsin, and chymotrypsin render these proteins more resistant to proteolytic degradation. Our results show that the lysozyme fraction with the highest content of carbohydrate was most resistant to inactivation by chymotrypsin. Assays of the proteolytic activity in the different incubation mixtures with lysozyme and its conjugates indicate that the results can not be due to variations in the activity of chymotrypsin.

The protective influence of carbohydrate constituents attached by the cyanogen bromide

Table 2. Proteolytic inactivation of protein-dextran conjugates and free proteins.

Incubation mixture No.	Composition	Enzyme assay	Remaining enzyme activity (%) Incubation time (days) 0	1	2	3	4	5
1	Lysozyme +T10 +chymotrypsin	Lysozyme	100	13	0	0	0	0
2	Lysozyme-T10-A +chymotrypsin	Lysozyme	100	52	45	42	40	40
3	Lysozyme-T10-B +chymotrypsin	Lysozyme	100	40	32	28	27	25
4	Lysozyme-T10-C +chymotrypsin	Lysozyme	100	35	27	22	20	17
5	Chymotrypsin +T10	Chymotrypsin	100	52	41	29	17	
6	Chymotrypsin-T10	Chymotrypsin	100	72	60	50	40	
			Incubation time (h) 0	1	2	5	24	
7	Trypsin +T10	Trypsin	100	24	16	2	0	
8	Trypsin-T10	Trypsin	100	50	40	24	20	

method is consistent with the results obtained with "natural" glycoproteins.[1-3] Thus it appears that the phenomenon may be a general one. To what extent the type of carbohydrate and the size of the polysaccharide groups affect the protective influence is not yet known, but experiments to clarify this point should be relatively simple in view of the convenient method available for preparation of glycosylated proteins.

The reason for the enhanced resistance to proteolysis is not definitely known although it seems plausible that steric hindrance is, at least partly, responsible for the effect. However, if carbohydrate constituents cause a reduction in the tendency for the polypeptide chain to oscillate between different conformational states, this might also affect the susceptibility to proteases.[11] An effect of this type might possibly be related to the increased resistance to thermal inactivation.[5]

Stabilisation of enzymes by attachment of carbohydrate might have applications for industrial processes or for enzymes used commercially as for example in detergents. Enzymes linked to soluble carbohydrate could subsequently be attached to an insoluble support and linkage between the carbohydrate part and the solid support might offer advantages.[12]

Increased half-life of therapeutic macromolecules by conjugation with polysaccharides is a possibility and it has been shown that the coupling of heparin to ficoll gave a complex with very good anticoagulant activity and the duration *in vivo* (rabbits) was significantly longer than that of free heparin.[13]

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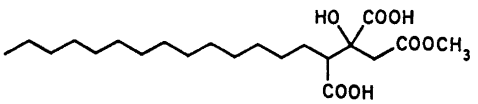
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The Structure of Caperatic Acid

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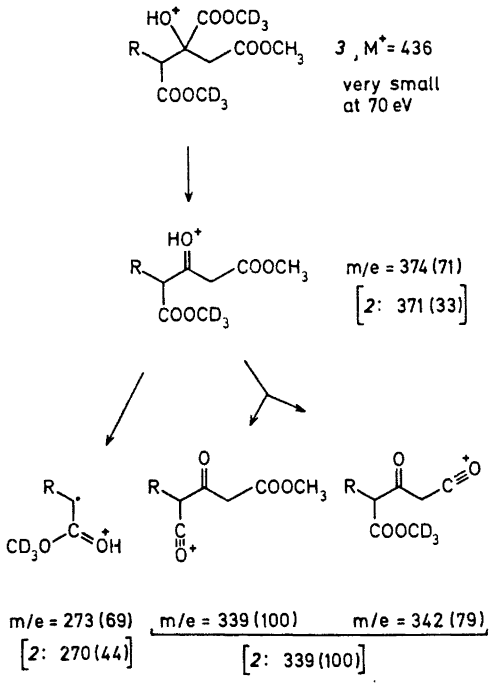
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The lichen compound caperatic acid (*1*) was first isolated from *Parmelia caperata* in 1897,[1] and has subsequently been found in many other lichens.[2–5] Zopf[5] isolated an acid believed to be *1* from *Cetraria glauca* and *Mycoblastus sanguinarius*, and we have now confirmed that *1* can be isolated from the former species. Asano and Ohta found that the acid is a monomethyl ester of 2-hydroxy-1,2,3-heptadecanetricarboxylic acid,[6] but the position of the methyl ester group has hitherto been unknown. We now report that caperatic acid has the structure *1*.



1

This result is based upon a comparison of the mass spectra of the trimethyl ester *2* and its corresponding d_6-ester *3* (Scheme 1), and also upon a chemical degradation of *1*. In the upper parts of the mass spectra of *2* and *3* there are, respectively, three and four prominent peaks, and from the *m/e* values of these peaks, the structure of caperatic acid was deduced (Scheme 1). Support for the degradations shown in Scheme 1 was obtained from high resolution mass spectrometry of *2* and from the existence of analogous prominent peaks in the mass spectra of homologous trimethyl alkylcitrates.[7]



Scheme 1.

In a corroborative chemical degradation, *1* was oxidized with sodium bismuthate, a reagent which oxidizes α-hydroxy carboxylic acids to aldehydes or ketones.[8] An NMR spectrum of the crude reaction product suggested that its major component was methyl 3-oxo-octadecanoate, the decarboxylation product of the initially formed β-keto carboxylic acid.

A CD investigation has shown that caperatic acid forms a complex with molybdate (VI) at pH 5.7.[7] This demonstrates that *1* contains a free α-hydroxy carboxylic acid group.[9]

Experimental. Melting points are corrected. The NMR spectrum was recorded on a Varian XL-100 spectrometer and the mass spectra on a Varian MAT 311 instrument using the direct inlet system.

Isolation of 1 *from* Cetraria glauca. From 300 g of fresh lichen, collected 30 km northeast of Stockholm, 1.6 g of *1* was isolated. After four recrystallisations from ethanol the product melted at 132.5–134 °C (lit.[6] m.p. 132–133.5 °C) $[\alpha]_D^{23}$ −15.5° ($c = 0.9$, dioxane). An authentic sample of *1*, isolated from *Parmelia caperata*, was obtained from L. Tibell, University of Uppsala, and this sample showed $[\alpha]_D^{23}$ −15.7° ($c = 0.6$, dioxane). The optical rotations of *1* have previously been measured in chloroform,[6,10] but we found that all samples of *1* were only slightly soluble in ordinary ethanol-stabilized chloroform ($c < 0.1$). Reaction with diazomethane in ether solution afforded the trimethyl ester *2*, m.p. 55–56 °C (from ethanol), lit.[6] m.p. 56.5–57.5 °C. MS: *m/e* (rel. intensity) 371 (33), 339 (100), 270 (44), 101 (48), 87 (49), 43 (37).

The d_6*-ester* 3 was prepared by reaction of *1*-d_3 with diazomethane-d_2. The former reactant was obtained by dissolving *1* in a mixture of CH_3OD (99 % isotopic purity) and dry ether, followed by evaporation of the solvent. Diazomethane-d_2 was prepared largely after Hecht and Kozarich,[11] but the *N*-nitrosomethylurea was replaced with *N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide and, instead of using the dimethoxyethane solution, dry ether was added and the diazomethane-d_2 was distilled together with ether into a flask containing a little D_2O (99.7 % isotopic purity). The water in the receiving flask was frozen out and the ethereal layer was then added to *1*-d_3. The resulting ether solution was washed with water, dried (Na_2SO_4) and concentrated. The product *3* was crystallised from ethanol, m.p. 55.5–

56.5 °C. MS: 374(71), 342(79), 339(100), 273(69), 101(33), 90(42).

Bismuthate oxidation of 1. A mixture of *1* (55 mg), sodium bismuthate (56 mg), and glacial acetic acid (2 ml) was warmed (35 °C, 80 min) with stirring in a stoppered flask. The resulting solution was poured into a mixture of cold water (100 ml) and ether (50 ml). The ether layer was repeatedly washed with sodium hydrogen carbonate solution and then twice with water. After drying (Na_2SO_4) and concentration of the ether layer, and finally drying in a vacuum desiccator over night, a residue (27 mg) was obtained. The NMR spectrum of this residue ($CDCl_3$) displayed singlets at $\delta = 3.66$ and 3.38, and triplet at $\delta = 2.46$ (integrals approximately 35, 18 and 21 mm, respectively), which indicate the partial structure $-CH_2-CH_2-CO-CH_2-COOCH_3$. No peaks from an isomeric β-keto ester could be detected.

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The Crystal Structures of Carbamoylcholine Chloride, Carbamoylcholine Iodide, and the 1:1 Complex Carbamoylcholine Picrate, Picric Acid

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The crystal structures of carbamoylcholine chloride, CARCHL, carbamoylcholine iodide, CARIOD, and the 1:1 complex carbamoylcholine picrate, picric acid, CARPCR, have been determined from diffractometer collected three-dimensional X-ray data and refined by full-matrix least-squares techniques to R values of 0.039, 0.043, and 0.056 for CARCHL, CARIOD, and CARPCR, respectively. In all of the three crystal structures the two possible hydrogen bonds N−H···A are found, but the contacts are generally rather weak. The conformations of the carbamoylcholine ions found in the crystals of the three salts are highly different, and *trans* as well as *gauche* arrangements of the choline moiety are seen.

The crystal structures of three carbamoylcholine salts, a chloride, an iodide, and a picrate complexed with picric acid, was investigated as part of conformational studies of choline derivatives in the solid state.



Since the carbamoylcholine ion is conformationally flexible, salts forming non-isostructural crystals have been investigated in order to examine the effect of different environments on the conformation of the carbamoylcholine ion. Here the possible formation of hydrogen bonds has been of special interest. Among the large number of salts of choline derivatives, of which the crystal structures are known, only very few have any possibility of formation of hydrogen bonds. The salts have most often been halides, and few of the choline derivatives examined can act as donors for hydrogen bonds.

EXPERIMENTAL

Carbamoylcholine chloride, CARCHL. A solution of commercial CARCHL in ethanol was cooled slowly to give colourless prisms of the compound. Preliminary Weissenberg- and precession X-ray diffraction photographs showed the crystals to be orthorhombic. The systematically absent reflections are $h0l$ when h is odd and $0kl$ when $k+l$ is odd, indicating that the space group is $Pna2_1$ or $Pnam$. The unit cell parameters were refined by least-squares techniques from diffractometer measured 2θ-angles (Mo$K\alpha$ radiation, $\lambda = 0.7107$ Å) for 17 independent reflections. The density of the crystals was measured by flotation in a mixture of chlorobenzene and bromobenzene. All melting points given in this paper were determined on a Leitz hot stage microscope. Table 1 lists the unit cell parameters and other crystal data.

Three-dimensional diffraction data were measured at room temperature on a Nonius three-circle automatic diffractometer using graphite monochromated Mo$K\alpha$-radiation. The ω scan technique with a scan speed of 1.2° min^{-1} was employed, and the scan angle was 1.2°. Background counts were taken for half the scanning time at each of the scan range limits. One standard reflection was measured after every 40 reflections. All the data were measured from a single crystal with approximate dimensions $0.31 \times 0.34 \times 0.42$ mm. The crystal was sealed in a glass capillary to avoid the humidity of the air and mounted with its elongated dimension, which was parallel to the

Table 1. Crystal data.

	CARCHL	CARIOD	CARPCR
Stoichiometry	$C_6H_{15}N_2O_2Cl$	$C_6H_{15}N_2O_2I$	$C_{18}H_{20}N_8O_{16}$
Formula weight	182.65	274.10	604.40
Z	4	4	2
$F(000)$	396	540	624
Space group	*Pnam*	$P2_1/c$	$P\bar{1}$
a	10.248(3) Å	6.073(2) Å	13.020(5) Å
b	12.850(4) Å	12.616(8) Å	13.736(4) Å
c	6.809(4) Å	13.688(5) Å	7.117(2) Å
α			96.39(3)°
β		95.79(3)°	96.77(2)°
γ			91.72(4)°
Cell volume	896.66 Å^3	1043.38 Å^3	1254.66 Å^3
D_x	1.35 g cm^{-3}	1.74 g cm^{-3}	1.60 g cm^{-3}
D_m	1.35(1) g cm^{-3}	1.74(1) g cm^{-3}	1.61(1) g cm^{-3}
$\mu_{(MoK\alpha)}$	3.83 cm^{-1}	30.62 cm^{-1}	1.54 cm^{-1}
Melting point	203–208 °C (decomp.)	193–195 °C	89–91 °C

crystallographic c-axis, along the ϕ-axis of the goniostat. Out of the 1289 independent reflections within the range $2.5° \leq \theta \leq 25.0°$, 979 had $I_{net} \geq 2.5\sigma(I)$, where $\sigma(I)$ is the standard deviation from counting statistics. These were regarded as observed reflections, whereas the remaining reflections were regarded as unobserved and excluded from the refinement procedure. Lorentz and polarization corrections were applied, but no absorption corrections were made.

The statistical distribution of normalized structure factor magnitudes ($|E|$'s) indicated that the crystal structure is centrosymmetric. Consequently the space group was assumed to be *Pnam*, and this assumption was corroborated by the final structure analysis. The trial structure was obtained by the heavy-atom method.

Carbamoylcholine iodide, CARIOD. CARIOD was prepared by mixing almost saturated aqueous solutions of carbamoylcholine chloride and potassium iodide. The resulting precipitate was purified by recrystallisation from 96 % ethanol. Single crystals (colourless prisms) were grown by slow cooling of a solution in aqueous ethanol. Preliminary Weissenberg- and precession photographs showed the crystals to be monoclinic. The systematically absent reflections are $h0l$ when l is odd and $0k0$ when k is odd, indicating that the space group is $P2_1/c$. The unit cell parameters were obtained by least-squares refinement of 2θ-angles (Mo$K\alpha$ radiation) for 48 reflections, measured from precession photographs. No corrections for film shrinkage were performed. The density of the crystals was measured by flotation in a mixture of bromobenzene and methyliodide. Crystal data are listed in Table 1.

Intensity data were measured from a single crystal with approximate dimensions $0.14 \times 0.16 \times 0.42$ mm. The crystal was sealed in a glass capillary and mounted with its elongated dimension, which was parellel to the crystallographic a-axis, along the ϕ-axis of the goniostat. The experimental conditions and treatment of the data were the same as described for CARCHL. Each of the 1831 independent reflections in the range $2.5° \leq \theta \leq 25.0°$ was measured twice. 2948 of the 3562 reflections ($hk \pm l$ and $h - k \pm l$) had $I_{net} \geq 2.5\sigma(I)$. The reflections for both quadrants were used independently in the least-squares refinement.

The trial structure was obtained by the heavy-atom method.

Carbamoylcholine picrate, picric acid, CARPCR. CARPCR was prepared by mixing aqueous solutions of carbamoylcholine chloride and picric acid. After standing for some minutes yellow prisms were formed. These were purified by recrystallisation from water, and single crystals were grown by slow cooling of an aqueous solution. Preliminary Weissenberg- and precession photographs showed the crystals to be triclinic. The unit cell parameters were refined by least-squares techniques from diffractometer measured 2θ-angles (Mo$K\alpha$ radiation) for 43 independent reflections. The density of the crystals was measured by flotation in a mixture of bromobenzene and methyl iodide. Crystal data are listed in Table 1.

Intensity data were measured from a single crystal with approximate dimensions $0.20 \times 0.22 \times 0.38$ mm. The crystal was mounted on a glass fiber with its elongated dimension, which was parallel to the crystallographic c-axis, along the ϕ-axis of the goniostat. The experimental conditions and treatment of the data were the same as described for CARCHL. Out of the 4436 independent reflections within the range $2.5° \leq \theta \leq 25.0°$ 2751 had $I_{net} \geq 2.5\sigma(I)$.

Table 2. Final positional and thermal parameters for non-hydrogen atoms. The estimated standard deviations, referring to the last significant figure, are given in parentheses. Thermal parameters are $\times 10^2$.
The temperature factor is defined by: $\exp[-2\pi^2(U_{11}h^2a^{*2}+\cdots+2U_{12}hka^*b^*+\cdots)]$.

Atom	x/a	y/b	z/c	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
CARCHL									
N(1)	.9997(2)	.3788(2)	.2500(−)	3.74(11)	2.94(10)	4.61(13)	−0.20(9)	0.0(−)	0.0(−)
C(2)	.9214(2)	.4617(2)	.2500(−)	3.35(11)	2.97(11)	2.27(11)	0.23(9)	0.0(−)	0.0(−)
O(3)	.8027(2)	.4593(1)	.2500(−)	2.96(8)	3.45(9)	5.23(12)	0.31(7)	0.0(−)	0.0(−)
O(4)	.9895(2)	.5523(1)	.2500(−)	2.85(8)	2.55(8)	4.81(11)	−0.02(7)	0.0(−)	0.0(−)
C(5)	.9071(2)	.6427(2)	.2500(−)	2.87(10)	2.48(11)	4.35(15)	0.06(9)	0.0(−)	0.0(−)
C(6)	1.0026(2)	.7334(2)	.2500(−)	2.48(10)	2.52(10)	3.43(13)	−0.09(9)	0.0(−)	0.0(−)
N(7)	.9378(2)	.8389(2)	.2500(−)	2.37(8)	2.52(9)	2.54(10)	0.14(7)	0.0(−)	0.0(−)
C(8)	1.0454(2)	.9193(2)	.2500(−)	3.16(11)	2.88(12)	4.10(15)	0.80(9)	0.0(−)	0.0(−)
C(9)	.8565(2)	.8551(2)	.0695(3)	3.67(8)	4.00(9)	3.51(10)	−0.23(7)	1.09(7)	0.45(8)
Cl^-	.8271(1)	.1558(1)	.2500(−)	3.39(3)	3.38(3)	4.10(4)	−0.12(3)	0.0(−)	0.0(−)
CARIOD									
N(1)	.9146(8)	−.0508(4)	.6144(3)	7.2(3)	6.3(3)	3.5(2)	1.1(2)	1.9(2)	0.7(2)
C(2)	.7587(8)	.0243(4)	.5916(3)	4.8(3)	3.7(2)	3.2(2)	−0.6(2)	0.6(2)	−0.4(2)
O(3)	.7301(6)	.0714(3)	.5153(2)	5.7(2)	4.9(2)	3.0(2)	0.2(2)	1.1(1)	0.4(1)
O(4)	.6356(6)	.0370(3)	.6671(2)	5.6(2)	4.7(2)	3.1(2)	1.1(2)	1.2(1)	0.3(1)
C(5)	.4693(9)	.1183(4)	.6551(3)	4.8(3)	4.1(2)	3.3(2)	0.8(2)	0.1(2)	0.1(2)
C(6)	.3361(8)	.1149(4)	.7423(4)	3.3(2)	4.2(2)	4.2(3)	0.0(2)	0.1(2)	0.1(2)
N(7)	.4527(7)	.1509(3)	.8398(3)	4.1(2)	4.2(2)	3.8(2)	0.2(2)	1.4(2)	−0.8(2)
C(8)	.6230(12)	.0700(7)	.8785(5)	6.3(4)	10.1(6)	4.1(3)	3.3(4)	−1.2(3)	−1.1(3)
C(9)	.5518(15)	.2570(6)	.8328(6)	10.1(6)	6.8(4)	7.0(4)	−4.9(4)	3.9(4)	−2.9(4)
C(10)	.2829(12)	.1563(6)	.9118(5)	7.2(4)	6.4(4)	6.0(4)	−0.5(3)	4.1(3)	−0.8(3)
I^-	−.05069(5)	.15367(2)	.13618(2)	4.47(2)	4.42(2)	3.93(2)	0.32(2)	0.63(1)	−0.14(2)
CARPCR									
N(1)	−.0214(2)	.0975(2)	.3450(4)	4.0(2)	4.7(2)	6.8(2)	0.5(1)	0.1(1)	1.1(1)
C(2)	.0815(2)	.1006(2)	.3608(5)	4.5(2)	3.0(2)	4.5(2)	0.3(1)	0.3(2)	−0.5(1)
O(3)	.1368(2)	.0452(2)	.4469(3)	4.2(1)	4.6(1)	6.5(2)	0.5(1)	−0.1(1)	1.2(1)
O(4)	.1180(2)	.1698(2)	.2644(3)	4.9(1)	4.5(1)	6.0(2)	0.6(1)	1.2(1)	1.0(1)
C(5)	.2276(3)	.1915(2)	.2886(5)	5.7(2)	4.5(2)	5.6(2)	−1.4(2)	1.2(2)	−0.8(2)
C(6)	.2665(3)	.1915(2)	.0971(5)	5.3(2)	3.1(2)	5.3(2)	−0.6(1)	1.2(2)	−0.1(1)
N(7)	.2903(2)	.0915(2)	.0020(4)	4.2(2)	3.4(1)	4.1(2)	−0.6(1)	0.2(1)	−0.3(1)
C(8)	.1961(3)	.0240(2)	−.0379(6)	5.1(2)	4.3(2)	7.1(3)	−1.2(2)	0.6(2)	−1.6(2)
C(9)	.3286(3)	.1057(3)	−.1845(6)	7.2(3)	7.0(3)	5.3(2)	−0.9(2)	1.8(2)	−0.2(2)

Table 2. Continued.

C(10)	.3740(3)	.0458(3)	.1226(6)	5.5(2)	5.0(2)	7.3(3)	0.8(2)	−0.7(2)	0.1(2)
C(11)	.2576(3)	.7813(2)	.3291(5)	6.0(2)	3.8(2)	4.1(2)	0.5(2)	1.5(2)	0.7(1)
C(12)	.1750(3)	.7160(2)	.2480(5)	5.1(2)	4.7(2)	3.9(2)	0.5(2)	1.1(2)	0.7(2)
C(13)	.1842(3)	.6159(2)	.2168(5)	7.3(3)	4.5(2)	4.2(2)	−0.5(2)	2.2(2)	0.2(2)
C(14)	.2791(3)	.5794(2)	.2712(5)	8.3(3)	3.7(2)	5.2(2)	0.8(2)	3.2(3)	0.6(2)
C(15)	.3633(3)	.6384(3)	.3469(5)	7.4(3)	5.5(2)	4.3(2)	1.7(2)	2.0(2)	1.0(2)
C(16)	.3526(3)	.7385(2)	.3721(5)	5.9(2)	4.8(2)	3.9(2)	0.5(2)	1.2(2)	0.5(2)
O(11)	.2516(2)	.8771(2)	.3680(4)	6.7(2)	3.9(1)	7.4(2)	0.7(1)	0.7(1)	0.4(1)
N(12)	.0733(2)	.7537(2)	.1891(4)	6.3(2)	6.3(2)	5.1(2)	−0.1(2)	0.7(2)	0.5(2)
O(121)	.0614(2)	.8427(2)	.2223(4)	6.9(2)	6.5(2)	8.2(2)	2.0(1)	0.3(2)	−0.1(1)
O(122)	.0055(2)	.6966(2)	.1104(5)	7.2(2)	8.6(2)	9.5(2)	−1.3(2)	−1.4(2)	0.3(2)
N(14)	.2901(3)	.4720(2)	.2392(5)	11.8(3)	4.9(2)	7.7(2)	2.3(2)	5.3(2)	1.2(2)
O(141)	.2210(3)	.4225(2)	.1357(5)	13.6(3)	4.8(2)	11.0(3)	−0.5(2)	4.9(2)	−1.1(2)
O(142)	.3686(3)	.4401(2)	.3175(6)	16.0(4)	6.4(2)	13.2(3)	5.0(2)	3.2(3)	1.7(2)
N(16)	.4472(2)	.7986(2)	.4459(4)	6.3(2)	6.7(2)	5.6(2)	0.4(2)	1.1(2)	−0.1(2)
O(161)	.5140(2)	.7603(2)	.5415(5)	7.2(2)	10.4(2)	9.3(2)	2.7(2)	−1.2(2)	−2.0(2)
O(162)	.4561(2)	.8796(2)	.4010(5)	8.3(2)	7.8(2)	11.8(3)	−2.0(2)	0.2(2)	1.6(2)
C(21)	.7947(2)	.3073(2)	.2220(4)	3.5(2)	3.3(2)	3.7(2)	0.1(1)	0.3(1)	0.1(1)
C(22)	.8536(2)	.3986(2)	.2863(4)	3.2(2)	3.6(2)	3.9(2)	−0.0(1)	0.2(1)	−0.0(1)
C(23)	.8141(3)	.4897(2)	.2894(5)	5.3(2)	3.4(2)	4.3(2)	−0.2(1)	0.6(2)	−0.0(1)
C(24)	.7086(3)	.4972(2)	.2368(5)	5.5(2)	3.6(2)	4.0(2)	1.1(2)	0.4(2)	0.1(1)
C(25)	.6443(2)	.4149(3)	.1804(5)	3.9(2)	5.8(2)	3.5(2)	1.0(2)	0.2(1)	0.3(2)
C(26)	.6857(2)	.3240(2)	.1763(4)	3.6(2)	4.2(2)	3.4(2)	−0.2(1)	0.4(1)	0.0(1)
O(21)	.8333(2)	.2265(2)	.1952(4)	4.7(1)	3.4(1)	7.2(2)	0.6(1)	−0.7(1)	−0.1(1)
N(22)	.9639(2)	.3932(2)	.3516(4)	4.0(2)	4.3(2)	5.4(2)	−0.4(1)	0.5(1)	−1.0(1)
O(221)	.9903(2)	.3306(2)	.4530(4)	4.9(2)	5.6(1)	7.5(2)	0.7(1)	−1.2(1)	0.1(1)
O(222)	1.0231(2)	.4546(2)	.3049(4)	4.9(2)	7.2(2)	8.8(2)	−2.1(1)	1.5(1)	0.1(1)
N(24)	.6656(3)	.5929(2)	.2387(4)	8.5(2)	5.0(2)	5.6(2)	2.7(2)	0.8(2)	0.0(1)
O(241)	.7250(3)	.6650(2)	.2963(4)	12.6(3)	4.0(1)	8.6(2)	1.9(2)	0.1(2)	−0.6(1)
O(242)	.5748(2)	.5992(2)	.1809(5)	8.2(2)	8.0(2)	10.7(3)	4.8(2)	−0.5(2)	0.2(2)
N(26)	.6155(2)	.2395(2)	.1209(4)	3.8(2)	5.7(2)	4.8(2)	−0.9(1)	0.3(1)	0.6(1)
O(261)	.5379(2)	.2466(2)	.0164(5)	6.7(2)	10.6(2)	10.4(3)	−3.2(2)	−3.4(2)	2.8(2)
O(262)	.6352(2)	.1619(2)	.1897(4)	6.0(2)	4.4(1)	10.5(2)	−0.6(1)	1.3(2)	−0.2(1)

The statistical distribution of normalized structure factor magnitudes indicated that the crystal structure is centrosymmetric, and the experimental density indicated that the unit cell contains two formula units of CARPCR. Consequently the space group was assumed to be $P\bar{1}$; this assumption was corroborated by the final structure analysis. A trial structure was obtained by direct methods with the use of MULTAN, *ed.* 1970,[1] in combination with intensive calculations by hand.

REFINEMENT OF THE TRIAL STRUCTURES

The trial structures were refined by full-matrix least-squares techniques. The quantity minimized was $\sum w(|F_o|-|F_c|)^2$ where weights were initially taken as unity but later changed as follows: For CARCHL $w=1/(1+((F_o-B)/A)^2)$ where $A=10.0$ and $B=15.0$. For CARIOD $w=(F_o/A)^2$ when $F_o \leq A$ and else $w=(A/F_o)^2$, where $A=25.0$. For CARPCR $w=1/(1+((F_o-B)/A)^2)$, where $A=10.0$ and $B=20.0$. The scattering factors for the iodide ion were those published by Cromer and Mann.[2] The scattering factors for all other atoms were taken from *International Tables for X-Ray Crystallography*.[3] All nitrogen and oxygen atoms were treated as uncharged. All hydrogen atoms were located in difference Fourier maps calculated during the latter stages of refinement. The final cycles of refinement included positional parameters for all atoms and anisotropic thermal parameters for all non-hydrogen atoms while temperature parameters of hydrogen atoms were fixed at arbitrary values. On the last cycles of refinement of the respective structures shifts of all parameters were less than one third of their estimated standard deviations. The final R index $(\sum||F_o|-|F_c||/\sum|F_c|)$ is 0.039 for CARCHL, 0.043 for CARIOD, and 0.056 for CARPCR. The calculations were performed on a GIER computer and an IBM 360/75 computer, using INDIFF,[4] a local version of *The N.R.C. 2A Picker Data Reduction Program*,[5] *The X-Ray System*,[6] and *ORTEP*.[7] The observed and calculated structure factor data are available from the author on request.

RESULTS AND DISCUSSION

Tables 2 and 3 list final parameters and estimated standard deviations for non-hydrogen atoms and for hydrogen atoms, respectively. The calculated bond lengths and angles involving only non-hydrogen atoms are given in Table 4.

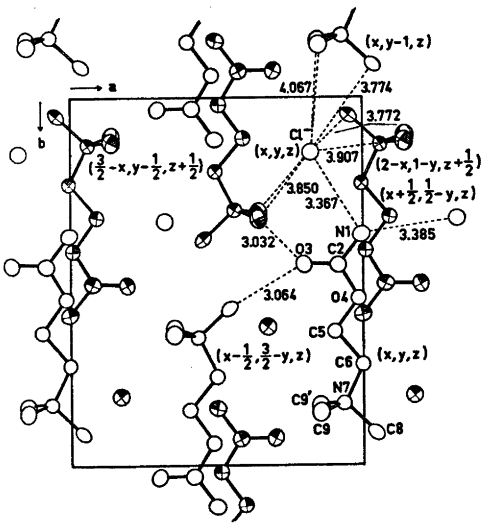


Fig. 1. CARCHL. The numbering system of the non-hydrogen atoms is given together with some *inter*molecular contacts. The structure is viewed in a direction deviating 5° from the direction of the c-axis to allow C(9) as well as C(9') to be seen. The thermal ellipsoids are drawn at the 50 % probability level. Atoms drawn as open ellipsoids are situated in or about the layer $z=1/4$, and the rest of the atoms are situated in or about the layer $z=3/4$.

Packing arrangements

CARCHL. Fig. 1 shows the crystal packing, the atomic numbering and some *inter*molecular contacts. The structure is very similar to that of 3-chloro- and 3-iodopropyltrimethylammonium iodide (mixed crystal),[8] and to that of carbamoylcholine bromide,[9] although the last compound was found to crystallize in the acentric space group $Pna2_1$. The chloride ion and all the non-hydrogen atoms of the carbamoylcholine ion with the exception of C(9) are situated in the mirror plane, while C(9) (and its mirror image C(9')), in the other carbamoylcholine salts named C(10)) occupy a general position. Two weak N–H···Cl⁻ contacts, N(1)–H(11)···$Cl^-_{(x,y,z)}$, and N(1)–H(12)···$Cl^-_{(x+\frac{1}{2},\frac{1}{2}-y,z)}$, link the ions together

Table 3. Final positional parameters for hydrogen atoms and the thermal parameters ($\times 10^2$) used. The estimated standard deviations (referring to the last significant figure) are given in parentheses.

Atom	*x/a*	*y/b*	*z/c*	*U*
CARCHL				
H(11)	.960(4)	.321(3)	.25(−)	3.8
H(12)	1.086(4)	.386(3)	.25(−)	3.8
H(5)	.849(2)	.643(2)	.132(4)	3.8
H(6)	1.058(2)	.731(2)	.124(4)	3.8
H(81)	.993(4)	.988(3)	.25(−)	3.8
H(82)	1.098(2)	.908(2)	.133(4)	3.8
H(91)	.819(2)	.927(2)	.080(4)	3.8
H(92)	.786(2)	.802(2)	.069(4)	3.8
H(93)	.914(2)	.846(2)	−.042(4)	3.8
CARIOD				
H(11)	1.083(11)	−.060(5)	.578(5)	5.1
H(12)	.882(12)	−.088(6)	.667(6)	5.1
H(51)	.549(13)	.185(6)	.643(5)	5.1
H(52)	.370(13)	.113(6)	.599(6)	5.1
H(61)	.217(13)	.164(5)	.737(5)	5.1
H(62)	.292(11)	.044(6)	.760(5)	5.1
H(81)	.519(11)	−.014(6)	.867(5)	5.1
H(82)	.720(13)	.075(6)	.837(6)	5.1
H(83)	.668(12)	.088(6)	.952(6)	5.1
H(91)	.616(13)	.280(6)	.893(5)	5.1
H(92)	.470(14)	.301(7)	.815(6)	5.1
H(93)	.681(12)	.241(6)	.790(5)	5.1
H(101)	.171(13)	.205(6)	.895(5)	5.1
H(102)	.205(12)	.083(6)	.917(5)	5.1
H(103)	.373(12)	.184(6)	.975(6)	5.1
CARPCR				
H(11)	−.054(3)	.144(3)	.299(6)	5.1
H(12)	−.053(3)	.051(3)	.410(6)	5.1
H(51)	.241(3)	.256(3)	.343(6)	5.1
H(52)	.260(3)	.141(3)	.363(6)	5.1
H(61)	.217(3)	.220(3)	−.002(6)	5.1
H(62)	.331(3)	.230(3)	.115(6)	5.1
H(81)	.174(3)	.012(3)	.082(6)	6.3
H(82)	.147(3)	.062(3)	−.105(6)	6.3
H(83)	.216(3)	−.035(3)	−.115(6)	6.3
H(91)	.270(3)	.135(3)	−.260(6)	6.3
H(92)	.387(3)	.152(3)	−.156(6)	6.3
H(93)	.346(3)	.035(3)	−.235(6)	6.3
H(101)	.435(3)	.095(3)	.147(6)	6.3
H(102)	.338(3)	.026(3)	.234(6)	6.3
H(103)	.396(3)	−.014(3)	.053(6)	6.3
H(13)	.127(3)	.577(3)	.168(6)	5.1
H(15)	.431(3)	.609(3)	.381(6)	5.1
H(111)	.176(4)	.891(4)	.332(7)	7.6
H(23)	.858(3)	.548(3)	.330(6)	5.1
H(25)	.575(3)	.418(3)	.145(6)	5.1

Table 4. Bond lengths (Å) and angles (°) involving only non-hydrogen atoms and their estimated standard deviations, referring to the last significant figure, in parentheses. The dimensions found for the carbamoylcholine ion in the crystal structure of carbamoylcholine bromide,[9] CARBRO, are given for comparison.

	CARCHL	CARIOD	CARPCR	CARBRO
N(1)–C(2)	1.334(3)	1.354(7)	1.329(4)	1.40(3)
C(2)–O(3)	1.217(3)	1.198(6)	1.227(4)	1.22(2)
C(2)–O(4)	1.358(3)	1.346(6)	1.340(4)	1.34(3)
O(4)–C(5)	1.436(3)	1.437(6)	1.435(4)	1.45(3)
C(5)–C(6)	1.522(3)	1.508(7)	1.509(5)	1.55(3)
C(6)–N(7)	1.509(3)	1.516(6)	1.520(4)	1.50(3)
N(7)–C(8)	1.511(3)	1.511(9)	1.495(4)	1.52(3)
N(7)–C(9)	1.500(2)	1.475(9)	1.502(5)	1.55(3)
N(7)–C(10)		1.498(8)	1.503(5)	1.51(3)
N(1)–C(2)–O(3)	125.5(2)	125.8(5)	124.8(3)	124(2)
N(1)–C(2)–O(4)	112.1(2)	109.6(4)	111.5(3)	112(2)
O(3)–C(2)–O(4)	122.4(2)	124.6(4)	123.6(3)	124(2)
C(2)–O(4)–C(5)	113.0(2)	115.8(4)	118.2(3)	112(2)
O(4)–C(5)–C(6)	104.0(2)	108.4(4)	110.2(3)	101(2)
C(5)–C(6)–N(7)	113.9(2)	116.7(4)	115.3(3)	114(2)
C(6)–N(7)–C(8)	107.1(2)	110.4(4)	111.8(3)	109(2)
C(6)–N(7)–C(9)	111.7(1)	111.8(4)	107.9(3)	113(2)
C(6)–N(7)–C(10)		107.6(4)	110.7(2)	110(2)
C(8)–N(7)–C(9)	108.1(1)	111.5(5)	108.2(3)	107(2)
C(8)–N(7)–C(10)		107.0(4)	109.9(3)	107(2)
C(9)–N(7)–C(10)	110.1(2)	108.3(5)	108.2(3)	110(2)

CARPCR

The picric acid molecule		The picrate ion	
C(11)–C(12)	1.406(5)	C(21)–C(22)	1.450(4)
C(12)–C(13)	1.378(5)	C(22)–C(23)	1.368(4)
C(13)–C(14)	1.377(6)	C(23)–C(24)	1.390(5)
C(14)–C(15)	1.364(5)	C(24)–C(25)	1.379(5)
C(15)–C(16)	1.379(5)	C(25)–C(26)	1.374(5)
C(16)–C(11)	1.401(5)	C(26)–C(21)	1.449(4)
C(11)–O(11)	1.321(4)	C(21)–O(21)	1.237(4)
C(12)–N(12)	1.468(5)	C(22)–N(22)	1.463(4)
N(12)–O(121)	1.237(4)	N(22)–O(221)	1.214(4)
N(12)–O(122)	1.205(4)	N(22)–O(222)	1.225(4)
C(14)–N(14)	1.481(5)	C(24)–N(24)	1.443(5)
N(14)–O(141)	1.228(5)	N(24)–O(241)	1.240(4)
N(14)–O(142)	1.222(6)	N(24)–O(242)	1.214(5)
C(16)–N(16)	1.471(5)	C(26)–N(26)	1.444(4)
N(16)–O(161)	1.210(5)	N(26)–O(261)	1.195(4)
N(16)–O(162)	1.200(5)	N(26)–O(262)	1.244(4)
C(11)–C(12)–C(13)	123.2(3)	C(21)–C(22)–C(23)	125.0(3)
C(12)–C(13)–C(14)	117.6(3)	C(22)–C(23)–C(24)	118.7(3)
C(13)–C(14)–C(15)	122.5(3)	C(23)–C(24)–C(25)	121.3(3)
C(14)–C(15)–C(16)	118.7(4)	C(24)–C(25)–C(26)	119.1(3)
C(15)–C(16)–C(11)	122.4(3)	C(25)–C(26)–C(21)	124.5(3)
C(16)–C(11)–C(12)	115.6(3)	C(26)–C(21)–C(22)	111.2(2)
O(11)–C(11)–C(16)	119.1(3)	O(21)–C(21)–C(26)	124.1(2)
O(11)–C(11)–C(12)	125.3(3)	O(21)–C(21)–C(22)	124.5(3)
N(12)–C(12)–C(11)	120.0(3)	N(22)–C(22)–C(21)	117.7(2)
N(12)–C(12)–C(13)	116.9(3)	N(22)–C(22)–C(23)	117.3(3)
O(121)–N(12)–C(12)	118.4(3)	O(221)–N(22)–C(22)	118.3(3)
O(122)–N(12)–C(12)	118.5(3)	O(222)–N(22)–C(22)	117.3(3)
O(121)–N(12)–O(122)	123.1(3)	O(221)–N(22)–O(222)	124.4(3)
N(14)–C(14)–C(13)	118.2(3)	N(24)–C(24)–C(23)	119.4(3)

Table 4. Continued.

N(14)–C(14)–C(15)	119.2(4)	N(24)–C(24)–C(25)	119.3(3)
O(141)–N(14)–C(14)	118.0(3)	O(241)–N(24)–C(24)	117.5(3)
O(142)–N(14)–C(14)	116.6(3)	O(242)–N(24)–C(24)	119.1(3)
O(141)–N(14)–O(142)	125.4(3)	O(241)–N(24)–O(242)	123.3(3)
N(16)–C(16)–C(15)	116.2(3)	N(26)–C(26)–C(25)	117.5(3)
N(16)–C(16)–C(11)	121.4(3)	N(26)–C(26)–C(21)	118.0(3)
O(161)–N(16)–C(16)	117.0(3)	O(261)–N(26)–C(26)	119.6(3)
O(162)–N(16)–C(16)	119.0(3)	O(262)–N(26)–C(26)	119.0(3)
O(161)–N(16)–O(162)	124.0(3)	O(261)–N(26)–O(262)	121.3(3)

Table 5. Distances and angles for possible hydrogen bonds (with estimated standard deviations referring to the last significant figure in parentheses).

	Donor	Acceptor	D···A	H···A	D-H···A Angle
CARCHL	N(1)–H(11)	$Cl^-_{(x,y,z)}$	3.367(2) Å	2.53(4) Å	176(4)°
	N(1)–H(12)	$Cl^-_{(x+\frac{1}{2},\frac{1}{2}-y,z)}$	3.385(3)	2.52(4)	162(3)
CARIOD	N(1)–H(11)	$O(3)_{(2-x,-y,1-z)}$	2.940(5)	1.79(7)	160(5)
	N(1)–H(12)	$I^-_{(1-x,-y,1-z)}$	3.669(5)	2.91(8)	144(6)
CARPCR	N(1)–H(11)	$O(21)_{(x-1,y,z)}$	2.829(4)	2.00(4)	163(4)
	N(1)–H(12)	$O(3)_{(-x,-y,1-z)}$	3.048(4)	2.11(4)	174(3)
	O(11)–H(111)	$O(121)_{(x,y,z)}$	2.578(4)	1.69(5)	145(4)
	O(11)–H(111)	$O(3)_{(x,1+y,z)}$	2.829(4)	2.28(5)	113(3)

within the plane. The nitrogen-chlorine distances are 3.367(2) Å and 3.385(3) Å for the two contacts. The geometry of the possible hydrogen bonds in all three crystals are given in Table 5. The carbonyl oxygen atom O(3) is involved in three close contacts to methyl-carbon atoms: one within the layer $O(3)\cdots C(8)_{(x-\frac{1}{2},1\frac{1}{2}-y,z)}$, 3.064(3) Å, and two to adjacent layers $O(3)\cdots C(9)_{(1\frac{1}{2}-x,y-\frac{1}{2},z-\frac{1}{2})}$ and $O(3)\cdots C(9)_{(1\frac{1}{2}-x,y-\frac{1}{2},z+\frac{1}{2})}$, 3.032(2) Å. A number of weak contacts between the chloride ion and methyl- and methylene-carbon atoms within the layer and in the adjacent layers are found, *cf.* Fig. 1.

CARIOD. The crystal packing is illustrated in Fig. 2 and in Fig. 3, in which the atomic numbering and some *inter*molecular contacts are also given. The iodide ions form puckered layers close to (100), and between these layers columns of carbamoylchline ions, related by the screw-axes, are situated. There are no close contacts between the carbamoylcholine ions within

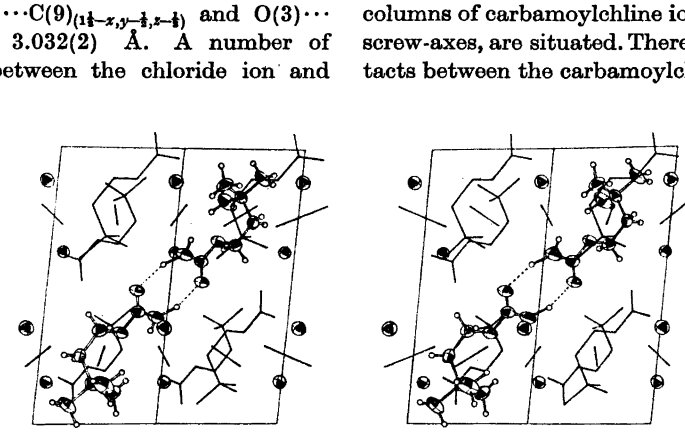


Fig. 2. CARIOD. Stereo drawing showing the packing. The *a*-axis is→, the *c*-axis is↙, and the $-b$-axis is pointing into the paper. The N–H···O hydrogen bonds are drawn with broken lines.

Table 6. Least squares planes and angles between them. The equations of the planes are in direct (unit cell) space. Distances (Å) to atoms defining the plane are asterisked.

Planes of parts of the molecule of picric acid (I) and the picrate ion (II)		Interplanar angles	
I	$-4.5975x-2.4453y+6.8828z+0.8469=0$		
I2	$-5.1159x-3.2127y+6.7506z+1.5206=0$	I–I2	4.3°
I4	$7.3710x+2.6615y-6.2410z-1.9056=0$	I–I4	11.4°
I6	$6.7592x-3.7225y-5.9515z+2.5851=0$	I–I6	27.1°
II	$-3.6191x-2.0992y+7.0122z+1.9399=0$		
II2	$-3.0300x+7.3597y+5.4908z-1.9155=0$	II–II2	41.2°
II4	$-4.4285x-2.0201y+6.9219z+2.5009=0$	II–II4	3.7°
II6	$7.7972x-2.9018y-5.7994z-3.3885=0$	II–II6	29.4°

Atom	I	I2	I4	I6
C(11)	0.017*			
C(12)	−0.001*	−0.000*		
C(13)	−0.014*			
C(14)	0.014*		0.001*	
C(15)	0.004*			
C(16)	−0.019*			0.005*
O(11)	0.078			
H(111)	0.14			
N(12)	−0.031	0.001*		
O(121)	0.034	−0.000*		
O(122)	−0.122	0.000*		
N(14)	0.005		−0.004*	
O(141)	−0.267		0.001*	
O(142)	0.261		0.001*	
N(16)	−0.093			−0.019*
O(161)	0.352			0.007*
O(162)	−0.641			0.007*
H(13)	0.01			
H(15)	−0.00			
	II	II2	II4	II6
C(21)	−0.025*			
C(22)	0.021*	0.003*		
C(23)	−0.005*			
C(24)	−0.008*		−0.002*	
C(25)	0.002*			
C(26)	0.014*			−0.004*
O(21)	−0.183			
N(22)	0.092	−0.012*		
O(221)	0.838	0.004*		
O(222)	−0.579	0.004*		
N(24)	−0.040		0.008*	
O(241)	−0.003		−0.003*	
O(242)	−0.130		−0.003*	
N(26)	0.057			0.015*
O(261)	−0.409			−0.005*
O(262)	0.631			−0.005*
H(23)	0.00			
H(25)	−0.00			

Planes through the four atoms N(1), C(2), O(3), and O(4) of the carbamoylcholine ions

CARCHL	$z-1.7023=0$
CARIOD	$3.6263x+8.9432y+4.2881z-5.4987=0$
CARPCR	$-0.9065x+7.7483y+5.3827z-2.6360=0$

Table 6. Continued.

Atom	CARBAC	CARIOD	CARPCR
N(1)	0.0*	−0.002*	−0.004*
C(2)	0.0*	0.007*	0.012*
O(3)	0.0*	−0.003*	−0.004*
O(4)	0.0*	−0.002*	−0.005*
C(5)	0.0*	0.067	0.195

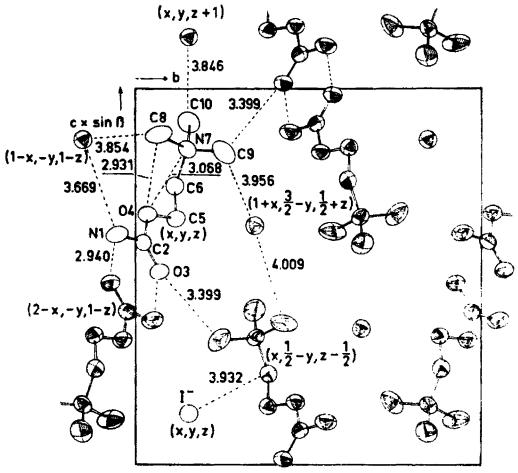


Fig. 3. CARIOD. Projection along the *a*-axis giving the numbering system of the non-hydrogen atoms together with some non-bonded distances. The ellipsoids are drawn at the 50 % probability level.

a column. Hydrogen bonds N(1)−H(11)···O(3) link carbamoylcholine ions related by a center of symmetry together in pairs. The distance $N(1)\cdots O(3)_{(2-x,-y,1-z)}$ is 2.940(5) Å. One more contact, $N(1)\cdots I^-_{(1-x,-y,1-z)}$, may be described as a hydrogen bond. The distance N(1)···I⁻ is 3.699(5) Å. A number of rather weak contacts between the iodide ion and methyl carbon atoms of adjacent carbamoylcholine ions are found, *cf.* Fig. 3. The packing in the CARIOD structure is at first glance remarkably similar to the packing in the crystal structures of succinylcholine perchlorate[10] and of (2-carbamoyloxyethyl)-*N*,*N*-dimethyl ethylammonium bromide.[9] This similarity is illustrated in Fig. 4. The space group of all three structures is $P2_1/c$. Only two of the structures are, however, isostructural, as the direction of the *b*- and *c*-axes are interchanged in the CARIOD structure compared to the directions of these axes in the two other structures.

CARPCR. The crystal packing is illustrated in Fig. 5 and in Fig. 6, in which the atomic numbering and some *inter*molecular contacts

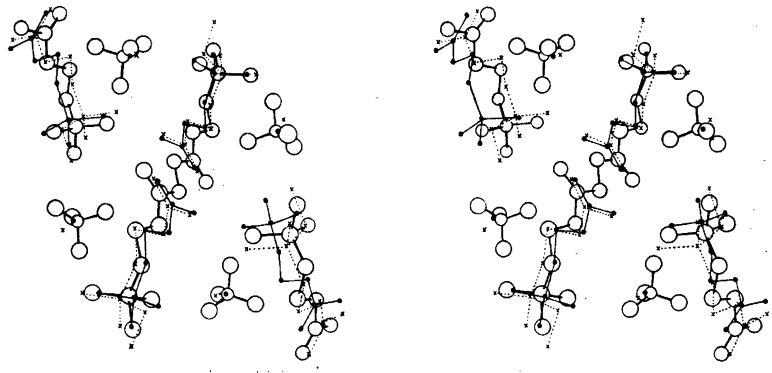


Fig. 4. Stereo drawing showing the structures of CARIOD, (●) succinylcholine perchlorate,[10] (O) and (2-carbamoyloxyethyl)-*N*,*N*-dimethylethylammonium bromide,[9] (×). The centers of symmetry in the middle of the drawing occupy a common point. All structures are viewed along the *a**-axis. For CARIOD the *b*-axis is →, and the *c*-axis is ↑. These directions are interchanged for the other two structures.

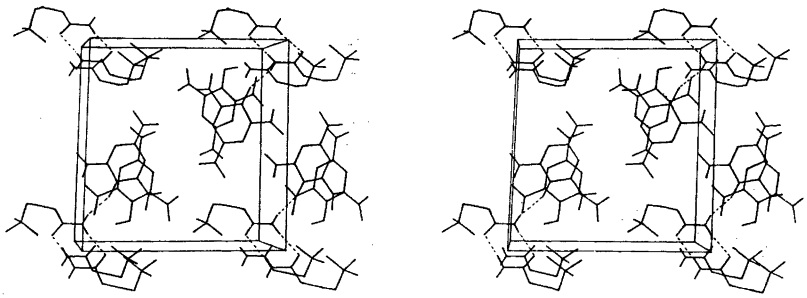


Fig. 5. CARPCR. Stereo drawing showing the packing. The *a*-axis is→, the *b*-axis is ↓, and the *c*-axis is pointing into the paper. Hydrogen bonds are indicated with broken lines. H(11) and H(12), the two hydrogen atoms attached to the amide nitrogen atom N(1) are drawn and so is H(111), the hydroxyl hydrogen atom of the molecule of picric acid. All other hydrogen atoms have been omitted.

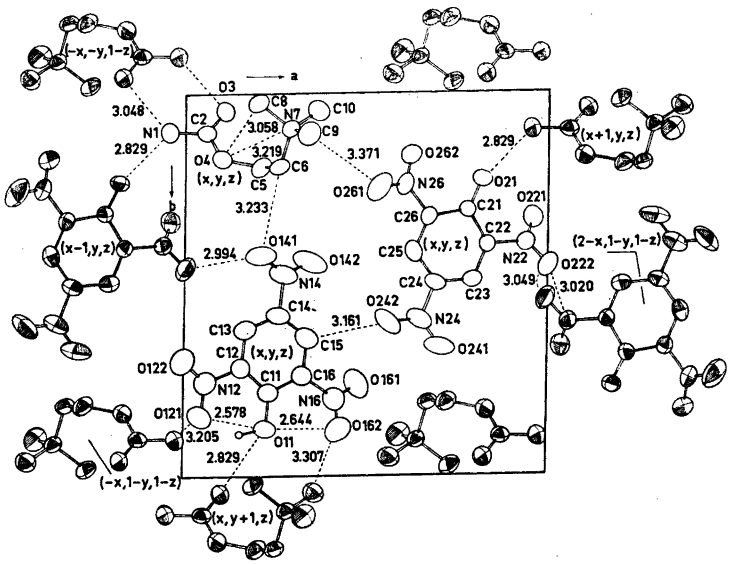


Fig. 6. CARPCR. Projection along the *c**-axis giving the numbering system of the non-hydrogen atoms together with some non-bonded distances. The ellipsoids are drawn at the 50 % probability level.

are also given. Carbamoylcholine ions related by a center of symmetry are linked together in pairs by weak hydrogen bonds N(1)−H(12)···O(3). The distance N(1)···O(3)$_{(-x,-y,1-z)}$ is 3.048(4) Å. The shortest distance between a carbamoylcholine ion and the one on top of it in the *c*-direction is O(3)···C(9)$_{(x,y,z+1)}$, 3.414(5) Å. A number of contacts between the carbamoylcholine ions and adjacent picrate ions and picric acid molecules are found. The distance between N(1) and the phenoxide oxygen atom O(21)$_{(x-1,y,z)}$ is 2.829(4) Å, and the position of H(11) is in agreement with the existence of a hydrogen bond N(1)−H(11)···O(21), *cf.* Table 5. The distance O(3)···O(11)$_{(x,y-1,z)}$ is also 2.829(4) Å, but here the dimensions given in Table 5 do not indicate the existence of a hydrogen bond O(11)−H(111)···O(3).[11]

The picrate ions are stacked alternating with

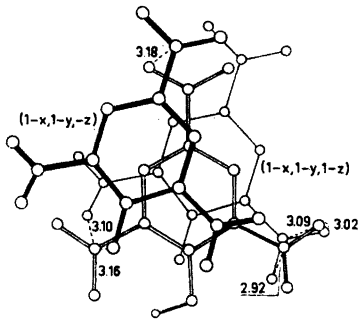


Fig. 7. One molecule of picric acid (O=O) together with the two neighbouring picrate ions. The stack is viewed perpendicular to the plane of the ring of the molecule of picric acid.

molecules of picric acid to form columns in the c-direction. The angle between ring plane I of the picric acid molecule and ring plane II of the picrate ion is 5.0°, while the angles between [001] and planes I and II are 23.6° and 18.7°, respectively. The overlap within a column is illustrated in Fig. 7. The *inter*planar spacing in the regions of overlap is approximately equal to 3.4 Å. A number of closer contacts between the atoms of the nitro groups of a picric acid molecule and of the adjacent picrate ions are pointed out in Fig. 7.

The picrate anion and the molecule of picric acid

The dimensions of the benzene ring of the picric acid molecule show some deviations from the dimensions of an unsubstituted benzene ring, *cf.* Table 4. The two bonds C(11)–C(12) and C(11)–C(16) are slightly longer than the ideal aromatic carbon-carbon distance,[12] and significant deviations from 120° are found for the valency angles in the ring. The same pattern is found for the dimensions of the picrate ion, although the deviations from the dimensions of benzene are greater here, as could be expected. The benzene rings of the picric acid molecule as well as of the picrate ion are slightly non-planar. The greater deviations from planarity are found for the picrate ion, *cf.* Table 6. The N(12) nitro group of the picric acid molecule is tilted only 4.3° out of the plane of the benzene ring, and one of the oxygen atoms of this nitro group is acceptor in an *intra*molecular hydrogen bond O(11)–H(111)···O(121). The distance O(11)···O(121) is 2.578(4) Å. The angles between the planes of all the nitro groups and the pertinent benzene rings are listed in Table 6.

The carbamoylcholine ion

The bond lengths and angles found for the carbamoylcholine ions of the three structures are very similar, but significant differences for a few of the valency angles are observed, *cf.* Table 4. The dimensions found for the carbamoylcholine ion in the crystal structure of carbamoylcholine bromide [9] are given for comparison. The bonds and angles found for the carbamoylcholine ions are hardly significantly different from those found for the corresponding bond lengths and angles of the acetylcholine ions [13–15] and of the succinylcholine ions.[10,16] The different conformations of the three carbamoylcholine ions of the present paper are visualized in Fig. 8, and Table 7 lists the torsion angles. Pullman and Courière [17] have studied the conformation of the carbamoylcholine ion and have performed PCILO calculations *based on the dimensions of CARBRO*. These authors predicted a pronounced flexibility of the car-

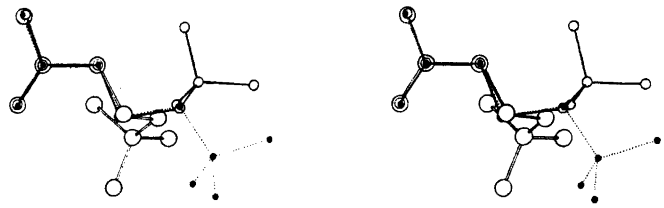


Fig. 8. A stereo view of the carbamoylcholine ions of CARCHL(●), CARIOD (o), and CARPCR (O). The ions are viewed perpendicular to the plane of the carbamoyl group, and C(2)–O(4) is defined to be common for the three ions.

Table 7. Selected torsion angles of the carbamoylcholine ions as found in the three crystal structures of the present paper and in the crystal structure of carbamoylcholine bromide.[9]

	CARCHL	CARIOD	CARPCR	CARBRO
N(1)–C(2)–O(4)–C(5)	180°	±177°	±171°	±177°[a]
C(2)–O(4)–C(5)–C(6)	180°	±175°	±131°	±173°
O(4)–C(5)–C(6)–N(7)	180°	±70°	±84°	±178°

[a] Calculated from the published [9] coordinates.

bamoylcholine ion. The conformations of the ions found in the four crystal structures of different carbamoylcholine salts are some of the many which could be expected from the theoretical energy map. The calculated energy barrier between the *gauche* and *trans* conformations of the carbamoylcholine ion was only 1 kcal/mol.[17] Thus weak interactions from the environments may cause a change from one conformation to another. It is a common feature in the crystal structures of the carbamoylcholine salts, and in salts of related compounds derived from carbamoylcholine by replacing one or more methyl groups by other substituents,[9] that although both of the two possible N–H···A hydrogen bonds are always found, these are generally of lengths indicating rather weak contacts. In accordance with the strong delocalisation of the positive charge of choline derivatives [18] no other single dominating contacts between the carbamoylcholine ion and the environments are seen in any of the structures, while a number of weaker contacts are always found.

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Studies of the Possible Effects of Adsorption on the Product Distribution from Organic Electrode Reactions. V. Stereochemistry of the Anodic Addition of Acetoxy Groups to 1-Alkylindenes

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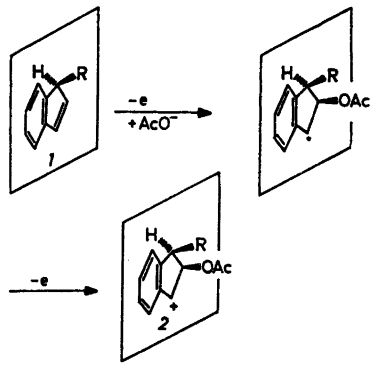
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A series of 1-alkylindenes (alkyl = Me, Et, Pr, i-Pr, Bu, and *t*-Bu) has been subjected to anodic oxidation in acetic acid/sodium acetate and oxidation by $Co(OAc)_3$ in acetic acid/sodium acetate solution. No significant differences in the isomer distribution of the 3-alkyl-1,2-indandiol diacetates formed were found. Thus it is concluded that the product-determining reactions do not take place at the electrode surface.

In previous studies of the anodic oxidation of substituted alkenes in HOAc/NaOAc, our main interest was focused on the stereochemical course of the addition of two acetoxy groups across a double bond.[1-3] Previous studies in this series [4-6] have aimed at elucidating the steric course of anodic substitution reactions of suitable alkylaromatic hydrocarbons. Since the results obtained were not conclusive as to whether the stereochemistry of these reactions is controlled by adsorption or not, we decided to investigate the addition of acetoxy groups to 1-alkylindenes (*1*). These compounds have attractive steric features for such a study (see below).

It has been shown [1] unambiguously that the anodic oxidation of an arylolefin is initiated by a one-electron transfer from the π-electron system to the anode. The radical cation thus formed will react very fast with any nucleophile present in the solution, *e.g.*, acetate ion, to give a radical. This radical can then be further oxidized to a cation which may be trapped by a nucleophile to give the end product(s). During the first two steps there are several possibilities for the orientation o the intermediates with respect to the anode surface. We shall consider here only the two extreme cases, *i.e.*, (1) the reaction occurs entirely at the anode surface due to adsorption, or (2) the reaction occurs entirely in the solution phase. By using a molecule in which one side is more crowded than the other, *e.g.*, a 1-alkylindene (*1*), adsorption at the least hindered side (eqn. 1) should be favoured. The radical cation formed in this orientation will then, after reaction with a nucleophile, give a sterically more crowded intermediate than if the reaction occurs in the solution.

If, on the other hand, the reaction occurs in the solution, the isomer ratio of the products should follow the same pattern as that from analogous homogeneous electron transfer oxidations.[7] *e.g.*, oxidation with $Co(OAc)_3$. Any



(1)

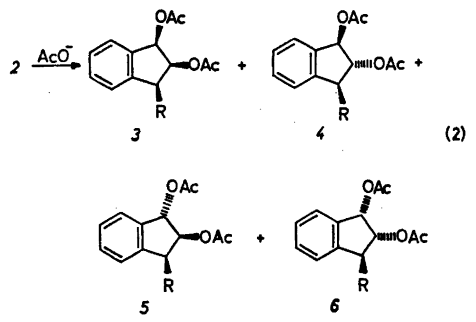
* To whom correspondence should be addressed.

large difference in product distribution between these reactions, specifically a stronger preference for a *cis* 2-acetoxy-/alkyl relationship in the electrochemical process, will thus be an indication of adsorption controlled stereochemistry.

RESULTS AND DISCUSSION

The mechanistic study made earlier [3] suggested that the first acetoxy group attacks position 2 in indene radical cation. The next reaction is removal of one electron and the creation of an acetoxycarbonium ion (*2*, eqn. 1, R=H).

Intermediate *2* can then react further with acetate ion to give the products. Accordingly, for the 1-alkylindene, formation of *3* and *5* from *2*, and 4 and *6* from a species adsorbed with R pointing toward the electrode surface, might be anticipated, *3* and *5* being the predominating products in the case of a sterically demanding R group (eqn. 2).



R=Me, Et, Pr, i-Pr, Bu, and *t*-Bu

The oxidation of the 1-alkylindenes was carried out in acetic acid, 1 M in sodium acetate. The anode material was graphite and the oxidation was run at a constant potential of +1.2 V *vs.* SCE. When the current fell below 10 mA (0.25 mA/cm²), coinciding with the passage of 2 F/mol of substrate, the electrolysis was interrupted and the reaction mixture worked up. To determine the product distribution of compounds *3*–*6* as accurately as possible, the reaction mixture was first treated with acetic anhydride and pyridine to convert any hydroxy acetate formed into diacetate.[3] Product distributions were determined by GLC. The results are summarized in Table 1. The main product, isolated by distillation, was a mixture of the four stereoisomers of 3-alkyl-1,2-indandiol diacetate, (*3*–*6*), previously synthesized and identified by unambiguous methods.[8]

These isolated total yields were in all cases reasonably high. There is however, no real synthetic value of preparing the individual diastereoisomers in this way, with two exceptions (see below), due to difficulties of separation.

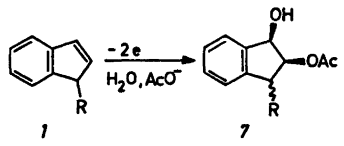
Analysis of the reaction mixture without prior acetylation disclosed an additional product *7** that could be converted to the diacetoxyalkylindans (*3* and *6*) on treatment with acetic anhydride and pyridine (eqn. 3).

* This is the most likely arrangement of —OH and —OAc; *cf.* Ref. 3.

Table 1. Product distribution from the anodic oxidation of 1-alkylindenes in HOAc/KOAc (1 M) at a carbon anode.

R	*n*/F	Product distribution, % [a]				Isolated yield, % [b]	*7* [c]
		3	*4*	*5*	*6*		
H	–	(63)[d]		(37)[e]		32	20
Me	1.80	20	28	52[f]		55	6
Et	1.87	12	35	9	44	75	10
Pr	1.95	12	34	8	46	73	20
i-Pr	1.94	2	41	2	55	58	16
Bu	2.03	11	31	7	50	62	14
t-Bu	1.89	0	38	1	62	73	18

[a] After treating the reaction mixture with acetic anhydride and pyridine, *i.e.*, including *7* after acetylation. GLC analysis normalized to 100 %. [b] Combined *3*–*6*, isolated by distillation, based on starting material. [c] Estimated from pre-acetylation data not shown here. [d] Combined yield of *3*+*6*, as previously reported (Ref. 3). [e] Combined yield of *4*+*5* as previously reported (Ref..3) [f] Combined yield of *5*+*6* as previously reported.



1 7 (3)

Accordingly, the amount of compound *7* in the reaction was estimated by determining the amount of *3* – *6* before and after treatment with acetic anhydride and pyridine. This made it possible to estimate the relative amount (Table 1) of *7* accurately without determining the GLC sensitivity factor of these compounds.

In order to investigate the influence of water on the product distribution, a series of runs with 1-isopropylindene was made involving varying concentrations of added water or acetic anhydride. The relative amount of (*7*) was essentially independent of the water concentration as has earlier been found for indene.[3] These reactions were performed at both platinum and carbon anodes under constant current conditions (Table 2).

In the cases of 1-*t*-butylindene and 1-isopropylindene, electrolysis is a useful way of preparing the isomers *4* and *6* in reasonable yields. So far no satisfactory conventional synthetic pathway for *4*, R = isopropyl or *t*-butyl has been reported. Separation of the two diacetates was performed by first hydrolyzing them to their parent *cis* and *trans* diols. The *cis* diol could be separated from the *trans* diol by reacting the mixture of diols with acetone and then extracting the *cis* acetone ketal into pentane.

The structure of *4*, R = *t*-butyl was demonstrated by the above mentioned reaction of its diol with acetone, its mass, NMR, and IR spectra, and assignment of structure to its stereoisomers. The stereochemistry of all the remaining compounds has been previously reported.[8] The stereochemistry of *7* was similarly established to be exlusively *cis* for the hydroxy/acetate groups.

In the mechanism outlined above (eqn. 2) the attack of the nucleophile was assumed to occur at position 2 of the radical cation. If the molecule is adsorbed on the electrode surface in its least hindered configuration during this reaction, an increasing ratio of *3*+*5* to *4*+*6* would be expected for an increasing bulk of R. These ratios are given in Table 4. It is obvious that the larger the alkyl group, the less is the proportion of *3*+*5*, contrary to predictions based upon an adsorption-controlled mechanism (eqn. 1). Instead, these results support the hypothesis that the reaction occurs in the solution phase, *i.e.*, not close enough to the surface for it to exert any stereochemical control.

The oxidation of 1-alkylindenes was also carried out with $Co(OAc)_3$ in acetic acid/sodium acetate (0.5 M). The products consisted of more than 70 % of the four isomers *3* – *6*. The isolated yield of these isomers increased by about 10 % when the crude reaction product was treated with acetic anhydride and pyridine prior to analysis (Table 3). Analytical difficulties prevented more exact evaluation of the crude reaction mixture. A comparison

Table 2. Electrolysis of 1-isopropylindene in HOAc/KOAc (1 M) in the presence of added water or acetic anhydride.

Anode	$[H_2O]$/[KOAc]	Product distribution, %[a]				
		3	*4*	*5*	*6*	*7*
C	[b]	3	43	2	37	15
C	0.0	4	42	3	34	17
C	1.1	2	38	2	38	20
C	2.7	2	37	2	38	21
Pt	[b]	5	40	5	32	18
Pt	0.0	5	38	5	32	20
Pt	1.1	4	36	3	33	24
Pt	2.7	4	33	4	35	24

[a] GLC analysis normalized to 100 %. Actual yield assumed about 73 % (see Table 1). [b] The reaction solution was a mixture of HOAc (30.0 g) and acetic anhydride (2.0 g).

Table 3. $Co(OAc)_3$ oxidation of 1-alkylindenes in HOAc/KOAc (0.5 M).

R	Product distribution, %[a] 3	4	5	6	Total yield, %
Me	6	37	57[b]		100
Et	6	41	9	44	99
Pr	6	34	6	54	86
i-Pr	1	37	3	59	79
Bu	6	41	6	47	85
t-Bu	1	44	1	55	85

[a] GLC analysis normalized to 100 %. [b] Combined yield of *5* + *6* as previously reported.

Table 4. A comparison of the product distributions from the anodic and the $Co(OAc)_3$ oxidation of 1-alkylindenes.

R	Electrolysis, % A[a]	B[a]	A/B	$Co(OAc)_3$, % A	B	A/B
Et	21	79	0.26	15	85	0.18
Pr	20	80	0.25	12	88	0.14
i-Pr	4	96	0.02	4	96	0.04
Bu	18	82	0.22	12	88	0.14
t-Bu	1	99	0.01	1	99	0.01

[a] *A* = *3* + *5*, *B* = *4* + *6*; from Tables 1 and 3.

between the anodic and the $Co(OAc)_3$ oxidations (Table 4) reveals a very close similarity in the product distribution pattern, confirming the conclusion reached above about the essentially homogeneous nature of the follow-up reactions of the anodic process.

It is pertinent to note that a study of the adsorption of aromatic compounds on a gold anode has been reported.[10] A plot of coverage against anode potential shows a bell-shaped curve with a maximum around +0.5 V *vs.* NHE. The coverage process was shown to take place slowly. As an example, maximum coverage of naphthalene was reached after about 800 s from a 10^{-6} M solution. This can be compared to the electrochemical and chemical steps proposed above, which under these circumstances must be fast. The adsorption of acetate ion [10] or acetic acid [11] is an irreversible process above +0.8 V *vs.* SCE and once such a layer is formed, it may be difficult to introduce an indene molecule.

EXPERIMENTAL

1-Alkylindenes [12,13] *and 3-alkyl-1,2-indandiol diacetates.*[8] These compounds were prepared as described earlier, except *trans, trans*-3-*t*-butylindandiol diacetate.

Preparative anodic oxidation of 1-alkylindenes. The controlled potential preparative electrolyses were carried out in a water-jacketed cylindrical cell (150 cm^3 volume), equipped with a carbon anode (40 cm^2) and a carbon cathode (40 cm^2). The saturated calomel electrode was used as a reference electrode and a Chemical Electronics Model TR 70/2A type potentiostat was employed.

A solution of a 1-alkylindene (0.1 mol), anhydrous sodium acetate (0.1 mol), and HOAc (106 g) was electrolyzed at a constant potential of +1.2 V *vs.* SCE until the current had dropped to less than 10 mA (0.25 mA/cm^2). This occurred when (1.9 ± 0.1) F/mol of 1-alkylindene had been passed.

The electrolyte was poured into ether (100 ml) and washed free of acetic acid with 5 × 200 ml of water. The content of 3-alkyl-1,2-indandiol monoacetate was analyzed by GLC.[8] The ether was evaporated and the residue was treated with a mixture of acetic anhydride (30 ml) and pyridine (1 ml) and refluxed for 15 min. After cooling and addition of water

(200 ml) the organic material was extracted into ether. The ether solution was washed free of acetic acid, dried, evaporated, and distilled. The 3-alkyl-1,2-indandiol diacetates were collected around 130 °C/1 mmHg (for more exact boiling points, see Ref. 8). The individual diastereoisomers were identified by comparison with authentic material. The results are found in Table 1.

Small-scale anodic oxidation of 1-isopropylindene. Small-scale controlled current electrolyses were carried out in a water-jacketed cylindrical cell (50 ml volume), equipped with a carbon anode (6 cm^2) or a platinum anode (3.7 cm^2) and a carbon cathode (6 cm^2), using a Radiak power supply.

A solution of 1-isopropylindene (3.2 mmol), analytical grade potassium acetate (3.0 g), and HOAc (30.0 g) was electrolyzed together with the additives in Table 2 at a constant current of 0.2 A. Work-up and identification were as described above. The results are summarized in Table 2.

Cobalt(III) acetate. This compound was prepared according to a literature procedure.[14]

Cobalt(III) acetate oxidation of 1-alkylindenes. A solution of 1-alkylindene (2.5 mmol), $Co(OAc)_3$ (5.3 mmol; iodometrically titrated value), acetic acid (75 ml), and sodium acetate (3.0 g) was refluxed for 30 min. After cooling and addition of water (200 ml), the organic material was extracted with ether and worked up as for small-scale electrolyses. The data are shown in Table 3.

Trans, trans-*3*-t-*Butylindandiol diacetate.* This compound was prepared from the diacetate mixture obtained in the electrolysis. The procedure is described in the text.

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Periodate Oxidation of Phenols. XVII.* Oxidation of 2-Methylphenols with Aqueous and Methanolic Periodic and Iodic Acids

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Treatment of 2,6-dimethylphenol (*1a*) and mesitol (*1b*) in H_2O-EtOH (4:1) with H_5IO_6 results in *ortho* and *para* oxidation similar to that effected by $NaIO_4$, although *para* oxidation is more pronounced with the former oxidant. Phenols *1a* and *1b* are almost stable towards $NaIO_3$, but are oxidized by HIO_3, mainly *para* oxidation products being obtained from *1b*.

Oxidation of *1a* and *1b*, as well as of 2,4- and 2,5-dimethylphenol, with HIO_4 in methanol produces the corresponding *o*-quinol methyl ethers which slowly dimerize by Diels-Alder reaction. With respect to stereospecificity and regiospecificity, these dimerizations are similar to those of *o*-quinols and their acetates.

From the reaction mixtures obtained with 2,4- and 2,6-dimethylphenol, minor amounts of products derived from iodinated *o*-quinols were isolated.

Methylation of the dimeric *o*-quinol *3* obtained from *1a* gave the expected dimethyl ether (*18a*) and, in addition, a compound (*27*) formed by deconjugation of the α,β-enone system initially present, as well as the C-7 methylation product (*28*) of the latter compound.

As reported earlier, oxidation of 2,4-, 2,5-, 2,6-, and 2,4,6-methyl-substituted phenols with aqueous sodium periodate gives the corresponding 6-hydroxy-6-methyl-2,4-cyclohexadienones ("*o*-quinols") as major products, which undergo rapid Diels-Alder dimerization.[1–3] Depending on the substitution pattern of the starting phenol, *o*- and *p*-quinones, as well as *p*-quinols, are also formed. If 2,4,6-trimethylphenol is treated with periodate or bismuthate in 80 % aqueous acetic acid, the *o* -and *p*-quinol acetates are obtained, in addition to the *o*-quinol dimer and the *p*-quinol.[4]

These results indicate that the solvent used (H_2O or HOAc) participates in the reactions, as earlier shown for the periodate oxidation of guaiacol and hydroquinone monomethyl ether in $H_2{}^{18}O$ which gave labelled *o*- and *p*-benzoquinone and unlabelled methanol.[5]

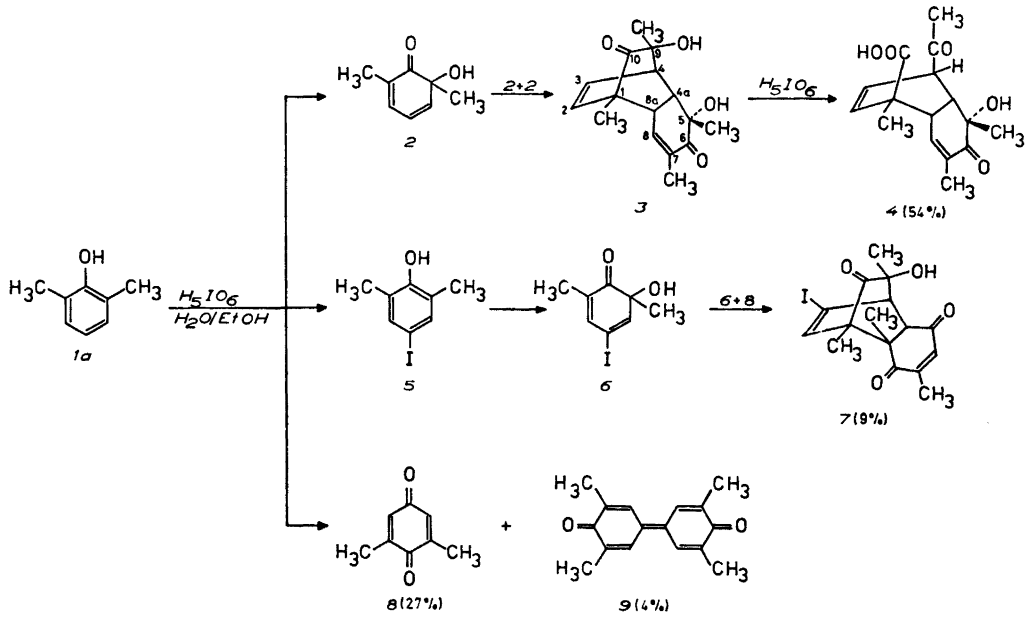
It then seemed of interest to examine the oxidation of the above-mentioned 2-methyl-substituted phenols in alcoholic solutions, which could be expected to afford *o*- and *p*-quinol alkyl ethers. Whereas several reports on the formation of *p*-quinol alkyl ethers have been published,[6–8] only three *o*-quinol analogues seem to have been described earlier.[7,8]

The present paper deals with the oxidation of the methyl-substituted *o*-cresols *1a–d* (see Scheme 4) in methanol. Since sodium periodate, which has been used as oxidant in our earlier studies, is only sparingly soluble in methanol, it was replaced by periodic acid. For comparison, the behaviour of two phenols (*1a*, *1b*) on treatment with the latter oxidant in an aqueous medium was also investigated; see following section.

Oxidation of 2,6-dimethylphenol and mesitol in aqueous solutions. The solvent used was a 4:1 mixture of water and ethanol, similar to that used in earlier experiments with sodium periodate as oxidant.[2]

Treatment of 2,6-dimethylphenol (*1a*) with periodic acid (H_5IO_6) in this solvent mixture

* Part XVI, see Ref. 10.



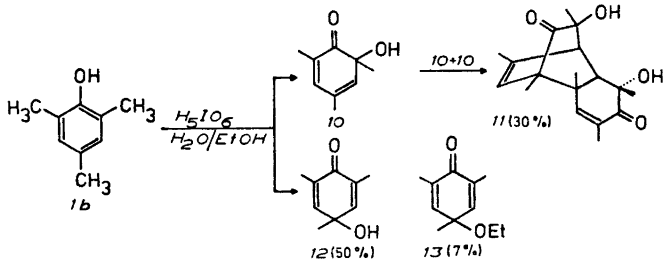
Scheme 1.

(Scheme 1) for 2 h gave the diketocarboxylic acid *4* as the major product. Its formation is due to cleavage, by excess H_5IO_6, of the 9,10-ketol bridge of dimer *3* arising by dimerization of the initially formed *o*-quinol *2*. Acid *4* has earlier been obtained either by treatment of *1a*, dissolved in water, with periodic acid for 2 h or by treatment of dimer *3* in aqueous acetic acid with sodium periodate.[9]

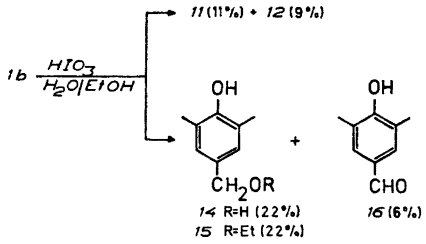
In addition, *para* oxidation of *1a* produced 2,6-dimethyl-*p*-benzoquinone (*8*), as well as a small amount of diphenoquinone *9*.

Finally, the Diels-Alder adduct *7* between the iodinated *o*-quinol *6* and *p*-quinone *8* has been isolated. The structural assignment of *7* is based on analytical and spectroscopic data. The UV spectrum of the compound was very similar to that of the corresponding iodine-free adduct,[10] and its NMR spectrum indicated the lack of a hydrogen atom in position 3. The oxidation of phenol *1a* with H_5IO_6 is accompanied by the formation of free iodine. This obviously causes iodination of some unconsumed *1a* to give *5*, the system iodine-periodic acid being an efficient iodinating agent.[11] The formation of adduct *7* then is readily understood as being due to oxidation of the iodinated phenol *5* to give *o*-quinol *6*, which adds to the dienophile *8*.

The liberation of iodine suggested that the starting phenol is attacked not only by the periodic acid, but also by iodic acid which is formed in the course of the reaction. It has been reported earlier that iodine is produced



Scheme 2.



Scheme 3. Yields are based on consumed *1b*.

when iodic acid is allowed to react with reducing substances such as reductones.[12]

In fact, iodic acid was found to oxidize phenol *1a*, although at a low rate. The *o*-quinol dimer *3* was obtained in a yield of 33 %, in addition to unchanged *1a* (55 %), after a reaction time of 24 h.

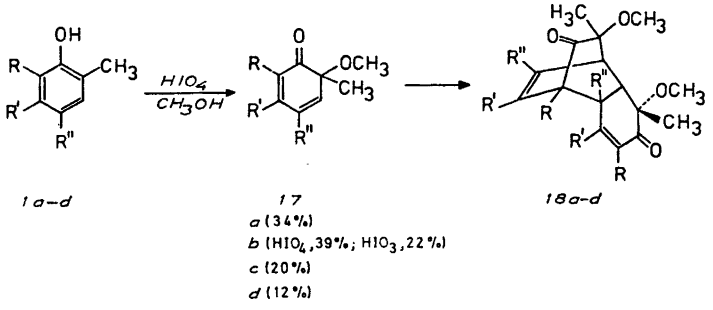
Mesitol (*1b*) reacted with H_5IO_6 as rapidly as with $NaIO_4$,[2] all phenol being consumed within 5 min. The *p*-quinol *12* was obtained as the major product (50 %); its ethyl ether *13* (7 %) and the *o*-quinol dimer *11*[2] (30 %) were also isolated (Scheme 2).

The reaction of mesitol with iodic acid was much faster than that of 2,6-dimethylphenol, which probably can be ascribed to the +I-effect of the additional methyl group present in mesitol. Only 11 % of unconsumed mesitol was recovered after 5 min reaction time. In addition to dimer *11* and *p*-quinol *12*, comparatively large amounts of the benzyl alcohol *14* and its ethyl ether *15*, as well as a small amount of benzaldehyde *16*, were isolated as reaction products (Scheme 3).

Compared with the oxidizing action of iodic acid, that of sodium iodate was much weaker. When $NaIO_3$ was allowed to act upon *1b* in the water-ethanol solvent, 80 % of the phenol could be recovered after four days and products *11* and *14* were obtained in yields of 7 and 2 %, respectively. It is known, however, that the oxidation of hydroquinone and its monomethyl ether,[13] as well as of its monoesters,[14,15] is faster with periodic acid than with periodate, and the behaviour of iodic acid and iodate, respectively, seems to be in analogy herewith.

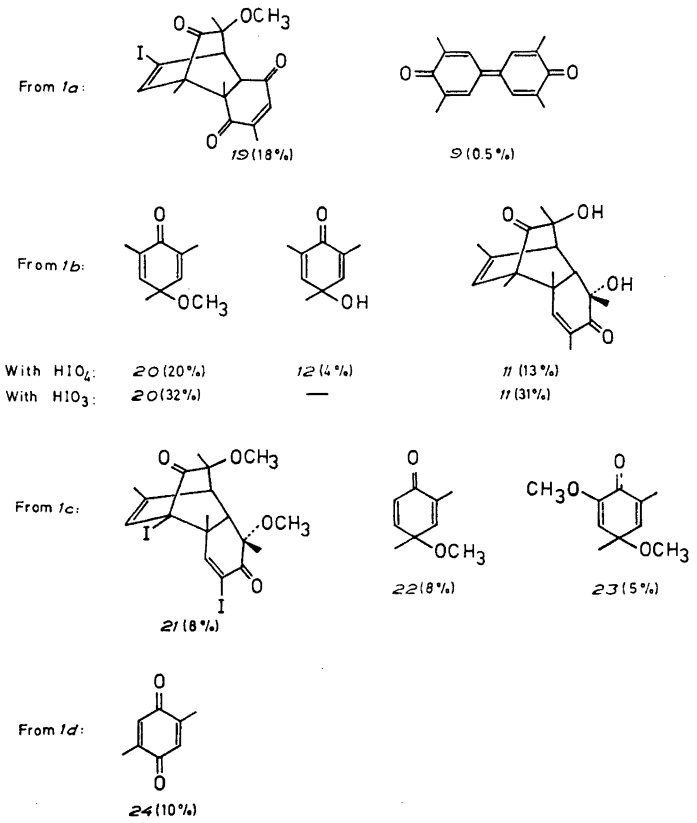
Qualitatively, the oxidation results obtained with periodic acid in H_2O/EtOH are similar to those previously obtained with sodium periodate. However, in the case of mesitol, periodic acid effects predominantly *para* oxidation, whereas *ortho* oxidation is dominant when periodate is used as oxidant.[2,3] Although less pronounced, a tendency towards increased *para* oxidation by periodic acid is also found in the case of 2,6-dimethylphenol.

Oxidation in methanol. As expected, oxidation of phenols *1a–d* (Scheme 4) with anhydrous periodic acid (HIO_4)[16] in methanolic solution produced the *o*-quinol methyl ethers *17a–d* as major products. They constituted yellow oils or solids exhibiting UV and IR characteristics closely similar to those of previously known 2,4-cyclohexadienones.[17–19] The monomers underwent Diels-Alder dimerization, conversion to *18a–d* at room temperature being complete within a few days. The freshly isolated monomers therefore already contained small amounts of the corresponding dimers. Monomer *17b*, derived from mesitol, dimerized comparatively slowly and was obtained essentially free from dimer from the oxidation mixture, as well as by retro-Diels-Alder reaction of dimer *18b* at 180 °C. The yields of the *o*-quinol



a R=CH_3, R'=R"=H; *b* R=R"=CH_3, R'=H; *c* R"=CH_3, R=R'=H; *d* R'=CH_3, R=R"=H

Scheme 4.



Scheme 5. Products formed in addition to *o*-quinol methyl ethers *17a–d* and their dimers *18a–d* on oxidation of phenols *1a–d* with HIO_4 and of *1b* with HIO_3 in methanol.

methyl ethers, determined as dimers, are given in Scheme 4.

As in the system H_5IO_6/H_2O-EtOH, iodine was liberated in the HIO_4/CH_3OH system, indicating that HIO_3 participated in the oxidation of the phenols. A separate experiment showed that mesitol on treatment with HIO_3 in methanol also gave *o*-quinol methyl ether *17b*.

In addition to the *o*-quinol methyl ethers (*17a–d*) and their dimers (*18a–d*), the compounds shown in Scheme 5 were isolated from the oxidation mixtures. They constitute by-products in the experiments using HIO_4, whereas in the oxidation of *1b* with HIO_3, the yields of both *20* and *11* exceeded the yield of *17b*.

The formation of the adduct *19* – the methyl ether of *7* (Scheme 1) – as well as of dimer *21*, derives from iodination of unconsumed phenol (*cf.* p. 910). As in the aqueous reaction systems, *para* oxidation is found to compete with *ortho* oxidation. Furthermore, the formation of products which must have involved the participation of water (*11*, *12*, *19*, *24*) is observed. Whereas *24* and the *p*-quinone incorporated in *19* may have been formed by hydrolysis of the corresponding 4,4-dimethoxy-2,5-cyclohexadienones, the occurrence of *11* and *12* indicates the direct participation of water in the oxidation sequence. (Concerning the availability of water in the systems used, see p. 915 and Scheme 7).

The comparatively low yields of products obtained from *1c* and *1d* (see Schemes *4* and *5*) may be partly due to the formation of the corresponding *o*-quinones and their conversion products. These were lost during the treatment of the crude product mixtures with alumina, which was carried out in order to remove free

iodine (see Experimental). The formation of 3,5-dimethyl-*o*-quinone on oxidation of *1c* with aqueous periodate has been reported earlier.[3]

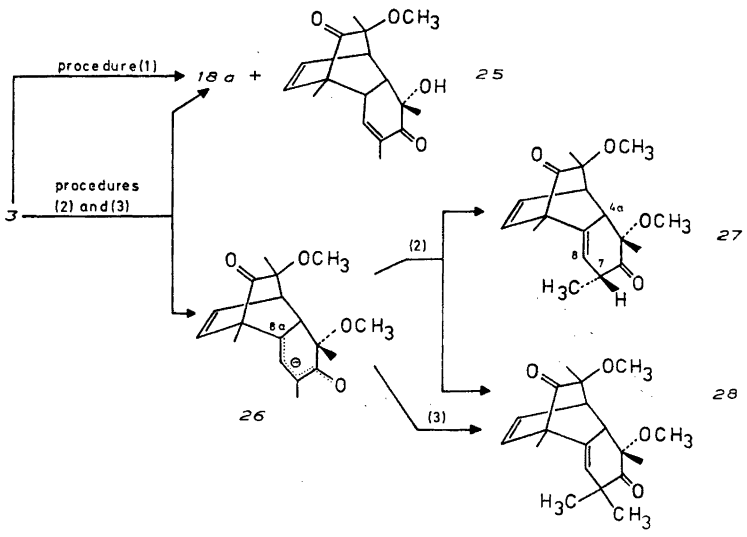
The formation of the dimethoxylated compound *23* from phenol *1c* is interpreted on p. 915.

Structures of dimeric *o*-quinol methyl ethers *18a – d*

The Diels-Alder dimerization of *o*-quinols has been shown to proceed in a stereospecific and regiospecific manner resulting in dimers with the steric orientation at C-5 and C-9, as well as the structural orientation of the addends, as represented for instance by formula *3*.[1,9,20,21] Analogous structures have been found for the dimers of spiro(oxirane-2,4-cyclohexadienones) which can be regarded as cyclic *o*-quinol ethers[1,22] and, therefore, the dimerization of *o*-quinol ethers *17a–d* could be expected to follow the same pattern. In fact, methylation of the *o*-quinol dimers *3*,[2] *11*[2] and *18c* (OH instead of OCH_3)[3] was found to give dimethyl ethers identical with those formed by spontaneous dimerization of *o*-quinol methyl ethers *17a–c*. This proves the correctness of formulae *18a–c* for the latter dimerization products. By analogy, the dimer of *17d* has been assigned structure *18d*.

The methylation of the tertiary hydroxyl groups of *o*-quinol dimers *11* and *18c* (OH instead of OCH_3) to give *18b* and *18c* was readily performed by means of a procedure described by B. Sjöberg and K. Sjöberg[23] using methyl iodide and sodium methylsulfinyl carbanion in DMSO. Methylation of *11* with methyl sulfate/BaO-$Ba(OH)_2$ in DMF[24] also gave *18b* in high yield, whereas *3* under the same conditions reacted more slowly and, in addition to 17 % of the desired dimethyl ether *18a*, gave the monomethyl ether *25* (62 %) (Scheme 6). The location of the methoxyl group in *25* at C-9 rather than C-5 became clear from a comparison of the stretching frequencies of both CO groups in dimeric *o*-quinols and their dimethyl ethers, respectively, which revealed that these frequencies are generally higher by 10 – 25 cm^{-1} for the methylated than for the nonmethylated dimers.

Methylation of dimer *3* by the procedure of Sjöberg and Sjöberg[23] produced the C-7 methyl derivative *28* (*90* % yield), whereas treatment of *3* with CH_3I/Ag_2O[25] afforded 31 % of *18a* and its isomer *27* (19 %), in addition to *28* (29 %).



Scheme 6. Procedure (1) $(CH_3O)_2SO_2$ / BaO-$Ba(OH)_2$ / DMF[24]

(2) CH_3I/Ag_2O/ DMF[25]

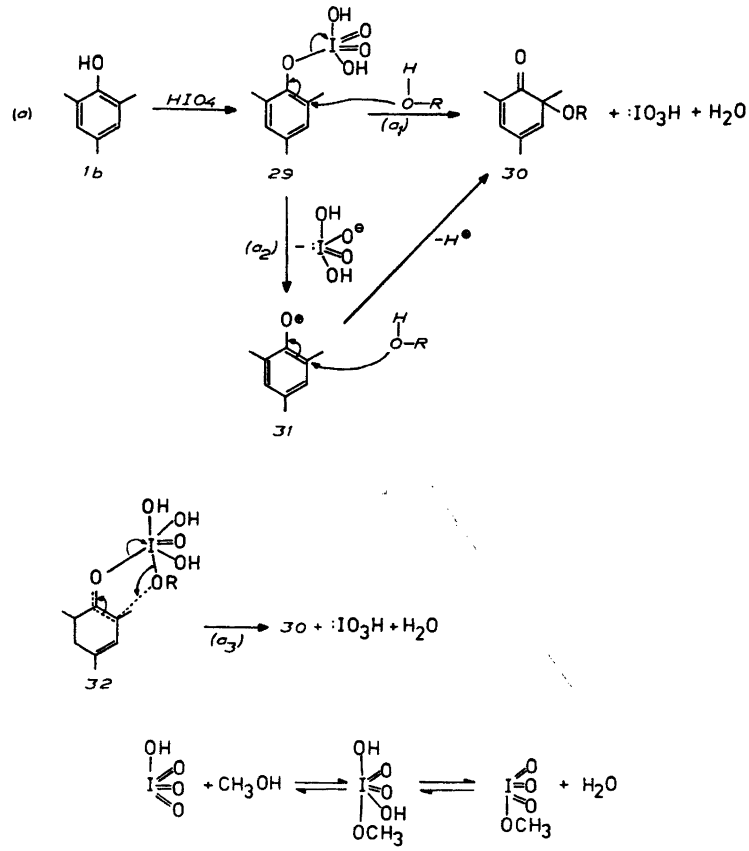
(3) $CH_3I/CH_3SOCH_2:^{\ominus}Na^{\oplus}$/ DMSO[23]

The formation of *27* and its *C*-methylation product *28* is analogous to earlier reported deconjugation reactions of α,β-conjugated ketone systems[26] and can be rationalized by assuming initial abstraction of a proton from C-8a to give anion *26* (Scheme 6; the sequence of *O*-methylation and C-7 methylation is unknown). In the NMR spectrum of *27*, the hydrogen atom at C-8 gives rise to a doublet due to coupling with the hydrogen at C-4a, the coupling constant being 2.5 Hz, which is the same value as found for $J_{4a,8}$ in compound *28*. The coupling constant $J_{7,8}$ however, is almost zero. This indicates that the hydrogen atom at C-7 in *27* is quasi-axial, the C-H bonds in positions 7 and 8 forming a dihedral angle of about 100° as shown by inspection of a Dreiding model.

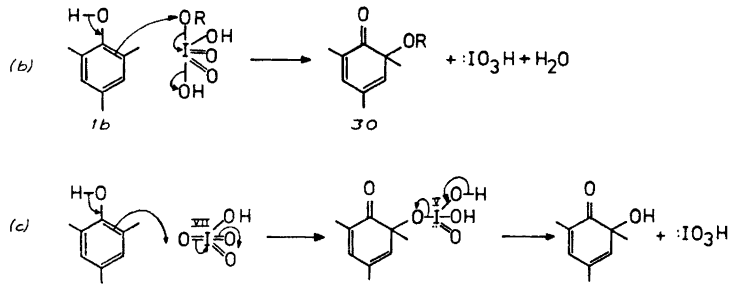
The NMR spectra of the compounds reported in this paper were in agreement with the assigned structures; in a few selected cases, NMR data are given in the experimental part.

Mechanism of phenol oxidation with periodic acid and similar oxidants

It has been suggested that the oxidation of phenols by periodate,[2] periodic acid[14] or bismuthate[27] is initiated by coordination of the phenolic hydroxyl group to the iodine or bismuthate atom. In the *ortho* oxidation of mesitol (*1b*), for instance, periodic acid is assumed to give the ester *29* (Scheme 7, route *a*), which may react further in three different ways ($a_1 - a_3$). In way a_1, two-electron transfer to iodine is concerted with nucleophilic attack of the solvent ROH (R=H, Ac or CH_3) upon one of the *o*-positions, yielding an *o*-quinol, *o*-quinol acetate,[4] or *o*-quinol ether (*30*). The alternative route a_2 involves two steps, *viz.*, formation of the phenoxonium ion (*31*) and subsequent attack of ROH. Reaction in *o*-posi-



Scheme 7.



Scheme 8.

tion may further be visualized to pass *via* a cyclic transition state (*32*). By this route (a_3), not only the formation of the *o*-quinol (*30*, R = H) in aqueous solution, but also that of the *o*-quinol ether (*30*, R = CH_3) in methanol may be possible, since, in methanol solution, coordination of solvent with HIO_4 may give periodic acid methyl esters. As shown in Scheme 7, these reversible coordination reactions involve the release of water, which may explain the formation of *o*-quinol dimer *11* (Scheme 5), in addition to the *o*-quinol methyl ether *17b*, when mesitol is treated with HIO_4 in dry methanol.

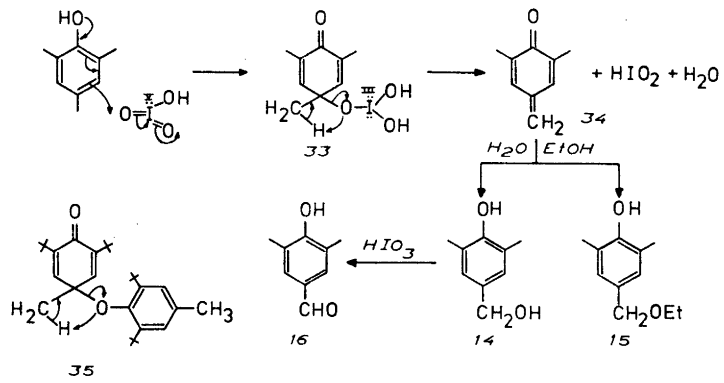
Two further two-electron mechanisms have been discussed (see the recent review article by Fatiadi[11]). In their adaptation to the phenol/periodic acid system they may be represented as shown in Scheme 8. Route *b* is analogous to a pathway proposed by Bubb and Sternhell[28] for the Wessely acetoxylation effected by lead tetraacetate and might explain the formation of *o*-(and *p*-)quinols (R = H), as well as that of their methyl ethers (R = CH_3). It seems doubtful, however, whether electrophilic attack by the group OR as depicted in this reaction scheme is acceptable, when R = CH_3. Route *c* is an adaptation of the mechanism suggested by Fatiadi[29] for the oxidation of polycyclic aromatic hydrocarbons, such as naphthalene, by periodic acid. It is not readily applicable, however, to the formation of quinol methyl ethers.

Routes a_1 and a_2, as well as *b* and *c*, naturally are applicable also to *para* oxidation, such as the formation of *p*-quinol *12* from mesitol (Scheme 2) and, at least the two first-mentioned routes, also to the analogous formation of p-quinol ether *20* (Scheme 5). Furthermore, attack upon a free *o*- or *p*-position according to *a* – *c* (R = H) will give *o*- or *p*-quinones, the primarily formed catechol or hydroquinone being readily oxidized by periodic acid.[5]

The formation of compound *23* (Scheme 5) from 2,4-dimethylphenol (*1c*) is certainly due to two consecutive oxidation steps, the first one involving the introduction of methoxyl into the free *o*-position of *1c* according to one of the reactions $a_1 - a_3$ (R = CH_3) and the second one being a *para* methoxylation of the 2-methoxy-4,6-dimethylphenol thus generated.

Fatiadi[11,30] recently reported the preparation of diphenoquinones of type *9* in yields of 60 – 94 % by treating highly concentrated solutions of 2,6-disubstituted phenols such as *1a* in dimethylformamide-water with H_5IO_6. He suggests a radical mechanism (cf. also Ref. 36) but does not exclude involvement of the phenoxonium ion. Waters[31] regards C – C-coupling in phenol oxidation to be the result of a phenoxonium ion attacking a phenol molecule. In our experiment with a dilute aqueous-ethanolic solution of *1a* and H_5IO_6 (Scheme 1) only a 4 % yield of *9* was obtained.

It seems reasonable to assume that the mechanism of phenol oxidation by iodic acid is analogous to that of the corresponding periodic acid reactions, at least in cases where the products generated by the two oxidants are similar. This is true for the oxidation of mesitol (*1b*) by the two acids in methanolic solution (see Schemes 4 and *5*). However, in the H_2O/EtOH solvent, periodic acid (Scheme 2) produced, from the same phenol, *p*-quinol *12* (50 %) and its ethyl ether *13* (7 %), in addition to the *o*-quinol dimer *11* (30 %), whereas in the iodic acid system (Scheme 3) benzyl alcohol *14* (22 %), its ethyl ether *15*



Scheme 9.

(22 %) and benzaldehyde *16* (6 %) together constituted the major type of products, quinols *11* and *12* being obtained in yields of *11* and *9* % only. This striking difference seems to indicate different mechanisms for the H_5IO_6 and the HIO_3 oxidations in the aqueous-ethanolic solvent. The formation of the aromatic compounds *14*–*16* suggests that the quinonemethide *34* (Scheme *9*) is an intermediate in the aqueous-ethanolic HIO_3 system, nucleophilic addition of H_2O or EtOH to *34* giving *14* and *15*, whereas *16* arises by slow oxidation of *14* as earlier found with $NaIO_4$ as oxidant.[4]

Initial electrophilic attack of HIO_3 to give ester *33*, similar to the first step of route (*c*)[29] in Scheme 8, seems to provide one of several possibilities for making the preferential formation of quinonemethide *34* understandable, see Scheme *9*. The formation of *33* already involves an oxidoreduction, the iodine atom changing its valence number from V to III. The structure of 33 is reminiscent of that of *p*-quinol aryl ether *35* which has been shown to decompose spontaneously into equimolar amounts of 2,6-di-*t*-butyl-4-methylphenol and the corresponding quinonemethide.[35] Similar heterolytic decomposition of *33* would give quinonemethide *34*.

Finally, it cannot be excluded that a one-electron transfer reaction between phenol *1b* and iodic acid produces a phenoxy radical which disproportionates, presumably *via* an intermediate of type *35*,[35] to give quinonemethide *34* in addition to starting phenol.

EXPERIMENTAL

UV, IR, and NMR spectra were recorded on a Cary Model 14, a Beckman 9A and a Varian A-60 instrument, respectively. Chemical shifts are given in δ units downfield from TMS (internal standard).

Oxidation in aqueous solution

General procedure. A solution of the phenol (2 g) in water-ethanol 4:1 (800 ml) was mixed at room temperature with a solution (200 ml) of H_5IO_6, HIO_3, or $NaIO_3$ in water; the molar ratio (R) of oxidant to phenol is given in the individual descriptions below. After the desired period of time (t), the mixture was extracted with five 100 ml-portions of dichloromethane. (In the experiments with H_5IO_6, uncomsumed oxidant was destroyed by addition of 2 g of ethylene glycol prior to the extraction.) The extracts obtained in the experiments with H_5IO_6 and HIO_3 were freed from iodine by washing with saturated aqueous sodium thiosulfate, then dried over anhydrous $CaSO_4$ and evaporated to dryness to give product mixture A.

Unless otherwise stated, separation of the reaction products present in A was performed by chromatography on a column (3 × 40 cm) of silica gel (Silicic Acid, Mallinckrodt, 100 mesh) using benzene-ethyl acetate 4:1 (solvent 1) or petroleum ether (b.p. 40–60 °C)-ethyl acetate 5:2 (solvent 2) as eluents. The location of the different compounds in the 15 ml fractions collected was determined by TLC on "Kieselgel 60 F 254" (E. Merck, Darmstadt), solvents 1 and 2, respectively, being used as mobile phases. In the following descriptions, the individual products are given in the order of their elution from the column.

Oxidation of 2,6-dimethylphenol (1a) *with aqueous* H_5IO_6. $R = 1.8$, $t = 2$ h. Prior to the extraction with dichloromethane, red crystals

of 3,3',5,5'-tetramethyldiphenoquinone (*9*)[10] were filtered off from the reaction mixture; yield 4 %. Product mixture A, a partially crystalline yellow oil, when treated with a small volume of ethyl ether, gave colourless crystals (1.27 g, 54 %) of *carboxylic acid 4*,[10] m.p. 183–184 °C after recrystallization from ethanol.

The ether solution was brought to dryness and the oily residue chromatographed on a column of neutral Al_2O_3 (Woelm) with ethyl acetate as solvent. The eluate fractions gave 27 % of *2,6-dimethyl-p-quinone (8)* and 9 % of *1,4,4a,8a-tetrahydro-9-hydroxy-3-iodo-1,7,8a,9-tetramethyl-1,4-ethanonaphthalene-5,8,10-trione (7)*, yellow crystals of m.p. 141–142 °C after recrystallization from isopropyl ether. (Found: C 48.22; H 4.31; I 31.48. Calc. for $C_{16}H_{17}O_4I$: C 48.02; H 4.28; I 31.71). UV (ethanol): λ_{max}, nm (log ε) 234 (4.09), 300 (2.67), 360 (1.93). IR (KBr): ν_{max}, cm^{-1} 1595 and 1625 (C=C), 1658 (conj. CO), 1718 (CO), 3400 (OH). NMR (acetone-d_6): δ 1.16, 1.23, 1.42 (s, 3 H each, 3 CH_3), 1.98 (d, 3 H, CH_3-7), 3.37 (t, 1 H, H-4), 3.54 (d, 1 H, H-4a), 5.12 (s, 1 H, OH), 6.41 (d, 1 H, H-2), 6.73 (m, 1 H, H-6). $J_{2,4} = J_{4,4a} = 2.5$ Hz $J_{6,CH_3-7} = 2$ Hz.

Oxidation of 2,6-dimethylphenol (1a) *with aqueous HIO_3*. (*a*) $R = 1.75$, $t = 5$ min. Chromatography of A (solvent 1) gave unreacted *1a* (87 %) in addition to *o-quinol dimer 3* (3 %). (*b*) $R = 1.75$, $t = 24$ h. Chromatography as under (*a*) gave unchanged *phenol 1a* (55 %), *2,6-dimethyl-p-benzoquinone (8)* (7 %) and *dimer 3* (33 %).

Oxidation of mesitol (1 b) *with aqueous H_5IO_6*. $R = 2$, $t = 5$ min. The partly crystalline product mixture A on treatment with cold hexane gave hexane-insoluble *o-quinol dimer 11*[2] in 30 % yield. The hexane-soluble fraction on column chromatography (solvent 1) provided *4-ethoxy-2,4,6-trimethyl-2,5-cyclohexadienone* (*13*) (7 %) and *4-hydroxy-2,4,6-trimethyl-2,5-cyclohexadienone* (*12*)[2,32] (50 %). Compound *13* was characterized as follows. M.p. *52–53* °C after sublimation (20 mmHg, 25 °C bath temp.) and recrystallization from isopropyl ether. Accurate mass determination, found 180.1170, calc. for $C_{11}H_{16}O_2$ 180.1150. UV (ethanol): λ_{max}, nm (log ε) 235 (4.06), sh 275 (3.01), sh 335 (1.76). IR (KBr): ν_{max}, cm^{-1} 1640, 1675. NMR ($CDCl_3$) δ1.12 (t, 3H, CH_2CH_3, $J = 7$ Hz), 1.35 (s, 3 H, CH_3-4), 1.88 (s, 6 H, CH_3-2 and CH_3-6), 3.33 (q, 2 H, CH_2CH_3, $J = 7$ Hz), 6.65 (broadened s, 2 H, H-3 and H-5).

Oxidation of mesitol (1b) *with aqueous HIO_3*. $R = 1.5$, $t = 5$ min. The oily product mixture A on chromatography (solvent 1) gave:

(*a*) Unchanged phenol *1b* (11 %).

(*b*) *4-Hydroxy-3,5-dimethylbenzyl ethyl ether* (*15*) (20 %), m.p. 36–37 °C (hexane). (Found: C 73.44; H 8.90. Calc. for $C_{11}H_{16}O_2$: C 73.30, H 8.95). In the NMR spectrum, the ethyl group gives rise to a triplet (3 H) at δ1.13 and a quartet (2 H) at δ3.43; $J = 7$ Hz. The compound was identical with a product obtained in 80 % yield by 4 h treatment of benzyl alcohol *14*[33] with ethanolic hydrochloric acid at room temperature.

(*c*) *4-Hydroxy-3,5-dimethylbenzaldehyde* (*16*) (5 %), m.p. 113–114 °C (lit.[34] 114–115 °C),

(*d*) p-*quinol 12*[2] (*8* %),

(*e*) o-*quinol dimer 11*[2] (10 %), and

(*f*) *4-hydroxy-3,5-dimethylbenzyl alcohol* (*14*) (20 %), m.p. 104–105 °C (lit.[33] 104–105 °C).

Oxidation of mesitol (1b) *with aqueous $NaIO_3$*. $R = 2.2$, $t = 96$ h. Chromatography of product A (solvent 2) gave unchanged *mesitol* (80 %), o-*quinol dimer 11*[2] (7 %) and *benzyl alcohol 14* (2 %).

Oxidation in methanolic solution

General procedure. A solution of the phenol (2 g) in anhydrous methanol (50 ml) was mixed with a solution of anhydrous periodic acid (HIO_4)[12] or HIO_3 in the same solvent (250 ml); molar ratio (R) of oxidant to phenol, see below. After the reaction time (t) water (300 ml) was added and the mixture extracted with five 100 ml-portions of dichloromethane. In the HIO_4 experiments, the combined extracts after drying over anhydrous $CaSO_4$ were evaporated and, in order to remove free iodine, the residual oil, dissolved in ethyl acetate, was passed through a column (4 × 15 cm) of neutral alumina (Woelm). The column was washed with the same solvent and the combined filtrates were evaporated to give product mixture A. For separation of the products present in A by silica gel chromatography, see preceding section. In the HIO_3 experiment, the alumina treatment was excluded.

Oxidation of 2,6-dimethylphenol (1a) *with methanolic HIO_4*. $R = 1.2$, $t = 4$ h. Chromatography of A (solvent 1) gave:

(*a*) *1,4,4a,8a-Tetrahydro-3-iodo-9-methoxy-1,7,8a,9-tetramethyl-1,4-ethanonaphthalene-5,8,10-trione* (*19*), yellow needles of m.p. 144–145 °C (isopropyl ether), in 18 % yield. (Found: C 49.14; H 4.70; OCH_3 8.08; I 31.21. Calc. for $C_{17}H_{19}O_4I$: C 49.29; H 4.62; OCH_3 7.49; I 30.64). The UV spectrum of the compound was very similar to that of *7*. IR (KBr): ν_{max}, cm^{-1} 1598 and 1632 (C=C), 1760 (conj. CO), 1728 (CO).

(b) A yellow oil, which according to its IR spectrum was *6-methoxy-2,6-dimethyl-2,4-cyclohexadienone* (*17a*) containing some dimer (*18a*). The oil crystallized within a few days at room temperature, the resulting product being identical with

(*c*) *1,4a,5,8a-tetrahydro-5,9-dimethoxy-1,5,7,9-tetramethyl-1,4-ethanonaphthalene-6,10(4H)-dione* (*18a*), colourless crystals of m.p. 134–135 °C (isopropyl ether). (Found: C 70.93; H 7.99; OCH_3 19.92. Calc. for $C_{18}H_{24}O_4$: C 71.02; H 7.95; OCH_3 20.39). UV (ethanol): λ_{max}, nm

(log ε) 207 (3.77), 238 (3.79), 310 (2.26). IR (KBr): ν_{max}, cm^{-1} 1697, 1725. NMR ($CDCl_3$): δ1.23, 1.32, 1.42 (s, 3 H each, 3 CH_3), 1.78 (d, 3 H, CH_3-7), 2.8–3.3 (m, 3 H, H-4, 4a, 8a), 3.35, 3.45 (s, 3 H each, 2 OCH_3), 5.50 (dd, 1 H, H-2), 6.05–6.4 (m, 2 H, H-3, H-8). $J_{2,3}$ = 8 Hz, $J_{2,4}$ = 1.5 Hz, $J_{CH_3-7,H-8}$ = 1.5 Hz. The yield of *18a* was 34 %.

Oxidation of mesitol (1b) *with methanolic* HIO_4. R = 1.1, t = 1 h. Product mixture A, a partly crystalline oil, was treated with cold hexane. The nonsoluble, colourless crystals were o-*quinol dimer 11*. Chromatography of the hexane-soluble material (solvent 2) gave:

(*a*) *4-Methoxy-2,4,6-trimethyl-2,5-cyclohexadienone* (*20*), m.p. 43–44 °C; yield, 19 %. (Found: C 72.45; H 8.47; OCH_3 18.66. Calc. for $C_{10}H_{14}O_2$: C 72.26; H 8.49; OCH_3 18.67). UV (ethanol): λ_{max}, nm (log ε) 234 (4.05), sh 270 (2.95), 345 (1.43). IR (KBr): ν_{max}, cm^{-1} 1648, 1680. NMR (benzene-d_6): δ 1.20 (s, 3 H, CH_3-4) 1.85 (d, 6 H, CH_3-2 and CH_3-6), 2.93 (s, 3 H, OCH_3), 6.19 (broadened s, 2 H, H-3 and H-5).

(*b*) *6-Methoxy-2,4,6-trimethyl-2,4-cyclohexadienone* (*17b*), yellow oil, containing a small amount of dimer *18b*, as revealed by IR and NMR spectra. B.p. 29 °C/0.1 mmHg, m.p. 13–16 °C. Yield, 39 %. UV (ethanol): λ_{max} 317 nm (log ε = 3.42). IR (KBr): ν_{max}, cm^{-1} sh 1650, 1666. NMR ($CDCl_3$): δ1.38 (s, 3 H, CH_3-6), 1.93 (s, 6 H, CH_3-2 CH_3-4), 3.15 (s, 3 H, OCH_3), 6.50 (broadened s, 2 H, H-3 and H-5). (Small peaks due to dimer *18b* have not been included in these IR and NMR data).

After one week, at room temperature, the oily *17b* had solidified. Recrystallization from benzene-hexane afforded *1,4a,5,8a-tetrahydro-5,9-dimethoxy-1,3,5,7,8a,9-hexamethyl-1,4-ethanonaphthalene-6,10*(4H)*-dione* (*18b*), colourless prisms, m.p. 118–119 °C (Found: C 72.45; H 8.36; OCH_3 18.72. Calc. for $C_{20}H_{28}O_4$: C 72.26; H 8.49; OCH_3 18.67). UV (ethanol): λ_{max}, nm (log ε) 212 (3.90), 228 (3.89), 313 (2.36). The IR spectrum of *18b* in KBr unexpectedly showed three strong peaks in the CO stretching region, ν = 1698, 1715, and 1730 cm^{-1}. In methanol solution, however, only two peaks appeared, ν = 1690 cm^{-1} (conj. CO) and 1720 cm^{-1} (CO).

(*c*) *4-Hydroxy-2,4,6-trimethyl-2,5-cyclohexadienone* (*12*)[2,32] (3 %).

(*d*) o-*Quinol dimer 11*.[2] Yield 13 %, including the portion which had crystallized out from A, see above.

Oxidation of mesitol (1b) *with methanolic* HIO_3. R = 1.5, t = 3 h. Product mixture A, a partly crystalline oil, on treatment with cold hexane, gave *dimer 11*[2] (31 %) as hexane-insoluble residue. From the hexane solution p-*quinol methyl ether 20* and o-*quinol methyl ether 17b* (see preceding experiment) were obtained in yields of 32 and 22 %, respectively, by chromatography using solvent 2.

Oxidation of 2,4-dimethylphenol (1c) *with methanolic* HIO_4. R = 1.15, t = 2 h. The yellow, oily product A was separated into four fractions by chromatography (solvent 2):

(*a*) Light-yellow needles of *1,4a,5,8a-tetrahydro-1,7-diiodo-5,9-dimethoxy-3,5,8a,9-tetramethyl-1,4-ethanonaphthalene-6,10*(4H)*-dione* (*21*), m.p. 155–157 °C (isopropyl ether); yield, 8 %. (Found: C 39.17; H 3.98; I 45.97; OCH_3 11.71. Calc. for $C_{18}H_{22}O_4I_2$: C 38.87; H 3.99; I 45.63; OCH_3 11.16). UV (ethanol): λ_{max}, nm (log ε) sh 205 (4.12), 257 (3.70), sh 300 (3.31). IR (KBr): ν_{max}, cm^{-1} 1710, 1730.

(*b*) Colourless crystals of *4-methoxy-2,4-dimethyl-2,5-cyclohexadienone* (*22*), after sublimation (25 °C/10 mmHg) and recrystallization from isopropyl ether m.p. 38–39 °C (lit.[6a] 40–40.5 °C). Yield, 8 %. M/e = 152 for parent peak, as expected for $C_9H_{12}O_2$. UV (ethanol): λ_{max}, nm (log ε) 227 (4.11), sh 265 (3.21). IR (KBr): ν_{max}, cm^{-1} sh 1620, 1645, 1672.

(*c*) Yellow oil, *6-methoxy-4,6-dimethyl-2,4-cyclohexadienone* (*17c*) containing a small amount of dimer *18c*; yield, 20 %. IR (KBr): ν_{max}, cm^{-1} 1556 (w), 1655 (s), 1676 (s) and an additional peak at 1720 (w) which can be ascribed to *18c*.

The oily monomer *17c* changed into a colourless solid on a few hours' heating on the steam bath or within some days at room temperature. Recrystallization from isopropyl ether gave *1,4a,5,8a-tetrahydro-5,9-dimethoxy-3,5,8a,9-tetramethyl-1,4-ethanonaphthalene-6,10*(4H)*-dione* (*18c*), m.p. 129–130 °C. (Found: C 71.03; H 7.94; OCH_3 20.73. Calc. for $C_{18}H_{24}O_4$: C 71.02; H 7.95; OCH_3 20.39). UV (ethanol): λ_{max}, nm (log ε) sh 205 (3.99), sh 227 (3.91), 310 (2.25). IR (KBr) ν_{max}, cm^{-1} 1700 (conj. CO), 1720 (CO).

(*d*) *2,4-Dimethoxy-4,6-dimethyl-2,5-cyclohexadienone* (*23*), m.p. 80–81 °C after recrystallization from isopropyl ether followed by sublimation (40 °C/5 mmHg); yield, 5 %. (Found: C 65.53; H 7.34; OCH_3 33.44. Calc. for $C_{10}H_{14}O_3$: C 65.91; H 7.74; OCH_3 34.06). UV (ethanol): λ_{max}, nm (log ε) 242 (4.03), 297 (3.05). IR (KBr): ν_{max}, cm^{-1} 1630, 1657, 1675. NMR(CCl_4): δ1.45 (s, 3 H, CH_3-4), 1.93 (d, 3 H, CH_3-6), 3.18 (s, 3 H, OCH_3-4), 3.71 (s, 3 H, OCH_3-2), 5.63 (d, 1 H, H-3), 6.55 (m, 1 H, H-5). $J_{3,5}$ = 3 Hz, $J_{5,6}$ *ca.* 1 Hz.

Oxidation of 2,5-dimethylphenol (1d) *with methanolic* HIO_4. R = 1.1, t = 4.5 h. Chromatography of the yellow oil A (solvent 1) gave:

(*a*) *2,5-Dimethyl*-p-*benzoquinone* (*24*). Yield, 10 %.

(*b*) *6-Methoxy-3,6-dimethyl-2,4-cyclohexadienone* (*17d*), a yellow oil, containing a small amount of dimer *18d*. IR (KBr): ν_{max}, cm^{-1} 1578 (w), 1649 (m) 1669 (s) and peaks due to *18d* at 1690 (w) and 1722 (w).

After a few days at room temperature, *17d* had dimerized to *1,4a,5,8a-tetrahydro-5,9-dimethoxy-2,5,8,9-tetramethyl-1,4-ethanonaphthalene-6,10*(4H)*-dione* (*18d*), m.p. 139–140 °C (isopropyl ether); yield, 12 %. Found: C 70.81; H 8.23; OCH_3 20.27. Calc. for $C_{18}H_{24}O_4$: C

71.02; H 7.95; OCH_3 20.39). UV (ethanol): λ_{max}, nm (log ε) sh 207 (3.90), 231 (3.99), 314 (2.31). IR (KBr): ν_{max}, cm^{-1} 1642 (w), 1692 (s), 1723 (s).

Methylation of o-quinol dimers

Methylation with $CH_3I/CH_3SOCH_2^-Na^+$/DMSO. The procedure of Sjöberg and Sjöberg [23] was followed. Dimer *11* [2] gave the *dimethyl ether 20b* in 85 % yield. Similarly, *dimethyl ether 20c* was obtained in 57 % yield from the corresponding *o*-quinol dimer.[3]

By the same method *o*-quinol dimer *3* gave a 90 % yield of *1,4a,5,7-tetrahydro-5,9-dimethoxy-1,5,7,7,9-pentamethyl-1,4-ethanonaphthalene-6,10(4H)-dione* (*28*), m.p. 100–101 °C (isopropyl ether). (Found: C 71.79; H 8.28; OCH_3 20.51. Calc. for $C_{19}H_{26}O_4$: C 71.67; H 8.23; OCH_3 19.49). UV (ethanol): Strong end absorption (log $\varepsilon = 4.02$ at λ 200 nm, probably due to intramolecular charge transfer in β,γ-enone systems), minimum at 270 nm (log $\varepsilon = 1.78$), max. at 315 nm (log $\varepsilon = 2.28$). IR (KBr): ν_{max}, cm^{-1} 1709 (s), 1722 (s). NMR ($CDCl_3$) δ1.14, 1.16, 1.32 (s, 3 H each, 3 CH_3), 1.35 (s, 6 H, 2 CH_3), 3.00 (s, 3 H, OCH_3), 3.03 –3.22 (m, 2 H, H-4, 4a), 3.30 (s, 3 H, OCH_3), 5.31 (d, 1 H, H-8), 5.71 (dd, 1 H, H-2), 6.31 (t, 1 H, H-3). $J_{2,3} = J_{3,4} = 7$ Hz, $J_{2,4} = 1.5$ Hz.

Methylation of o-quinol dimer 3 with CH_3I/Ag_2O/DMF.[25] Methyl iodide (6.1 g) and silver oxide (6.75 g) were added to a solution of dimer *3* (2.0 g) in DMF (25 ml) and the mixture was stirred for 18 h. After addition of chloroform (150 ml) the mixture was filtered and the filtrate, after being dried over anhydrous $CaSO_4$, was brought to dryness under vacuum. Chromatography of the resulting oil on a silica gel column (benzene-ethyl acetate 4:1) provided compounds *28* ($R_F = 0.5$), *27* ($R_F = 0.4$) and *18a* ($R_F = 0.3$) in yields of 29, 19, and 31 %, respectively.

Compound *27*, *1,4a,5,7-tetrahydro-5,9-dimethoxy-1,5,7,9-tetramethyl-1,4-ethanonaphthalene-6,10(4H)-dione*, had m.p. 179–180 °C (isopropyl ether). (Found: C 70.93; H 7.69; OCH_3 20.69. Calc. for $C_{18}H_{24}O_4$: C 71.02; H 7.95; $OCH_3$20.39). UV (ethanol): Strong end absorption (log $\varepsilon = 3.97$ at λ 200 nm), minimum at 280 nm (log ε = 2.12), maximum at 313 nm (log ε = 2.38). IR (KBr): ν_{max}, cm^{-1} 1706 (s), 1735 (s). NMR ($CDCl_3$): δ1.20–1.42 (m, 12 H, 4 CH_3), 2.96 (s, 3 H, OCH_3), 2.98–3.25 (m, 3H, H-4, 4a, 7), 3.36 (s, 3 H, OCH_3), 5.31 (d, 1 H, H-8), 5.64 (dd, 1 H, H-2), 6.31 (t, 1 H, H-3). $J_{2,3} = J_{3,4} = 7.5$ Hz, $J_{2,4} = 1.5$ Hz, $J_{4a,8} = 2.5$ Hz.

Methylation with $(CH_3O)_2SO_2/BaO-Ba(OH)_2$/DMF.[24] A suspension of BaO (4.4 g) and $Ba(OH)_2.8H_2O$(4.4 g) in a solution of *o*-quinol dimer *3* (2.0 g) in *N,N*-dimethylformamide (25 ml) was cooled to 0 °C, and dimethylsulfate (9 ml) was added dropwise without further cooling. The mixture was then stirred for 16 h at room temperature, conc. ammonia (6 ml) was added and stirring continued for one further hour. Extraction with chloroform gave a crystalline solid (1.73 g) consisting of two components according to TLC. Separation on a silica gel column (light petroleum b.p. 40–60 °C–ethyl acetate 5:2) gave *dimethyl ether 18a* (17 %) and *monomethyl ether 25* [*1,4a,5,8a-tetrahydro-5-hydroxy-9-methoxy-1,5,7,9-tetramethyl-1,4-ethanonaphthalene-6,10(4H)-dione*] (62 %). M.p. 157–158 °C (isopropyl ether). (Found: C 70.54; H 7.70. Calc. for $C_{17}H_{22}O_4$: C 70.32; H 7.64). UV (ethanol): λ_{max}, nm (log ε) 208 (3.79), 240 (3.79), 310 (2.27). IR (KBr): ν_{max}, cm^{-1} 1670 (conj. CO), 1730 (CO), 3480 (OH). The NMR spectrum ($CDCl_3$) shows one OCH_3 singlet at δ 3.38 and one OH singlet at δ 4.04 (exchangeable with D_2O).

o-Quinol dimer *11* was similarly methylated to give a 96 % yield of *dimethyl ether 18b*.

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Bacterial Carotenoids, XLVI.* C_{50}-Carotenoids, 14.**

C_{50}-Carotenoids from *Arthrobacter glacialis*

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From the psychrophilic bacterium *Arthrobacter glacialis* have been isolated three C_{50}-carotenoids with molecular formulae $C_{50}H_{72}O_2$: the bicyclic decaprenoxanthin (*1a*, 7 % of total carotenoids), the aliphatic bisanhydrobacterioruberin (*2a*, 10 %) and the monocyclic *A.g.* 470 (*3a*, 83 %).

Decaprenoxanthin (*1*) and bisanhydrobacterioruberin (*2*) were in all respects, including chiroptical properties, identical with known carotenoids.

The constitution of the previously undescribed *A.g.* 470 (*3a*) followed from its spectral properties (electronic, ^{1}H NMR including Eu-shift experiments and mass spectra) and derivatization to *3b* and *3c*. *3a* suffered remarkable elimination to the tridecaene *4a* ($C_{47}H_{64}O$) upon DMSO/KOMe/MeOH treatment. Judged by CD data *A.g.* 470 (*3a*) also in stereochemical respect *3a* appears to be half decaprenoxanthin (*1a*) + half bisanhydrobacterioruberin (*2a*).

The intensity ratios of the M − 92/M − 106 ions on electron impact of *3a,b,c* and *4a,b* are consistent with the general theory.

So far altogether fifteen different C_{50}-carotenoids have been encountered in Nature.[1–4] They may all be considered as 2,2′-isopentenylated C_{40}-carotenoids and represent bicyclic or monocyclic modifications with β- or ε-rings [5] and aliphatic carotenoids of varying chromophore. The oxygen functions are restricted to primary allylic or tertiary alcohols and glycosides thereof.[1–4]

Their known occurrence indicates that C_{50}-carotenoids may be restricted to spheric or rod-shaped, aerobic, gram-positive, non-photosynthetic bacteria.[1–4,6]

We now report the isolation of two known and a major, previously undescribed monocyclic C_{50}-diol with ε-ring from a psychrophilic bacterium. Its structure and occurrence fit into the general pattern described above.

RESULTS AND DISCUSSION

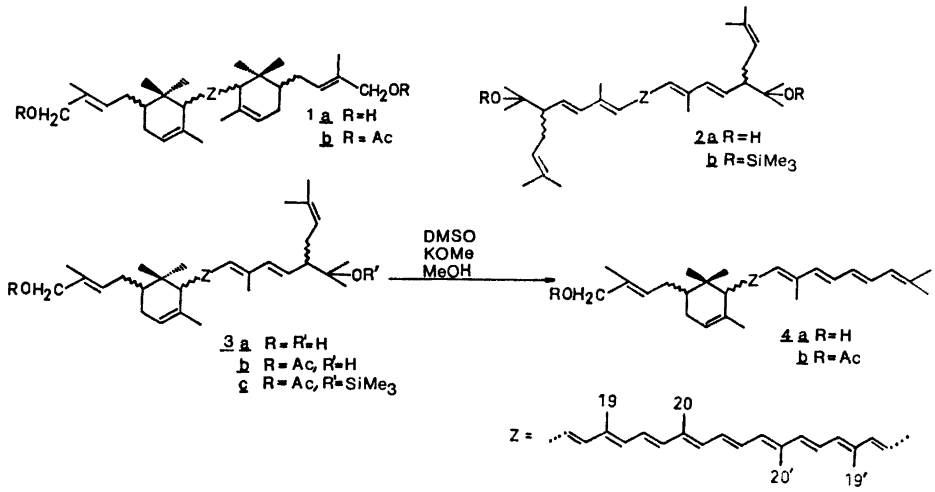
Arthrobacter glacialis Moiroud and Gounot is a yellow, gram-negative (or gram-variable), polymorph, psychrophilic bacterium of the family Corynebacteriaceae, isolated from morainic mud.[7] The pigmentation is due to carotenoids, previously subjected to preliminary studies.[8,9]

In the present work *A. glacialis*, cultivated on 6500 Petri dishes in a glucose/trypton enriched medium at 6 °C, contained 78 mg total carotenoid.

Colourless lipids were removed by repeated precipitation from acetone. Column chromatography followed by TLC on silica gel provided decaprenoxanthin (*1a*, 4.5 mg, 7 % of total), bisanhydrobacterioruberin (*2a*, 6.5 mg, 10 % of total) and *A.g.* 470 (*3a*, 55 mg, 83 % of total).

Decaprenoxanthin (*1a*) was identified on the basis of its electronic spectrum, mass spectrum (*m/e* 704 = M, consistent with $C_{50}H_{72}O_2$, M − 18, M − 92, M − 106, M − 140 (RDA-fragmentation)), co-chromatography with *1a ex Flavobacterium*

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dehydrogenans [10] and transformation to the diacetate *1b* (*m/e* 788=M, compatible with $C_{50}H_{70}(OCOCH_3)_2$, M−60, M−92, M−106, M−182 (RDA-fragmentation)). The CD spectrum of *1b* was fully consistent with that of decaprenoxanthin (*1a*) *ex F. dehydrogenans*,[11] demonstrating the same, so far unestablished, chirality of decaprenoxanthin from the two sources.

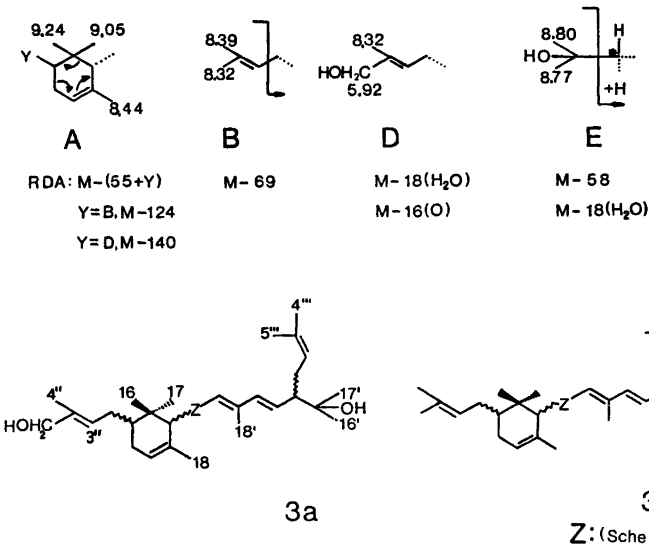
Bisanhydrobacterioruberin (*2a*) was identified by the following criteria. Its electronic spectrum and mass spectrum (*m/e* 704=M, compatible with $C_{50}H_{72}O_2$, M−18, M−18−18, M−58, M−69, M−92, M−106) corresponded to those of *2a ex Corynebacterium poinsettiae*.[12] Cochromatography gave no separation. Upon silylation *2a* gave a di(trimethylsilyl) ether (*m/e* 848=M, corresponding to $C_{50}H_{70}(OSi(CH_3)_3)_2$, M−15, M−58, M−72, M−92, M−106, 131). Finally the CD spectrum of *2a* corresponded to that of bisanhydrobacterioruberin (*2a*) *ex C. poinsettiae* [11] demonstrating the same, unknown, chirality at C-2,2′ for bisanhydrobacterioruberin from the two sources.

A.g. 470 (*3a*) exhibited electronic spectra characteristic of an aliphatic undecaene chromophore. Its polarity (Table 1) was indicative of a C_{50}-diol. Acetylation resulted in a monoacetate *3b* and silylation of the monoacetate gave a monoacetate-mono(trimethylsilyl) ether *3c*, demonstrating the presence of one primary or secondary and one tertiary hydroxy group. The diol assignment was supported by the mass spectra of *3a* (*m/e* 704=M, compatible

Table 1. Adsorptive properties of the carotenoids from *A. glacialis* and their derivatives.

Carotenoid	Schleicher & Schüll 287 kieselguhr paper				
	0 %[a]	1 %	2 %	5 %	10 %
Decaprenoxanthin (*1a*)	0		0.06	0.46	0.65
diacetate (*1b*)	0.13	0.46	0.74		
A.g. 470 (*3a*)	0			0.21	0.60
monoacetate (*3b*)			0.13	0.66	
monoacetate mono TMS ether (*3c*)			0.88		
Bisanhydrobacterioruberin (*2a*)					0.51
di TMS ether (*2b*)	0.81				

[a] Acetone in petroleum ether.



with $C_{50}H_{72}O_2$), *3b* (*m/e* 746=M; $C_{50}H_{70}$-$(OCOCH_3)OH$) and *3c* (*m/e* 818=M; $C_{50}H_{70}$-$(OCOCH_3)OSi(CH_3)_3$). Molecular ions were confirmed by fragment ions (M−92, M−106, M−158) associated with losses from the polyene chain.[13] Characteristic fragment ions attributed to the end groups were observed for the diol *3a* (M−18, M−76=M−18−58?, M−124=M−106−18?, M−140=M−16−124?, M−142=M−18−124?) and the monoacetate *3b* (M−18, M−42, M−58, M−60, M−78=M−18−60?, *m/e* 622.474=M−124=M−C_8H_{10}−H_2O, M−142=M−18−124?, M−164=M−106−56?, M−182).

^{1}H NMR data ($CDCl_3$) of the free diol showed the presence of four in-chain methyl groups (δ 1.98, *ca.* 12 H) and one near-end-of-chain methyl group (δ 1.93, 3 H), defining the chromophore. Methyl signals typical of an ε-end group A, Scheme 2: δ 0.76 (3 H), 0.95 (3 H) and 1.56 (3 H), of an isopropylidene end group B: δ 1.62 (3 H) and 1.68 (3 H), of a hydroxylated isopropylidene group D: δ 1.68 (3 H) and 4.02 (2 H) and of end group E: δ 1.20 (3 H), 1.23 (3 H) were further found for *3a*. Corresponding signals were observed for the monoacetate *3b* with the expected downfield shift of the acetylated end group D signal at δ 4.02 (2 H) to δ 4.46 (2 H).

However, unequivocal differentiation between *3a* and *3d* (Scheme 2) for *A.g.* 470 could not be made from these ^{1}H NMR and mass spectra. Thus fragment ions which may be attributed to RDA-fragmentation of *3d* (M−124) and *3a* (M−140) could also represent combination losses (M−124=106−18? and M−140=124−16?) from other functionalities present. For the monoacetate *3b* accurate mass measurements demonstrated that the M−124 ion indeed represented such a combined loss (M−toluene−H_2O) rather than a one-step loss of a hydrocarbon fragment.

This ambiguity with the two hydroxy functions located at the same or opposite extremity of the molecule readily lends itself to analysis by the ^{1}H NMR Eu-shift technique.[14,15a] Stepwise addition of Eu(dpm)$_3$ to *A.g.* 470 demonstrated (Fig. 1) induced shifts of the 16,17 *gem.* dimethyl groups under conditions where no induced shift occurred for signals from the C-18,18′ range of the molecule, in preference of *3a*. As expected the highest induced shifts were connected with the protons of D and E (Scheme 2, Fig. 1) for *A.g.* 470 (*3a*). *trans*-Configuration of the $\Delta 3''$-bond is assumed on the basis of identical chemical shift of the $-CH_2OH$ signal (δ 4.02) in *A.g.* 470 (*3a*) and decaprenoxanthin (*1a*), for which *trans*-configuration has been demonstrated.[15b]

The (M−92)/(M−106) intensity ratio observed in the mass spectrum of *A.g.* 470 (*3a*) is within the range predicted[16,17] for this chromophore with bulky C-2′ substituents. Also the magnetic non-equivalence of the two

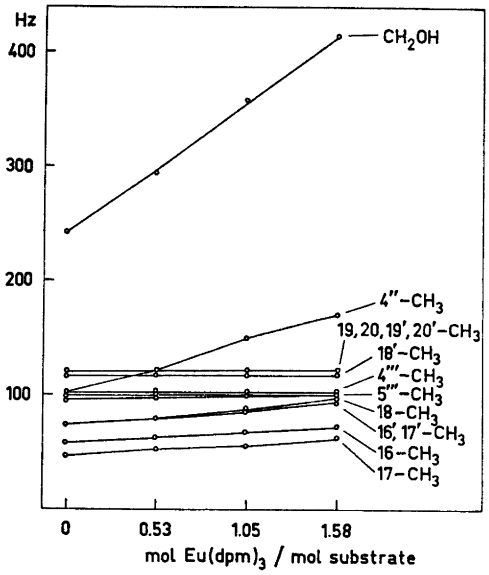


Fig. 1. Induced chemical shifts (Hz) on stepwise addition of Eu(dpm)$_3$ to *A.g.* 470 (*3a*) in $CDCl_3$.

gem. dimethyl groups 16',17' supports the branching point at C-2'.[13] Location of the second extra C_5-unit to C-2 mainly rests on the RDA fragmentation on electron impact (M − 140 for *3a*, M − 182 for *3b*).

Formally [5] *A.g.* 470 (*3a*) is 2-(4''-hydroxy-3''-methyl-2''-butenyl), 2'-(3'''-methyl-2'''-butenyl)-3',4'-didehydro-1',2'-dihydro-ε,ψ-carotene-1'-ol, and in principle *3a* = half *1a* + half *2a*.

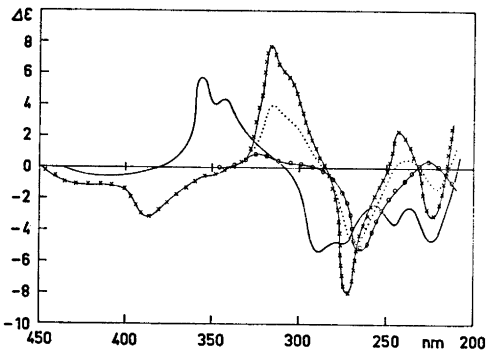


Fig. 2. CD spectra in EPA (diethyl ether-isopentane-ethanol 5:5:2) solution of carotenoids *ex A. glacialis:* O decaprenoxanthin diacetate (*1a*) × bisanhydrobacterioruberin (*2a*), ——— *A.g.* 470 (*3a*) obs., ··· *A.g.* 470 (*3a*) calc. from half CD *1a* + half CD *2b*.

If, as expected from biosynthetic considerations, the ε-end group of *3a* possessed the same chirality at C-2 and C-6 as *1a* and the aliphatic end group the same chirality as the corresponding end group of *2a*, the CD-curve of *A.g.* 470 (*3a*) should be half CD of *1a* + half CD of *2a*, provided the additivity rule of Klyne and co-workers [18] for ORD spectra of bicyclic carotenoids could be expanded to CD spectra of monocyclic carotenoids. So far the additivity rule has proved valid for CD spectra of various bicyclic carotenoids.[19,20] Fig. 2 gives the CD spectra of decaprenoxanthin (*1a*), bisanhydrobacterioruberin (*2a*) and *A.g.* 470 (*3a*), all *ex. A. glacialis*, and of *3a* calculated on the basis of the additivity hypothesis. Only a rough agreement (negative Cotton effect below *ca.* 290 nm and positive Cotton effect above) is found for the observed and calculated values for *3a*. The 20 − 40 nm bathochromic displacement of the observed CD spectrum relative to the calculated one may reflect chromophoric differences, a phenomenon not yet well understood.

In an attempt to isomerize the ε-end group to a β-end group by the procedure of Andrewes [21] for stereochemical studies, a remarkable elimination was observed. Thus treatment of the diol *3a* with KOMe/MeOH/DMSO provided in 15 % yield *4a* with aliphatic tridecaene chromophore; *m/e* 644 = M, corresponding to $C_{47}H_{64}O$; M − 92, M − 106. Upon acetylation *4a* gave a monoacetate *4b* (*m/e* 686 = M, corresponding to $C_{47}H_{63}OCOCH_3$; M − 60, M − 92, M − 106). *4b* failed to give a trimethylsilyl ether on silylation, confirming that the tertiary hydroxy function was lost during the elimination. Two-three times increase in the M − 92/M − 106 intensity ratios on electron impact of *4a, b* relative to *3a,b* is consistent with the general hypothesis.[16,17,22] Thus one extra mode of xylene elimination is introduced when the steric hindrance from the C − 2' substituent is removed. However, this may be counteracted by two extra modes of toluene elimination by prolongation of the chromophore.

Attempts to prepare the analogous pentadecaene di-elimination product from bisanhydrobacterioruberin (*2a*) failed, since *2a* decomposed under the reaction conditions employed.

EXPERIMENTAL

Biological material. Arthrobacter glacialis Moiroud and Gounot [7] was grown in 6500 Petri dishes at 6 °C for 2 months at pH 7 on a solid medium [23] containing: trypticase 5 g, liver extract 2.5 g, K_2HPO_4 1 g, $MgSO_4$ 0.1 g, $CaCl_2$ 0.05 g, glucose 5 g, trace element solution 0.1 ml (containing $ZnSO_4$ 0.02 g, $MnSO_4$ 0.02 g, H_2BO_3 0.5 g, Na_2MoO_4 0.5 g, $CoCl_2$ 0.02 g, and $FeCl_3.6\ H_2O$ 3 ml per 100 ml) and H_2O to 1000 ml.

Other materials and methods were as commonly employed in the Norwegian laboratory.[24] CD spectra were recorded in EPA solution on a Roussel-Jouan dicrographe. Acetylation and silylation were effected by standard methods.[25] Adsorptive properties of the pigments studied are compiled in Table 1.

Isolation of the carotenoids. The cells were removed from the agar surface by means of the blade of a knife and extracted with acetone at room temperature: estimated yield 78 mg (E(1 %, 1 cm) = 2500 at λ_{max}). Colourless lipids were removed by five consecutive precipitations from a concentrated acetone solution; estimated lipid content > 20 (carotenoid content). Since column chromatography on deactivated alumina gave unsatisfactory pigment recovery, chromatography on the weaker, but less selective silica gel columns, was resorted to. Due to additional lipids some carotenoids were eluted with benzene – 30 % chloroform in benzene. However, 96 % of the total carotenoids were eluted as a mixed fraction with 40 % chloroform in benzene. The mixture contained decaprenoxanthin (*1a*, *ca.* 4.5 mg; 7 % of total), *A.g.* 470 (*3a*, *ca.* 55 mg; 83 % of total) and bisanhydrobacterioruberin (*2a*, *ca.* 6.5 mg; 10 % of total) judged by TLC data below. Further lipids were removed by three successive TLC purifications on silica gel (developer 50 % ether in petroleum ether) also effecting quantitative separation of *1a*, *2a*, and *3a*.

Decaprenoxanthin (*1a*)

Decaprenoxanthin (1a), available *ca.* 3 mg had λ_{max} (acetone) 417, *441* and 470.5 nm, % III/II [26] = 98. Main maxima are given in italics. The mass spectrum had *m/e* 704 = M, M – 18, M – 79, M – 92, M – 106, M – 140. No separation was observed on co-chromatography with decaprenoxanthin *ex Flavobacterium dehydrogenans*,[10] *cf.* Table 1.

Decaprenoxanthin diacetate (1b), prepared by acetylation of *1a*, yield *ca.* 1 mg, had *m/e* 788 (M), M – 92, M – 106, M – 82, % (M – 92)/M – 106 [24,25] = 3.1. The CD spectrum is given in Fig. 2.

Bisanhydrobacterioruberin (*2a*)

Bisanhydrobacterioruberin (2a), available *ca.* 4 mg had λ_{max} (hexane) 370, 388, 465, *494*, 527, % III/II = 61; *m/e* 704 = M, M – 18, M – 18 – 18, M – 58, M – 69, M – 92, M – 106), 69 (base peak); % (M – 92)/(M – 106) = 0.13. The CD spectrum is given in Fig. 2. Co-chromatography with *2a ex Corynebacterium poinsettiae* [12] gave one zone, *cf.* Table 1). *2a* gave no acetylated product under standard acetylation conditions. *2a* was destroyed under the conditions [21] where *3a* was converted to *4a* below.

Bisanhydrobacterioruberin di(trimethylsilyl) ether (2b) had *m/e* 848 = M, M – 15, M – 58, M – 72, M – 92, M – 106, 131 (base peak).

A. g. 470 (*3a*)

A.g. 470 (*3a*), available *ca.* 40 mg containing some colourless lipid impurities, had λ_{max} (hexane) 345, 362, (420), 443, *470* and 502 nm, % III/II = 77; *m/e* 704 = M, M – 2, M – 18, M – 76, M – 92, M – 106, M – 128, M – 140, M – 142, M – 158, % (M – 92)/M – 106 = 0.35; δ ($CDCl_3$) 0.76 (3 H), 0.95 (3 H), 1.20 (3 H), 1.23 (3 H), 1.56 (3 H), 1.62 (3 H), 1.68 (6 H), 1.93 (3 H), 1.98 (*ca.* 12 H), 4.02 (2 H), 5.2 – 5.7 (3 H) and 6.0 – 6.7 (*ca.* 17 H). Stepwise addition of 2 mg $Eu(dpm)_3$ to final concentrations of 2, 4, and 6 mg $Eu(dpm)_3$/8 mg *3a* in $CDCl_3$ (0.4 ml) caused induced shifts as presented in Fig. 1. The CD spectrum of *3a* is given in Fig. 2.

A.g. 470 monoacetate (3b), prepared by standard acetylation [25] of *3a*, available *ca.* 7 mg not lipid-free, m.p. 85 °C (evacuated tube) exhibited electronic spectrum as *3a* with E (1 %, 1 cm) = 2065 at 470 nm in hexane (ε = 112 000); *m/e* 746 (M), M – 18, M – 58, M – 60, M – 78, M – 87, M – 92, M – 106, M – 118, M – 142, M – 150, M – 158, M – 164, % M – 92/106 = 0.27; δ ($CDCl_3$) 0.75 (3 H), 0.93 (3 H), 1.20 (6 H), 1.55 (3 H), 1.62 (3 H), 1.67 (6 H), 1.92 (3 H), 1.97 (*ca.* 12 H), 2.05 (*ca.* 4 H), 4.46 (2 H), 5.3 – 5.7 (3 H) and 6.0 – 7.0 (*ca.* 17 H).

A.g. 470 monoacetate mono(trimethylsilyl) ether (3c), prepared by standard silylation [25] of *3b* had electronic spectrum as *3a*, *m/e* 818 (M), M – 15, M – 58, M – 60, M – 92, M – 106, M – 124, M – 131, M – 140, M – 142, 131 (base peak), % M – 92/M – 106 = 0.29.

Elimination product 4a. 3a (1 mg) in DMSO (2 ml), benzene (0.5 ml), methanol (0.5 ml, containing 4.5 % KOMe) was kept under nitrogen at 110 °C for 15 min.[21] After cooling, methanol (2.5 ml) was added and the pigments extracted with ether in the usual manner. No *3a* was recovered. Upon TLC a purple product (*4a*, yield *ca.* 15 %), less polar than *3a*, was isolated. *4a* had λ_{max} (acetone) (370) 389, 468, *494*, 528 nm, % III/II = 44; *m/e* 644 (M), M – 92, M – 106, % M – 92/M – 106 = 0.72.

Acetylated elimination product 4b. 4b, prepared

from *4a* had electronic spectrum as *4a* and *m/e* 686 (M), M−60, M−84, M−92, M−106, % M−92/M−106 = 0.66. *4b* gave no trimethylsilyl ether on silylation.

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Intermolecular Hydride Transfer Reactions. III. Reactions of 1,1-Diarylethylenes with Flavylium Perchlorate, 2,6-Dimethoxycarbonylpyrylium Perchlorate and 2,6-Dimethoxycarbonyl-4*H*-pyran

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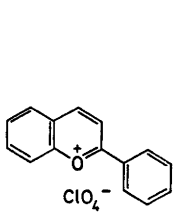
The reaction of flavylium perchlorate with 1,1-di(*p*-methoxyphenyl)ethylene to yield 4-[2,2-di-(*p*-methoxyphenyl)vinyl]flavylium perchlorate has been reinvestigated. Formation of 1,1-di(*p*-methoxyphenyl)ethane was observed in addition to the flavylium salt. The reaction of 2,6-dimethoxycarbonylpyrylium perchlorate with 1,1-diarylethylenes resulted in the corresponding 4-vinyl substituted pyrylium salts. Treatment of 2,6-dimethoxycarbonyl-4H-pyran and 1,1-di-(*p*-methoxyphenyl)ethylene with perchloric acid yielded 4-[2,2-di(*p*-methoxyphenyl)vinyl]2,6-dimethoxycarbonylpyrylium perchlorate and 1,1-di(*p*-methoxyphenyl)ethane. Electrophilic substitutions and intermolecular hydride transfer reactions accounted for the observed products.

The reactions of flavylium perchlorate (*1a*) with 1,1-diarylethylenes *2* have been studied by Wizinger *et al.*[1] Dehydrogenated adducts (*3*) were reported to be formed. The fate of the missing hydrogen atoms was not explained (Scheme 1A).

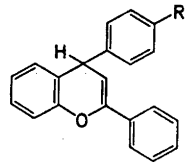
Recently examples of intermolecular hydride transfer reactions leading to related adducts have been studied. These are summarized in Scheme 1B,C. 2,6-Dimethoxycarbonylpyrylium perchlorate (*4a*) was shown to react with mono-substituted benzene derivatives *5* (R = Me, OMe, $N(Me)_2$) leading to the introduction of the 4H-pyranyl group into the *para* position of *5*. The 4H-pyranyl group was further oxidized into the corresponding pyrylium cation by intermolecular hydride transfer reaction. The pyrylium cation *4a* which acted as hydride acceptor was reduced to the 4H-pyran *6*.[2] Similar reactions between the flavylium perchlorate (*1a*) and dimethylaniline (*5*, R = $N(Me)_2$) yielded the dehydrogenated adduct *7* (R = $N(Me)_2$) and the parent flavene *8*.[3]

Considering the flavylium cation *1a* as a potential hydride acceptor, it was felt that similar intermolecular hydride transfer may be involved in the formation of the dehydrogenated adducts *3* (eqn. I, Table 1).

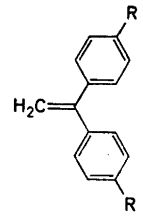
The reaction of flavylium perchlorate (*1*) with dianisylethylene (*2*, R = OMe) in boiling acetic acid solution was repeated[1] and after concentration of the reaction mixture the residue was triturated with ether. The ether insoluble material was removed by filtration. However, the expected flavene *8* could not be detected by ^1H NMR or GLC of the concentrated ether solution. The ^1H NMR spectrum of the residual oil showed a doublet centered at δ 1.6 (J 7.5, 3 H), a singlet at δ 3.7 (6 H), a quartet centered at δ 4.0 (J 7.5, 1 H) and the usual pattern due to a *para*-substituted benzene derivative centered at δ 7.0 (8 H). The spectrum suggested that dianisylethane (*9*, R = OMe) was formed, the presence of which was further verified by GLC. A possible explanation for the formation of *9* (R = OMe) and the dehydrogenated adduct *3*, is given in Scheme 2A,B. Considering the first step as a substitution reaction, perchloric acid will be liberated. Protonation of unreacted dianisylethylene (*2*, R = OMe) would give the



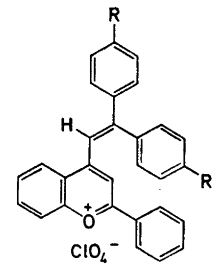
1a



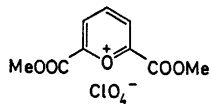
1b



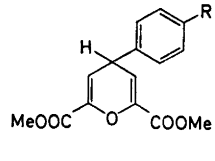
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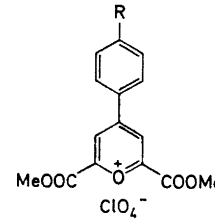
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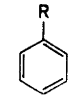
4a



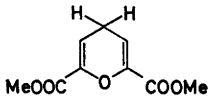
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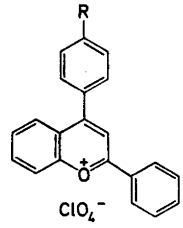
4c



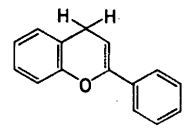
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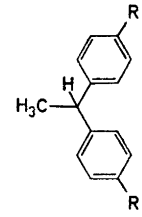
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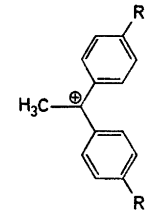
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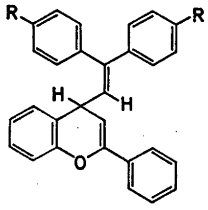
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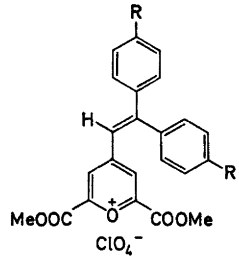
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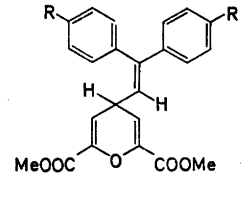
10



11



12



13

carbonium ion *10* (R = OMe) which subsequently oxidizes the substitution product *11* (R = OMe) to yield the dehydrogenated adduct *3* (R = OMe) and the reduction product *9* (R = OMe) by hydride transfer. Nearly equimolecular amounts of *1a* and *2* (R = OMe) were used in this reaction. According to the steps outlined above, only 50 % of the substrate *1a* should be transformed into *3* (R = OMe) assuming a quantitative reaction. The ether insoluble material from the

$$\text{A}\quad 1a+2 \xrightarrow{} ? \xrightarrow{-2H} 3$$

$$\text{B}\quad 4a+5 \xrightarrow{-HClO_4} 4b \xrightarrow{+4a} 4c+6$$

$$\text{C}\quad 1a+5 \xrightarrow{-HClO_4} 1b \xrightarrow{+1a} 7+8$$

Scheme 1.

$$\text{A}\quad 1a+2 \xrightarrow{-HClO_4} 11 \xrightarrow{+10} 3+9$$

$$\text{B}\quad H^+ + 2 \rightleftharpoons 10$$

$$\text{C}\quad 4a+2 \xrightarrow{-HClO_4} 13 \xrightarrow{+10} 12+9$$

Scheme 2.

$$6+2+HClO_4 \rightleftharpoons 4a+9$$

Scheme 3.

Table 1. Eqns. I – VI.

I:	2 (*1a*) +	(*2*)	=	(*3*) +	(*8*) + $HClO_4$
II:	(*1a*) + 2	(*2*)	=	(*3*) +	(*9*)
III:	(*4a*) + 2	(*2*)	=	(*12*) +	(*9*)
IV:	2 (*4a*) +	(*2*)	=	(*12*) +	(*6*) + $HClO_4$
V:	(*6*) +	(*2*) + $HClO_4$	=	(*4a*) +	(*9*)
VI:	(*6*) + 3	(*2*) + $HClO_4$	=	(*12*) + 2	(*9*)

above reaction was dissolved in trifluoroacetic acid. The ^{1}H NMR spectrum of this solution indicated a mixture of *3* (R = OMe) and the substrate *1a*. Only traces of unreacted dianisylethylene (*2*, R = OMe) could be identified from analysis of the ether soluble and insoluble fractions.

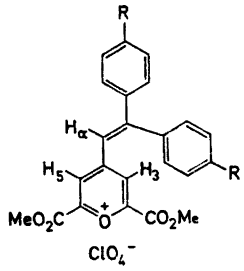
When the reaction was repeated with *1a* and *2* (R = OMe) in molar ratio 1:2, respectively, the dehydrogenated adduct *3* (R = OMe) was formed in a yield corresponding to 93 % transformation of substrate *1a* into *3* (R = OMe). Dianisylethane (*9*, R = OMe) was the only reduction product which could be identified. The stoichiometry of the reaction is given by eqn. II, Table 1.

Similar reactions were carried out between the pyrylium salt *4a* and the diphenylethylenes *2* (R = H, Me, OMe) in acetonitrile solution at 20 °C using molar ratio 1:2, respectively. Pyrylium salts *12* (R = H, Me, OMe) were isolated in high yields and the reduction products *9* (R = H, Me, OMe) were identified (Scheme 2B,C). The stoichiometry of the reaction is given by eqn. III, Table 1.

The reaction of *4a* with dianisylethylene (*2*, R = OMe) in the molar ratio 2:1, respectively, was studied in trideuterioacetonitrile solution (eqn. IV, Table 1). ^{1}H NMR analysis of the reaction mixture clearly established the formation of the dehydrogenated adduct *12* (R = OMe) and the 4H-pyran *6*. Furthermore signals due to dianisylethane *9* (R = OMe) and unreacted pyrylium salt *4a* were observed. Formation of *6* and *9* (R = OMe) indicated hydride transfer both to the pyrylium salt *4a* and to the carbonium ion *10* (R = OMe). With two hydride acceptors present in the reaction between *4a* and *2* (R = OMe), both possibly capable of oxidizing the substitution product *13* (R = OMe), the redox reaction depicted in Scheme 3 had to be considered (eqn. V, Table 1). The ^{1}H NMR spectrum of dianisylethylene (*2*, R = OMe) in deuteriochloroform solution showed a singlet at δ 5.3 (2 H), a singlet at δ 3.8 (6 H) and the pattern due to the aromatic protons centered at δ 7.1 (8 H). In trifluoroacetic acid solution the aromatic and the methoxy proton signals were shifted downfield to δ 7.8 (8 H) and δ 4.2 (6 H), respectively. A singlet at δ 3.3 (3 H) was interpreted as being due to the methyl group of the carbonium ion *10* (R = OMe) but no signal due to the ethylenic protons was observed (Scheme 2B). The downfield shifts mentioned above supported the idea of the electron deficient species. In deuteriotrifluoroacetic acid solution the singlet at δ 3.3 could not be detected. Only signals due to the methoxy groups and the aromatic protons were observed. This indicates that complete deuterium incorporation had taken place in the methyl group of *10* (R = OMe) during the preparation of the sample. The exchanged protons appeared as a singlet at δ 11.4, which is the normal shift position of trifluoroacetic acid. The deuterium/hydrogen exchange is supposed to take place through the equilibrium given in Scheme 2B. Furthermore, it is assumed that the concentration of *2* (R = OMe) in trifluoroacetic acid solution is low, since its presence was not detectable in the ^{1}H NMR spectrum of the equilibrium mixture. When an equimolecular amount of the 4*H*-pyran *6* was added to the solution of *2* (R = OMe) in trifluoroacetic acid, the ^{1}H NMR spectrum showed that the redox reaction corresponding to the reaction given in Scheme 3 was displaced to the right. However, it should

be pointed out that the observed signals were due to dianisylethane (*9*, R = OMe) and the cation *12* (R = OMe). The formation of the latter compound suggests that the pyrylium cation *4a* formed, immediately reacts with *2* corresponding to the reaction given in Scheme 2C. The observation of signals due to unreacted 4*H*-pyran *6*, supported this conclusion.

The above mentioned conclusion indicates that a reaction between one mol of the 4*H*-pyran *6* and three mol of dianisylethylene (*2*, R = OMe) in acidic solution would be expected to proceed quantitatively into the pyrylium cation *12* (R = OMe) and dianisylethane *9* (R = OMe). One mol of *6* and one mol of *2* (R = OMe) should give one mol of pyrylium cation *4a* according to Scheme 3 and the additional two mol of *2* (R = OMe) should give the pyrylium cation *12* (R = OMe) according to Scheme 2C. Evidently, the ^{1}H NMR spectrum of this reaction mixture in trifluoroacetic acid after 4 days at 20 °C exhibited mainly signals due to the pyrylium cation *12* (R = OMe) and dianisylethane (*9*, R = OMe). Furthermore the pyrylium salt *12* (R = OMe) was obtained in 90 % yield from a reaction carried out in a mixture of acetic acid and perchloric acid. The yield was calculated according to eqn. VI, which is the sum of eqns. V and III (Table 1).



12

The 4H-pyran *6* was not identified from reactions carried out between the pyrylium cation *4a* and dianisylethylene (*2*, R = OMe) in 1:2 ratio. Direct hydride transfer from the substitution product to the protonated dianisylethylene *10* (R = OMe) accounted for the observed products *12* (R = OMe) and *9* (R = OMe) (eqn III, Table 1). However, since eqn. III is the sum of IV and V, it seems possible that hydride transfer to the pyrylium salt *4a* may occur to some extent with formation of *6* which is subsequently reoxidized through a second hydride transfer (eqn. V). This may explain the formation of only reduction product *9* (R = OMe). Kinetic studies of these reactions have not been carried out. The intermediate substitution product *11* and *13* could not be detected from the reactions between the heterocyclic cations *1a*, *4a*, and dianisylethylene (2, R = OMe). This indicates that the hydride transfer reactions are faster than the substitution reactions. Furthermore, it should be pointed out that in the hydride transfer between the substitution products and the cation *10* (R = OMe), two tertiary carbon atoms are involved. In hydride transfer reactions between the substitution products and the heterocyclic cations and in redox reactions as shown in Scheme 3, tertiary and secondary carbon atoms are involved. With respect to sterical effects, it seems likely that the latter examples of hydride transfer reactions should be favoured.

The ^{1}H NMR shift values of the pyrylium salt *12* are listed in Table 2.

The increased stabilizing effect of substituent R on the pyrylium cation through the series H, Me, OMe is reflected in chemical shift positions of the heterocyclic ring protons. The upfield shifts of these protons are in agreement with a

Table 2. ^{1}H NMR spectra of pyrylium perchlorate *12* in TFA (δ).

R	H_3	H_5	H_α	$H_{Ar.}$	R	CO_2Me
H	8.05[a]	8.05[a]		7.3 – 7.9[b,c]		4.13[a]
Me	7.90[a]	7.90[a]	7.66[a,c]	7.3 – 7.7[b,c]	2.21[a]	4.13[a]
OMe	7.65[a,c]	7.65[a,c]	7.49[a]	7.3[d], 7.7[d,c]	4.10[a,c]	4.10[a,c]

[a] singlet. [b] multiplet. [c] superimposed. [d] AA'BB'system, J_{AB} = 9.0 Hz.

decreased positive charge of the pyrylium nucleus.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Varian A-60A instrument. A Perkin-Elmer F-11 instrument equipped with an OV-17 column was used for GLC analysis.

*4-[2,2-Di(*p*-methoxyphenyl)vinyl]flavylium perchlorate (3, R=OMe)* [1] *and 1,1-di(*p*-methoxyphenyl)ethane (9, R=OMe).* A mixture of flavylium perchlorate (*1a*) [4] (1.53 g, 0.005 mol) and 1,1-di(*p*-methoxyphenyl)ethylene (*2*, R=OMe) [5] (2.4 g, 0.01 mol) in acetic acid solution (100 ml) was refluxed for 12 h. The precipitated material was collected by filtration after 24 h and washed with dry ether. The yield of *3* (R=OMe) was 2.48 g (93 %), m.p. 237 °C (decomp.), λ_{max} 568 nm (AcOH). ^{1}H NMR spectrum of *3* (R=OMe) (trifluoroacetic acid solution): δ 4.08 (6 H, s), 7.7–8.3 (19 H, m). The filtrate was evaporated and the residue was extracted with ether (200 ml). The ether solution was concentrated and *9* (R=OMe) was identified by the ^{1}H NMR spectrum of the residual oil in deuteriochloroform solution: δ 1.58 (*J* 7.5 Hz, 3 H, d), 3.75 (6 H, s), 4.05 (*J* 7.5 Hz, 1 H, q), 6.8 (J_{AB} 9.0, 8 H, AA'BB'-system). Authentic samples of *9* (R=OMe) [6] and 2-flavene (*8*) [7] were prepared for purposes of identification. GLC analysis verified the formation of *9* (R=OMe). The flavene *8* could not be detected.

4-(2,2-Diarylvinyl)-2,6-dimethoxycarbonylpyrylium perchlorate (12) and 1,1-diarylethane (9). General procedure. 2,6-Dimethoxycarbonylpyrylium perchlorate (*4a*) [8] (0.0025 mol) was added to a solution of 1,1-diarylethylene (*2*) (0.005 mol) in dry acetonitrile (25 ml) at 20 °C. After stirring for 24 h the solution was evaporated. The residue was triturated with ether (500 ml). The pyrylium salt *12* was collected by filtration and recrystallized from acetic acid. The filtrate was evaporated and the residue was examined by ^{1}H NMR spectroscopy for reduction products.

*4-[2,2-Di(*p*-methoxyphenyl)vinyl]-2,6-dimethoxycarbonylpyrylium perchlorate (12, R=OMe)* was prepared from the pyrylium salt *4a* and 1,1-di(*p*-methoxyphenyl)ethylene (*2*, R=OMe) [5] in 90 % yield, m.p. 140 °C (decomp.), λ_{max} 580 nm (AcOH). ^{1}H NMR spectrum, see Table 2. (Found: C 56.01; H 4.39; Cl 6.82. Calc. for $C_{25}H_{23}O_{11}Cl$: C 56.13; H 4.30; Cl 6.64).

*1,1-Di(*p*-methoxyphenyl)ethane (9, R=OMe)* [6] was identified from the reaction of *4a* with *2* (R=OMe). ^{1}H NMR spectrum ($CDCl_3$): δ 1.58 (*J* 7.5 Hz, 3 H, d), 3.75 (6 H, s), 4.05 (*J* 7.5 Hz, 1 H, q), 6.8 J_{AB} 9.0 Hz, 8 H, AA'BB'-system).

4-(2,2-Diphenylvinyl)-2,6-dimethoxycarbonylpyrylium perchlorate (12, R=H) was prepared from the pyrylium salt *4a* and 1,1-diphenylethylene (*2*, R=H) in 70 % yield, m.p. 186 °C (decomp.), λ_{max} 493 nm (AcOH). ^{1}H NMR spectrum, see Table 2. (Found: C 58.08; H 4.11. Calc. for $C_{23}H_{19}O_9Cl$: C 58.16; H 4.00).

1,1-Diphenylethane (9, R=H) was identified from the reaction of *4a* with *2* (R=H) by comparison with a sample prepared from catalytic hydrogenation of *2* (R=H) in ethyl acetate (10 % Pd/$CaCO_3$). ^{1}H NMR spectrum ($CDCl_3$): δ 1.58 (*J* 7.5 Hz, 3 H, d), 4.08 (*J* 7.5 Hz, 1 H, q), 7.17 (10 H, narrow m).

*4-[2,2-Di-(*p*-methylphenyl)vinyl]-2,6-dimethoxycarbonylpyrylium perchlorate (12, R=Me)* was prepared from the pyrylium salt *4a* and 1,1-di-(*p*-methylphenyl)ethylene (*2*, R=Me),[5] m.p. 178 °C (decomp.). ^{1}H NMR spectrum, see Table 2. (Found: C 59.52; H. 4.50. Calc. for $C_{25}H_{23}O_9Cl$: C 59.70; H 4.58).

*1,1-Di(*p*-methylphenyl)ethane (9, R=Me)* was identified from the reaction of *4a* with *2* (R=Me) by comparison with a sample prepared from catalytic hydrogenation of *2* (R=Me) in ethyl acetate (10 % Pd/$CaCO_3$, 3 atm.). ^{1}H NMR spectrum ($CDCl_3$): δ 1.58 (*J* 7.5 Hz, 3 H, d), 2.29 (6 H, s), 4.05 (*J* 7.5 Hz, 1 H, q), 7.1 (8 H AA'BB'-system).

*4-[2,2-Di-(*p*-methoxyphenyl)vinyl]-2,6-dimethoxycarbonylpyrylium perchlorate (12, R=OMe).* 2,6-Dimethoxycarbonyl-4H-pyran (*6*) [8] (0.495 g, 0.0025 mol) and 1,1-di(*p*-methoxyphenyl)ethylene (*2*, R=OMe) [5] (1.8 g, 0.0075 mol) were added to a mixture of acetic acid (50 ml) and 70 % perchloric acid (0.5 g). The reaction mixture was left at 25 °C for 4 days with stirring, diluted with ether (300 ml) and after 12 h the precipitated material was collected by filtration. Yield 90 % (1.2 g), m.p. 140 °C (decomp.), λ_{max} 580 nm (AcOH), ^{1}H NMR spectrum, see Table 2.

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Putrescine-insensitive S-Adenosyl-L-methionine Decarboxylase from *Tetrahymena pyriformis*

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Extracts of *Tetrahymena pyriformis* contain a soluble *S*-adenosyl-L-methionine decarboxylase which, in contrast to the enzyme from most eukaryotic organisms, is not stimulated by putrescine or spermidine. The protozoan adenosylmethionine decarboxylase, unlike the putrescine-insensitive enzyme form *Escherichia coli*, did not require any metal ions for catalytic activity either. Adenosylmethionine decarboxylase from *Tetrahymena* resembled the prokaryotic enzyme as far the inhibition by methylglyoxal bis(guanylhydrazone) was concerned, but behaved more like putrescine-activated enzyme in regard to the inhibition by 4-bromo-3-hydroxy benzyl-oxyamine. Adesylmethionine decarboxylase from rat liver, baker's yeast, *E. coli* and *Tetrahymena* were strongly inhibited by *S*-methyladenosylhomocysteamine (decarboxylated adenosylmethionine), the product of the reaction. The function of adenosylmethionine decarboxylase from *Tetrahymena* like that of the enzymes from other organisms appears to be closely connected to the synthesis of spermidine.

The synthesis of spermidine both in prokaryotic and eukaryotic organisms is accomplished by two separate enzymes: a *S*-adenosyl-L-methionine decarboxylase (AMD; EC 4.1.1.50) catalyzing the formation of *S*-methyladenosylhomocysteamine (decarboxylated adenosylmethionine) and an aminopropyltransferase (spermidine synthase, EC 2.5.1.16) transferring the propylamine moiety from decarboxylated adenosylmethionine to putrescine.[1-4] These two enzymes appear to function very closely together in the formation of spermidine in all organisms studied. Thus crude extracts from most animal tissues catalyze a stoichiometric formation of spermidine and carbon dioxide from adenosylmethionine in the presence of putrescine.[5] A fundamental difference between AMD from prokaryotic and eukaryotic organisms seems to be the fact that the enzyme from the latter sources is intensively and specifically stimulated by minute concentrations of putrescine.[5,6,3] AMD appears to be activated by putrescine at least in all animal tissues studied so far, including livers from reptilia, crustacae, amphibia, birds, and mammals.[7] The enzyme from baker's yeast also shows an almost absolute requirement for putrescine.[7,8]. In contrast to the AMD from the eukaryotic sources mentioned, the enzyme from bacteria is totally insensitive to putrescine but instead requires Mg^{2+} ions for its activity. Thus AMD from *E.coli* [1,7] and *Azotobacter vinelandii* [7] exhibits virtually absolute requirement for divalent cations. The classification of AMD to prokaryotic putrescine-insensitive and putrescine-activated eukaryotic enzymes is, however, not universal, since AMD activity in extracts of an eukaryotic plant (mung bean sprouts) is not stimulated by putrescine but requires Mg^{2+}.[7] An even more important exception of this general rule is the AMD from the lower eukaryotic plasmodium *Physarum polycephalum*. AMD activity from the latter organism does not appear to be stimulated either by putrescine or Mg^{2+}, however, still functioning in the synthesis of spermidine.[9]

The present communication deals with some characteristics of a putrescine-insensitive AMD from extracts of *Tetrahymena pyriformis*. The enzyme did not require any metal ions

for the catalytic activity. The protozoan AMD resembled the prokaryotic enzyme in regard to the inhibition by methylglyoxal bis (guanylhydrazone), a potent inhibitor of putrescine-activated enzyme.[10]

MATERIAL AND METHODS

Chemicals. Unlabelled and labelled *S*-adenosyl-L-methionine was synthesized enzymically by the method of Pegg and Williams-Ashman.[5] Decarboxylated adenosylmethionine was prepared with the aid of AMD partially purified from *E.coli* cells. The product of the enzymic decarboxylation was purified and finally separated from adenosylmethionine by preparative paper electrophoresis.[8]

Methylglyoxal bis(guanylhydrazone) was purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A., and 4-bromo-3-hydroxybenzyloxyamine (NSD-1055) was a product of Smith and Research Ltd., Harlow, Essex, England.

Putrescine-1,4^{14}C dihydrochloride (sp. radioactivity 17.5 mCi/mmol) was purchased from New England Nuclear Corp., Dreieichenhain, West Germany, and purified before use on a column (H^+ form; 1 cm × 5 cm) of Dowex 50.[12]

Organisms and growth conditions. Tetrahymena pyriformis, strain W (obtained from Dr. G. Rølle, Department of Nutritional Biochemistry, The Royal Danish School for Educational Studies, Copenhagen, Denmark), was maintained in stock cultures of peptone-yeast medium at pH 8.4. For experimental purpose 3 – 7 day-old stock cultures were used to inoculate peptone-yeast medium (containing 2 % proteose-peptone, 0.1 % yeast extract, 0.5 % glucose and 0.1 % NaCl) in a Roux-bottle with a surface area of 200 cm^2. The cultures were incubated at 25 °C without shaking, and the cells were harvested at the early stationary phase (72 h) by centrifugation at 10 000 *g* for 10 min. The yield (wet wt.) was about 3 g/100 ml per 72 h.

Preparation of cell extracts. The cells of *Tetrahymena pyriformis* W, *E. coli* (strain ATCC 4157; harvested at the stationary phase) and *Saccharomyces cerevisiae* (obtained from Oy Alko, Helsinki, Finland) were suspended in an equal volume (wt./vol) of 25 mM Tris-HCl, pH 7.1, containing 0.1 mM EDTA and 1 mM dithiothreitol (standard buffer) and extruded twice through a chilled (– 25 °C) pressure cell (X-press, Biox AB, Nacka, Sweden) at 1500 kg/cm^2. After the disintegration of the cells. the homogenates were centrifuged at 15 000 *g* for 15 min and the supernatant fractions were used for a further purification or as the source of the AMD.

Liver cytosol fraction was prepared by homogenizing rat liver with two volumes of the standard buffer and centrifuging as above.

Analytical methods. The activity of AMD was assayed essentially as described earlier.[1,3] The activity of spermidine synthase was assayed in the presence of decarboxylated adenosylmethionine and radioactive putrescine as described elsewhere.[4,8] Protein was measured by the method of Lowry *et al.*[11]

Partial purification of adenosylmethionine decarboxylase from Tetrahymena pyriformis. A crude extract prepared from 10 g of *Tetrahymena* cells was fractionated with solid ammonium sulfate (special enzyme grade, Schwarz/ Mann, Orangeburg, N.Y., U.S.A) at 0 °C. The proteins precipitated between 0.45 – 0.70 saturation of ammonium sulfate were collected and dissolved in a small volume of the standard buffer and dialyzed for 16 h against the same buffer. The dialyzed protein fraction was subjected to chromatography on a DEAE cellulose column (1.1 cm × 17 cm; DE-52 Whatman), previously equilibrated against the standard buffer. The column was washed with 70 ml of the equilibration buffer and connected to a linear gradient of 0 to 0.4 M NaCl in the standard buffer (the total gradient volume was 200 ml). AMD activity emerged from the column at about 0.3 M NaCl. The most active fractions were pooled and concentrated in an ultrafiltration cell to 5 ml. This partial purification of the enzyme activity resulted in a 17-fold purification with an overall

Table 1. Effect of various amines, metals, pyridoxal phosphate, and nucleotides on AMD activity from *Tetrahymena pyriformis*. Dialyzed extract (0.8 mg protein) was used in Experiment I and partially purified enzyme (DEAE fraction; 0.016 mg protein) in Experiment II as the source of the enzyme. The activities are expressed as pmol of CO_2 formed per 30 min.

Compound added	Conc. (mM)	AMD activity Exp. I	Exp. II
None	–	62.0	29.2
Putrescine	2.5	70.3	33.8
Spermidine	2.5	49.0	32.7
Spermine	2.5	49.0	32.3
Diaminopropane	2.5	50.0	
Cadaverine	2.5	52.8	
Pyridoxal phosphate	0.1	39.7	19.4
Mg^{2+}	10	46.9	17.5
Mn^{2+}	10	16.3	
Na^+	100	38.0	
K^+	100	38.0	
ATP	1.0		21.1
GTP	1.0		21.8
CTP	1.0		21.4
UTP	1.0		20.7

yield not more than 0.6 %. The poor recovery of the enzyme activity was due to the extreme lability of the enzyme, so that all attempts at more extensive purifications were considered unrealistic.

RESULTS AND DISCUSSION

As shown in Table 1, the activity of AMD from cells of *Tetrahymena pyriformis* was not stimulated to any appreciable extent by putrescine, spermidine, spermine, 1,3- diaminopropane, or cadaverine. Divalent and monovalent cations appeared to be inhibitory for the enzyme. It did not matter whether dialyzed crude extract or partially purified enzyme (DEAE fraction) was used.

AMD from the protozoan was extremely labile. The partially purified enzyme was inactivated with an apparent half-life of about 2 h when dialyzed at 3 °C against the standard buffer. A dialysis of the crude extract for 16 h resulted in a loss of AMD activity up to 80 %. It was unlikely that the rapid inactivation was caused by the removal of a dialyzable small molecule since the combination of the partially purified enzyme with undialyzed crude extracts from *Tetrahymena* only resulted in a simple addition of the enzyme activity. Pyridoxal-5'-phosphate was slightly inhibitory, and neutralized nucleotides were without any effect whatsoever (Table 1). The enzyme thus resembled AMD from dialyzed extracts of the plasmodium *Physarum polycephalum* in being insensitive to putrescine (and other polyamines) as well as to divalent cations.[9]

Table 2. Inhibition of AMD activity from *Tetrahymena pyriformis* by carbonyl reagents. Dialyzed extract (0.8 mg protein) was used as the source of the enzyme. The enzyme activity is expressed as pmol of CO_2 formed per 30 min.

Inhibitor	Conc. (mM)	Enzyme activity	Inhibition %
None	–	41.8	0
KCN	5	4.6	89
Isonicotinic acid hydrazide	5	38.8	7
Semicarbazide	5	16.1	61
$NaBH_4$	10	16.6	60

The activity of AMD from extracts of *Tetrahymena* was inhibited by a number of carbonyl reagents like KCN, semicarbazide and borohydride but not by isonicotinic acid hydrazide (Table 2). The latter finding together with the apparent ineffectiveness of added pyridoxal phosphate might indicate that carbonyl groups other than pyridoxal phosphate are required for the catalytic activity of AMD from *Tetrahymena pyriformis*. The nature of the prosthetic group of mammalian putrescine-activated adenosylmethionine decarboxylase still remains open, whereas the magnesium-activated enzyme from *E. coli* contains covalently bound pyruvate probably functioning as the prosthetic group.[1]

Methylglyoxal bis(guanylhydrazone) is known to be a specific and powerful inhibitor of putrescine-activated AMD from rat ventral prostate,[10] baker's yeast,[10] and rat liver.[13] It also inhibits the putrescine-insensitive adenosylmethionine decarboxylase from the plasmodium *Physarum polycephalum*,[9] whereas relatively high concentrations are needed for a similar inhibition of the putrescine-insensitive AMD from *E. coli*.[10] The mechanism of the inhibition could involve interactions with the putrescine binding site since the inhibitor appears to diminish the magnitude of the stimulation by putrescine.[10,13] The finding that diamine oxidase from rat tissues (using putrescine as the substrate) is also extremely sensitive to methylglyoxal bis(guanylhydrazone) also points in this direction.[13]

As seen in Fig. 1, the activity of AMD from *Tetrahymena* was also inhibited by methyl-

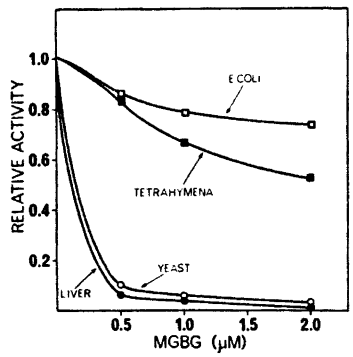


Fig. 1. Inhibition of AMD activity from various organisms by methylglyoxal bis(guanylhydrazone) (MGBG). Dialyzed crude extracts were used as the source of the enzyme in each case.

glyoxal bis(guanylhydrozone) but in this respect the enzyme resembled AMD from *E. coli* being considerably less sensitive than the enzyme from extracts of rat liver or baker's yeast.

4-Bromo-3-hydroxy benzyloxyamine (NSD-1055) is known to inhibit some pyridoxal phosphate requiring enzymes like histidine decarboxylase from rat tissues.[14,15] The compound apparently forms an oxime with pyridoxal phosphate.[15] Pegg and Williams-Ashman [5] reported that AMD from rat ventral prostate was powerfully inhibited by the drug, whereas the enzyme from *E. coli* cells was relatively insensitive. The inhibition of AMD from rat ventral prostate could be reversed by a prior incubation of the enzyme preparation in the presence of pyridoxal phosphate but hardly in the presence of free pyridoxal.[5] The latter finding has been taken as a suggestive evidence that the prosthetic group of mammalian AMD would be pyridoxal phosphate. However, no direct evidence has been obtained to prove or disprove this hypothesis. If anything, the data available at present rather suggest that the carbonyl group obviously needed for the decarboxylation of adenosylmethionine by eukaryotic AMD would not be pyridoxal phosphate.[16] As also seen in the present study, AMD from *Tetrahymena* was not affected by the inclusion of pyridoxal phosphate in the incubation mixture (Table 1), neither was the enzyme activity inhibited by 5 mM isonicotinic acid hydrazide (Table 2).

As illustrated in Fig. 2, AMD from rat liver most markedly inhibited by NSD-1055 whereas the enzyme from *E. coli* was hardly affected by the compound at concentrations up to 1 mM. The degree of inhibition of putrescine activated AMD from baker's yeast and that of the putrescine-insensitive enzyme from protozoan *Tetrahymena* was somewhere between the two extremities.

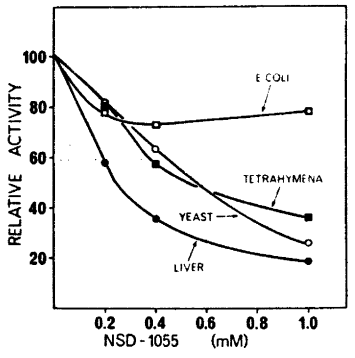


Fig. 2. Inhibition of AMD activity from various organisms by 4-bromo-3-hydroxy benzyloxyamine (NSD-1055). Dialyzed crude extracts were used as the source of the enzyme in each case.

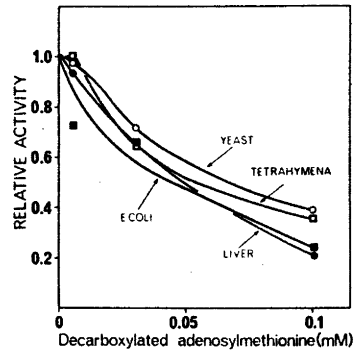


Fig. 3. Inhibition of AMD activity from various organisms by *S*-methyladenosylhomocysteamine (decarboxylated adenosylmethionine). The source of the enzymes as in Figs. 1 and 2.

The product of the decarboxylation reaction, decarboxylated adenosylmethionine, S-methyladenosylhomocysteamine, is a potent inhibitor for both eukaryotic and prokaryotic AMD.[8] In fact, it has been reported that putrescine-activated enzyme from rat ventral prostate or baker's yeast would be more sensitive to the product inhibition than the enzyme from *E. coli*.[8] As shown in Fig. 3, AMD from all four organisms was inhibited by the product of the reaction in a similar fashion with an half-maximal inhibition occurring at a concentration of approximately 50 μM. The reason for this apparent discrepancy with the earlier report is not known. This might conceivably be due to slightly different experimental conditions, different rat tissue (liver *versus* ventral prostate) and a different strain of *E. coli* used in the present study.

When the formation of spermidine from adenosylmethionine was followed simultaneously with the formation of carbon dioxide from the latter compound a close stoichiometry was found in the presence of putrescine (not tabulated).

It appears that the stimulation of AMD activity by putrescine can be no longer consid-

ered as a general property of eukaryotic organisms. There exist at least three eukaryotic AMD's which are not stimulated by putrescine or by any other amine: (i) AMD from bean sprouts,[7] (ii) that of the plasmodium *Physarum polycephalum*,[9] and (iii) that from protozoan *Tetrahymena pyriformis*. The enzyme from the plasmodium and protozoan also is insensitive to divalent cations (which might even be inhibitory) whereas the activity of AMD from bean sprouts is markedly enhanced by Mg^{2+}.[7]

It is quite impossible to decide what really determines the sensitivity of AMD to putrescine. However, it also appears somewhat unlikely that the presence of real nuclear membrane should change the properties of a cytosolic enzyme. Nevertheless, there seems to exist an interesting difference between the two types of AMD. All rat tissues [17] and baker's yeast [18] contain substantial amounts of spermine and, at least rat tissues, also contain relatively active spermine synthase activity [19,20] that catalyzes the transfer of the propylamine group of decarboxylated adenosylmethionine to spermidine. On the contrary, *E. coli* cells do not contain any spermine, the only polyamines in this organism are putrescine and spermidine.[18] The cells of *Physarum polycephalum* [9] and those of *Tetrahymena pyriformis* [21] only contain almost unmeasurable traces of spermine as compared to the levels of putrescine and spermidine. It is thus possible that the stimulation of AMD by putrescine is an evolutionary change in the enzyme appearing at the time when the organism starts to synthesize spermine. The stimulation of AMD by putrescine would thus assure that sufficient amounts of decarboxylated adenosylmethionine are available for the synthesis of spermidine by spermidine synthase.

However, more extensive systematic studies obviously are needed to elucidate the possible relations between the stimulations of AMD activity by putrescine and the ability of the organism to synthesize and accumulate spermine.

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Proteolysis of Some Synthetic Poly-α-amino Acids

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The hydrolysis of poly-L-glutamic acid (PLG) and poly-L-lysine (PLL) by a number of commercial proteolytic enzymes was followed by thin layer chromatography. On the basis of these chromatograms, pH activity curves were made by a viscosimetric method. The chromatograms as well as the pH curves showed significant variations between the different enzymes although their pH optima were around 5 for PLG and between 6.7 and 10.1 for PLL. The degradation products were analyzed by thin layer chromatography at pH 5, 6.5, and 8 for PLG and at pH 5, 7, and 8.5 for PLL. The chromatograms from the actual enzymes were different. Among the enzymes tested, thermolysin and proteinase Novo did not hydrolyse poly-L-glutamic acid.

Poly-L-ornithine was split by pronase and subtilisin with pH optima at 10.6 and 10.2, respectively.

Streptomyces protease fixed to agarose or to carboxymethyl cellulose showed weak hydrolysis of poly-L-glutamic acid around pH 5 and of poly-L-lysine at pH 8.3 – 8.7. Insolubilized agarose trypsin hydrolysed poly-L-lysine more rapidly, but more than 50 % of the hydrolysis was due to autolysis of the enzyme conjugate.

Carboxymethyl cellulose trypsin was stabile and hydrolysed poly-L-lysine at pH 7.75 – 8.75.

Some bacterial enzymes and pancreas extracts tested for poly-D-glutamic acid hydrolysis were found to be inactive.

Very weak hydrolysis of poly-D-lysine around pH 8 was found in extracts of pancreas protease Merck and pancreatin Sigma, Grade III.

Some years ago one of the present writers (G.L.) studied the hydrolysis of poly-α-L-lysine and poly-α-L-glutamic acid.[1] The enzymes trypsin, chymotrypsin, ficin, pronase, and pepsin were investigated as well as laboratory extracts from thyroid glands, sea urchin eggs, the fungus *Aspergillus fumigates* and the culture medium of *Staphylococcus aureus*. The hydrolysis at different pH was studied by a viscosimetric method, and the breakdown was followed by means of gel chromatography.

In the present work the investigation of the influence of pH on the hydrolysis of some poly-α-L-amino acids by commercial enzymes was extended and the split products were followed by means of thin layer chromatography.

The hydrolysis of poly-L-glutamic acid and poly-L-lysine by insolubilized trypsin, *i.e.* trypsin bound to agarose or carboxymethyl cellulose and by *S. griseus* protease bound to agarose, was also studied.

In 1956 Tsuyuki, Tsuyuki and Stahmann [2,3] reported the breakdown of poly-α-D-lysine by strong extracts of pancreas powder. These results have not been repeated — as far as the present authors know — and therefore the susceptibility to hydrolysis of poly-D-lysine and poly-D-glutamic acid by different proteolytic enzyme preparations was investigated in the present paper. A number of proteinases such as subtilisin, pronase (*S. griseus* protease) and Nagarse (subtilisin BPN'), extracts of fresh bovine and swine pancreas and of different commercial pancreas powders were tested as well as *Helix pomatia* digestive juice, *Myxococcus virescens* culture medium, and amnion from hen's egg.

MATERIAL AND METHODS

Substrates. Poly-L-lysine hydrobromide (mol. wt. 200 000), poly-D-lysine (mol. wt. 60 000), poly-L-glutamic acid sodium salt (mol. wt. 148 000), poly-D-glutamic acid sodium salt (mol. wt. 94 000), poly-L-arginine (mol. wt. 60 000), and poly-L-ornithine hydrobromide (mol. wt. 70 000) were purchased from Pilot Chemicals Inc., Watertown, Mass. USA. Poly-L-lysine hydrobromide (mol. wt. 104 000, lot

Ly 172) was from Miles Lab. Ltd., Slough, England.

Commercial enzymes. Acid protease (*Rhizopus chinensis* Batch 7015, Miles Lab. Ltd., England); Alcalase (Subtilisin Carlsberg, cryst. 27.5 A.U./g, batch No. 1 x 16, Novo Industri A/S, Copenhagen); *Aspergillus* protease I (*A. oryzae* Astra, Sweden); *Aspergillus* protease II (*A. oryzae* type II, Crude, 0.15 μmol tyrosine/(min mg) pH 7.5 and 37 °C from casein, Sigma Chem. Co., St. Louis); *Bacillus subtilis* protease (*B. subtilis* type VIII, Sigma); Bacterial proteinase Novo (cryst. 25.9 A.U./g, batch No. 58, Novo Ind.); Bromelain (Mann Research Lab., Inc. USA); Crystalline subtilisin (27.5 A.U./g, batch No. 1 x 16, Novo Ind.); Nagarse (Subtilisin BPN′, lyophilized, cryst. bacterial proteinase, potency 150 000 P. U. N. in vial, batch DAF 0406, Teikoku Chem. Ind., Osaka, Japan); Pronase B grade (*Streptomyces griseus* 45 000 PUK/g, lot 53997, Calbiochem, La Jolla, Calif.); *Streptomyces* protease (*S. griseus*, type V, purified, lot 17B-1740, 1.7 μmol tyrosine/(min mg), Sigma); Thermolysin (*B. thermoproteolyticus* Rokko proteinase 50 PU/mg, lot TA8C91, Daiva Kasei, K.K., Japan). Enzite-agarose-trypsin (3 x cryst. bovine trypsin bound to agarose), Enzite-trypsin (trypsin bound to carboxymethyl cellulose), and Enzite-agarose-protease (*S. griseus* protease bound to agarose) were purchased from Miles-Seravac, Miles Lab. Ltd., England.

Snail digestive juice, batch No. 61310 was from Koch-Light Lab., England.

Crude enzyme preparations

1. *Pancreatin extract.* Pancreatin powder from Weddel Pharmaceuticals Ltd., The British Drug House Ltd., Schwarz-Mann, Merck, Sigma and Koch-Light Laboratories Ltd., was used. A suspension of 5 g in 100 ml dist. water was stirred for 3 h at + 4 °C and then centrifuged at 5000 *g* for 20 min in the cold.

2. *Extract of fresh pancreas.* Fresh pancreas from ox or swine was freed from fat and connective tissue, cut in pieces, ground in a mincing-machine and disintegrated in a "VirTis" blender in 0.9 % NaCl for 90 s. After centrifugation at 10 000 *g* for 30 min, a second centrifugation of the supernatant was made at 20 000 *g* for 30 min. All operations were carried out in the cold. The supernatant was used for proteolytic test.

3. *Amnion* fluid from hen's egg was tested immediately for proteolytic activity.

4. Culture medium "enzyme 1 and 3" from *Myxococcus virescens* was obtained from Dr. G. Haskå, University of Lund, Sweden.

Assay of proteolytic activity

1. *The viscosimetric method* elaborated by Hultin [4-7] was used. The determinations were made in Ostwald viscosimeters at 35.5 °C, and the enzyme activity was calculated according to Hultin's formula [1] and expressed in Hultin Units (HU).

2. *Potentiometric determination.* A continuous potentiometric (pH-stat) titration [8] of the carboxyl groups, liberated by the action of the enzyme at various constant pH values, was made for the determination of the enzyme activity.[9,10] A pH meter model 26 with Titrator 11 and Autoburette ABU12 from Radiometer, Copenhagen, was used for the assay which was carried out in a thermostated vessel under constant magnetic stirring in a nitrogen atmosphere.

3. *Thin layer chromatography.* The hydrolysis of poly-amino-acids was followed by chromatography on DC-Fertigplatten Kieselgel F254 (Merck). Methanol-chloroform-ammoniak 2:2:1 was used as solvent. Each sample was divided into two parts and frozen. One part was incubated at 35.5 °C for 48 or 72 h, and 20 μl was then applied to the plate and the front left to move for 3 h about 15 cm. After drying in air the spots were developed by 0.1 % ninhydrin solution in butanol. The other part used as a control was directly applied to the plate after thawing.

RESULTS

I. Hydrolysis of poly-α-L-amino acids by some commercial enzymes

1. *Investigation of poly-L-glutamic acid.* The hydrolysis of poly-L-lysine (PLL) and poly-L-glutamic acid (PLG) by some commercial proteolytic enzyms was followed at different pH values by means of thin layer chromatography (TLC). The results of the TLC on the split products of poly-L-glutamic acid are shown in Figs. 1 – 3, and it can be seen that all enzymes, except thermolysin and proteinase Novo, degraded poly-L-glutamic acid.

As the L-glutamic acid oligopeptides were not commercially available the PLG chromatograms were compared with those of other reports.[10,11] As can be seen in Fig. 1, three of the four enzymes tested gave rise to an extensive degradation of the poly-L-amino acid. At pH 5 and 6.5 mono-, di-, tri- and tetra-glutamic acids were formed, at pH 8.0 only pronase formed split products such as tri-, tetra- and pentaglutamic acids. The controls for subtilisin and bromelain (Fig. 1 b and d) show an advanced degradation of the substrate at pH 5, indicating a rapid enzymatic activity to have

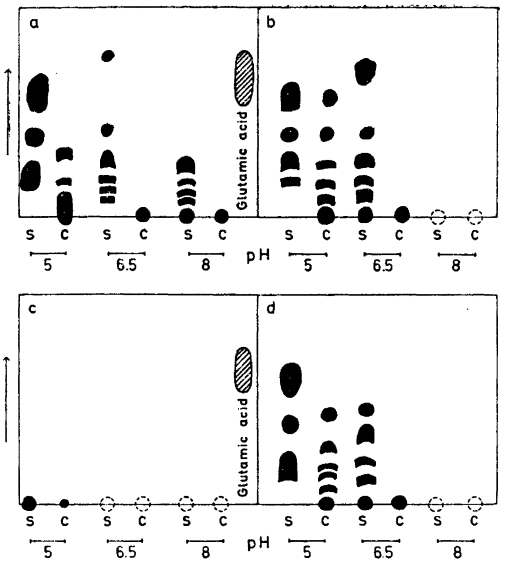


Fig. 1 a–d. Thin layer chromatograms from the action of some proteolytic enzymes on poly-L-glutamic acid (PLG). The reaction mixture contained 0.5 ml enzyme (1 mg/ml), 0.5 ml McIlvaine buffer and 1.0 ml 1 % PLG. Incubation at 35.5 °C: 48 h for a and b; 72 h for c and d. For other data see Methods. a, Pronase B grade; b, cryst. subtilisin (Novo); c, thermolysin, and d, bromelain. S = sample and C = control.

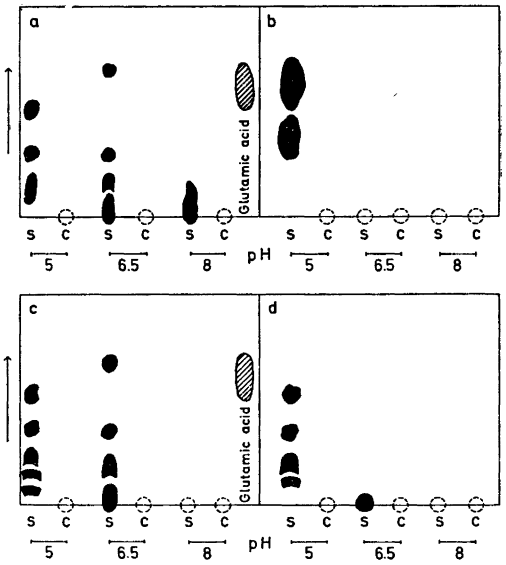


Fig. 2 a–d. Thin layer chromatograms from the action of some proteolytic enzymes on PLG. Incubation 48 h at 35.5 °C. For other data see Fig. 1 and Methods. a, *Streptomyces* protease (Sigma); b, *Aspergillus* protease II (Sigma); c, *B. subtilis* protease (Sigma), and d, *Aspergillus* protease I (Astra).

taken place between the time of mixing and freezing of the control samples. Figs. 2 a, c, and d show the breakdown of PLG to different oligoglutamic acids by protease from *Streptomyces*, *B. subtilis* and *Aspergillus* (protease I, Astra), respectively. *Aspergillus* protease II (Sigma) was more active and degraded the substrate to glutamic and diglutamic acid, as can be seen in Fig. 2 b. The enzyme alcalase (Fig. 3 b) digested PLG so quickly that also the control gave an advanced degradation, whereas Nagarse (Fig. 3 a) and acid protease (Fig. 3 d) gave normal controls. The bacterial proteinase (Novo) gave ninhydrin stainable spots at pH 5 and 6.5 which means that only relatively few peptide bonds were split.

Based on these results the influence of the active enzymes on this substrate was studied viscosimetrically as shown in Figs. 4 and 5. It can be seen that the pH optimum for the proteolytic degradation of poly-L-glutamic acid lies around pH 5 for all the enzymes and for

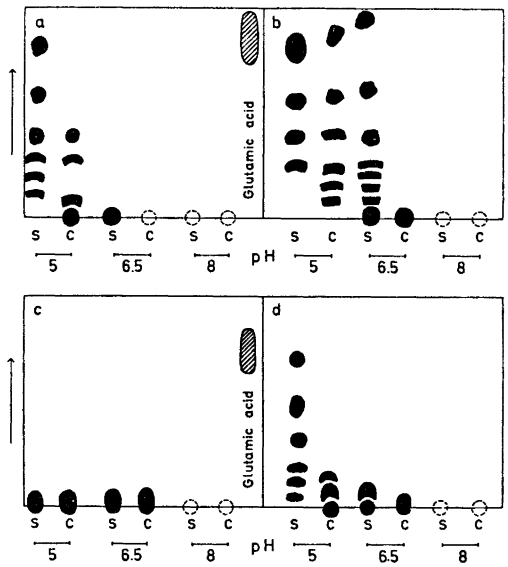


Fig. 3 a–d. Thin layer chromatograms from the action of some proteolytic enzymes on PLG. Incubation 48 h at 35.5 °C. For other data see Fig. 1 and Methods. a, Nagarse (subtilisin BPN′); b, Alcalase; c, bacterial proteinase (Novo), and d, acid protease (Miles).

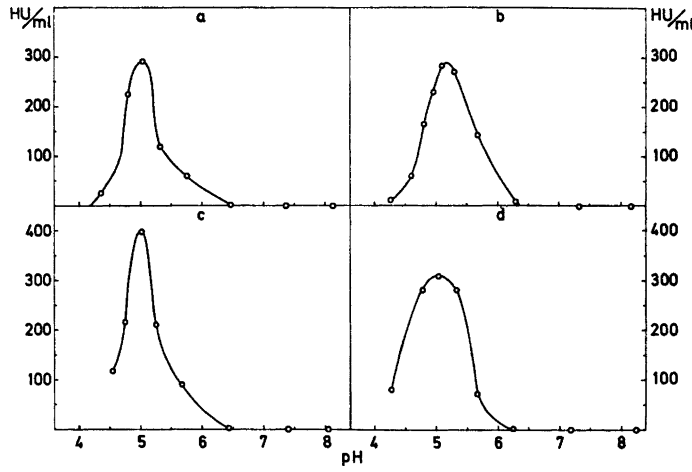


Fig. 4 a–d. Influence of pH on the hydrolysis of poly-L-glutamic acid by: a, Alcalase; b, *Streptomyces* protease (Sigma); c, *B. subtilis* protease (Sigma) and d, *Aspergillus* protease II (Sigma). Incubation mixture: 0.5 ml enzyme (1; 100; 5 and 100 μg/ml, resp.) + 1.0 ml McIlvaine buffer + 1.0 ml 1.5 % substrate. The proteolytic activity was followed viscosimetrically at 35.5 °C and expressed in HU/ml.

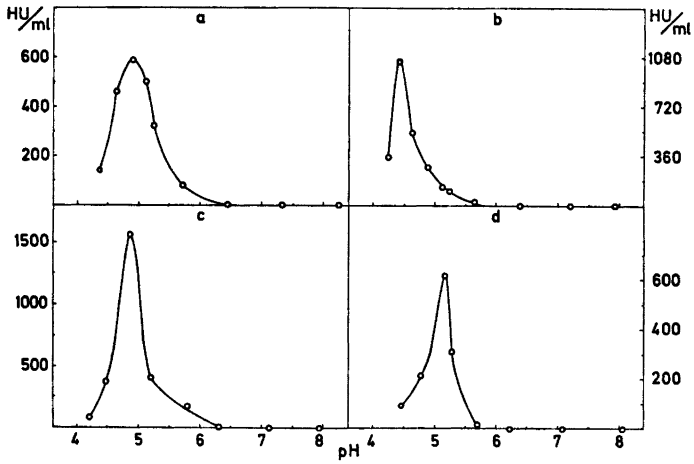


Fig. 5 a–d. Influence of pH on the hydrolysis of poly-L-glutamic acid by: a, *Aspergillus* protease I (Astra); b, acid protease (Miles); c, bromelain and d, Nagarse. Incubation mixture: 0.5 ml enzyme (10; 100; 100 and 10 μg/ml, resp.) + 1.0 ml McIlvaine buffer + 1.0 ml substrate. Enzymatic assay as in Fig. 4.

crystalline subtilisin Novo, not shown in the figures. For acid protease shown in Fig. 5 b, the pH optimum lies at 4,4. The form of the pH activity curves and the extent of the active interval varies for the different enzymes, however.

2. *Investigation of poly-L-lysine and poly-L-ornithine.* The thin layer chromatograms of proteolytically hydrolyzed poly-L-lysine (PLL) are shown in Figs. 6 and 7. Identification of the split products by control samples was made but is not shown here. The results agree with those of other authors.[10,12,13] *Aspergillus* protease II degraded PLL completely at the three pH values investigated: 5, 7 and 8.5 (Fig. 6 a), whereas *Aspergillus* protease I (Fig. 6 e) had

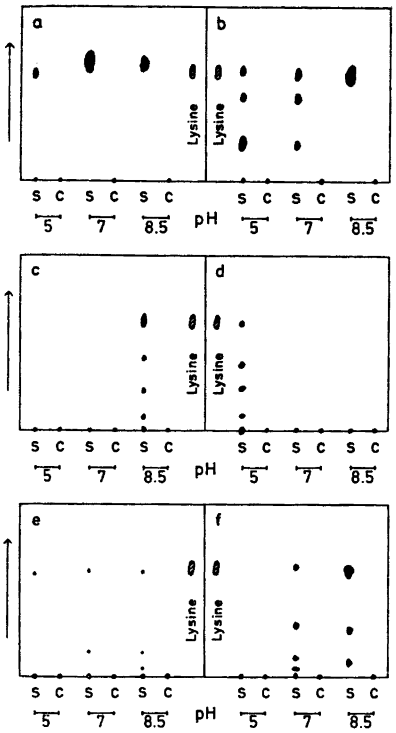


Fig. 6 a–f. Thin layer chromatograms from the action of some proteolytic enzymes on poly-L-lysine (PLL). The reaction mixture contained 0.5 ml enzyme (1 mg/ml), 0.5 ml McIlvaine buffer and 1.0 ml PLL. Incubation time 48 h at 35.5 °C. For other data see Methods. a, *Aspergillus* protease II (Sigma); b, *Streptomyces* protease (Sigma); c, *B. subtilis* protease (Sigma); d, acid protease (Miles); e, *Aspergillus* protease I (Astra); f, Nagarse. S = sample and C = control.

a less strong effect. *Streptomyces* protease split PLL to lysine at pH 8.5 and partly to di- and tetralysine at pH 5 and 7 (Fig. 6 b). *B. subtilis* protease hydrolysed the substrate at pH 8.5 (Fig. 6 c) and acid protease at pH 5 (Fig. 6 d), both enzymes giving the same split products. Nagarse exerted degradation at pH 8.5 and less at 7, the hydrolysis resulting in lysine, trilysine, and oligolysines but no dilysine. Alcalase degraded PLL to lysine and tetra- and pentalysine at pH 8 (Fig. 7 a), and bromelain split the substrate to lysine, trilysine and pentalysine (Fig. 7 d). Bacterial proteinase (Fig. 7 b) and crystalline subtilisin (Fig. 7 c)

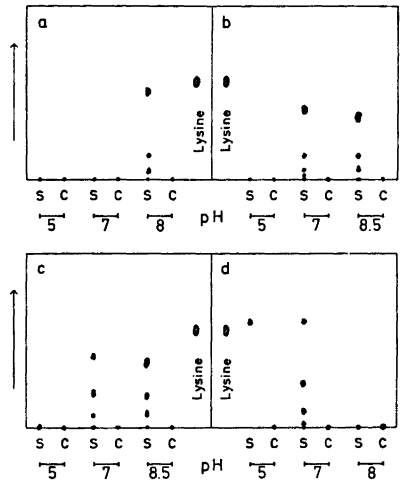


Fig. 7 a–d. Thin layer chromatograms from the action of some proteolytic enzymes on PLL. a, Alcalase; b, Bacterial proteinase (Novo); c, cryst. subtilisin (Novo); d, bromelain. For data see Fig. 6 and Methods.

hydrolysed PLL to di-, tri- and tetralysine at pH 7 and 8.5.

The pH optima for the hydrolysis of PLL by the investigated enzymes varied between pH 6.7 and 10.1 as shown in Figs. 8–9. *Aspergillus* protease II gave an optimum around 9.2 (Fig. 8 a), whereas the other three enzymes shown in Fig. 8 b–d had optima around pH 9.7. *Streptomyces* protease had its optimum around pH 9.5 (Fig. 9 a), bromelain around 6.7 and *B. subtilis* protease and alcalase around pH 10 (Fig. 9 c–d).

Poly-L-ornithine which is not degraded by trypsin [14] can be split by pronase and subtilisin as is seen in Fig. 10 a–b. The pH optima are around 10.6 and 10.15, respectively.

II. Effect of insolubilized trypsin and *S. griseus* protease on the hydrolysis of poly-L-glutamic acid and poly-L-lysine

Suspensions of insolubilized enzyme conjugate were mixed with the actual poly-amino acids and buffer, and the consumption of NaOH was assayed by pH-stat titration during magnetic stirring at 30 °C in a nitrogen-atmosphere. *Streptomyces* protease attached to

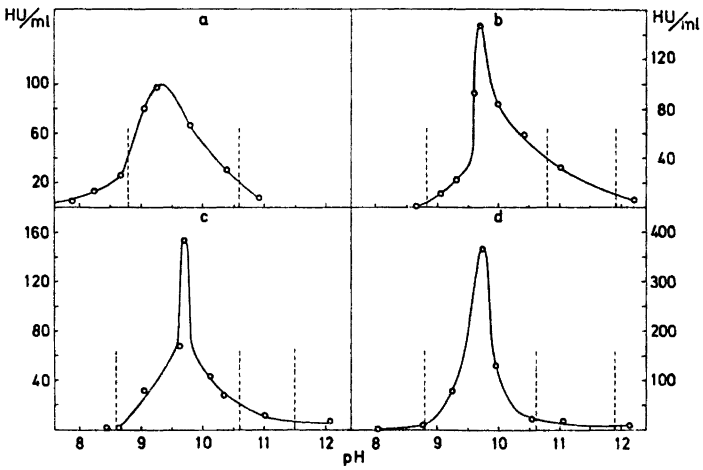


Fig. 8 a – d. Influence of pH on the hydrolysis of poly-L-lysine by: a, *Aspergillus* protease II (Sigma); b, cryst. subtilisin (Novo); c, bacterial proteinase (Novo); d, Nagarse. Incubation mixture: 0.5 ml enzyme (50; 0.13; 0.05 and 12.5 μg/ml, resp.) + 1.0 ml buffer (below pH 5.6 acetate; pH 5.6 – 7.8 phosphate; pH 7.6 – 8.8 Tris-HCl; pH 8.8 – 10.6 glycine-NaOH; pH 10.6 – 11.9 phosphate-NaOH, and higher than pH 11.9 NaOH) + 1.0 ml 0.5 % poly-L-lysine. Enzymatic assay as in Fig. 4. The dotted lines indicate the intervals of the buffers.

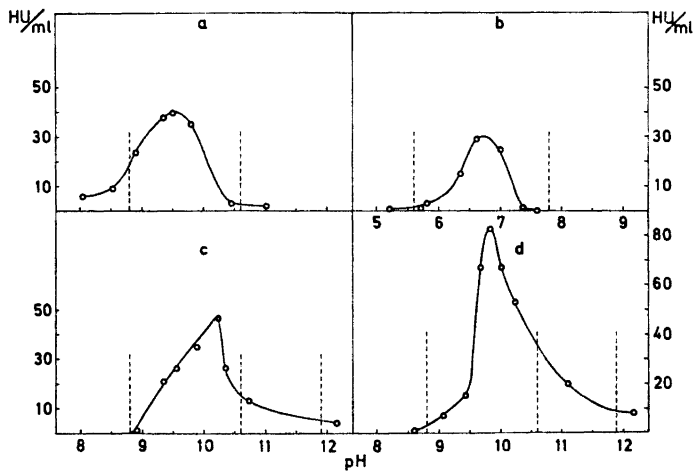


Fig. 9 a – d. Influence of pH on the hydrolysis of poly-L-lysine by: a, *Streptomyces* protease (Sigma); b, bromelain; c, *B. subtilis* protease (Sigma); d, Alcalase. Incubation mixture: 0.5 ml enzyme (20; 500; 0.25 and 0.25 μg/ml, resp.) + 1.0 ml buffer + 1.0 ml 0.5 % poly-L-lysine. Enzymatic assay as in Fig. 4. The dotted lines indicate the intervals of buffers described in Fig. 8.

agarose exerted very low hydrolysis of poly-L-glutamic acid at pH 4.9 and 5.2. The autolysis of the enzyme conjugates could be left out of consideration in the actual pH interval. Poly-L-lysine was weakly hydrolyzed at pH 8.3 – 8.7 by the insolubilized enzyme. At this pH interval, however, an equally strong autolysis of the enzyme conjugate occurred.

The Enzite-agarose-trypsin conjugate hydrolyzed poly-L-lysine to a higher degree than the insolubilized *Streptomyces* protease. The optimal splitting of this substrate was

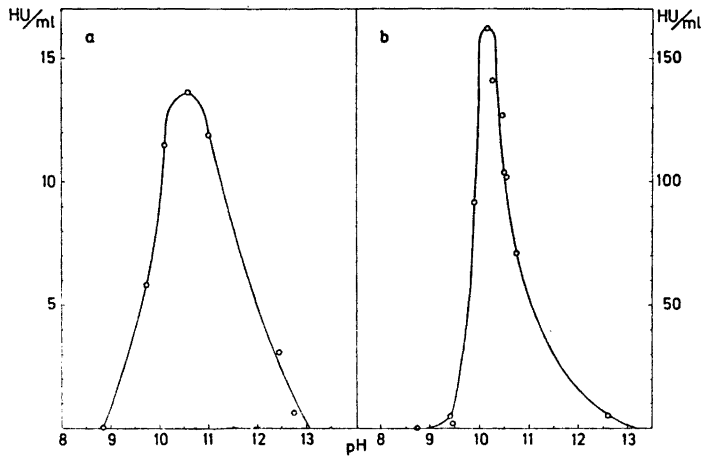


Fig. 10 a–b. Influence of pH on the hydrolysis of poly-L-ornithine by: a, Pronase; b, cryst. subtilisin (Novo). Incubation mixture: 0.25 ml enzyme (50, resp. 10 μg/ml) + 1.00 ml buffer (0.1 – 1 M glycine-NaOH, Sørensen) + 0.25 ml 5.0 % poly-L-ornithine. The proteolytic activity was followed viscosimetrically at 30.0 °C and expressed in HU/ml.

around pH 9 as shown in Fig. 11. However, the autolysis of the Enzite-agarose-trypsin in a system free from substrate was between 50 and 65 % of the degradation of the complete enzyme-conjugate-substrate-buffer system.

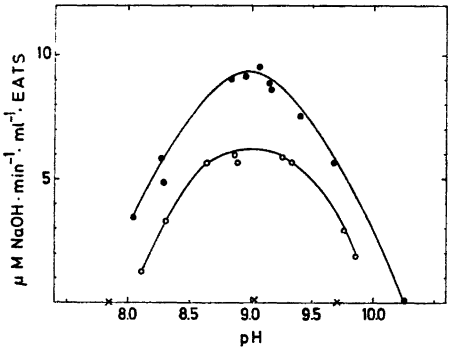


Fig. 11. Influence of pH on the hydrolysis of poly-L-lysine by Enzite-agarose-trypsin suspension (EATS). Incubation mixture: 0.50 ml EATS (diluted 1:6) + 3.00 ml 0.01 M glycine-NaOH buffer (Sørensen) + 1.00 ml poly-L-lysine (2 % in 0.45 % NaCl, pH 7.85) + 1.00 ml 0.5 mM $CaCl_2$. pH-stat titration under constant stirring at 30 °C in N_2-atmosphere. Alkali concentration 0.0445 M. For control of autolysis of enzyme conjugate and spontaneous hydrolysis of the substrate the excluded component was replaced by 0.45 % NaCl. For other data see Methods. ●, hydrolysis of poly-L-lysine by EATS; ○, autolysis of EATS; ×, autolysis of poly-L-lysine.

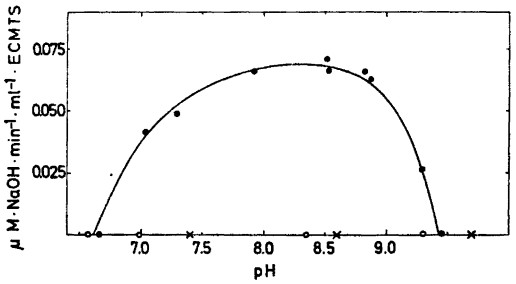


Fig. 12. Influence of pH on the hydrolysis of poly-L-lysine by Enzite carboxymethyl cellulose trypsin (ECMTS). Incubation mixture: 0.50 ml ECMTS (10.4 mg/ml) + 3.00 ml buffer (0.01 M McIlvaine for pH 6.67 – 7.29; 0.01 M collidine-HCl for pH 7.00 and 0.01 M glycine-NaOH for pH 7.90 – 9.80) + 1.00 ml poly-L-lysine (2 % in 0.45 % NaCl, pH 7.77) + 1.00 ml 0.5 mM $CaCl_2$. pH-stat titration under constant stirring at 30 °C in N_2-atmosphere. For control of autolysis of enzyme conjugate and spontaneous hydrolysis of the substrate the excluded component was replaced by 0.45 % NaCl. For other data see Methods. ●, hydrolysis of poly-L-lysine by ECMTS; ○, autolysis of ECMTS; ×, autolysis of poly-L-lysine.

The Enzite carboxymethyl cellulose trypsin was stable in the pH interval 6.7 – 9.8 as can be seen in Fig. 12. The optimal hydrolysis of poly-L-lysine was at a broader pH interval, 7.75 – 8.75, than the agarose-trypsin shown in Fig. 11.

Table 1. Enzyme preparations tested for hydrolysis on poly-D-glutamic acid. Reaction mixture: 1.00 ml enzyme + 0.50 ml 2.0 % poly-D-glutamic acid + 1.50 ml 10 mM McIlvaine buffer. pH-stat titration in N_2-atmosphere with 5 mM NaOH at 23 °C under constant stirring.

Enzyme preparation	Manufacturer	Concentration mg/ml	Concentration ml/ml	Poly-D-glutamic acid Manufacturer	Poly-D-glutamic acid Mol.wt.	Endpoint pH
Pancreas, swine	Fresh extract	14.8		Pilot	94.000	5.52
Pancreatin	Schwarz/Mann	6.6		Pilot	94.000	5.83
Pancreatin	Sigma, Grade III	7.0		Miles	29.000	5.47
Nagarse (subtilisin, BPN)	Teikoku, Japan	1.67		Pilot	94.000	5.45
Nagarse	Teikoku, Japan	3.34		Pilot	94.000	5.49
Snail digestive juice	Koch-Light		0.17	Pilot	94.000	5.47
Pronase	Calbiochem, B-grade	0.8		Pilot	94.000	5.30
Myxococcus vir. cultivation medium	University, Lund		0.08	Sigma	60.000	5.00

Table 2. Enzyme preparations tested for hydrolysis of poly-L-lysine (PLL) and poly-D-lysine (PDL) by pH-stat titration. Reaction mixture: 1.00 ml enzyme + 0.50 ml 2 % substrate + 1.50 ml 10 mM glycine-NaOH buffer.

Enzyme preparation	Enzyme mg/ml	Substrate		Endpoint pH	Consumption of NaOH nmol/(mg/min)
Extract from calf pancreas	0.13	PLL		8.43	156
	0.26	PLL		8.38	250
	0.13	–		8.41	11
	3.3		PDL	8.40	6.1
	3.3		–	8.30	14.1
Nagarse (subtilisin, BPN)	3.3	PLL		8.26	26.8
„	3.3		PDL	8.33	0
Pancreatin Sigma	0.14	PLL		8.60	432
	6.7		PDL	8.22	16.7
	6.7		PDL	8.22	18.3
	6.7		PDL	8.32	23.3
	6.7		–	8.29	13.3
	6.7		–	8.17	13.5
Pronase, Calbiochem, B-grade	0.17	PLL		8.11	31
	1.67		PDL	8.10	0 (pH-
	0.8		PDL	8.60	0 in-
	0.8		PDL	7.60	0 crease)

III. Effect of some proteolytic enzymes and extracts on the hydrolysis of poly-α-D-amino acids

The enzymatic hydrolysis of poly-D-glutamic acid and poly-D-lysine was investigated by pH-stat titration (regulated 'endpoint') or by incubation for 24 h at buffered pH and assay of the decrease in pH after incubation. Before the enzyme preparations were tested on their ability to hydrolyse poly-D-amino acids, the poly-L-amino acid degradation was always controlled.

In Table 1 there are data for some bacterial enzymes and digestive extracts tested on poly-D-glutamic acid under pH regulated conditions. None of these enzyme samples hydrolysed the

D-form. That means that the hydrolysis estimated as NaOH consumption of a complete enzyme substrate sample was not greater than the one without enzyme under in other respects identical conditions. The L-form was enzymatically split at the corresponding pH.

The hydrolysis of poly-D-lysine assayed by pH-stat titration resulted in a significant difference for pancreatin Sigma Grade III as shown in Table 2. For the other preparations tested no hydrolysis of poly-D-lysine occurred. Neither was this the case by viscosimetric assay on pancreatin extract (Weddel, 10 mg/ml reaction mixture) which strongly hydrolysed poly-L-lysine with a maximum around pH 7.

Studies of prolonged hydrolysis, *i.e.* 24 h incubation of pancreatic enzyme and poly-D-lysine, were also performed. After incubation the samples were titrated back to the starting pH under controlled conditions. Pancreatin Sigma did not hydrolyse poly-D-lysine, *i.e.* the decrease in pH was about the same also in the absence of substrate, which means that the autolysis of the pancreatin was high. Pancreas protease Merck seemed to split poly-D-lysine, the difference in NaOH consumption being 4 – 5 μM.

DISCUSSION

Hydrolysis of poly-L-amino acids. The action of some bacterial and fungal enzymes and of bromelain on poly-L-lysine and poly-L-glutamic acid has been studied. The influence of pronase and subtilisin on the hydrolysis of poly-L-ornithine was also investigated. Thermolysin did not split poly-L-lysine or poly-L-glutamic acid, and bacterial proteinase Novo had no effect on poly-L-glutamic acid. For the other proteinases tested the pH optimum for splitting of poly-L-glutamic acid was around pH 5. Miller [15] has shown that, at this pH, the substrate undergoes a sharp helix-random coil transition at which approximately half of the sidechain carboxyl groups are ionized. At pepsin hydrolysis of poly-L-glutamic acid at least some α-helix form seems to be necessary.[16]

The thin layer chromatograms (Figs. 1 – 3) show that there are characteristic differences in the formation of split products of PLG by the different enzymes investigated. Also the affinity to the substrate, *i.e.* the concentration of enzyme necessary for an appropriate time for viscosimetric assay (20 – 30 min), varied considerably between the enzymes tested (Figs. 4 – 5).

The proteolytic hydrolysis of poly-L-lysine (PLL) by the enzymes tested also resulted in different patterns of split products (Figs. 6 – 7) and in different shapes of the pH-activity curves (Figs. 8 – 10). Also the affinity for PLL among the enzymes tested varied.

Hydrolysis of poly-L-amino acids by insolubilized trypsin and a bacterial protease. Several investigation have been published on proteolytic activity of insolubilized proteinases. Insolubilized trypsin bound to different carriers with use of appropriate techniques has been prepared and studied for several years.[17–25] Stability, influence of pH on the enzyme activity as well as different physico-chemical constants have been determined and compared with those of soluble trypsin. No reports on the pH dependent autolysis of the enzyme-conjugate have been found, however. As described above the Enzite-agarose-trypsin conjugate, when assayed as control without substrate, showed a pH dependent autolysis as did Enzite-agarose protease. The pH optima of the enzyme conjugates were the same as those of complete enzyme-poly-L-lysine systems. Carboxymethyl cellulose-trypsin showed, however, no autolysis in the actual pH intervals. It seems as if just agarose bound enzymes are instable. As the titration of enzymic activity in the present study is based on decrease in pH, *i.e.*, liberation of protons, an enzymic assay based on spectrophotometric, viscosimetric or other methods might not reveal autolysis.

Contrary to the autolysis of the insolubilized agarose-trypsin, as demonstrated in the present study, Bar-Eli and Katchalski [17,18] reported that the relatively high enzymic stability of the water-insoluble polytyrosyl trypsin may be attributed mainly to the prevention of autodigestion as a result of fixation of the enzyme to the insoluble carrier. Porath *et al.*[26] who introduced the fixation of enzymes to agarose found good stability for chymotrypsin-agarose gel, but agarose-rennin was reported to release active enzyme in solution.[27] *Arthrobacter* proteinase fixed to agarose was studied by Gable and v. Hofsten [28] who found good stability and no leakage of enzyme from the gel into the

solution under various conditions. As the pH dependent autolysis curve is determined by the consumption of hydroxyl ions, *i.e.* caused by the liberation of protons, it cannot be caused by small amounts of negatively charged groups from the gel reported by Carlsson *et al.*[29]

Hydrolysis of poly-D-amino acids. In nature no proteins or polypeptides entirely built up by D-amino acids have been found. Different D-amino acids occur, however, not only in bacteria[30] but also in several insect species and even in higher animals, *e.g.* in guinea pig blood and shark liver.[31] The existence of D-peptides has been reported by Hanson and others.[32,33]

Due to lack of D-forms of proteins and polypeptides, the possibility to find D-peptide bond splitting enzymes in nature could not be demonstrated until the synthesis of poly-D-amino acids. The reports by Tsuyuki, Tsuyuki and Stahmann [2,3] seem to be the first in this field and the only published so far.

The present work, being a continued report on proteolytic hydrolysis of some polyamino acids, gave room only for a limited study on poly-D-amino acid hydrolysis. The enzyme preparations were tested on poly-D-glutamic acid and poly-D-lysine near the pH optimum for proteolytic splitting of the corresponding L-forms. No indications for enzymatic splitting of poly-D-glutamic acid were found (Table 1). The results seem, however, to indicate the existence of poly-D-lysine splitting enzyme(s). Pancreatin (Sigma Grade III) showed a weak hydrolysis of poly-D-lysine assayed by pH-stat titration (Table 2).

Evidences in the present paper for the existence of proteolytic enzyme(s) that split poly-D-amino acid are not strong. However, Gill, Papermaster and Mowbray [34] demonstrated that poly-D-Glu 55, D-Lys 39, D-Tyr 6 is degraded by rabbits very slowly, *i.e.* to about 25 to 30 % of the injected D-polymer over 15 to 20 days. The amount of degraded D-polymer was a constant fraction of the injected dose. The authors propose that constitutive D-proteases and D-peptides in the rabbit are involved in the metabolism and are not induced by the injection of polypeptide. The L-polymer, on the other hand, is completely degraded within 3 to 4 days. Other experiments in this field [35] showed that a D-polymer [247, p(D-Tyr, D-Glu, D-Ala)] breaks down twenty-two times more slowly than the L-polymer when injected in mice. The complicated nature of the metabolic mechanism is apparent from the fact that the D-polymer is broken down slowly and retained in large quantities in the tissues, especially in the kidneys.[36,37] This persistence of the D-polymer indicates that the enzymes necessary for its degradation are lacking in the kidneys. The L-polymer is not retained in the liver or kidneys and is thus rapidly broken down.

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Periodate Oxidation of Phenols. XVIII.* Oxidation of 2-Methoxyphenols with Periodic Acid in Methanol

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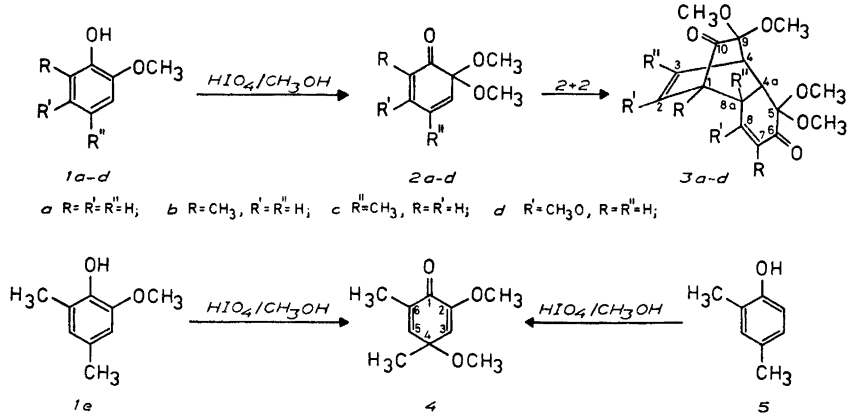
Treatment of the 2-methoxyphenols *1a—d* with methanolic periodic acid gives the corresponding 6,6-dimethoxy-2,4-cyclohexadienones (*o*-quinone dimethyl ketals) *2a—d*, which more or less rapidly undergo Diels-Alder dimerization to the bis(ketals) *3a—d*. The competitive formation of 4-methoxy-4-methyl-2,5-cyclohexadienones (*4*, *11*) from the 4-methyl substituted phenols *1c* and *1e*, as well as the formation of iodinated by-products (*10*, *13*), is also described.

In Part XVII[1] of this series the oxidation of various 2-methylphenols with periodic acid (HIO_4) in methanolic solution to give 6-methoxy-6-methyl-2,4-cyclohexadienones ("*o*-quinol methyl ethers") has been reported. The present paper describes the analogous formation of the 6,6-dimethoxy-2,4-cyclohexadienones *2a—d* ("*o*-quinone dimethyl ketals"; the term "*o*-quinals" has also been proposed[2]) on similar treatment of the 2-methoxyphenols *1a—d*.

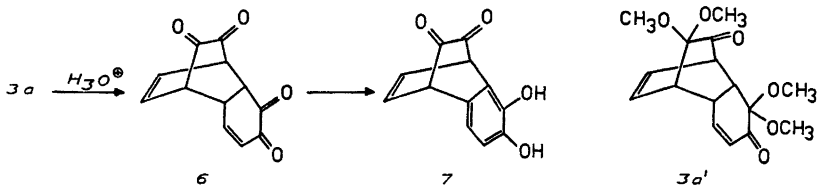
The ketals *2a*, *2b*, and *2c* could not be isolated as monomers, since they underwent Diels-Alder dimerization during the reaction or during the work-up to give *3a*, *3b*, and *3c*, respectively (Scheme 1). From the mixture obtained on oxidation of *1d*, however, the monomeric ketal *2d* was isolated as a yellow oil which showed the spectroscopic characteristics of a 2,4-cyclohexadienone. During a period of two days it changed into the crystalline dimer *3d*.

Although a few cyclic diaryl ketals (spiroketals) of *o*-quinones,[3] as well as ketals of *p*-quinones,[3b,4] have been described, only one *o*-quinone dialkyl ketal of type *2* seems to have been reported earlier.[3b] The tetramethyl ketals of *o*- and *p*-benzoquinone have been obtained by electrochemical oxidation of veratrol and hydroquinone dimethyl ether, respectively.[5]

* Part XVII, see Ref. 1.



Scheme 1.

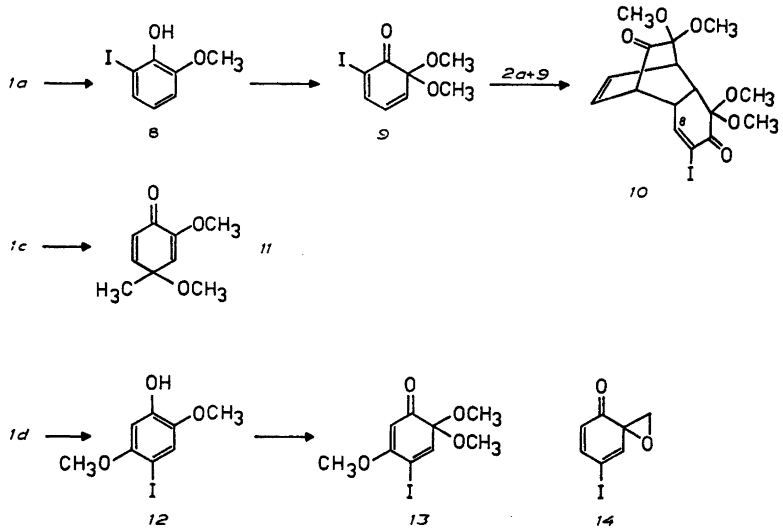


Scheme 2.

The UV, IR, and NMR data of dimers *3a–d* were in accord with those of previously investigated dimeric 2,4-cyclohexadienones (see Part XVII[1] and preceding papers in this series). Furthermore, chemical support for structure *3a* was provided by acid hydrolysis of the ketal groups of *3a*, which yielded the expected catechol *7*[7] *via* the dimeric *o*-benzoquinone *6*.[6,7] (Scheme 2). Admittedly, neither the spectroscopic data available nor the result of the hydrolysis experiment permit a decision to be made between *3a* and its regioisomer *3a'*. Structure *3a* and, correspondingly, structures *3b–d* are preferred, however, because dipole measurements[8] and X-ray crystallography,[9] as well as chemical evidence,[10,11,1] have shown that the dimerization of various types of 2,4-cyclohexadienones exclusively gives the regioisomers of type *3a*.

The occurrence of *para* oxidation has been demonstrated in two of the cases investigated. Thus, 2-methoxy-4-methylphenol (*1c*) gave the *p*-quinol ether *11* (Scheme 3) in addition to the dimeric *o*-quinone ketal *3c*, and the homologous compound *4* was the only product isolated from the reaction mixture obtained on oxidation of phenol *1e* with the methanolic periodic acid (Scheme 1). As reported recently,[1] *p*-quinol ether *4* is also a by-product in the oxidation of 2,4-dimethylphenol (*5*) with methanolic periodic acid, a twofold oxidative methoxylation taking place in this case.

Furthermore, the liberation of iodine in the course of the oxidation (*cf.* Ref. 1) gave rise to iodophenols which were converted to the corresponding iodinated *o*-quinone ketals. The oxidation of guaiacol (*1a*) gave a small amount of compound *10*, which is the Diels-Alder adduct between the noniodinated ketal *2a* acting as a diene and its 2-iodinated analogue *9*, which originates from iodophenol *8*, acting as dienophile (Scheme 3).



Scheme 3. By-products (*10*, *11*, *13*) obtained from phenols *1a*, *1c*, and *1d*.

The location of the iodine in *10* was derived from a comparison of spectral data for *3a* and *10*. Whereas the $\pi \rightarrow \pi^*$ band of the α,β-conjugated carbonyl system of *3a* has λ_{max} at 232 nm (calc.[12] 227 nm), that of *10* is found at 264 nm. The bathochromic shift of 32 nm is in accord with the presence of a halogen atom in the α- or β-position of the system (an α- or β-bromine substituent causes a bathochromic shift of 25 or 30 nm, respectively[12]). The IR strectching frequency of the conjugated carbonyl group of *10* (1705 cm^{-1}) is 15 cm^{-1} higher than that of *3a* (1690 cm^{-1}) which indicates that the iodine is located in the α-position (C-7) (*cf.* Ref. 13). Finally, the NMR spectrum of *10* shows a doublet (1 H) at δ 7.10 which must be ascribed to the hydrogen atom at C-8 ($J_{8,8a} = 4.5$ Hz).

From the oxidation of 2,5-dimethoxyphenol (*1d*), the iodinated *o*-quinone ketal *13*, obviously formed *via* the iodophenol *12*, was obtained in addition to the noniodinated ketal *2d*. Contrary to *2d*, its 4-iodo derivative *13* does not dimerize; this can be ascribed to steric hindrance exerted by the iodo substituent. The similar 4-iodo-2,4-cyclohexadienone *14* has earlier been found to be stable as monomer.[14]

The total yields of products isolated from the reaction mixtures obtained on oxidation of phenols *1a—d* were in the range of 20—34 %. During the oxidation, the solutions acquired an intense red colour indicating the formation of considerable amounts of *o*-quinones. The latter reaction can be ascribed to the presence of water arising by the interaction between periodic acid and methanol (*cf.* Ref. 1). No efforts were made to isolate the red products; they were removed, together with the inorganic acids present, by filtering the solution through a column of an anion-exchange resin in the acetate form,[15] or by chromatography on a column of aluminium oxide which firmly adsorbs *o*-quinones.

On the basis of experiments performed in $H_2{}^{18}O$, the degradation of monoethers of catechols and hydroquinones by aqueous periodate to give *o*- and *p*-quinones, in addition to the corresponding alcohol or phenol, has been interpreted to be due to the intermediate formation of the quinone hemiketals.[16] The analogous formation of *o*-quinone dimethyl ketals on treatment of 2-methoxyphenols with methanolic periodic acid lends further support to this interpretation. It may be noted that both periodic acid and iodic acid in aqueous media also were found to effect the oxidative demethoxylation of 2-methoxyphenols.[17]

EXPERIMENTAL

UV, IR, and NMR spectra were recorded on a Cary Model 14, a Beckman 9A, and a Varian A-60 instrument, respectively. Chemical shifts are given in δ units, TMS being used as internal standard.

Oxidation. A solution of the phenol (10—25 mmol) in absolute methanol (50 ml) was mixed with the solution of an equimolar amount of anhydrous periodic acid[18] in the same solvent (450 ml). After 2 h at room temperature, the deep red reaction mixtures were worked up by one of the following three procedures.

Procedure 1. The reaction mixture was passed through a column of Amberlite IRA-400 (600 g) in the acetate form.[15] After the addition of water, the yellow filtrate was extracted with five 50 ml-portions of dichloromethane. The combined extracts were dried over anhydrous $CaSO_4$ and evaporated under vacuum, leaving "residue A". The latter was chromatographed on a column (3 × 110 cm) of silica gel (Mallinckrodt, 100 mesh), benzene-ethyl acetate (4:1) being used as eluent.

Procedure 2. Residue A obtained as above was dissolved in ethyl acetate and the brown solution passed through a column (4 × 15 cm) of aluminium oxide ("neutral", Woelm). Dark brown material remained firmly adsorbed in the upper part of the column. The filtrate, on evaporation, gave "residue B" which was chromatographed on silica gel as in procedure 1.

Procedure 3. The red reaction mixture, after dilution with water, was extracted with dichloromethane (5 × 50 ml), the combined red extracts were washed with saturated aqueous $NaHCO_3$ and with water and dried over $CaSO_4$. Evaporation gave "residue C", which was purified with Al_2O_3 (see procedure 2) to give "residue D" which, in turn, was chromatographed on silica gel according to procedure 1.

Thin layer chromatography was performed on silica gel with benzene-ethyl acetate (4:1) as mobile phase.

Oxidation of guaiacol (1a). The reaction mixture obtained from 2.90 g of the phenol was worked up according to procedure 3 to give "residue C" (2.90 g) as a brown oil and "residue D" as a slightly yellow crystalline solid (1.03 g), which was separated on silica gel into the following two compounds:

(a) *1,4a,5,8a-Tetrahydro-7-iodo-5,5,9,9-tetramethoxy-1,4-ethanonaphthalene-6,10(4H)-dione* (10). $R_F = 0.4$. Colourless rods, m.p. 210 °C (dec.) after recrystallization from chloroform-hexane. Yield, 3 %. (Found: I 29.04. Calc. for $C_{16}H_{19}O_6I$: I 29.23). UV (ethanol): λ_{max}, nm (logε) 205 (4.05), 264 (3.56), sh 300 (3.29). IR (KBr): ν_{max}, cm^{-1} 1595 (w), 1705 (s), 1740

(s). NMR ($CDCl_3$): δ 3.10, 3.26, 3.45 and 3.50 (12 H, 4 CH_3O) overlapping a multiplet (4 H, H-1, H-4, H-4a, H-8a), 5.88–6.43 (m, 2 H, H-2, H-3), 7.10 (d, 1 H, H-8). $J_{8,8a}=4.5$ Hz.

(b) *1,4a,5,8a-Tetrahydro-5,5,9,9-tetramethoxy-1,4-ethanonaphthalene-6,10(4H)-dione* (3a). $R_F=0.2$, m.p. 187–190 °C (yield, 23 %). After recrystallization from ethyl acetate colourless needles, m.p. 191–192 °C. (Found: C 62.41; H 6.46; OCH_3 40.32. Calc. for $C_{16}H_{20}O_6$: C 62.33; H 6.54; OCH_3 40.26). UV (ethanol): λ_{max}, nm (log ε) 205 (3.88), 232 (3.89), 324 (2.25). IR (KBr): ν_{max}, cm^{-1} 1625 (w), 1690 (s), 1735 (s). NMR ($CDCl_3$): δ 3.08, 3.27, 3.42 and 3.48 (4 CH_3O) overlapping a multiplet (4 H, H-1, H-4, H-4a, H-8a), 5.75–6.64 (m, 4 H, H-2, H-3, H-7, H-8).

1,4-Dihydro-5,6-dihydroxy-1,4-ethanonaphthalene-9,10-dione (7). Dimer *3a* (100 mg) was dissolved in methanol (10 ml), and after the addition of water (30 ml) and conc. hydrochloric acid (1 ml), the mixture was refluxed for 15 min. The yellow solution was extracted with 5 × 15 ml of ethyl acetate. The extract was thoroughly washed with water, dried over $CaSO_4$ and evaporated to dryness. The semi-solid yellow residue on recrystallization from ethyl acetate-hexane gave 35 % of *7*, m.p. 194–195 °C, identical by mixed m.p. with authentic material (m.p. 195–196 °C, Ref. 7).

1,4a,5,8a-Tetrahydro-5,5,9,9-tetramethoxy-1,7-dimethyl-1,4-ethanonaphthalene-6,10(4H)-dione (*3b*). Oxidation of 2-methoxy-6-methylphenol (*1b*) and work-up according to procedure 1 gave a light-brown, partly crystalline "residue A". On silica gel chromatography, the first eluate fractions gave small amounts of an amorphous, brown material. According to TLC, subsequent fractions contained only *3b* ($R_F=0.34$), colourless crystals of m.p. 152–155 °C (yield, 34 %); recrystallization from isopropyl ether raised the m.p. to 156–157 °C. (Found: C 64.49; H 7.45; OCH_3 36.84. Calc. for $C_{18}H_{24}O_6$: C 64.27; H 7.19; OCH_3 36.90). UV (ethanol): λ_{max}, nm (log ε) 205 (3.79), 245 (3.89), 324 (2.27). IR (KBr): ν_{max}, cm^{-1} 1648 (w), 1701, 1730. NMR ($CDCl_3$): δ 1.31 (s, 3H, CH_3-1), 1.82 (t, 3 H, CH_3-7), 3.04, 3.22, 3.40, 3.47 (12 H, 4 CH_3O) overlapping a multiplet (3 H, H-4, H-4a, H-8a), 5.52 (dd, 1 H, H-2), 6.06–6.22 (m, 2 H, H-3, H-8). $J_{CH_3\text{-}7,\ H\text{-}8} = J_{CH_3\text{-}7,\ H\text{-}8a}$ *ca.* 1 Hz, $J_{2,3}=8$ Hz, $J_{2,4}$ *ca.* 1.5 Hz.

Oxidation of 2-methoxy-4-methylphenol (1c). Procedure 1 gave a light-brown, partly crystalline "residue A". Silica gel chromatography provided two crystalline products:

(a) *1,4a,5,8a-Tetrahydro-5,5,9,9-tetramethoxy-3,8a-dimethyl-1,4-ethanonaphthalene-6,10(4H)-dione* (3c). $R_F=0.3$, m.p. 131–135 °C (yield, 11.5 %), after recrystallization from isopropyl ether colourless prisms of m.p. 133–135 °C. (Found: C 63.96; H 6.92; OCH_3 36.58. Calc. for $C_{18}H_{24}O_6$: C 64.27; H 7.19; OCH_3 36.90). UV (ethanol): λ_{max}, nm (log ε) 206 (3.92), 229 (3.91), 324 (2.36). IR (KBr): ν_{max}, cm^{-1} 1625 (w), 1698, 1736. NMR ($CDCl_3$): δ 1.39 (s, 3 H, CH_3-8a), 1.76 (d, 3 H, CH_3-3), 2.80 (d, 1 H, H-1), 2.87 (broadened s, 2 H, H-4, H-4a), 3.08, 3.32, 3.40, 3.42 (s, 3 H each, 4 CH_3O), 5.53 (dq, 1 H, H-2), 5.95 and 6.20 (pair of doublets, 1 H each, H-7, H-8). $J_{1,2}=6.5$ Hz, $J_{H\text{-}1,CH_3\text{-}3}$ *ca.* 1 Hz, $J_{7,8}=10$ Hz.

(b) *2,4-Dimethoxy-4-methyl-2,5-cyclohexadienone* (11). $R_F=0.2$, m.p. 108–110 °C (yield, 10.5 %), after recrystallization from isopropyl ether and sublimation at 40 °C/0.01 mmHg colourless plates of m.p. 110–111 °C. (Found: C 64.00; H 7.02; OCH_3 36.98. Calc. for $C_9H_{12}O_3$: C 64.27; H 7.19; OCH_3 36.90). UV (ethanol): λ_{max}, nm (log ε) 231 (3.99); 295 (3.22). IR (KBr): ν_{max}, cm^{-1} 1615 (s, enol ether), 1648, 1672 (both s).[19] NMR ($CDCl_3$): δ 1.48 (s, 3 H, CH_3-4), 3.18 (s, 3 H, CH_3O-4), 3.70 (s, 3 H, CH_3O-2), 5.63 (d, 1 H, H-3), 6.33 (d, 1 H, H-6), 6.78 (dd, 1 H, H-5). $J_{3,5}=3$ Hz, $J_{5,6}=10$ Hz.

Oxidation of 2,5-dimethoxyphenol (1d).[20] Procedure 2 gave a brown, oily "residue A" (R_F values 0.0, 0.2, and 0.4). The subsequent purification on Al_2O_3 gave a yellow oil ("residue B") which on silica gel chromatography afforded the following compounds:

(a) *4-Iodo-3,6,6-trimethoxy-2,4-cyclohexadienone* (13). $R_F=0.4$, yellow oil (yield, 10 %). UV (ethanol): λ_{max}, nm (log ε) 333 (3.37). IR (KBr): ν_{max}, cm^{-1} 1580 (s, enol ether), 1625 (w), 1670 (s). NMR ($CDCl_3$): δ 3.41 (s, 6 H, $(CH_3O)_2$-6), 3.85 (s, 3 H, CH_3O-3), 5.42 (s, 1 H, H-2), 7.20 (s, 1 H, H-5).

(b) *3,6,6-Trimethoxy-2,4-cyclohexadienone* (2d). $R_F=0.2$, light-yellow oil (10 %) containing traces of dimer *3d* ($R_F=0.1$). The spectra of the freshly prepared oil were as follows: UV (ethanol): λ_{max} 315 nm. IR (KBr): ν_{max}, cm^{-1} 1590 (s, enol ether), 1668 (s). NMR ($CDCl_3$): δ 3.38 (s, 6 H, $(CH_3O)_2$-6), 3.79 (s, 3 H, CH_3O-3), 5.40 (d, 1 H, H-2), 6.20 (dd, 1 H, H-4), 6.41 (d, 1 H, H-5). $J_{2,4}=2.5$ Hz, $J_{4,5}=10$ Hz.

1,4a,5,8a-Tetrahydro-2,5,5,8,9,9-hexamethoxy-1,4-ethanonaphthalene-6,10(4H)-dione (3d). The yellow oil *2d* had solidified after two days at room temperature. Recrystallization from ethyl acetate gave *3d*, m.p. 199–200 °C. (Found: C 58.58; H 6.42; OCH_3 50.48. Calc. for $C_{12}H_{24}O_8$: C 58.69; H 6.57; OCH_3 50.54). UV (ethanol): λ_{max}, nm (log ε) 250 (4.07), sh 302 (2.63). IR (KBr): ν_{max}, cm^{-1} 1623 and 1649 (enol ethers), 1690 (conj. CO) 1740 (CO), all strong. NMR ($CDCl_3$): δ 3.05, 3.22, 3.38 (9 H, 3 CH_3O) overlapping multiplet (4 H, H-1, H-4, H-4a, H-8a), 3.42 (s, 6 H, 2 CH_3O), 3.61 (s, 3 H, CH_3O), 4.78 (dd, 1 H, H-3), 5.32 (s, 1 H, H-7). $J_{1,3}$ *ca.* 1.5 Hz, $J_{3,4}=7.5$ Hz.

2,4-Dimethoxy-4,6-dimethyl-2,5-cyclohexadienone (4). Oxidation of 6-methoxy-2,4-dimethylphenol (*1e*, preparation see below) and work-up according to procedure 1 yielded "residue A" as a brownish, partly crystalline product which showed only one distinct spot on TLC ($R_F=$ 0.25). Silica gel chromatography gave *4*, colour-

less crystals of m.p. 77 – 79 °C (11 % yield); the m.p. was raised to 80 – 81 °C on sublimation at 40 °C/5 mmHg. The substance was identical by m.p., mixed m.p. and IR spectrum with a by-product obtained on oxidation of 2,4-dimethylphenol with methanolic periodic acid.[1]

6-Methoxy-2,4-dimethylphenol (1e).* A solution of 2-hydroxymethyl-6-methoxy-4-methylphenol[21] (10.8 g) in ethanol (100 ml) was shaken in a hydrogen atmosphere over a 10 % Pd/C catalyst (1 g). The required amount of hydrogen was consumed after 30 min. Filtration over Celite and evaporation of the filtrate gave an oil (9.3 g) which crystallized on cooling. M.p. 35 °C, $b.p._{13}$ 111 °C, after recrystallization from acetic acid-water (Lit.[22] m.p. 36 °C, $b.p._{12}$ 110 °C).

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Formation of 1,2,3-Triazoles, Secondary Amides, α-Aminoketones, and Vinylketones in the Acid-catalyzed Decomposition of 5-Hydroxy-Δ^2-1,2,3-Triazolines

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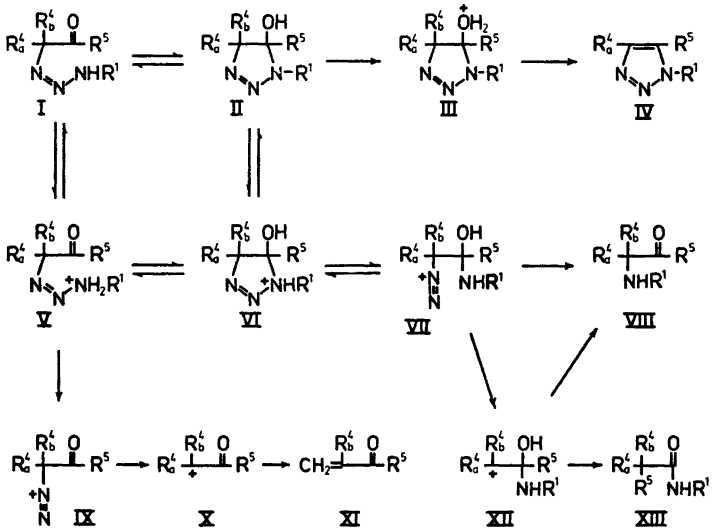
On treatment with acid 5-hydroxy-Δ^2-1,2,3-triazolines are converted into 1,2,3-triazoles, amides, α-aminoketones, or vinylketones, depending on the substituents. Aliphatic diazonium ions are proposed as intermediates in the last three cases.

Nucleophiles, such as benzoyl chloride or BF_3/CH_2N_2, react at N-3, giving derivatives of the corresponding ketotriazenes.

As described previously [1-4] 5-hydroxy-Δ^2-1,2,3-triazolines undergo various conversions on treatment with base. Acid also induces reactions which may be rather vigorous. The products are (Scheme 1) 1,2,3-triazoles (IV), amides (XIII), α-aminoketones (VIII), or vinylketones (XI). In a number of experiments, one equivalent of trifluoroacetic acid (TFAA) was added to a 0.2 M solution of the hydroxytriazoline in $CDCl_3$. The product distribution as determined by ^1H NMR spectroscopy is shown in Table 1. Here is also given the yields obtained in some other experiments where the products were isolated and identified after treatment of the solid triazoline or a solution of it in an organic solvent with hydrochloric acid.

Formation of 1,2,3-triazoles (IV). With the exception of case v, 1,2,3-triazoles are only

* Present address.



Scheme 1.

Table 1. Product distributions in acid-catalyzed decompositions of 5-hydroxy-Δ^2-1,2,3-triazolines, II (or α-ketotriazenes, I).

Case	Substituents				Products detected by NMR ($CDCl_3$/TFAA)				Isolated products (aqueous acid)			
	R^1	R_b^4	R_a^4	R^5	IV	XIII	VIII	XI	IV	XIII	VIII	Procedure
a	Me	H	Me	Me	22	79						
b	Me	H	Me	iPr	42	53						
c	Me	H	Me	*t*Bu	33	47				35		B
d	Me	H	$-(CH_2)_4-$		66				25	43		B
e	Me	H	Me	Ph	5	90				97		B
f	Me	Me	Me	iPr		32	34	16				
g	Me	Me	Me	*t*Bu		26	38	10				
h	Me	Me	Me	Ph						97		B
i	$PhCH_2$	H	H	Me	52		44					
j	$PhCH_2$	H	H	Ph	12	33	41					
k	$PhCH_2$	H	Me	Me	20	75						
l	$PhCH_2$	H	Me	Et						94		D
m	$PhCH_2$	H	Me	iPr	43	50						
n	$PhCH_2$	H	Me	*t*Bu	30	63						
o	$PhCH_2$	H	$-(CH_2)_4-$		83	13			77	15		C
p	$PhCH_2$	H	Me	Ph	5	91						
q	$PhCH_2$	Me	Me	iPr		27	28	24		32	62	D
r	Ph	H	Me	Me	< 1	96				93		D
s	Ph	H	Me	Et						97		B
t	Ph	H	Me	iPr	< 1	> 90						
u	Ph	H	Me	*t*Bu	< 1	55				49	14	C
v	Ph	H	$-(CH_2)_4-$						15	46		C
w	Ph	H	Me	Ph	< 1	94						
x	Ph	Me	Me	iPr		36	27	33		47	31	A
y	Ph	Me	Me	*t*Bu				89				
z	Ph	Me	Me	Ph		97				100		B
ü	pNO_2Ph	Me	Me	iPr						40	36	C
ö[a]	Ts	Me	Me	iPr						63	7	

[a] This triazoline decomposes spontaneously.

formed in a significant amount when R^1 is aliphatic. On the other hand the size of R^1 does not seem to influence the yields, as they are almost the same when R^1 is methyl and benzyl (*cf.* cases a and k, b and m, c and n, e and p). This is in contrast to the influence of R^5, where the yield of triazole increases in the series Ph, Me, *t*-Bu, iPr, $-(CH_2)_4-$ for a given alkyl group at N-1.

The yield of triazole is relatively high when the triazoline is unsubstituted at C-4 (*cf.* cases i and j with cases k and p).

The effect of the solvent has only been examined in case a. The yield of 1,4,5-trimethyl-1,2,3-triazole was almost the same in chloroform, benzene, methanol, and dimethyl sulfoxide, whereas it was more than doubled in pyridine (55 %).

From a synthetic point of view the formation of 1,2,3-triazoles is of limited value, since dehydration of hydroxytriazolines is normally better accomplished by treatment with methanolic potassium hydroxide.[3] Only when the groups to be eliminated (H and OH) are prevented from being *trans* to each other (cases d, o, and v) the acid catalyzed dehydration may be valuable.

Formation of amides (XIII), α-aminoketones (VIII), and vinylketones (XI). Amides are formed in almost all cases, most often as the main product. Hence, this reaction provides a realistic synthetic route to highly branched

carboxylic acids by way of their amides, particularly when R^1 is aromatic. With regard to R^5 the yield of amide follows the reverse trend of that observed for the triazole formation.

Formation of α-aminoketones and vinylketones only takes place in a few instances and is therefore less important.

The reactions in question are closely related to the spontaneous decomposition of 1-tosyl-Δ^2-1,2,3-triazolines (*cf.* references in Ref. 4). Accordingly, the *tert*-butoxide-catalysed reaction of tosyl azide with diisopropyl ketone (case ö) gives a mixture of XIII and VIII by way of the triazoline.

Proposed mechanism for the acid-catalyzed conversions of 5-hydroxy-Δ^2-1,2,3-triazolines. The proposed mechanism for the acid-catalyzed conversions of hydroxytriazolines is outlined in Scheme 1. Although the triazoline nucleus in the presence of acid is most likely to be protonated at N-3 (*cf.* the section on reactions with other nucleophiles), this is not shown in the scheme because it does not manifest itself by starting irreversible processes. In contrast, protonation at N-1 may cause ring-opening to give the aliphatic diazonium ion VII and subsequently XIII and VIII by elimination of nitrogen and migration of a group from C-5 to C-4.

The higher yield of products (XIII and VIII) derived from VII relative to that of triazole, when R^1 is aromatic rather than aliphatic, is in accordance with the supposition that the ring-opening is reversible with the equilibrium lying more to the right in the former case.

An unsettled question, which is closely related to the long standing discussion of the mechanism for the reaction of aliphatic amines with nitrous acid,[5-7] is whether the diazonium ion (VII) or the carbocation (XII) is the branching point for further reactions, *i.e.* whether migration of the C-5 group takes place concerted with or after elimination of nitrogen.

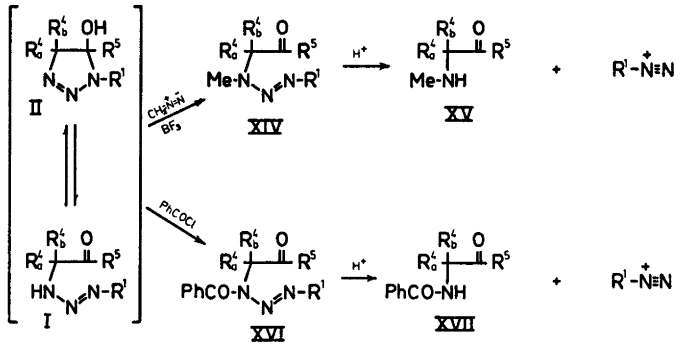
The concerted mechanism presumably applies when there are two hydrogens at C-4. This will explain the high yield of VIII relative to XIII in cases i and j (*cf.* Ref. 8, concerning 5-alkoxytriazolines). The relatively high stability of primary diazonium ions (VII, $R_a^4 = R_b^4 = H$) assumingly demands intramolecular assistance – at which the amino group is most effective [9] – in the expulsion of nitrogen.[10] This theory is in accordance with the concurrent high yield of IV (R_a^4 = Me reduces it to less than half). Not decomposing spontaneously into XII, VII has the possibility of reverting to the closed structure, VI, and eventually end up as IV, if it misses conversion into VIII.

In contrast, with two methyl groups present at C-4, decomposition of VII will lead to a tertiary carbocation, XII ($R_a^4 = R_b^4$ = Me). Hence the non-concerted mechanism would be expected in this case. The relatively high yield of VIII in cases f, g, q, and x must here be due to steric hindrance toward migration of the bulky R^5 in XII.

With only one methyl group at C-4 the situation is less clear. The stereochemistry of the acid-catalyzed conversion of II into XIII was studied in case s. IIs – like some other hydroxytriazolines – forms a racemic mixture on crystallization and could hence be obtained in optically active form by mechanical separation of the two kinds of crystals. Treatment of the levorotatory form with TFAA gave (+)-XIIIs of the same optical purity as the starting material. Thus the reaction is completely stereospecific and presumably involves inversion at C-4.[7] However, this does not differentiate clearly between the two mechanisms, since inversion may result from the S_N2-like reaction in the concerted mechanism as well as from steric control of the carbocation conformation in the two-step mechanism.[7]

Vinylketones, XI, are formed from sterically encumbered triazolines with two methyl groups at C-4. They are most likely formed by way of the protonated triazene, V, which may be generated either by protonation of the keto-triazene, I, or, when R^1 is aliphatic, more likely by cleavage of the N-1···C-5 bond of the protonated triazoline, VI. On deamination, V leads to a tertiary α-ketodiazonium ion, IX, which is apt to loose nitrogen spontaneously [11] to give the carbocation X and finally the vinylketone. XI.

The trend in the yield of triazole from 1,5-dialkyltriazolines largely follows the capability of R^5 at hindering protonation of N-1, which would lead to XIII or VIII. For instance the accessibility of N-1 in the 4,5-tetramethylene triazolines (cases d, o, and v) in the assumed envelope conformation [12] is particularly poor, and protonation of the hydroxy group, leading



Scheme 2.

to dehydration, is therefore strongly favored.

The increased yield of triazole when running the reaction in pyridine may be related to this phenomenon. Thus it may be more difficult for the large proton donor (*cf.* Ref. 13) in this solvent to reach the rather hindered N-1 lone-pair than the more exposed axial hydroxyl group.

Reaction of 5-hydroxy-Δ²-1,2,3-triazolines with other electrophiles. Also electrophiles other than protons will react with the triazolines (Scheme 2). Thus treatment of II l, s, and x with diazomethane and boron trifluoride in ether [14] gave products whose structure appeared to be that of methylated ketotriazenes (XIV). The position of the methyl group, N-3 rather than N-1, follows from the fact that for example the product formed in case l, on treatment with boiling hydrochloric acid,[15] gives a mixture of benzyl alcohol, benzyl chloride, and the α-aminoketone XVl. Besides, the product formed in case x, XIVx, could be synthesized by coupling the α-aminoketone XVx with phenyl diazonium chloride (*cf.* Ref. 15, p. 695).

Benzoylation in pyridine of 5-hydroxy-Δ²-1,2,3-triazolines (again represented by cases l, s, and x) normally gives the corresponding benzoylated ketotriazene, XVI. The structure appears from the NMR and IR spectra (two C=O bands present in the product in cases l and s) and from the fact that benzoylated α-aminoketones (XVII) are obtained on treatment of the benzoyl derivatives, XVI l and x, with acid.

Benzoylation of IIf followed by treatment with sulfuric acid gave, by way of XVIf, the benzoylated α-aminoketone XVIIf (identical with XVIIx) in a 49 % yield. *N*-Methylbenzamide, the product to be expected if reaction occurred at N-1, could not be detected.

The methyl and benzoyl derivatives might of course come from the ketotriazene (only one of the two possible tautomeric forms is shown in Scheme 2). However, if this were the case, one would expect reaction to occur also at N-1, at least when both substituents of the triazene are aliphatic. In case f, reaction at N-1 should even be greatly favored – for steric reasons – but still only reaction at N-3 is observed.

An explanation to the exclusive formation of N-3 derivatives would be that it is the triazoline (II), and not the ketotriazene itself, that reacts with the electrophilic reagent. Thus the positive charge generated in II in the electrophilic attack will be much more effectively delocalized when reaction takes place at N-3 rather than at N-1.

EXPERIMENTAL

NMR-spectra were recorded on Varian A-60 and JEOL JNM-C-60HL instruments, using TMS as an internal standard ($CDCl_3$ if not otherwise specified). IR-spectra were recorded on a Perkin-Elmer model 421 instrument. Preparative thin-layer chromatography (TLC) was carried out using Merck Kieselgel PF_{254}. Melting points are uncorrected. All hydroxytriazolines have been described in previous papers. Other starting materials are commercially available. Microanalyses were performed by Dr. A. Bernhardt, Mikroanalytisches Laboratorium.

Decomposition by TFAA of 5-hydroxy-Δ²-1,2,3-triazolines in $CDCl_3$ and determination of the product distribution by NMR spectroscopy (cf. *Tables 1 and 3 – 6*). 0.1 mmol of triazoline is dissolved in 0.5 ml of $CDCl_3$, and 0.1 mmol (7.6 μl) of TFAA is added. Heat generation is considerable when the N_2 evolution is so. 0.1 mmol (10.5 μl) of 1,1,2,2-tetrachloroethane is added to provide a quantitative internal

standard, and the mixture is shaken with saturated $NaHCO_3$. The NMR spectrum is recorded and integrated and the yields are calculated from the relative signal intensities of products and $CHCl_2CHCl_2$.

δ-Values of signals from compounds in parenthesis in Tables 3–6 have been read directly from the NMR spectra of the reaction mixtures after shaking with $NaHCO_3$. These compounds have not been isolated.

The same procedure, except for the treatment with $NaHCO_3$, was used in the decomposition of IIa in benzene, dimethyl sulfoxide, and pyridine. In methanol it proved necessary to first remove the solvent (with compressed air) due to interference of the OH and reference signals. HCl in ether was first added in order to prevent loss of triazole. After neutralization ($NaHCO_3$) and dissolution in $CDCl_3$, standard was added and the NMR spectrum recorded.

Acid-catalyzed decompositions of 5-hydroxy-Δ^2-1,2,3-triazolines and isolation of the products (Tables 1–6). Procedure A (case x) The hydroxytriazoline (*ca.* 2 mmol) is added to 5 ml of ice cold 3 N hydrochloric acid. The mixture is stirred for half an hour at 0 °C and then for half an hour at room temperature. The precipitated amide (XIII) is filtered off and recrystallized from ethyl acetate-pentane.

The filtrate is neutralized with $NaHCO_3$ and extracted with ether; the extract is dried over $MgSO_4$. Evaporation and recrystallization from pentane gives the α-aminoketone (VIII).

Procedure B (cases c, d, e, h, s, and z). The hydroxytriazoline (1–2 mmol) is dissolved in 10 ml of methylene chloride, and 2 ml of 3 N hydrochloric acid is added. The mixture is shaken for a few minutes, until the evolution of nitrogen has ceased. The aqueous phase is washed with 4 ml of methylene chloride (plus 2 × 5 ml in case d). The combined organic phases are dried over $MgSO_4$ and evaporated. The amide (XIII) is usually left in a very pure state. It may be recrystallized from ethyl acetate-pentane (or cyclohexane in case z).

In case d the triazole, IVd, is obtained from the aqueous phase after neutralization ($NaHCO_3$) and extraction with methylene chloride. It is purified by way of its picrate, which is recrystallized from ethanol.

Procedure C (cases o, u, v, and ü). The hydroxytriazoline (1–2 mmol) is dissolved in 10 ml of methylene chloride, and 1 ml of 3 N hydrochloric acid is added. The mixture is shaken for a few minutes and then neutralized with $NaHCO_3$ and extracted with methylene chloride. After drying ($MgSO_4$) and evaporation, the resulting mixture is subjected to TLC

Table 2. Melting points and analyses of isolated products.

Structure	Case	M.p. Found	M.p. Reported	Formula	Analyses Found C	Found H	Found N	Calc. C	Calc. H	Calc. N
IV	d(picrate)	157–59		$C_{13}H_{14}N_6O_7$	42.47	3.99	23.10	42.62	3.86	22.94
	o	79–80	77[16]	$C_{13}H_{15}N_3$	73.17	7.15	19.63	73.23	7.09	19.63
	v	112–13	117–18[17]	$C_{12}H_{13}N_3$	72.30	6.55	21.32	72.35	6.57	21.10
XIII	c	85–86		$C_8H_{17}NO$	67.30	12.04	9.74	67.10	11.96	9.79
	d	50–53	58–59[18]							
	e	78–80		$C_{10}H_{13}NO$	74.30	8.20	8.75	73.59	8.03	8.58
	h	64–65		$C_{11}H_{15}NO$	74.72	8.64	8.06	74.51	8.52	7.90
	l	45–46	47.5–48.5[19]							
	o	94–95		$C_{13}H_{17}NO$	76.73	8.32	7.03	76.80	8.43	6.89
	q	44–45		$C_{14}H_{21}NO$	76.50	9.54	6.32	76.68	9.65	6.39
	r	102–102.5	105.5[20]							
	s	109–110	110–110.5[21]							
	u	109–110	104.5[22]							
	v	159–160	160[23]							
	x	78–79	78–79[24]							
	z	99–100		$C_{16}H_{17}NO$	80.46	7.25	6.03	80.30	7.16	5.86
	ü	119–120		$C_{13}H_{18}N_2O_3$	62.25	7.12	11.02	62.37	7.25	11.19
	ö	160–164		$C_{14}H_{21}NO_3S$	59.18	7.35	4.82	59.33	7.47	4.94
VIII	q(hydrochloride)	196–204		$C_{14}H_{22}NOCl$	65.50	8.58	5.39	65.73	8.67	5.47
	u(benzoylated)	91–93		$C_{20}H_{23}NO_2$	77.78	7.48	4.75	77.63	7.49	4.53
	x	65–66		$C_{13}H_{19}NO$	75.97	9.38	6.70	76.05	9.33	6.82
	ü	163–164		$C_{13}H_{18}N_2O_3$	62.26	7.41	11.03	62.37	7.25	11.19
	ö	155–156		$C_{14}H_{21}NO_3S$	59.24	7.32	4.98	59.33	7.47	4.94
XI	y	liq.		$C_8H_{14}O$				76.13	11.18	

Table 3. NMR data of amides (XIII). Compounds in parentheses have not been isolated; the NMR data were obtained directly from the reaction mixtures after shaking with saturated $NaHCO_3$ solution. The coupling constant between CONH and the adjacent CH_3 or $PhCH_2$ protons is 4–6 Hz.

	R^1	R_b^4	R_a^4	R^5
(a)	2.82d	2.40 sept.		1.16d
(b)	2.84d		1.12d	0.90d
c	2.78d	2.07q	1.09d	0.97s
d	2.83 broad s	2.1–2.9 m		1.5–2.2 m
e	2.73d	3.56q	1.53d	7.3"s"
(f)	2.80d		1.08s	0.85d
(g)	2.79d			
h	2.73d		1.59s	7.37"s"
(j)	4.39d 7.28s		3.60s	7.0–7.5m
(k)	4.45d 4.50s	2.52sept.		1.19d
l	4.44d 7.30s		1.14d	0.7–2.4m
(m)	4.45d			
(n)	4.43d	1.98q	1.07d	0.97s
o	4.41d 7.28s	2.1–3.0m		1.3–2.2m
(p)	4.39d	3.61q	1.55d	
q	4.44 7.30s		1.08s	0.84d 2.02 sept.
r	7.0–7.7m	2.54sept.		1.24d
s	6.9–8.0m		1.21d	0.7–2.6m
(t)	7.0–7.7m		1.20d	0.97d 0.99d
u	6.9–7.7m	2.15q	1.22d	1.04s
(w)	6.9–7.7m	3.85q	1.62d	
x	6.9–7.8m		1.21s	0.92d 2.08sept.
z	6.9–7.6m		1.66s	
ü	7.78"d" 8.26"d"		1.24s	0.94d 2.08sept.
ö	7.39"d"8.02"d"2.47s		1.04s	0.74d 1.85sept.

Table 4. NMR data of α-aminoketones (VIII) (*cf.* comments to Table 3).

VIII	R^1	R_b^4	R_a^4	R^5
(f)[a]	2.26s		1.28s	1.07d
(g)	2.21s		1.25s	1.14s
(i)	3.52s?		3.79s?	2.12s
q	3.59s, 7.3–7.7m		1.37s	1.11d, 3.32sept.
u	6.5–7.4m	4.66q	1.32d	1.20s
benzoylated u	7.0–7.7m	5.80q	1.13d	1.35s
x	6.4–7.3m		1.49s	1.03d, 3.39sept.
ü	6.54"d", 8.10"d"		1.58s	1.06d, 3.29sept.
ö	7.30"d", 7.81"d", 2.42s		1.46s	1.08d, 3.13sept.

[a] *Cf.* data for 2-methylamino-2,4-dimethyl-3-pentanone (*v.i.*).

(eluents: case o: ether-pentane 2:1; case u: ethyl acetate-pentane 1:6; case v: ethyl acetate-pentane 1:2; case ü: ethyl acetate-pentane 1:3). The products are recrystallized from ethyl acetate-pentane, VIIIu after benzoylation in pyridine.

Procedure D (cases l, q, and r). The hydroxytriazoline (1–2 mmol) is dissolved in 10 ml of ether, and 0.3 ml of 3 N hydrochloric acid is added. The mixture is shaken for a few minutes until evolution of nitrogen has ceased. The amide, XIII, is left when the dried ($MgSO_4$) ether phase is evaporated.

In case q, the ether phase was first washed with 3 × 4 ml of 3 N hydrochloric acid, and the amide was recrystallized from cyclohexane-pentane. The combined aqueous phases were neutralized with $NaHCO_3$ and extracted with

Table 5. NMR data of vinylketones (XI) (*cf.* comments to Table 3).

XI	$CH_2=C<$	R_b[4]	R[5]
(f,q,x)	5.79m, 5.97m	1.89m	1.10d
y,(g)	5.40m	1.91m	1.24s

methylene chloride. Drying and evaporation gave the α-aminoketone, VIIIq, which was converted into its hydrochloride by HCl in ether.

Decomposition of Iy by TFAA in chloroform. Iy (0.433 g) dissolved in 10 ml of chloroform was decomposed by 0.15 ml of TFAA. The reaction mixture was washed with 10 ml of 4 N hydrochloric acid, dried over $MgSO_4$, and evaporated at 60 mmHg. Distillation yielded 0.193 g (87 %) of the vinylketone, XIy. Owing to the presence of a trace of chloroform a satisfactory elementary analysis could not be obtained. In the mass spectrum, however, the molecular ion was present at 126 *m/e*.

Reaction of tosyl azide with diisopropyl ketone. Diisopropyl ketone (1.42 ml, 0.01 mol) was added to a solution of 0.5 g of potassium in 10 ml of *tert*-butyl alcohol. Tosyl azide [25] (1.55 ml) was added dropwise and with stirring and cooling so that the temperature did not exceed 30 °C. Stirring was continued for 4 min. The mixture was poured into 200 ml of ice-water, whereby considerable nitrogen evolution occurred. The mixture was extracted with 4 x 25 ml of methylene chloride; it was necessary to let the mixture stand overnight for separation the first time. The extract was dried over $MgSO_4$ and evaporated. A semisolid mixture was left, which was converted into a colorless, crystalline product, VIIIö, on trituration with ether-pentane. Yield: 191 mg (7 %), m.p. 147 – 151 °C. Recrystallization from ethyl acetate two times gave the pure product (*cf.* Table 2 for m.p. and analysis and Table 4 for NMR data).

Acidification of the aqueous phase with hydrochloric acid caused precipitation of 1.78 g (63 %) of XIIIö as slightly discolored crystals, which were recrystallized from ethyl acetate (*cf.* Table 2 for m.p. and analysis and Table 3 for NMR data). The structure of XIIIö was confirmed by its conversion into p-toluenesulfonamide and 2,2,3-trimethylbutanoic acid on hydrolysis: 97 mg of XIIIö was heated to 100 °C for a few minutes with a mixture of 0.2 ml of water and 0.4 ml of conc. sulfuric acid, until the mixture became clear. The reaction mixture was extracted with pentane (3 x 3 ml). The extract was washed with water, dried over $MgSO_4$, and evaporated, leaving 43 mg (96 %) of 2,2,3-trimethylbutanoic acid as low melting crystals. NMR data: 0.90 (d, 6 H), 1.12 (s, 6 H), and 2.02 (sept., 1 H). Water (1 ml) was added to the inorganic phase, causing crystallization of 42 mg (72 %) of *p*-toluenesulfonamide, identified through mixed melting point with an authentic sample.

Treatment of optically active IIs with TFAA. Some hydroxytriazolines crystallize as a racemic mixture and may hence be obtained in optically active form by mechanical separation of (+)- from (−)-crystals. Slow evaporation of a solution of IIs in ether dried over sodium produced single crystals weighing up to 2 mg. The individual crystals were visually indistinguishable, and in order to separate (+)- from (−)-crystals the rotation of each crystal had to be measured. In several cases $[\alpha]_D$ 199° (*c*, *ca.* 0.1 in acetone) was measured, and this value is therefore considered to apply for optically pure IIs. The specific rotation was often lower due to the presence of twins.

A larger amount of optically active IIs was obtained by cooling a saturated ethereal solution. The resulting crystal aggregates were sorted according to sign of rotation in acetone. After collecting a sufficient amount, recrystallization of the (−)-fraction from ether-pentane gave a specific rotation of −188.3°, corresponding to an optical purity of 95 %. 17.4 mg of this material was dissolved in *ca.* 1 ml of $CDCl_3$, and 10 μl of TFAA was added. The reaction was completed after a few seconds. After a few minutes the solution was evaporated and the residue subjected to TLC (ethyl acetate-pentane 1:6), resulting in 15.3 mg of pure XIIIs. The specific rotation was +37.9° (*c*, 1.5 in acetone), corresponding to an optical purity of 96 %.[26] Thus the reaction seems to be completely stereospecific.

Methylation of 5-hydroxy-Δ^2-1,2,3-triazolines (*cf. Tables 7 and 8*). The triazoline (2 mmol) is

Table 6. NMR data of 1,2,3-triazoles (IV), not reported in Ref. 3 (*cf.* comments to Table 3).

IV	R^1	R_a[4]	R[5]
(b)	3.96s	2.34s	1.31 (d, 6 H)
d	3.92s	1.7 – 2.2(m, 4 H), 2.4 – 3.0(m, 4 H)	
(m)	5.49 (s, 2 H)	2.37s	2.98(sept., 1 H)
v	7.52"s"	1.7 – 2.1 (m, 4 H), 2.6 – 3.1(m, 4 H)	

Table 7. Yields, melting points, and analyses of methyl- and benzoyl-derivatives.

		Yield %	M.p. °C	Formula	Analyses Found C	H	N	Calc. C	H	N
XIV	l	92	liq.	$C_{13}H_{19}N_3O$	66.80	8.32	17.82	66.92	8.21	18.02
	s	90	liq.	$C_{12}H_{17}N_3O$	65.52	7.93	19.13	65.72	7.81	19.16
	x	47	60–61	$C_{14}H_{21}N_3O$	67.94	8.66	16.96	68.00	8.56	16.99
XVI	l	89	liq.	$C_{19}H_{21}N_3O_2$	70.40	6.57	12.61	70.56	6.54	12.99
	s	100	liq.	$C_{18}H_{19}N_3O_2$	69.83	6.27		69.87	6.19	
	x	78	67–68	$C_{20}H_{23}N_3O_2$	71.03	6.83	12.54	71.20	6.87	12.45

Table 8. NMR and IR data of methyl- and benzoyl-derivatives.

		NMR data Me–N	PhCO–N	R^1	R_b^4	R_a^4	R^5	IR data PhCO–	R^5–CO
XIV	l	3.03s		7.31s 4.79s	4.58q	1.37d	1.01t 2.43q		1715
	s	3.25s		7.0–7.7m	4.78q	1.48d	1.07t 2.51q		1720
	x	3.18s		7.0–7.7m	1.57s		1.08d 3.13sept.		1710
XVI	l		7.30(s, 5 H) 7.1–7.8(m, 5 H) 4.85s		5.43q	1.45d	0.91t 2.28q	1672	1720
	s		7.41(s, 5 H) 7.2–8.0(m, 5 H)		5.68q	1.60d	1.08t 2.46q	1675	1720
	x		7.30(s, 5 H) 7.3–8.0(m, 5 H)		1.81s		1.16d 3.18sept.	1690	

dissolved in 40 ml of ether, and a solution of ca. 10 mmol of diazomethane [27] in 40 ml of ether is added. The mixture is cooled to 0 °C, and two drops of boron trifluoride etherate are added. Some nitrogen evolution occurs. After *ca.* 30 min the mixture has become colorless. After washing with saturated $NaHCO_3$ the ether phase is evaporated. Pentane (25 ml), Na_2SO_4, and a little activated carbon are added, and the mixture is stirred for a few minutes. The solid materials are filtered off and the pentane solution is evaporated on a rotary evaporator. The product is usually left as a colorless oil.

The purity was checked by NMR spectroscopy. In case x purification by preparative TLC (ethyl acetate-pentane 1:4) followed by recrystallization from pentane was necessary to obtain a pure product.

Benzoylation of 5-hydroxy-Δ^2-1,2,3-triazolines (*cf. Tables 7 and 8*). The triazoline (4 mmol) is dissolved in 10 ml of pyridine, and the mixture is cooled to 0 °C. Benzoyl chloride (0.92 ml, 8 mmol) is added, and the mixture is let stand at room temperature for an hour. After cooling to 0 °C 0.2 ml of water is added, and the mixture is removed from the ice bath and left for 30 min. Ether (40 ml) is added, and after washing twice with diluted sulfuric acid (30 ml and 10 ml), once with water (10 ml), and once with saturated $NaHCO_3$ (20 ml), the ether phase is dried over $MgSO_4$ and evaporated. The products are left in an almost pure state, but for microanalysis, XVIx was recrystallized from pentane and XVIs subjected to TLC, using ethyl acetate-pentane 1:7 as eluent.

Benzoylation of 1,4,4-trimethyl-5-isopropyl-5-hydroxy-Δ^2-1,2,3-triazoline (*IIf*). The benzoylation and hydrolysis of excess PhCOCl was done as in the general procedure. Instead of adding the 40 ml portion of ether, pyridine was removed on a rotary evaporator. The residue was refluxed for 2 min with 2 N H_2SO_4. Ether was added, and after shaking and separation, the organic phase was washed with 2 N H_2SO_4, water, and saturated $NaHCO_3$ and dried over

$MgSO_4$. Evaporation gave a residue from which 31.8 mg of XVIIf (identical with XVIIx, *vide infra*) could be crystallized from ethyl acetate-pentane. From the mother liquor additional 15.1 mg could be isolated by TLC (ethyl acetate-pentane 1:3) and crystallization from pentane. The total yield, 46.9 mg, corresponds to 49 %.

Treatment of XIV l with hydrochloric acid. XIV l (0.117 g) was dissolved in 0.5 ml of 3 N hydrochloric acid, and the mixture was refluxed for 3 min. After cooling, the mixture was extracted with methylene chloride (3 × 1 ml). Drying over $MgSO_4$ and evaporation gave 67 mg of an oil, the major part of which consisted of an equimolecular mixture of benzyl chloride and benzyl alcohol, as seen from the IR and NMR spectra.

Evaporation of the aqueous phase gave 71 mg of a colorless syrup, the hydrochloride of XV l. Benzoylation in pyridine (standing for two days) afforded 49 mg of almost pure (NMR) benzoylated XV l as a yellowish oil. It was further purified by preparative TLC, using ethyl acetate-pentane 1:2. (Found: C 70.23; H 7.82; N 6.29. Calc. for $C_{13}H_{17}NO_2$: C 71.19; H 7.81; N 6.39). NMR data ($CDCl_3$, 100 MHz): 1.09 ("t", 3 H), 1.44 (d, 3 H), 2.1 – 3.0 (m, 2 H), 2.90 (s, 3 H), 4.0 – 5.6 (m), and 7.44 (s, 5 H).

Treatment of XVI l with TFAA in chloroform. XVI l (0.117 g) and TFAA (50 µl) dissolved in ethanol free chloroform (3 ml) was heated to 40 – 50 °C for 15 min. The solvent was removed in vacuum and the residue subjected to TLC (ether-pentane 2:1). Two components showed up, one of which (the slowest running) consisted of 47 mg (63 %) of XVII l. (Found: C 69.10; H 7.45; N 6.59. Calc. for $C_{12}H_{15}NO_2$: C 70.23; H 7.37; N 6.82). NMR data ($CDCl_3$, 100 MHz): 1.12 (t, 3 H), 1.46 (d, 3 H), 2.61 (dq, 2 H), 4.81 (quintet, 1 H), and 7.0 – 8.0 (m, 5 H).

Treatment of XVIx with HCl in ether. XVIx (0.117 g) was added to 5 ml of ether saturated with HCl, and the mixture was stirred for 30 min. A white, crystalline product separated. The mixture was cooled to 0 °C and shaken with 5 ml of water. The organic phase was dried and evaporated, yielding 73 mg (90 %) of crystalline XVIIx. After recrystallization from ethyl acetate-pentane the m.p. was 120 – 121 °C. (Found: C 71.99; H 8.34; N 6.22. Calc. for $C_{14}H_{19}NO_2$; C 72.07; H 8.21; N 6.00). NMR data: 1.18 (d, 6 H), 1.74 (s, 6 H), 3.24 (sept., 1 H), and 7.3 – 8.1 (m, 5 H).

The aqueous phase was added to a solution of 0.5 g of β-naphthol in 25 ml of 2 N NaOH. The separating red product was filtered off, washed with water, and dried, giving 66 mg (77 %) of red crystals with m.p. 124 – 127 °C. Recrystallization from ethanol raised the m.p. to 130 – 130.5 °C, undepressed in mixture with a sample prepared from phenyldiazonium chloride and β-naphthol.[28]

Preparation of reference compounds

2-Bromo-2,4-dimethyl-3-pentanone. Diisopropyl ketone (14.2 ml, 0.1 mol) was cooled in ice-water, and bromine (5.1 ml, 0.1 mol) was added dropwise and with stirring. After a short time the mixture became colorless. The product was purified by addition of 50 ml of ether, washing with saturated $NaHCO_3$, drying over $MgSO_4$, and distillation. Yield 18.4 g (95 %), b.p. 167 – 168 °C (reported [29] 166 – 168 °C). NMR data: 1.19 (d, 6 H), 1.89 (s, 6 H), and 3.49 (sept, 1 H).

2-Anilino-2,4-dimethyl-3-pentanone (cf. *Ref. 30*). 2-Bromo-2,4-dimethyl-3-pentanone (1.93 g), aniline (0.47 g), and $NaHCO_3$ (2.1 g) were heated in an oil bath (160 – 175 °C) for 7 h. Water (10 ml) was added, and the product was extracted with methylene chloride. Preparative TLC (ethyl acetate-pentane 1:5) and recrystallization from pentane afforded 55 % of a crystalline product, identical with VIIIx, as seen from the NMR spectrum and melting point.

2-Methylamino-2,4-dimethyl-3-pentanone. Methylamine (*ca.* 1.5 ml) was added to 2-bromo-2,4-dimethyl-3-pentanone (1.0 g) at −76 °C in a tube. The tube was sealed and left at room temperature for 3 h. Excess of methylamine was allowed to evaporate, and the solution was acidified with hydrochloric acid. After washing with methylene chloride (3 × 2 ml) the solution was made alkaline with sodium hydroxide, and the product was extracted with methylene chloride (5 × 20 ml). Drying over $MgSO_4$ and evaporation yielded 0.68 g of product. NMR data: 1.06 (d, 6 H), 1.25 (s, 6 H), 2.23 (s, 3 H), and 3.16 (sept., 1 H). In addition, irrelevant signals were present at 1.08 (d), 2.39 (s), and 2.6 – 3.1 (m). The product was, however, used in the next step without further purification.

1-Phenyl-3-methyl-3-(1,1,3-trimethyl-2-ketobutyl)triazene (cf. *Ref. 31*). Aniline (93 mg, 1 mmol), dissolved in 1 ml of 3 N hydrochloric acid, was diazotized at 0 °C with 69 mg of sodium nitrite. 2-Methylamino-2,4-dimethyl-3-pentanone (143 mg) was added, and the solution was made alkaline with 2 N NaOH. The mixture was stirred at room temperature for 30 min. The oily product was extracted with methylene chloride, and the solution dried over Na_2SO_4 and evaporated, yielding 0.22 g of a reddish brown syrup. TLC (ether-pentane 1:4) afforded 147 mg (60 %) of a product, which was identical (IR, NMR, and m.p.) with XIVx.

2-Azido-2,4-dimethyl-3-pentanone. 2-Bromo-2,4-dimethyl-3-pentanone (3.86 g, 0.02 mol) and sodium azide (2.6 g, 0.04 mol) were dissolved with stirring in dimethyl sulfoxide (10 ml). Cooling was necessary in order to keep the temperature below 40 °C. The mixture solidified after a few minutes, but was allowed to stand at room temperature for 2 h before it was worked up. Water (10 ml) and ether (15 ml)

were added, and the ethereal phase was washed with water (2 × 10 ml), dried over $MgSO_4$, and evaporated. The azide was left in a 95 % yield as a liquid containing a trace of ether (NMR). NMR data: 1.11 (d, 6 H), 1.43 (s, 6 H), and 3.22 (sept., 1 H).

2-Benzoylamino-2,4-dimethyl-3-pentanone. A mixture of 2-azido-2,4-dimethyl-3-pentanone (155 mg, 1 mmol), conc. hydrochloric acid (0.3 ml), platinum oxide catalyst (5 mg), and methanol (5 ml) was hydrogenated at 1 atm. hydrogen pressure for 2 h. Platinum and methanol were removed, and the resulting α-aminoketone was treated with benzoyl chloride (1 ml) and 2 N NaOH (20 ml). Extraction with methylene chloride, drying over $MgSO_4$, evaporation, and washing with pentane gave 0.221 g (96 %) of benzoylated α-aminoketone. After recrystallization from ethyl acetate-pentane a product was obtained, which was identical with XVIIx (NMR, mixed m.p.).

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Properties of Plasma Membranes from Granulation Tissue with Reference to Extracellular Matrix

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Treatment with neuraminidase decreased the activity of Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase in plasma membranes isolated from experimental granulation tissue but not that of 5′-nucleotidase or leucine-β-naphthylamidase. A temporary lowering of the pH of the plasma membrane suspension to 2 – 3 inactivated all three enzymes, which remained inactive after the pH had been readjusted to 7.4. Addition of dextran preparations to the membrane suspension decreased the activity of adenosine triphosphatase. Ethanol (0.4 %) had a similar effect. These marker enzymes of plasma membranes were not affected by additions of hyaluronate, chondroitin sulfate, protein polysaccharide or soluble collagen. Serotonin stimulated the adenosine triphosphatase activity slightly.

About 10 – 20 % of the protein in the plasma membrane preparation was extracted with EDTA. This "fuzzy coat" fraction yielded a distinct gel-electrophoretic protein pattern. Hyaluronidase was not helpful in cleaving this surface layer from the plasma membranes.

The main purpose of the present work was to explore the effects of extracellular components on the isolated plasma membranes of the granulation tissue. The activities of the marker enzymes were considered as the most sensitive parameters of the membrane functions. The extracellular matrix should be important in any modification of the activities of the connective-tissue cells, especially when the composition or the properties of the intercellular space have been altered, *e.g.*, in rheumatoid disease or after an injury. Since the fibroblast is the main source of the extracellular matrix, the relations of collagen and acidic mucopolysaccharides to the plasma membranes are of special interest. The accessibility of the receptors for hormones and for the "inflammation mediators" regulates the activation of the connective tissue to the granuloma formation and fibrosis. The chemical anatomy of the cell membrane with its "fuzzy coat" [1,2] is relevant for the social functions of the cell such as adhesion, contact inhibition, charge, transport and intercellular communication.[3]

MATERIAL AND METHODS

Plasma membrane preparations and their treatment. The plasma membranes were prepared without the use of enzymes or heavy metals from experimental granulation tissue as described in detail elsewhere.[4] Mature granulation tissue was pressed through a sieve plate, the homogenization completed with a Dounce homogenizer and the bulk of the heavy plasma membranes isolated by an isopycnic centrifugation in discontinuous sucrose gradient where the main fraction accumulated to the interphase of 28/38 %, w/v, sucrose. Both fresh and frozen (at −70 °C) membranes were used, though during storage some of the enzymic activities were lost. The concentration of the membranes in the suspension did not affect the specific enzymic activities calculated per protein. The activity of Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase was about 15 – 20 % higher in 40 mM Tris-HCl buffer than in Krebs-Ringer medium buffered with 20 mM Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid; Sigma, St. Louis, Mo., USA).

Interaction of the plasma membranes with various substances. The membrane suspension (about 1 mg protein/ml), either in Tris-HCl buffer or Krebs-Ringer-Hepes medium, was preincubated with several concentrations of the additive for 30 – 60 min at 37 °C (Table 1). Then the substrate solutions of the marker enzymes were added with the appropriate cofactors for

the determination of enzymic activities (see below).

In the experiments with neuraminidase from *Vibrio comma* (Behringwerke, Marburg, Germany; ORKD No. T 1070S and 572S) the membranes were treated for various periods and with various concentrations of the enzyme (Fig. 1). Collagenase and hyaluronidase were commercial preparations, not homogeneous in polyacrylamide-gel electrophoresis.

Dextran preparations were obtained from Pharmacia, Uppsala, Sweden. Bradykinin (B-1377), ganglioside (Type III: G-2375), lysolecithin (L-6626), spermine (S-2876) and spermidine (S-2501) were from Sigma, serotonin (No. 85030) from Fluka AG (Buchs, Switzerland) and histamine from E. Merck AG (Darmstadt, Germany). The other preparations were made in our laboratory. Prostaglandins were a gift from The Upjohn Company through Mr. O. Oinonen, Helsinki, Finland.

Separation of the "fuzzy coat". The plasma membranes were treated with 0.01 M EDTA in 0.05 M Tris-HCl buffer, pH 6.8, for 5 h at +4° C(the control samples without EDTA) and centrifuged at 100 000 *g* for 45 – 60 min. In some experiments the membrane suspension was subjected to mechanical shear in Couette's rotation viscometer at +4 °C. The gap between the cylinder and the jacket was 0.75 mm, the diameter 1.8 cm, and the speed 150 rpm. The control samples were kept in the same condition without shear. Both the pelleted materials and the supernatants were analyzed for proteins and the enzyme activities. In the supernatants the values were so small that they are not considered reliable.

Analytical. The marker enzymes determined were 5′-nucleotidase (EC 3.1.3.5),[5] Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase (EC 3.6.1.3),[6] and leucine-β-naphthylamidase (EC 3.4.11.1).[7] Also the following components were analyzed: liberated inorganic phosphate,[8] protein,[9] cholesterol,[10] and *N*-acetylneuraminic acid.[11] The polyacrylamide-gel electrophoresis has been described in detail earlier [4] and the protein staining was performed according to Lenard.[12]

RESULTS

Effects of extracellular constituents and pertinent enzymes. Connective-tissue components had no clear effect on the marker enzymes of

Table 1. Effects of various additions on the activities of the characteristic enzymes of plasma membranes.[a]

(0) No effect, (+) activation, (−) inhibition

Addition and the largest final concentration	5′-Nucleotidase	Na^+,K^+ activated ATPase	Leucine-β-naphthylamidase
Hyaluronate, 1 %	(−)	(−)	0
Chondroitin sulfate-4, 4 %	0	0	0
Proteoglycan, 4 %	0	0	0
Heparin, 0,1 %	0	0	0
Dextran T10 (Fig. 3)	0	–	0
Dextran T200 (Fig. 3)	0	–	0
Dextran sulfate (Fig. 3)	(−)	–	0
Soluble collagen, 2 %	0	0	0
Serum albumin, 2 %	0	0	0
Immunoglobulin, crude, 0.5 %	0	0	0
Lipoproteins (HDL, LDL, VLDL,) 100 μg protein/ml	0	0	0
Ganglioside, crude, 0.1 mg/ml	–	0	0
Lysolecithin, 0.1 mg/ml	0	–	0
Hyaluronidase*	0	0	0
Collagenase*	0	(−)	(−)
Neuraminidase* (Fig. 1)	0	–	0
Spermine, spermidine, 10 mM	0	0	0
Bradykinin,* 0.1 mM	0	0	0
Prostaglandins E_1, E_2, $F_2\alpha$, 10 μg/ml	0	0	0
Serotonin,* 10^{-4} M	0	(+)	0
Histamine,* 10^{-2} M	0	0	0
Ethanol, 0.4 %	0	–	0

[a] All the experiments were carried out in Tris-HCl buffer, except those marked * performed in Krebs-Ringer medium buffered with Hepes. Serotonin and histamine were studied with both solvents.

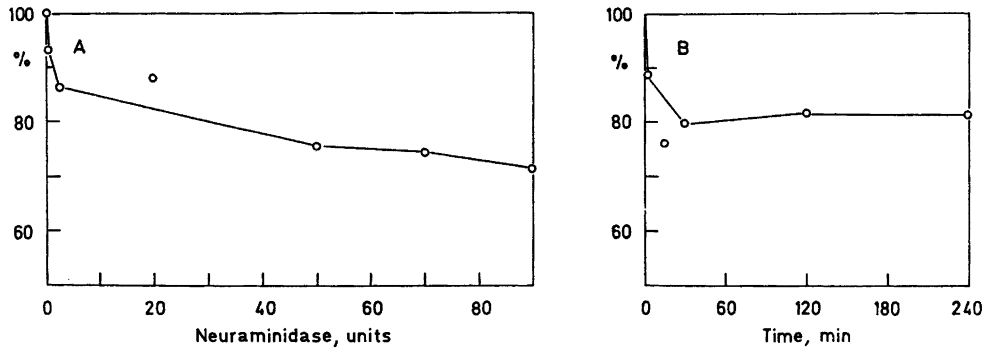


Fig. 1. Effect of a pretreatment of the plasma membranes with neuraminidase on the activity of Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase. A. Effect of various amounts of the enzyme (duration 15 min); B. Effect of the duration of the treatment (neuraminidase 10 U/sample). The solvent was Krebs-Ringer-Hepes medium.

the plasma membrane preparations (Table 1), presumably because the membranes contained some acidic mucopolysaccharides and collagen already. Only hyaluronate (0.5 – 2 %) may have had some inhibitory effect, at least more than chondroitin sulfate, both on Na^+,K^+-activated adenosine triphosphatase and on 5′-nucleotidase although it is difficult to assess the effect of hyaluronate quantitatively. The membranes stained with Alcian blue, which was partially removed with 0.2 M $MgCl_2$.

Experiments were made therefore with pertinent hydrolytic enzymes. The effects of hyaluronidase and collagenase were slight and variable, and the presence of impurities in the enzyme preparations cannot be ruled out. Neuraminidase had a clear effect on Na^+,K^+-activated adenosine triphosphatase, but only 20 – 25 % of the total activity could be inhibited (Fig. 1).

Prompted by an occasional observation that even a short exposure to low pH decreased the subsequent enzymic activities of plasma membranes at neutral environment, a systematic experiment was carried out (Fig. 2). All the marker enzymes proved sensitive although at different pH.

Rheumatoid synovial fluid, human serum, and immunoglobulins were inert with respect to the enzymic activities of the plasma membranes.

Effects of artificial macromolecules. High-molecular-weight dextran and dextran sulfate depressed the activity of Na^+,K^+-activated

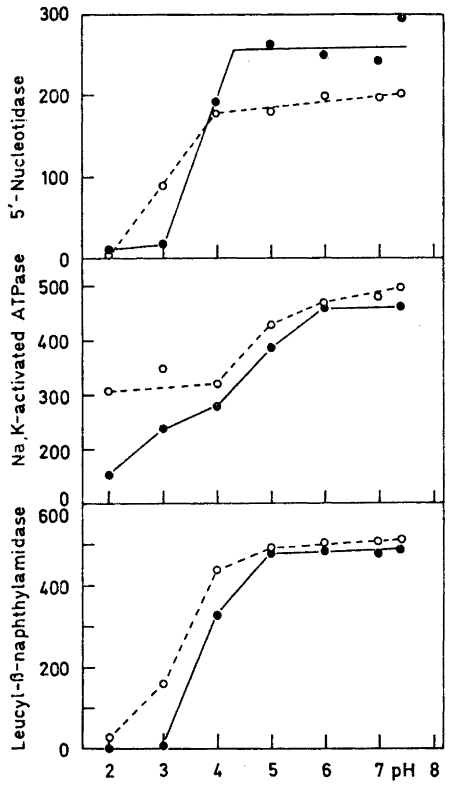


Fig. 2. Effect of the temporary lowering of pH on the subsequent activity of 5′-nucleotidase, Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase, and leucyl-β-naphthylamidase of plasma membranes. The duration at the indicated pH (●) 30 min or (○) 5 min. The solvent was Krebs-Ringer-Hepes medium.

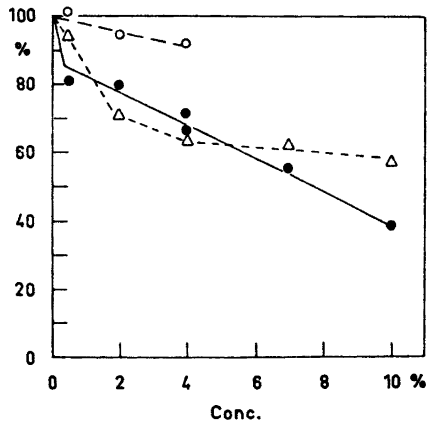


Fig. 3. Effect of dextran preparations on the activity of Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase of plasma membranes. (△) Dextran sulfate, (○) dextran T10 (mol wt. 10 000) and (●) dextran T2000 (mol wt. 2 000 000). The solvent was Tris-HCl buffer.

Mg^{2+}-adenosine triphosphatase (Fig. 3). The effect was not due to an increase of colloid osmotic pressure because low-molecular-weight dextran was less effective. With dextran sulfate the inhibitory effect levels off at low concentration but with dextran it is more concentration-dependent.

Low-molecular-weight substances. The polyamines which one would expect to combine with the acidic groups in the membrane did not have any effect.

The effect of ethanol in "physiological" concentrations, a decrease by 13 – 27 % in the activity of Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase, has not been described earlier on isolated membranes but on slices and microsomal preparations.[13] The effect of serotonin was investigated very thoroughly because serotonin is alleged to stimulate fibroblast functions at low concentrations (*cf.* Ref. 14). In these preparations and with the present parameters the stimulating effect of serotonin at $10^{-5} - 10^{-4}$ M is very small (about + 10 %) if any.

5′-Nucleotidase is sensitive to gangliosides and Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase to lysolecithin (0.1 mg/ml). Several detergents in the concentrations of 1 mg/ml activate the insoluble 5′-nucleotidase of these

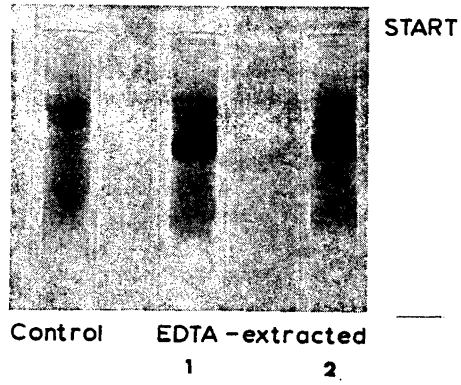


Fig. 4. Effect of the extraction with EDTA with (2) and without (1) mechanical shear on the gel-electrophoretic distribution of proteins in supernatant material detached from plasma membrane preparations. The samples were obtained from the experiment No. 1, Table 2.

Table 2. Effects of EDTA and the treatment with mechanical shear on the plasma membranes.

Conditions of treatment	Protein mg/ml	Enzyme activities, μmol/(h/mg protein) 5′-Nucleotidase	Na^+,K^+-act. ATPase	Leucine-β-naphthylamidase
Exp. 1 (duration 5 h)				
Control	2.15	3.9	26.2	2.6
Extraction with EDTA	2.00	2.7	30.9	2.8
Extraction with EDTA at shear	1.90	2.1	29.6	2.9
Exp. 2 (duration 2 h)				
Control	0.36	7.5	28.0	5.5
Extraction at shear	0.32	5.8	28.9	4.9

The solvent was 0.05 M Tris-HCl, pH 6.9 and the temperature +4 °C. The data refer to the pelleted material.

plasma membranes (T. Vihersaari, personal communication). The inactivating effect of EDTA may have bearing on the finding that 5′-nucleotidase is located on the cell surface (Table 2).

"*Fuzzy coat*". The membranes were treated with hyaluronidase, extracted with EDTA and other solutions, and subjected to increased mechanical shear in a rotation viscometer. Hyaluronidase did not detach a significant amount of protein from the membranes, affect the enzymic activities or change the gel-electrophoretic distribution of membrane proteins. Instead, EDTA liberated to the supernatant proteins having a specific gel-electrophoretic pattern (Fig. 4). The membranes lost to the solution mainly 5′-nucleotidase. The mechanical shear liberated additional protein and further inactivated 5′-nucleotidase (Table 2). No cholesterol or *N*-acetylneuraminic acid was released.

DISCUSSION

The preparation of granulation-tissue plasma membranes is not sensitive to substances that are intimately associated with the cell membranes in the tissues or to enzymes that degrade the components of the extracellular matrix. Either the characteristic marker enzymes are not in this case the proper probes of the membrane function or their sensitivity is lost during the preparation of the membranes. Other enzymes, *e.g.*, adenylate cyclase, may be more sensitive, or the membrane may respond only *in situ*, as part of a living cell.

The effect of dextran preparations may depend on exclusion phenomena [15] which interfere with the formation of a complex between Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase and its substrate. It has been observed that dextran increases the zeta-potential on the surface of erythrocytes.[16] It remains to be investigated whether the activity of adenosine triphosphatase is in any way related to the double layer. The effect of neuraminidase is in agreement with the fact that Na^+,K^+-activated Mg^{2+}-adenosine triphosphatase is a sialoprotein.[17] Ethanol presumably disturbs the relationships between adenosine triphosphatase protein and the stimulating phospholipid in the membrane, or interferes with the local structure of water.

The irreversible inactivation at low pH is similar for 5′-nucleotidase and leucine-β-naphthylamidase. The dissociation point of dicarboxylic amino acids [18] and hexuronic acids is at pH 3–4 and the secondary structures of macromolecules may be lost and inactive complexes formed between adjacent substances. The mechanism for the inactivation of adenosine triphosphatase may be different. The curves in Fig. 2 do not resemble those for the pH dependence of the activities of the enzymes.

The "fuzzy coat" [19] of cells has attracted considerable attention because it is suggested to be different in transformed cells.[20] Its preparation from the plasma membranes originating from solid tissue, obtained without destroying the membranes or covalent bonds, has not been described earlier. This material contains a characteristic protein pattern as seen in Fig. 4. The EDTA-liberated acidic mucopolysaccharides as well as glycoproteins from erythrocyte membranes are heterogenous, markedly different from those shown in Fig. 4.[21] A growth-enhancing protein has been obtained from cell surface of cultured fibroblasts with urea-EDTA.[22]

As the next step in this study we suggest a more sensitive and specific analysis of the composition of the EDTA-liberated fraction. This plasma membrane has been prepared without enzymes and heavy metals and represents the native state as closely as possible. The "fuzzy coat" fraction is neither a part of the plasma membrane proper nor does it belong to the matrix. It conceivably contains the substances just leaving the cell as well as material influencing the social functions of the cells.

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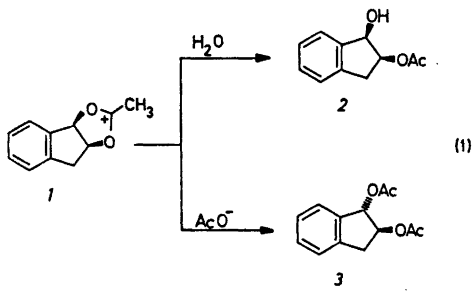
On the Mechanism of the Additive Anodic Acetoxylation of Indene

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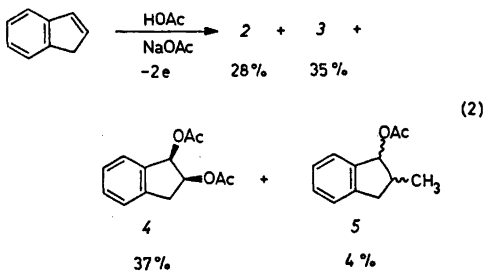
The anodic acetoxylation of indene has been performed in acetic acid/potassium acetate (1 M) with water or acetic anhydride as additives. The influence of these additives on the product distribution is negligible. INDO calculations have been carried out on the neutral molecule and its radical cation. On basis of these results a simple acetoxycarbonium ion mechanism is suggested to operate simultaneously with the previously suggested acetoxonium ion mechanism.

The mechanism of the anodic addition of acetoxy groups to an arylolefin has been studied earlier. [1,2] According to these investigations, the key intermediate formed in the anodic process is an acetoxonium ion which reacts as exemplified by eqn. (1)[3] by the acetoxonium ion derived from indene, *i.e.* with formation of *trans* diacetate (*3*) in the reaction with acetate ion and a *cis* hydroxy acetate (*2*)** in the reaction with water (eqn.1).



(1)

The additive acetoxylation of indene in HOAc/NaOAc (1 M) gave,[4] however, a 32 % yield of a mixture of addition products that contained an appreciable amount of the *cis* diacetate (*4*) in addition to the products expected on the basis of eqn. 1 (*2* and *3*). A small yield of the CH_3/OAc addition product (*5*) was also isolated.



(2)

Since the formation of *4* is not satisfactorily accounted for by the acetoxonium ion mechanism, we have performed further experiments designed to elucidate its possible mode of formation. The results indicate that a mechanism involving a benzylic cation may be invoked to account satisfactorily for its appearance. In addition it appears rather improbable that water plays any role in the route to the *cis* hydroxy acetate (*2*). Finally, as will be discussed below, the formation of the corresponding *trans* hydroxy acetate (*7*) in water-containing electrolyte solutions cannot be reconciled with the cyclic acetoxonium ion mechanism.

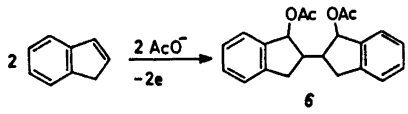
RESULTS

Experiments. A large-scale run (2 mol of indene) using a capillary gap cell [5–7] with a graphite anode gave a mixture of *2*, *3*, and *4*

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** NMR comparison between the compounds in Ref. 18 and compound *2* suggests the proposed structure.

in a combined yield of 34 %. The yield of methyl derivative (*5*) was the same as that obtained before.[4] Using a slightly different work-up procedure than that employed earlier,[4] an 8 % yield of an additive dimer of indene and acetate ion was isolated (eqn. 3). This product was assigned the structure of 2,2'-additive dimer (*6*) on the basis of its mass, NMR and IR spectral properties. It consisted of two diastereoisomers. The yield of the dimer



(3)

6

did not increase significantly upon increasing the indene concentration from 1.6 to 10.5 M. A run with sodium perchlorate as the supporting electrolyte was unsuccessful due to the formation of a polymeric coating on the anode. With platinum as the anode material the combined current yield of *2*, *3*, and *4* increased to 47 %.

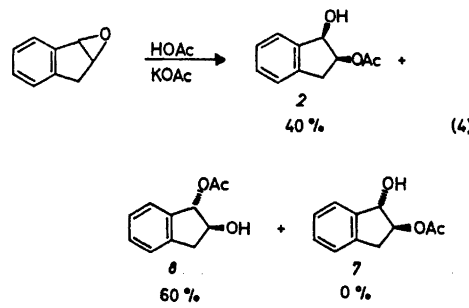
In order to trace the origin of the cis hydroxy acetate (*2*), a series of semi-micro scale runs with either acetic anhydride or water added at graphite or platinum anodes was carried out. As indicated in Table 1, no significant change in product distribution was observed when the solvent was changed from 50 % acetic anhydride/acetic acid to 9 % water/acetic acid, independently of whether graphite or platinum was used as anode material. This observation would seem to rule out the reaction between *1* and water as a possible source of *2* (eqn. 1). No effect upon product distribution was noted when the current density on a platinum anode was changed from 13 to 850 mA/cm².

Table 1. Product distribution from electrolysis of indene in HOAc/KOAc (1 M) in the presence of added water or acetic anhydride.

Anode	Additive [H_2O]/[KOAc]	Product distribution % (*3*)	(*4*)	(*2*)	(*7*)
C	[b]	46	40	14	0
C	0.0	45	40	15	0
C	1.1	44	38	15	3
C	2.7	43	38	16	4
C	5.5	38	36	20	6
Pt	[b]	39	44	18	0
Pt	0.0	40	42	18	0
Pt	1.1	41	40	18	1
Pt	2.7	40	37	19	4
Pt	5.5	38	34	20	8

[a] GLC analysis normalized to 100 %. [b] The reaction solution was a mixture of acetic anhydride (15.0 g) and of acetic acid (15.0 g).

With varying amounts of water added to the electrolyte solution, a new product (*7* in Table 1) was observed. The identity of this compound was established from the following experiments. A mixture of *2* and *7* in the proportions 85:15, isolated from the product mixture from the anodic oxidation of indene by repeated recrystallization from hexane, reacted with acetic anhydride/pyridine to give *3* and *4* in the proportions 15:85. The NMR spectrum of *7* was similar to that of *2* (see experimental part). Ring-opening of 1,2-epoxyindan in HOAc/KOAc gave two products, one of which was identical with *2* and the second one was the *trans* hydroxy acetate *8*, compound *7* not being observed. Exactly how this reaction occurs has not been established.[8] The mixture

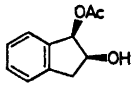


(4)

of hydroxy acetates from this experiment upon treatment with acetic anhydride/pyridine was converted to a mixture of (*3*) and (*4*) in the proportions of 60:40.

A further complication was noted when the electrolysis was performed in a neutral medium. In this experiment dichloromethane was used as the solvent with solid potassium acetate and dicyclohexyl-18-crown-6 present to generate crown-ether solubilized KOAc as the supporting electrolyte. Under these conditions a new *cis* hydroxy acetate, *9*, was formed in addition to *2*, *3*, and *4*. The composition of the mixture was *2*:*3*:*4*:*9* equal to *36*:*22*:*23*:*19*. The structure of *9* was inferred from its non-identity with *2* and conversion to *4* by treatment with

acetic anhydride/pyridine. It is well documented that *9* rearranges to *2* in acidic solution [9] (probable route for the formation of *2* from the epoxide in eqn. 4), so that *9* cannot be ruled out as an intermediate in the electrolyses performed in acetic acid.



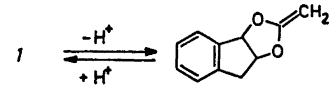
9

It is known that indene reacts with oxygen to give diindanylperoxide. [10] In order to eliminate this possible source of ambiguity, indene was carefully distilled in an argon atmosphere and a middle fraction collected. Two reactions with this sample were performed, one under argon and one under carbon dioxide atmosphere and both with platinum as the anode material. No influence on the product composition was noted, however.

With reference to the somewhat curious fact that the relative amount of *2* formed from indene under conditions ranging from a completely anhydrous medium (with 50 % acetic anhydride present) to one containing nearly 10 % of water does not change at all (Table 1), we performed a few experiments in order to find the relative rate of cation trapping by acetate ion and water, respectively. As a model reaction benzylamine was reacted with sodium nitrite in HOAc/KOAc with water present in varying concentrations and the ratio benzyl acetate/benzyl alcohol determined. A ratio of about 300 was calculated for k_{OAc^-}/k_{H_2O}. That acetate is a stronger nucleophile than water by a factor of 525 was established by Swain and Scott [21] for S_N2 reactivity. One would therefore expect cation capture by acetate ion to predominate to a large extent even in the electrolysis experiments with added water.

Check experiments in which diacetates *3* and *4* were anodically oxidized under the same conditions as those employed for indene, showed that no *cis* hydroxy acetate (*2*) was formed in a secondary process.

Finally, in order to see if the work-up procedure might influence the product composition (due to survival [3] of the acetoxonium ion or its corresponding base, the ketene acetal (*10*), or both during electrolysis; see eqn. 5) two different work-up techniques were tried.



(5)

10

After a charge of 2 F/mol of indene had passed, the electrolyte solution was divided into two equal parts. The first one was poured into a large excess of water, and the second one into absolute ethanol. Capture of *1* or *10* by water would be expected to take place to a larger extent in the former case. However, no difference in product composition was observed between the two procedures.

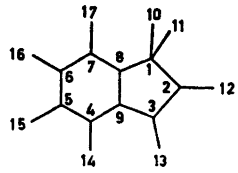
INDO calculations. The first step in the anodic oxidation of an arylolefin is to remove an electron from the highest occupied molecular orbital and thus form a radical cation.[11] In order to get an idea of the most probable reactions of the indene radical cation (I^+), INDO calculations were carried out for this ion and its parent hydrocarbon. It has been shown that this type of calculations describes charge densities almost as well as STO-3G *ab initio* calculations.[12] The results are shown in Table 2, from which the following picture emerges. In the radical cation both charge and spin densities are predominantly located at positions 2 and 6, whereas positions 4 and 8 have relatively low charge densities. Using bond orders, as defined by Ehrenson and Seltzer,[13] as a measure of bond strength, it is seen that the strength of the bond between atoms 3 and 2 decreases and that of the bond between atoms 3 and 9 increases when one electron is removed from indene.

Thus, these calculations predict that the coupling of two I^+ should give the 2,2′-, 2,6′-, and/or 6,6′-dimers rather than the 2,3′- or 3,3′-dimer. The electrophilic attack of I^+ on a neutral indene molecule should likewise occur at position 2 and thus give a 2,2′- and/or 2,6′-dimer.

As shown above for the dimerization of indene and demonstrated by others,[14] coupling *via* the 2,2′-mode is predominant. No coupling *via* the 6-position was observed in either case.

If the radical cation is trapped by acetate ion, the calculations predict that this should

Table 2. INDO calculation on indene (I) and indene radical cation (I^+). The numbers 1 – 9 refer to carbon and 10 – 17 to hydrogen atoms.



Atom No.	Charge density (I)	(I^+)	Radical density	Bond	Bond order (I)	(I^+)
1	0.0607	0.0243	−0.0305	1 – 2	0.7329	0.7375
2	−0.0070	0.1580	0.4035	1 – 8	0.7226	0.7210
3	0.0055	0.0718	0.0588	2 – 3	1.0674	1.0025
4	0.0130	0.0814	0.1489	3 – 9	0.7858	0.8295
5	0.0254	0.0275	−0.0772	4 – 9	0.9238	0.9088
6	0.0228	0.1506	0.2889	4 – 5	0.9666	0.9787
7	0.0223	0.0228	−0.0803	5 – 6	0.9589	0.9392
8	0.0046	0.0794	0.1510	6 – 7	0.9641	0.9432
9	0.0314	0.0941	0.1424	7 – 8	0.9536	0.9675
10	−0.0116	0.0503	0.0134	8 – 9	0.9224	0.8855
11	−0.0116	0.0503	0.0134			
12	−0.0099	0.0532	−0.0164			
13	−0.0240	0.0401	−0.0033			
14	−0.0317	0.0194	−0.0065			
15	−0.0290	0.0289	0.0025			
16	−0.0276	0.0259	−0.0115			
17	−0.0331	0.0223	0.0023			

also occur to a large extent at positions 2 and/or 6. Experimentally, acetoxylation takes place at the olefinic bond of indene and no nuclear acetoxylation is observed. It appears reasonable that the attack should occur at the 2-position.

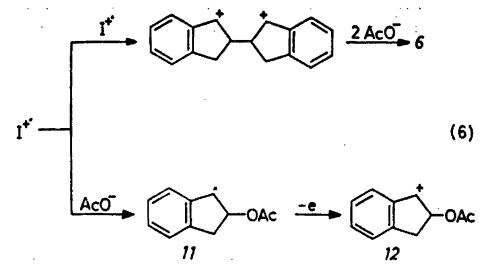
The reasoning above is based upon the simple view that the reaction between two radicals should take place at the position(s) of highest spin density, and that the attack of acetate ion should occur at the position(s) of the highest positive charge density. In both cases an early transition state is implied. Should one, however, adopt the view that the transition state is late – resembling the first intermediate – resonance theory predicts that attack on position 6 should lead to rather unfavourable intermediates (in which the aromatic π-electron system is lacking) as compared to those originating at the 2-position.

DISCUSSION

Considering the results described above, the simple acetoxonium ion mechanism previously proposed [1] cannot be extended to indene system. We would therefore like to suggest a somewhat modified mechanism, still involving the acetoxonium ion as a critical intermediate, but with elements of a second mechanism involving a benzylic cation added.

The first step is formation of I^+, which either reacts with another "indene species" or with acetate ion (eqn. 6), in both cases at position 2. In the former case, the "indene species" can either be the indene molecule or I^+; we prefer the latter alternative in view of the findings from earlier investigations [2] and the fact that the relative yield of additive dimer (*6*) is independent of the concentration of indene in the range of 1.6 to 10.5 M. Other types of anodic couplings, such as biaryl and diphenylmethane coupling, show a strong dependency of relative product distribution with substrate concentration [15–17] and this has been taken as evidence for a substrate-radical cation reaction as the product-determining step.

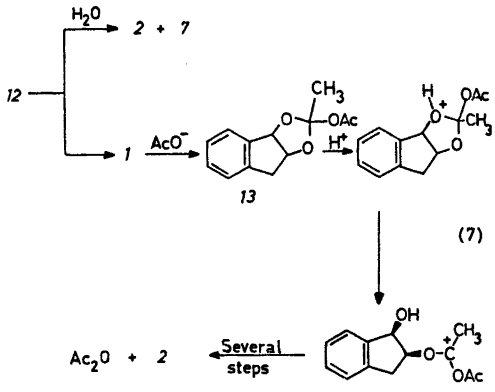
The acetoxy species (*11*), a benzylic radical and hence easily oxidizable, will lose an electron to the anode to give cation *12* in a very fast reaction. From this species one can in principle



derive all the addition products that have been obtained by invoking its capture by acetate ion or water to give *2*, *3*, *4*, *7*, and *8*. However, the results in the anhydrous media clearly do not support such a simple mechanism; especially the formation of *2* cannot be accounted for in this way. We therefore propose that *12* is indeed partially converted to acetoxonium ion *1* which reacts further with acetate ion to give *13* (eqn. 7).[3] Protonation of *13* at the benzylic oxygen produces a species that might undergo heterolytic cleavage at the bond between the protonated oxygen atom and the former carboxyl carbon atom. This would ultimately produce *2* and one molecule of acetic anhydride.

Acetic anhydride was specifically sought for (GLC analysis with a detection limit of 1 %) in a run with no additive present but not detected. This negative result does not add much to our knowledge of the reaction mechanism, since acetic anhydride is oxidizable at the platinum anode and hence may disappear as soon as it is formed.[15]

The formation of *3* might likewise partially occur *via* *1*, whereas both *4* and *7* most likely are derived directly from *12*.



EXPERIMENTAL

Large-scale anodic oxidation of indene. A large-scale electrolysis was carried out in a capillary gap cell equipped with a graphite rod anode (800 cm^2) and a steel cathode. The cell construction has been described earlier.[5–7]

A solution of indene (2.0 mol), KOAc (1.0 mol), acetic anhydride (20 ml), and glacial acetic acid (1200 ml) was electrolysed at a current of 10 A and a temperature between 20 and 27 °C until 2 F/mol of indene has passed.

The solution was poured into ether (3000 ml) and successively washed with 5 × 1 l of water. At the end of this procedure a black-grey solid appeared at the interface and was filtered off. The solid (6.6 g) consisted of a 2,2′-dimer and fragments from the carbon rod. The dimer was separated and purified by recrystallization from ethanol, m.p. 188 °C. NMR ($CDCl_3$): δ 2.1 (s, 6 H, CH_3CO-), 2.5–3.3 (m, 6 H, $-CH_2-CH-$), 6.2 (d, 2 H, $-CH-O$), and 7.3 (br s, 8 H, Ar); ($CDCl_3$ and $Eu(fod)_3$/substrate = 0.36): δ 3.6 (s, 6 H, CH_3CO-), 3.6–3.8 (m, 6 H, $-CH_2-CH-$), 7.2–7.5 (m, 6 H, Ar), 7.9–8.3 (m, 2 H, Ar), and 10.3 (d, 2 H, $-CH-O-$). The mass spectrum had the highest *m/e* at 230 (elimination of 2 molecules of HOAc from the parent ion). The IR spectrum showed a carbonyl band at 1745 cm^{-1}.

The ether was evaporated and the residue was treated with a mixture of acetic anhydride (200 ml) and pyridine (5 ml) and refluxed for 15 min. After cooling and addition of water (200 ml) the organic material was extracted into ether. The ether solution was washed free of acetic acid, dried, and evaporated. The residue (310 g) was continuously extracted with pentane to separate the "monomeric" products from oligomers and polymers. From the crude product 260 g of material was extracted into the pentane solution. A second crop of 2,2′-dimer (2.6 g) was collected from the filtrate after concentration. This second crop consisted of two of the ten possible isomers and had NMR, IR, and MS characteristics similar to the first one. By distillation a mixture of *3* and *4* (145 g, 31 %) was collected at 101–104 °C/0.15 mmHg. The residue was treated with a mixture of KOH (50 g), ethanol (400 ml), and water (20 ml) and refluxed for 30 min. After cooling and addition of water (2000 ml) the crystals were filtered off and washed with acetone. Treatment with acetic anhydride (20 ml) and pyridine (1 ml) gave a third crop of 2,2′-dimer (18.5 g), similar to the second one.

Small-scale anodic oxidation of indene. The small-scale electrolyses were carried out in a water- jacketed cell (volume 50 ml) equipped with a carbon or platinum anode, a carbon cathode, and a magnetic stirrer. The electrolyses were performed at constant current supplied from a 60 V/2 A power supply from Radiak, Sweden.

A solution of indene (0.5 g), and KOAc (3.0 g) in the solvent mixtures (30 g) given in Table 1 was electrolysed until 1.6 F/mol of indene had passed. The solution was poured into water (100 ml) and extracted with ether (100 ml) containing bimesityl (100 mg) as an internal standard. The ether solution was concentrated and analyzed by GLC.

The electrolysis with dicyclohexyl-18-crown-6 present was performed in the following way: A solution of indene (0.5 g), dicyclohexyl-18-crown (0.4 g), and dichloromethane (30 ml) was mixed with solid potassium acetate (3.0 g) and electrolysed until 1.6 F/mol of indene had passed. The dark black-brown solution was worked up as described above.

In order to determine the product *7*, the crude electrolyte from a run of indene (10 g), acetic acid (95 g), water (10 g) and potassium acetate was worked up as described above except that no bimesityl was added. By repeated recrystallization from hexane, and careful washing with pentane, a mixture of *2* and *7* was isolated in better than 80 % purity, as analyzed by GLC. The remainder consisted of the two diacetates *3* and *4*. The NMR spectrum was strongly dominated by *2* but δ values of protons C*H*−OH and C*H*−OAc of *7* would be expected to come close to the corresponding ones of *2*, which was the case (compare with δ values of the 2-formate of *cis-* and *trans-*indan-1,2-diol).[18]

Reaction of 1,2-epoxyindan with acetic acid/potassium acetate. A solution of 1,2-epoxyindan [8] (0.5 g), HOAc (10 ml), and KOAc (1.0 g) was allowed to stand for 24 h at room temperature. The reaction mixture was worked up as described above. NMR, δ ($CDCl_3$) of *2*: 5.1 (d, 1 H, C*H*−OH) and 5.4 (q, 1 H, C*H*−OAc); and of *8*: 4.5 (q, 1 H, C*H*−OH) and 5.9 (d, 1 H, C*H*−OAc). After reflux for 30 min with acetic anhydride (10 ml) and pyridine (1 ml) the products were identified as *3* and *4*.

The INDO calculations. The INDO calculations were performed on the UNIVAC 1108 computer of Lund University Computing Center using the CNINDO program by Pople, Beveridge, and Dobosh.[19] Bond distances for indene, determined by electron diffraction [20] were applied to the radical cation without any effort to minimize the energy. The results appear in Table 2.

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Oxygen—Alkali Treatment of Cellobiose

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C-(2,3-Dihydroxypropyl)tartronic acid is a major dicarboxylic acid formed during O_2-NaOH and O_2-$NaHCO_3$ treatment of cellobiose. The observation that this acid is the main reaction product after alkali treatment of ascorbic acid in O_2-free medium supports the conclusion that it is formed *via* an aglycon moiety related to ascorbic acid. Other dicarboxylic acids from cellobiose are oxalic, tartronic, deoxytetraric, *C*-(hydroxymethyl) tartronic, and succinic acids.

The temperature, pH and additions of iron and cobalt salts strongly influence the product composition. The formation of aldobionic acids from cellobiose parallels the formation of aldonic acid end groups during oxygen bleaching of cellulose.

Determinations of monocarboxylic acids and sugars formed by treating cellobiose with oxygen in sodium hydroxide solution have given valuable information about reactions during oxygen-alkali treatment of cellulose.[1–2] We now report on the dicarboxylic acids formed from cellobiose and on the effect of catalysts upon the product composition. Since the cellulose reactions with oxygen in bicarbonate solution have attracted attention in recent years, a study of the behaviour of cellobiose in this medium is included.

RESULTS AND DISCUSSION

Sugars and monocarboxylic acids. In the experiments with sodium hydroxide, the amount of alkali was chosen so that the sodium hydroxide was consumed. In the experiments carried out for 6 min at 97 °C the final pH was about 9. Among non-volatile compounds, monocarboxylic acids constituted the major fraction, but large amounts of sugars were also formed (Table 1). The presence of a large

Table 1. Recovery of non-volatile solutes (g per 100 g cellobiose) after oxygen-alkali treatment under various conditions.

	0.125 M Sodium hydroxide					$NaHCO_3$
	No addn 6 min 97 °C	Fe 6 min 97 °C	Co 6 min 97 °C	Co 10 min 50 °C	No addn 60 min 97 °C	$+Na_2CO_3$ No addn 90 min 97 °C
4-*O*-(β-D-Glucopyranosyl)-D-glucose	6.0	3.5	1.8	12.9	2.6	25.2
4-*O*-(β-D-Glucopyranosyl)-D-mannose	2.5	1.4	1.1	7.5	1.8	17.2
4-*O*-(β-D-Glucopyranosyl)-D-fructose	tr[a]	tr	tr	0.8	tr	0.8
D-Glucose	4.8	3.7	4.6	6.4	6.4	20.7
D-Mannose	2.0	1.7	2.0	0.2	2.9	0.5
D-Fructose	2.7	0.7	1.5	1.0	2.0	2.2
Monocarboxylic acids	66.8	62.4	55.0	46.8	66.0	20.2
Dicarboxylic acids	4.9	5.8	8.2	3.7	5.4	3.3
Total recovery	89.7	79.2	74.2	79.3	87.1	90.1

[a] tr = less than 0.2.

amount of glucopyranosylmannose shows that Lobry de Bruyn-Alberda van Ekenstein rearrangements occurred. As expected with regard to its lower stability much smaller amounts of glucopyranosylfructose were present. The results confirm that following the introduction of a carbonyl group at C-2 in the reducing glucose moiety by isomerization or oxidation, glucose is liberated by β-elimination. Glucose is further attacked and converted mainly to monocarboxylic acids.[3] It is noteworthy that an increased reaction time from 6 min to 60 min resulted in an increased glucose concentration demonstrating that in weakly alkaline solution the β-elimination is more rapid than the following destruction of glucose. This was confirmed by an experiment in bicarbonate-carbonate solution. Under these conditions the hexoses constituted the largest fraction of the products. From the amounts of hexoses and disaccharides it was estimated that 0.84 mol of hexoses were present per mol of destructed disaccharide. Since hexoses are not formed from the reducing glucose moiety, this experiment shows that in weakly alkaline medium β-elimination and conversion of the reducing moiety to carboxylic acids occur much more rapidly than the attack of oxygen on the liberated glucose.

Moreover, the glucose was converted to mannose and fructose whereas other reactions known to occur in alkaline medium [4] occurred only to a slight extent. The results are similar to those obtained by oxygen-alkali cooking of wood meal in bicarbonate solution where the depolymerization of the carbohydrates results in a liberation of terminal reducing end groups which suffer little oxidation [5] in comparison to that occurring during oxygen-sodium hydroxide treatment of cellulose [6] and xylan.[7]

Anion exchange chromatography of the non-volatile monocarboxylic acids produced during the treatment in bicarbonate solution showed that the *erythro* and *threo* forms of 3-deoxy-2-*C*-(hydroxymethyl)pentonic acids together constituted 15 % (by weight) of this fraction. Evidently the benzilic acid rearrangement of 4-deoxy-2,3-hexodiulose formed from the reducing glucose moiety was of great importance although the experiment was made under oxygen pressure. Other acids derived in part from this dicarbonyl intermediate were 3,4-dihydroxybutanoic (28 %) and glycolic (39 %) acids. The presence of 3-deoxypentonic acid (*threo* and *erythro*, together 12 %) indicated an oxidation of the reducing glucose moiety in cellobiose to a hexosulose moiety at low alkalinity.[8] Glyceric acid which is an important oxidation product formed from glucose in sodium hydroxide[3] was obtained in small amounts (4 %) during the oxidation of cellobiose in bicarbonate solution. These results confirm that the hexoses were only slightly attacked at low pH and that the monocarboxylic acids were mainly formed from the reducing glucose moiety.

Dicarboxylic acids. As can be seen in Table 1 the dicarboxylic acids constituted about 10 % of the non-volatile carboxylic acids formed. No attempt to determine and identify the dicarboxylic acids had been previously made. All dicarboxylic acid fractions were therefore isolated and analysed.

C-(2,3-Dihydroxypropyl)tartronic acid was the major dicarboxylic acid formed during oxygen-sodium hydroxide treatment of cellulose. The large amount of this acid from cellobiose both in hydroxide and bicarbonate media (Table 2) was therefore expected. The results indicate that the acid decomposes on prolonged treatment. According to the reaction scheme proposed for its formation,[6] the acid is formed *via* terminal 2-hexulosonic acid (or the corresponding lactone) groups. The keto group at C-2 gives rise to a β-elimination followed by a benzilic acid rearrangement. A fragmentation of the intermediate dicarbonyl compound leads to the formation of oxalic and 3,4-dihydroxybutanoic acids which were both found after treatment of cellulose and cellobiose.

If the postulated reaction scheme is correct, it should be possible to prepare *C*-(2,3-dihydroxypropyl)tartronic acid from ascorbic acid in alkaline medium (Fig. 1). An experiment with ascorbic acid was therefore made in lime-water. A suspension of calcium hydroxide in 15 ml of boiled water was covered with paraffin oil to prevent dissolution of oxygen. Ascorbic acid (100 mg) in boiled water (4 ml) was then injected into the suspension. After 89 h at 95 °C, the solution contained the expected acid together with unreacted ascorbic acid and its diastereomer (molar ratio ≈2:2:1).

Table 2. Relative amounts (mol %) and retention in gas chromatography (trimethylsilyl derivatives) of dicarboxylic acids from cellobiose.

Acids	Retention rel. to the glucitol derivative QF-1, 120 °C	0.125 M Sodium hydroxide					$NaHCO_3$ + Na_2CO_3
		No addn 6 min 97 °C %	Fe 6 min 97 °C %	Co 6 min 97 °C %	Co 10 min 50 °C %	No addn 60 min 97 °C %	No addn 90 min 97 °C %
C-(2,3-Dihydroxypropyl)-tartronic	1.365	28	17	15	24	15	16
C-(3-Hydroxypropyl)-tartronic [a]	0.689			3	20		
Deoxytetraric	0.280	13	7	5	5	15	7
C-(Hydroxymethyl)tartronic	0.275	6	3	9	3	3	3
Tartronic	0.187	19	44	30	25	22	8
Succinic	0.141	6	4	3	3	4	11
Oxalic	0.075	28	25	38	21	41	55

[a] Tentative identification based on mass spectrum of the Me_3Si derivative.

These acids were identified by GC and GC-MS The remaining material (15 %) consisted of a wide range of other products. At 23 °C the reaction was very slow and even after 310 h the starting material was virtually unchanged. The results indicate that hexulosonic acid end groups are formed during oxygen-alkali treatment of cellulose and cellobiose and that *C*-(2,3-dihydroxypropyl)tartronic acid is derived from these.

Tartronic, deoxytetraric, and *C*-(hydroxymethyl)tartronic acids were also produced during oxygen-alkali treatment of cellulose.[6] With cellobiose the relative amount of oxalic acid was much larger in the experiment in bicarbonate medium than in the short-time treatments in sodium hydroxide whereas with tartronic acid the opposite holds true. These observations indicate that oxalic acid is formed mainly from the reducing end group while an oxidation of the liberated glucose is mainly responsible for the formation of tartronic acid.

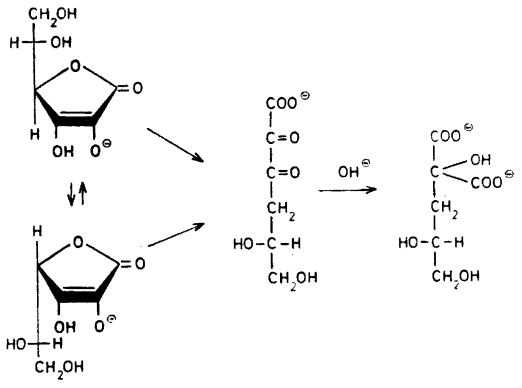


Fig. 1. Schematic representation of the formation of *C*-(2,3-dihydroxypropyl)tartronic acid from ascorbic acid in alkaline medium.

This hypothesis was confirmed in a separate experiment with glucose (0.53 % in 0.125 M NaOH, 97 °C, 30 min). The dicarboxylic acid fraction obtained in a yield of 4.6 % (compared to 74.0 % non-volatile monocarboxylic acids and 8.3 % remaining monosaccharides) was studied by GC and GC-MS. Tartronic and oxalic acids in a molar ratio of 6:1 were the major dicarboxylic acids. A large number of other acids together constituting about 25 % of the fraction were recorded. Among these deoxytetraric and *C*-(hydroxymethyl)tartronic acids were identified.

Effect of catalysts on the product composition. During oxygen-alkali treatment of cellobiose at low temperature and high alkali concentration aldobionic acids are the dominant products.[1] By lowering the alkali concentration and increasing the temperature, their yield decreases[2] whereas the fraction of acids formed after β-elimination increases markedly. Similarly, the number of aldonic acid end groups in cellulose relative to the amount of soluble carboxylic acids is lower after oxygen-alkali

Table 3. Aldobionic acids produced by oxygen-alkali treatment of cellobiose in 0.125 M sodium hydroxide. Weights refer to 100 g cellobiose.

Acids	No addn 6 min 97 °C g	Fe 6 min 97 °C g	Co 6 min 97 °C g	Co 10 min 50 °C g
4-*O*-(β-D-Glucopyranosyl)-D-mannonic	0.50	0.29	0.68	0.37
4-*O*-(β-D-Glucopyranosyl)-D-gluconic	0.17	0.22	0.35	0.22
3-*O*-(β-D-Glucopyranosyl)-D-arabinonic	2.29	1.72	2.71	3.67
2-*O*-(β-D-Glucopyranosyl)-D-erythronic	0.75	0.30	0.31	0.86
2-*O*-(β-D-Glucopyranosyl)-D-threonic	0.19	0.07	0.15	0.08
Total	3.9	2.6	4.2	5.2

treatment of cellulose under conditions simulating oxygen bleaching[6] (low alkali concentration, high temperature) than that observed after ageing of alkali cellulose. The results given in Table 3 show that, as expected, a fairly small fraction of the cellobiose was converted to aldobionic acids during the oxygen treatment in 0.5 % sodium hydroxide at 97 °C, whereas at 50 °C the abundance of aldobionic acids was significantly larger. The results confirm that under the applied conditions cleavage of the glycosidic bond by β-elimination is favoured compared to oxidation. In accordance with earlier findings, glucopyranosylmannonic acid was formed in larger amounts than glucopyranosylgluconic acid.

It is seen that the addition of catalysts had a significant effect on the relative amounts of the two diastereomers with terminal hexonic acid groups. This was also observed with cellulose where in experiments without added catalysts the number of mannonic acid end groups was much larger than that of gluconic acid groups while the opposite was found in experiments with additions of cobalt and iron.[6] These results together with those given in Table 3 indicate that with both cellulose and cellobiose the formation of *arabino*-hexosulose end groups is less important in the presence of cobalt and iron than in their absence. It is reasonable to assume that in the presence of catalysts the major portion of gluconic acid end groups is formed by some other reaction mechanism than by benzilic acid rearrangement of *arabino*-hexosulose moieties.

As can be seen from Tables 1 and 2 the amounts of other products were also affected by the presence of cobalt and iron. In the experiments made under otherwise unchanged conditions, the total amount of recovered non-volatile compounds was significantly lower than in the blank and the amount of dicarboxylic acids was higher. Among these, tartronic acid was formed in larger amounts in the presence of the metal compounds. In the experiments with cobalt at 50 °C a large amount of an acid tentatively identified as *C*-(3-hydroxypropyl)tartronic acid was recorded. This acid was also present after the experiment with cobalt at 97 °C but was neither detected in the presence of iron nor in the blank. Inspection of the tables shows that in addition there are several other differences between cobalt (*e.g.* larger formation of volatile compounds and oxalic acid) and iron (*e.g.* larger relative amount of tartronic acid). Moreover, the amount of fructose was much lower in the presence of iron than in the run with cobalt or in the blank. Evidently, metal compounds exert a great influence not only on the rate of cleavage of the glycosidic bond[9] but also on the consecutive reactions.

EXPERIMENTAL

The oxygen-alkali treatments were performed in a Teflon reactor.[10] A preheated cellobiose solution (1 g in 10 ml water) was injected into 90 ml of a preheated sodium hydroxide solution in equilibrium with oxygen at a gauge pressure of 0.6 MPa. The concentration of sodium hydroxide in the reaction mixture was 0.125 M and that of transition metals 0.05 mM. The metal compounds [$FeSO_4$ and $Co(NO_3)_2$] were added to the preheated cellobiose solution. In one experiment the sodium hydroxide was

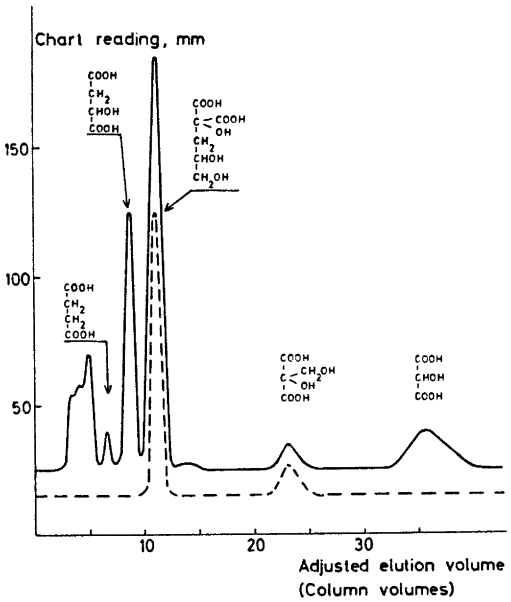


Fig. 2. Ion exchange chromatography of dicarboxylic acids (0.05 mmol) in 0.3 M sodium acetate in 2 M acetic acid at 30 °C. Column: 4 × 815 mm, Dowex 1 − X8; 14 − 17 μm. Nominal linear flow: 4.5 cm min^{-1}. (————), chromic acid channel, (– – – –), periodate-formaldehyde channel (succinic acid recorded in the chromic acid channel by oxidation in the presence of silver sulfate).

replaced by a mixture of sodium bicarbonate (0.043 M) and sodium carbonate (0.009 M). After the experiments the reaction mixture was cooled in ice-water and kept at − 18 °C.

The yields of non-electrolytes, monocarboxylic and dicarboxylic acids were determined after group separation of an aliquot of the reaction solution (pH 9) on an anion exchanger in the acetate form (Dowex, 1-X8). The non-electrolytes were displaced with 7 column volumes of water, the monocarboxylic acids eluted with 17.5 column volumes of 2 M acetic acid and the dicarboxylic acids with 15 column volumes of 0.3 M magnesium acetate. After cation exchange (Dowex 50 − X8, H^+) the fractions (Table 1) were evaporated to dryness.

The non-electrolyte fraction was analysed by partition chromatography on an anion exchanger in the sulfate form.[11] The monocarboxylic acid fraction was chromatographed on an analytical anion exchange column[12] in 0.5 M acetic acid and in 0.08 M sodium acetate. The glucosylaldonic acids were isolated by applying an aliquot of the alkaline reaction solution to a preparative anion exchange column and eluting the monocarboxylic acids with 0.08 M sodium acetate (pH 5.9). The aldobionic acids which appeared in a single band before the other acids[1] were hydrolyzed[13] and the resulting aldonic acids analysed by GC as trimethylsilyl (Me_3Si) derivatives.

The dicarboxylic acid fraction obtained from the experiment in sodium hydroxide (6 min) without catalysts was studied by anion exchange chromatography (Fig. 2). The chromatographic bands from a preparative run were further analyzed by GC.[14] Only GC was used for the separation of the individual acids in the other experiments. Each of the acids (Table 2) was identified by MS in at least one of the experiments. Deoxytetraric and *C*-(hydroxymethyl)tartronic acids were not resolved on QF-1 but could be analyzed on OV-17. In addition to the major products listed in Table 2 at least five minor compounds together constituting less than 15 % of the total fraction were recorded by GC. The quantitative determinations were based on the assumption that the GC peak areas were proportional to the weight of the derivatives.

To confirm the identity of *C*-(2,3-dihydroxypropyl)tartronic acid, a sample isolated by anion exchange chromatography was dissolved in water and evaporated to dryness in 3 M HCl. The resulting esters (including lactones) were reduced with borohydride.[15] Analysis of the reaction products by GC-MS gave 3-deoxy-2-*C*-hydroxymethyl-*erythro*-pentonic acid, 3-deoxy-2-*C*-hydroxymethyl-*threo*-pentonic acid, and 3-deoxy-2-*C*-(hydroxymethyl)pentitol (molar ratio ≈3:1:2). Less than 1 % of the starting material remained unchanged. The results confirm (*cf.* Ref. 6) that the acid was *C*-(2,3-dihydroxypropyl)tartronic acid. The presence of the corresponding alditol among the reduction products indicates that, in addition to lactones, intermolecular esters were formed during the evaporation in acid medium.

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Short Communications

The Synthesis of 3-Bromo-2-fluoronitrobenzene and its Reduction by Copper(I)

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Many efforts were made by various methods to prepare 3-bromo-2-fluoronitrobenzene, which was desired for the purpose to study its reaction with copper(I) chloride.

An attempt to decompose thermally the 2-bromo-6-nitrobenzenediazonium fluoroborate, which was obtained in 59 % yield from 2-bromo-6-nitroaniline (available from previous work [1]) by the method of Lamm and Andersson,[2] was unsuccessful. The decomposition of the fluoroborate in aqueous acetone medium with copper(I) chloride following a description of Bergmann *et al.*[3] as well as the diazotization of 2-bromo-6-nitroaniline in anhydrous hydrogen fluoride followed by heating and decomposing according to Ferm and van der Werf [4] also failed to give the fluoro compound.

A halogen exchange of 3-bromo-2-chloronitrobenzene with potassium fluoride in dimethylformamide by a method described by Finger and Kruse [5] was found to be favourable and gave a yield of 76 % of the 3-bromo-2-fluoronitrobenzene. Finger and Kruse [5] reported that aryl halide-potassium fluoride exchanges are at least 10^3 times faster in a dipolar aprotic solvent (DMF) than in a protic one (methanol). Fluoride ion is much less solvated and thus more powerful as a nucleophile in dipolar aprotic solvents.

The reaction of 3-bromo-2-fluoronitrobenzene in aqueous hydrochloric acid-acetic acid with copper(I) chloride was carried out under an argon atmosphere at 90 °C as described in Ref. 1. After 23 h, 16 % of 3-bromo-2-fluoroaniline was obtained, as determined by GLC and mass spectrum, which had a 1:1 doublet at *m/e* 189 and 191. The remaining part was unreacted 3-bromo-2-fluoronitrobenzene. No fluorine-chlorine exchange products were detected. This is in full agreement with the observation of Bacon and Hill [6] who noticed "no reaction" between 1-fluoronaphthalene and copper(I) chloride in pyridine. In previous work [7] the copper(I) catalysed bromine-chlorine exchange in halonitrobenzenes was found to be about three times faster than the iodine-chlorine exchange. Bacon and Hill [6] give the following order of reactivity for 1-halonaphthalenes with copper(I) chloride in pyridine:
$ArI \approx ArBr > ArCl \gg ArF$.
This agrees with the tendency of different halide ions to formation of the copper(I) halide complexes in aqueous solution; the stability of the complexes increases with increasing atomic number of the halogen. Actually complex formation between copper(I) ion and fluoride ion is unknown. However, in our case, with the assumed approximately tetrahedral copper halide-aryl halide transition state, one must also consider the most favourable geometry.[1]

The observed reduction of the nitro group, which always occurs as a side reaction of about 2 – 3 % in all copper(I) catalysed bromine-chlorine and iodine-chlorine exchange reactions studied by the present author, will be more fully discussed in a following paper [8] concerning alkyl substituted bromonitrobenzenes and experiments with some free radical initiators and inhibitors. With the present compound, 3-bromo-2-fluoronitrobenzene, only one experiment was carried out: 1,4-dihydroxybenzene, known as an inhibitor, was added in equimolecular amount to the nitro compound in the reaction mixture, but no change in the degree of reduction was noticed. In the absence of copper(I) chloride in the dark, neither exchange nor reduction was observed. When the reaction mixture with copper(I) chloride present was irradiated at 30 °C for 2 h at 350 nm, the rate of reduction increased compared to the reduction at 30 °C in the dark. Mechanistic aspects of these results will be discussed in the following paper.[8]

Experimental. Melting points were determined with a Kofler Hot-Stage Microscope.

The GLC investigations were performed on a Perkin Elmer F 11 Hot Wire Gas Chromatograph with a 3 mm o.d. 2 m SE-30 column at 120 °C. Helium was carrier gas and the instrument was equipped with a Varian Model 480 Electronic Digital Integrator.

The ^{19}F NMR spectrum was recorded on a Varian XL-100-15 spectrometer at Instrumentstationen, The Chemical Center, University of Lund.

The irradiation was carried out in a photochemical reactor, Rayonet RPR-100.

The mass spectra were obtained on an LKB 9000 instrument at Crystallography group,

Mass Spectrometric Service at the University of Göteborg.

3-Bromo-2-chloronitrobenzene prepared from 2-bromo-6-nitroaniline by the same method as described for 2-chloro-3-fluoronitrobenzene,[9] gave a yield of 81 % after steam distillation and one recrystallization from hexane, m.p. 57.0 – 58.5 °C, lit.[1] 57.0 – 58.0 °C.

3-Bromo-2-fluoronitrobenzene. 3-Bromo-2-chloronitrobenzene (14.2 g, 0.06 mol, dried over P_2O_5) was boiled for 7 days at 150 °C with 6.95 g (0.12 mol) of commercial anhydrous potassium fluoride (which was heated before use in order to remove all moisture) in 25 ml of dimethylformamide, dried over molecular sieves (4 Å). The reaction was followed by GLC. After 7 days the reaction had gone to 96 – 97 % completion. The yield was 10.0 g (76 %) after steam distillation. After two recrystallizations from hexane and two from methanol, the yield was 2.4 g, m.p. 29.5 – 30.5 °C. 3-Bromo-2-fluoronitrobenzene has apparently not been described in the literature. The mass spectrum revealed a 1:1 doublet at *m/e* 219 and 221, as expected for the desired compound.

^{19}F NMR spectrum for 3-bromo-2-fluoronitrobenzene in $CDCl_3$: 111 ppm upfield from CCl_3F (octet). J_{F-H_5} 1.48 Hz, J_{F-H_4} 5.75 or 6.45 Hz and J_{F-H_6} 6.45 or 5.75 Hz.

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The Role of Oxaloacetate as Feed-back Inhibitor of Isocitrate Lyase in Baker's Yeast

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It has been shown that oxaloacetate competitively inhibits purified isocitrate lyase from *Chorella pyrenoidosa*,[1] *Brevibacterium flavum*,[2] and *Candida guillermondii*.[3] For oxaloacetate to function as a feed-back inhibitor of isocitrate lyase (EC 4.1.3.1) *in vivo*, it must inhibit the lyase in the conditions existing inside the cell. Because the Michaelis constant for *threo*-D_s-isocitrate of isocitrate lyase from *Chlorella* is smaller (23 μM) than the *in vivo* concentration of isocitrate, John and Syrett [1] concluded that the enzyme was normally saturated with its substrate, and therefore that oxaloacetate, a competitive inhibitor, cannot cause significant inhibition *in vivo*. However, the Michaelis constant is much higher (300 μM) for the enzyme from *C. guillermondii*, and Hildebrandt and Weide [3] have concluded that oxaloacetate may be an effective regulator of isocitrate lyase in this organism. For both enzymes inhibition constants are small (37 and 50 μM, respectively). We have investigated the inhibition of isocitrate lyase from baker's yeast, *Saccharomyces cerevisiae* by several intermediates of the TCA cycle. Our results are quite similar to those of Hildebrandt and Weide for *C. guillermondii*,[3] except in the case of oxaloacetate. Reaction rates were estimated by a method which prevented contact between the oxaloacetate and phenylhydrazine reagent used to estimate the product, glyoxylic acid.

Material and methods. A_4 growth-stage baker's yeast from Alko Yeast Factory, Rajamäki, was used. The culture has been described in detail elsewhere.[4] Sodium glyoxylate was from Fluka AG (Buchs, SG., Switzerland), trisodium-DL-isocitrate from the Sigma Chemical Company (St.Louis, Mo., USA) and oxaloacetate from C. F. Boehringer & Soehne GmbH (Mannheim, West-Germany). All other reagents were of analytical grade from E. Merck AG (Darmstadt, West-Germany).

Isocitrate lyase was purified as follows. 100 g baker's yeast was suspended in 250 ml 0.2 M potassium phosphate buffer pH 7.5 Ballotini beads (40 ml, No. 31/12, diameter 0.25 mm) were added to 25 ml fractions and the cells were treated for 6 min in a Mini-mill disintegrator (Gifford-Wood Co., New Jersey, USA) using a rotor speed of 3300 rpm. The cup of the apparatus was kept in an ice-bath during the disintegration.

The protamine sulfate and ammonium sulfate precipitations, dialysis, and chromatography on

Mass Spectrometric Service at the University of Göteborg.

3-Bromo-2-chloronitrobenzene prepared from 2-bromo-6-nitroaniline by the same method as described for 2-chloro-3-fluoronitrobenzene,[9] gave a yield of 81 % after steam distillation and one recrystallization from hexane, m.p. 57.0 – 58.5 °C, lit.[1] 57.0 – 58.0 °C.

3-Bromo-2-fluoronitrobenzene. 3-Bromo-2-chloronitrobenzene (14.2 g, 0.06 mol, dried over P_2O_5) was boiled for 7 days at 150 °C with 6.95 g (0.12 mol) of commercial anhydrous potassium fluoride (which was heated before use in order to remove all moisture) in 25 ml of dimethylformamide, dried over molecular sieves (4 Å). The reaction was followed by GLC. After 7 days the reaction had gone to 96 – 97 % completion. The yield was 10.0 g (76 %) after steam distillation. After two recrystallizations from hexane and two from methanol, the yield was 2.4 g, m.p. 29.5 – 30.5 °C. 3-Bromo-2-fluoronitrobenzene has apparently not been described in the literature. The mass spectrum revealed a 1:1 doublet at *m/e* 219 and 221, as expected for the desired compound.

^{19}F NMR spectrum for 3-bromo-2-fluoronitrobenzene in $CDCl_3$: 111 ppm upfield from CCl_3F (octet). J_{F-H_5} 1.48 Hz, J_{F-H_4} 5.75 or 6.45 Hz and J_{F-H_6} 6.45 or 5.75 Hz.

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The Role of Oxaloacetate as Feed-back Inhibitor of Isocitrate Lyase in Baker's Yeast

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It has been shown that oxaloacetate competitively inhibits purified isocitrate lyase from *Chorella pyrenoidosa*,[1] *Brevibacterium flavum*,[2] and *Candida guillermondii*.[3] For oxaloacetate to function as a feed-back inhibitor of isocitrate lyase (EC 4.1.3.1) *in vivo*, it must inhibit the lyase in the conditions existing inside the cell. Because the Michaelis constant for *threo*-D_s-isocitrate of isocitrate lyase from *Chlorella* is smaller (23 μM) than the *in vivo* concentration of isocitrate, John and Syrett [1] concluded that the enzyme was normally saturated with its substrate, and therefore that oxaloacetate, a competitive inhibitor, cannot cause significant inhibition *in vivo*. However, the Michaelis constant is much higher (300 μM) for the enzyme from *C. guillermondii*, and Hildebrandt and Weide [3] have concluded that oxaloacetate may be an effective regulator of isocitrate lyase in this organism. For both enzymes inhibition constants are small (37 and 50 μM, respectively). We have investigated the inhibition of isocitrate lyase from baker's yeast, *Saccharomyces cerevisiae* by several intermediates of the TCA cycle. Our results are quite similar to those of Hildebrandt and Weide for *C. guillermondii*,[3] except in the case of oxaloacetate. Reaction rates were estimated by a method which prevented contact between the oxaloacetate and phenylhydrazine reagent used to estimate the product, glyoxylic acid.

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The protamine sulfate and ammonium sulfate precipitations, dialysis, and chromatography on

a DEAE column were performed according to Olson.[5] No heat treatment or ethanol precipitation was performed. The buffer solutions used were essentially the same as those of Olson,[5] but were 3 mM in $MgCl_2$, and 1 mM in cysteine and EDTA. A purification of about 40 fold was obtained.

The isocitrate lyase activity was determined at pH 6.0[5] and 30 °C, by the method of Dixon and Kornberg.[6] The reaction was initiated by adding DL-isocitrate. In the inhibition experiments the enzyme and inhibitor were incubated together for 10 min at 30 °C before the substrate was added. However, to prevent contact between oxaloacetate and phenylhydrazine when determining the effect of oxaloacetate on the enzyme activity, gloxylate formed in the reaction was estimated colorimetrically as follows.[7] At the end of the enzymic reaction 0.3 ml 0.5 M oxalic acid and 0.2 ml 1 % phenylhydrazine were added to 1 ml of reaction mixture, which contained 65 μM potassium phosphate buffer, pH 6.0, 2 μM cysteine, 1 – 5 μM oxaloacetate, 2.5 μM $MgCl_2$, about 5 μl enzyme and 0.5 – 5 μM DL-isocitrate. The mixture was held for 5 min at 105 °C and then in ice for at least 5 min. To the chilled mixture was added 1 ml concentrated hydrochloric acid and 0.5 ml 3 % potassium ferricyanate. The absorbance at 520 nm was measured on a Perkin-Elmer 124 double-beam spectrophotometer between 2 and 5 min after adding ferricyanate. At the same time an individual gloxylate standard was determined for each inhibitor concentration.

The protein content was estimated from the equation: protein content (mg/ml) = $0.77A_{280} - 0.38A_{260}$, where A_{280} and A_{260} are the absorbances at 280 and 260 nm, respectively.[8] The enzyme preparation used produced 3 μmol glyoxylate min^{-1} (mg protein)$^{-1}$ at 30 °C.

Results. The method of Dixon and Kornberg [6] gave a value of 0.71 mM for the Michaelis constant (Fig. 1). According to the manufacturer, *threo*-D_S-isocitrate, the real substrate for the enzyme, constitutes 49.5 % of the DL-isocitrate preparation used, and so the corrected value for the Michaelis constant for this substrate is 0.35 mM. Olson [5] reported that *threo*-L_S-isocitrate does not inhibit isocitratelyase. In Fig. 2a are Lineaweaver-Burk plots obtained at different concentrations of oxaloacetate. In Fig. 2b the slopes of the straight lines in Fig. 2a are plotted as a function of the inhibitor concentration. It can be seen that oxaloacetate causes linear competitive inhibition, with an inhibition constant of 1.1 mM.

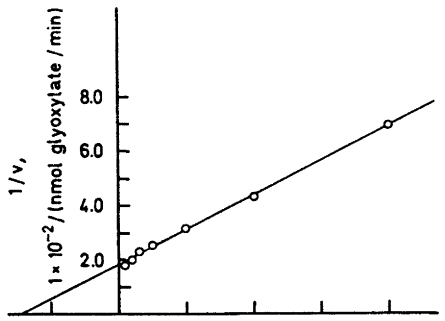


Fig. 1. Michaelis constant for DL-isocitrate at pH 6.0 and 30 °C. The data were obtained by the method of Dixon and Kornberg.[6]

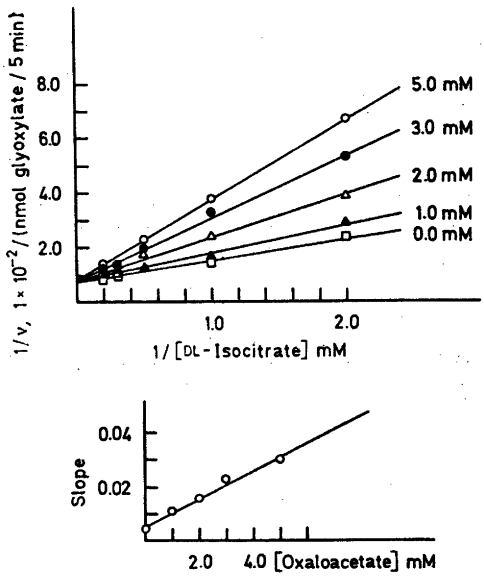


Fig. 2a. Oxaloacetate inhibition of inhibitor concentrations of 0, 1, 2, 3, and 5 mM.
Fig. 2b. Gradients of the straight lines obtained in Fig. 2a as a function of inhibitor concentration.

Discussion. The Michaelis constant obtained for *threo*-D_S-isocitrate (0.35 mM) is only a third the size of that reported (1.2 mM) by Olson for baker's yeast,[5] but is in good agreement with the value (0.3 mM) obtained by Hildebrandt and Weide.[9]

The oxaloacetate inhibition constant obtained is an order of magnitude larger than those reported by John and Syrett [1] and Hildebrandt and Weide,[3] and slightly larger than that (1 mM) of Ozaki and Shiio.[2] The experimental method employed differs from that of Hildebrandt and Weide [3] in that a different yeast species was used and that the phenylhydrazine reagent was not allowed to come in contact with the oxaloacetate until the enzymic reaction was complete. Ozaki and Shiio [2] used a scheme corresponding to the one described here.

Although they determined the inhibition at only one oxaloacetate concentration, Hildebrandt and Weide [3] supposed that the competitive inhibition by oxaloacetate of isocitrate lyase from *C. guillermondii* was linear. Our results show this to be correct for the enzyme from *S. cerevisiae*. When the inhibition of isocitrate lyase caused by oxaloacetate is small, the regulation mechanism suggested by Hildebrandt and Weide [3] for *C. guillermondii* is not possible for baker's yeast. However, according to the inhibition constant for oxaloacetate of 1.1 mM reported here, and a value for the concentration of oxaloacetate of less than 25 μM in baker's yeast grown on ethanol,[10] the maximum inhibition of isocitrate lyase by oxaloacetate (*i.e.*, at very low isocitrate concentrations) is only 2.5 %. Further, at 25 °C and pH 7.4 citrate and isocitrate make up 91 and 6 %, respectively, of the aconitase reaction equilibrium mixture.[11] The citrate concentration in baker's yeast grown on ethanol is about 2.5 mM,[12] so that the concentration of isocitrate may well be as high as 0.15 mM, which is comparable with, but rather lower than, its Michaelis constant. From these calculations we conclude that oxaloacetate cannot act as a feed-back inhibitor of isocitratelyase in baker's yeast.

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Evidence for an Ionic Mechanism for the Reaction of 2-Cyclopropylpyridine *N*-Oxide with Acetic Anhydride

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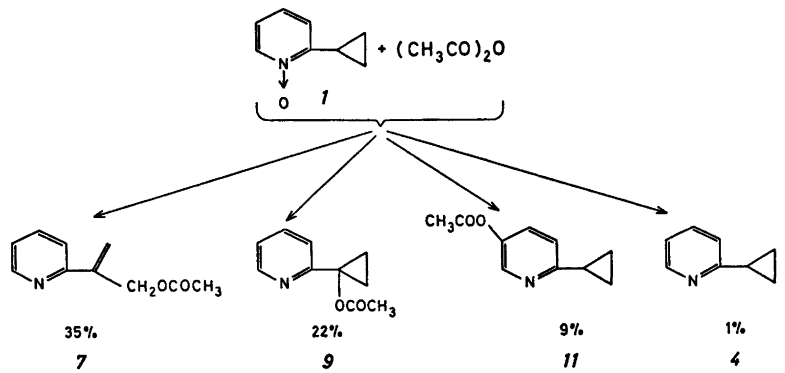
The rearrangement of 2-cyclopropylpyridine *N*-oxide with acetic anhydride afforded 2-(2-pyridyl)-2-propenyl acetate and 2-(1-acetoxycyclopropyl)pyridine as the main products; 5-acetoxy-2-cyclopropylpyridine was also isolated in moderate yield. The opening of the cyclopropyl ring provides evidence for an ionic mechanism. This was supported by experiments with ^{18}O-labeled acetic anhydride.

Reaction of 2-alkylpyridine *N*-oxides with acetic anhydride proceeds with rearrangement to the corresponding 2-acetoxyalkylpyridines. The mechanism is still controversial;[1–7] the results seem best accommodated by a tight ion pair mechanism but neither radical pairs nor concerted reaction paths can be entirely excluded.

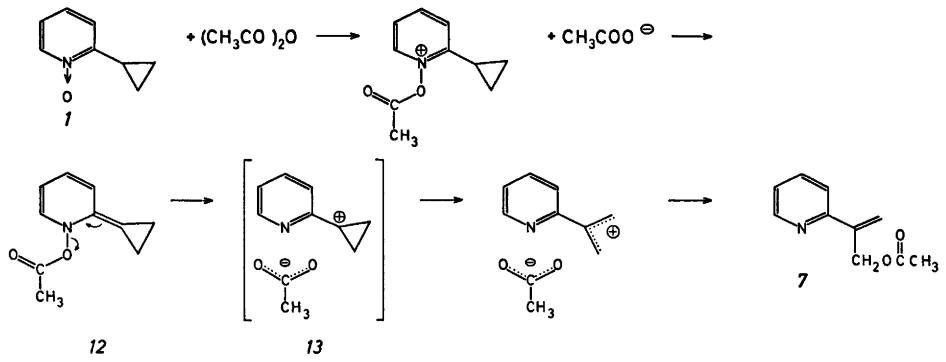
The *N*-oxide of 2-cyclopropylpyridine (*1*) should lend itself particularly well for obtaining direct proof for an ionic reaction path since the cyclopropyl cation would only be expected to have a transient existence [8] while the corresponding radical is expected to survive the reaction conditions. [9]

When the present work was undertaken, no satisfactory method for the preparation of 2-cyclopropylpyridine was available.[10–13] The double bonds of 2-vinylpyridine (*2*) and 4-vinylpyridine (*3*) are electron deficient and might therefore react with sulfur ylids to yield the corresponding cyclopropane derivatives. Indeed both isomers *2* and *3* reacted readily with dimethylsulfonium methylide to give 2-(*4*) and 4-cyclopropylpyridine (*5*) in 63 and 60 % yields, respectively. After this work was completed Levine and Patrick [14] reported an extensive study on the same reaction with similar results. Compounds *2* and *3* reacted also with dimethyloxosulfonium methylide, but the yields of the cyclopropanes *4* and *5* were only 7 and 19 %, respectively.

Peracetic acid oxidation of 2-cyclopropylpyridine (*4*) afforded the *N*-oxide *1* in 88 % yield. When a solution of the latter in acetic



Scheme 1.



Scheme 2.

anhydride was heated to 160 °C for 2 h a product consisting of essentially three isomeric compounds was obtained in 66 % yield. They were separated by preparative gas chromatography, and identified on the basis of spectroscopic data. The major product (35 %) was shown to be 2-(2-pyridyl)-2-propenyl acetate (*7*) which on basic hydrolysis gave the corresponding alcohol *8*. The second product (22 %) was identified as 2-(1-acetoxycyclopropyl)pyridine (*9*); in this case basic hydrolysis led to the known 1-(2-pyridyl)-1-propanone (*10*).[15] The spectral properties of the minor product (9 %) were in acoordance with the structure of 5-acetoxy-2-cyclopropylpyridine (*11*); the 3-acetoxy isomer could not be detected in the reaction mixture. No interconversion of the esters *7* and *9* took place under the reaction conditions. In addition to the above compounds the product mixture contained a small amount of 2-cyclopropylpyridine (1 %) which was most probably formed from the *N*-oxide *1* by a deoxygenation process.

The formation of compound *7* is particularly interesting because it provides evidence for an ionic mechanism as depicted in Scheme 2.

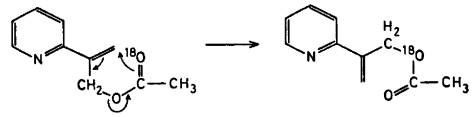
The heterolytic cleavage of the N – O bond in the anhydro base *12* affords a cyclopropyl cation *13* which undergoes ring-opening before recombining with an acetate anion.

The formation of compound *9* is surprising because the cyclopropyl ring is preserved. It cannot easily be accepted as a result of an ionic mechanisn; a radical pair mechanism or a concerted rearrangement of the anhydro base would appear to be more reasonable explanations. On the other hand, compound *11* may originate from any of the three mechanistic pathways. In an attempt to distinguish between these alternatives the reaction was carried out with ^{18}O-labeled acetic anhydride (*14*) and the products were analysed by mass spectrometry.

No molecular ion was obtained from the anhydride *14* and therefore the atom % of ^{18}O was calculated from *m/e* 43 and 45 and was found to be 8.1 %. The excess acetyl chloride from the preparation of the anhydride *14* contained approximately the same amount of ^{18}O (7.8 %) and equilibration of all oxygen atoms in the anhydride therefore must have occurred, *e.g.* 8.1 % labeling per oxygen.

The ^{18}O-content of the esters *7*, *9*, and *11* was calculated from *m/e* 43 – 45 for the carbonyl oxygen and *m/e* 134 – 136 and 135 – 137 for the ether oxygen; peaks corresponding to the molecular ions were either lacking or of too low intensity for the desired accuracy. The results are presented in Table 1 as a mean of three to eight measurements; owing to random distribution the error is estimated to be ± 0.3.

An oxy-Cope type rearrangement of the ester *7* in the mass spectrometer would result in scrambling of the labeled oxygens; however, analyses of the corresponding alcohol *8* gave the same amount of labeling as in the corresponding oxygen of ester *7*; such a rearrange-



Scheme 3.

Table 1. Percentages of ^{18}O in acetyl chloride, acetic anhydride, and the pyridines studied calculated from peaks at *m/e* 43–45 and *m/e* 134–136 and 135–137 in their mass spectra after correction for ^{13}C-abundance.

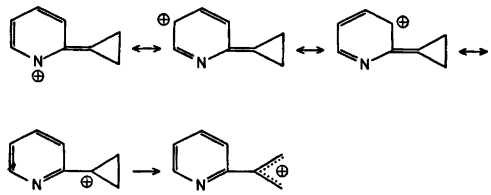
Compound	*m/e* 43–45	*m/e* 134–136 135–137	Total
CH_3COCl[a]	7.8		
$(CH_3CO)_2O$ (*14*)	8.1		
CH_3COOH[b]	7.2		
7	6.3	6.1	12.4
8		6.2	
9	5.6	2.4	8.0
11	6.5	4.4	10.9
7[c]	4.8	5.2	10.0
CH_3COOH[bc]	4.9		

[a] Excess acetyl chloride distilled off from the reaction mixture from the preparation of the ^{18}O-labeled acetic anhydride (*14*). [b] Acetic acid and excess acetic anhydride distilled off from the reaction mixture after the rearrangement reaction. [c] From the control experiment starting with unlabeled ester *7* and 7.2 % labeling per oxygen in the acetic anhydride/acetic acid mixture.

ment cannot therefore have altered the isotopic distribution.

Since one acyl group adds to the *N*-oxide an intramolecular rearrangement would require a total labeling in the resulting esters of 8.1 %,* the same amount as in one oxygen atom of the anhydride. Higher ^{18}O-content in any of the esters could be due to contributing ionic intermolecular processes since the acetate anion formed from the acetic anhydride contains 16.2 % of labeled oxygen. However, an alternative explanation is possible. If an acetate exchange takes place between the labeled acetic anhydride/acetic acid and either of the esters a higher than 8.1 % content of ^{18}O would result. Such an exchange took place in the case of the allylic ester *7* (Table 1), and we can therefore not conclude that the high content of ^{18}O measured for this compound is due to an intermolecular process.

The total ^{18}O-content of the ester *9* is 8.0 % as expected for an intramolecular process and the distribution reveals that the process is not concerted. In 5-acetoxy-2-cyclopropylpyridine (*11*), however, the total ^{18}O-content of 10.9 % is too high to originate from an intramolecular process exclusively; hence, the formation of compound *11* could partly involve an ionic intermolecular pathway. The positive charge in the 2-cyclopropylpyridine cation intermediate *13* is resonance stabilized by the pyridine ring thus giving this intermediate the possibility to exist without ring opening sufficiently for both the esters *9* and *11* to be formed through ionic intermediates (Scheme 4).



Scheme 4.

The uneven ^{18}O-distribution in the esters *9* and *11* shows that in both cases the rate of recombination and that of rotation of the acetate anion are comparable. The predominance of isotopic labeling in the carbonyl oxygen confirms the findings of Oae *et al.*[4,16]

Our work with 2-cyclopropylpyridine *N*-oxide gives ample evidence for an ionic mechanism for ~53 % of the products. There is also good reason for assuming essentially an ionic intramolecular recombination for the remaining 47 %, even if traces of a concerted reaction cannot be entirely excluded.

EXPERIMENTAL

Melting points were determined on a micro hot stage. Preparative gas chromatography was carried out on a Varian Aerograph Model 711 with 15 % SE 52 on Chromosorb W 30–60 mesh. UV spectra were recorded on Cary 14 Spectrophotometer in ethanol, IR spectra on a Perkin-Elmer 457 Grating Infrared Spectrophotometer (film or KBr), NMR spectra on Varian A-60 and HA 100 Spectrometers with TMS as internal standard and the mass spectra on an AEI/EC MS 902 instrument with indirect inlet, 70 eV, high resolution and absolute intensities which had to be corrected for ^{13}C-abundance. All glassware used in ^{18}O-labeled work was dried at 150 °C for 20 h before use.

* The naturally occurring isotope in the *N*-oxide *1* is only 0.2 % which is less than the estimated error and is therefore neglected.

Elemental analyses were performed by I. Beetz, West Germany.

2-Cyclopropylpyridine (4). To sodium hydride 50 % dispersed in oil (8 g, 167 mmol) dimethyl sulfoxide (60 ml) (distilled from calcium hydride) was slowly added with stirring under nitrogen. The reaction mixture was kept at 75 °C for 30 min until evolution of hydrogen had ceased. Dry tetrahydrofuran (60 ml) was added and the solution was cooled on ice before addition of trimethylsulfonium iodide (35 g, 172 mmol) in dimethyl sulfoxide (180 ml). After 1 min 2-vinylpyridine (*2*) (17 g, 162 mmol) was added and the ice bath was removed after 45 min allowing the reaction mixture to attain room temperature. Water was added and the solution was extracted with ether. The combined extracts were dried and distillation gave 12 g (63 %) of 2-cyclopropylpyridine (*4*), b.p. 26–28 °C/0.05 mmHg, (lit.[14] b.p. 67–70 °C/20 mmHg). The UV, IR, and NMR spectra were consistent with those previously reported.[13]

2-Cyclopropylpyridine N-*oxide (1).* A mixture of 2-cyclopropylpyridine (*4*) (7 g, 59 mmol) and hydrogen peroxide (30 %, 10 ml, 86 mmol) in acetic acid (70 ml) was kept at 70 °C for 20 h. The acetic acid was removed and the residue distilled to give 7 g (88 %) of compound *1*, b.p. 100–113 °C/0.02 mmHg, m.p. ~30 °C. NMR ($CDCl_3$): δ 0.58–1.37 and 2.47–3.98 (cyclopropyl H, complex signal), 6.72–7.38 and 8.13–8.34 (aromatic H, complex signal). IR: 1240 cm^{-1} s. MS: *m/e* 135 (M^+), 119, 118 (base peak) and 116. (Found: C 70.95; H 7.07. Calc. for C_8H_9NO: C 71.07; H 6.81).

*Rearrangement of 2-cyclopropylpyridine-*N-*oxide.* The *N*-oxide *1* (6 g, 44 mmol) was dissolved in acetic anhydride (220 ml) and heated to 160 °C for 2 h. The excess anhydride was removed at reduced pressure and the residue distilled to give 4.5 g (66 %) of a mixture of three products, *7*, *9*, and *11*, b.p. 100–103 °C/0.25 mmHg. These were separated by preparative gas chromatography.

2-(2-Pyridyl)-2-propenyl acetate (7). The yield was 35 % of a colourless liquid. NMR (CCl_4): δ 2.07 (s, Ac), 5.15 (CH_2), 5.52 and 5.96 (olefinic H), 7.00–7.73 and 8.90 (aromatic H, complex signal). IR: 1732 cm^{-1}. MS: *m/e* 135, 134 (base peak) and 118. UV: λ_{max} 234.5 nm (log ε 3.8) and 275 (log ε 3.6). (Found: C 67.90; H 5.85. Calc. for $C_{10}H_{11}NO_2$: C 67.73; H 6.26).

2-(2-Pyridyl)-2-propenol (8). The acetate *7* (100 mg) was refluxed in a 10 % sodium hydroxide solution (3 ml) for 1 h, the mixture was extracted with ether, dried with sodium sulfate and the ether evaporated to give 60 mg (79 %) of compound *8*; NMR (CCl_4): δ 4.16 (s, OH), 4.58 (methylene H), 5.78 and 5.50 (olefinic H), 7.06–7.71 and 8.33–8.67 (aromatic H, complex signal). IR: 3300 cm^{-1} broad and strong relationship in the fingerprint region to the IR spectrum of compound *7*. MS: *m/e* 135 (M^+), 134, 106 (base peak), 104, and 78. UV: λ_{max} 233.5 nm (log ε 3.2) and 275 nm (log ε 3.5).

2-(1-Acetoxycyclopropyl)pyridine (9). The yield was 22 % of a while solid, m.p. 34–36 °C. NMR (CCl_4): δ 1.34 and 1.58 (m, cyclopropyl H), 2.15 (s, Ac), 6.90–7.70 and 8.01 (aromatic H, complex signal). IR: 1750 cm^{-1} s. MS: *m/e* 135, 134 (base peak), 79, and 78. UV: λ_{max} 268 nm (log ε 3.5). (Found: C 68.23; H 6.14. Calc. for $C_{10}H_{11}NO_2$: C 67.73; H 6.26.)

1-(2-Pyridyl)-1-propanone (10). The ester *9* (100 mg) was refluxed in a 10 % sodium hydroxide solution (3 ml) for 4 h, the solution was extracted with ether, dried with sodium sulfate and the ether evaporated to give 50 mg (65 %) of compound *10*. NMR ($CDCl_3$): δ 1.27 (t) and 3.23 (q, J 7.5 Hz, ethyl H), 7.01–8.33 (aromatic H, complex signal). IR: 1689 cm^{-1} s. Phenylhydrazone: m.p. 128–129 °C. (lit.[15] m.p. 129–131 °C).

5-Acetoxy-2-cyclopropylpyridine (11). The yield was 9 % of a colourless liquid. NMR ($CDCl_3$): δ 1.00 (d, methylene cyclopropyl H), 2.20 (q, J 6 Hz, methine cyclopropyl H), 2.33 (s, Ac), 7.12 (d, H3), 7.33 (2d, H4), 8.14 (d, H6); $J_{3,4}$ 8.5 Hz and $J_{4,6}$ 3 Hz. IR: 1760 cm^{-1} s, 1040 m, 1020 m. MS: *m/e* 177 (M^+), 135, 134 (base peak) and 59. UV: λ_{max} 226.5 nm (log ε 3.8) and 275.5 nm (log ε 3.8)

^{18}O-Labeled acetic anhydride (14). From a pressure equalized dropping funnel placed on top of a reflux condenser acetyl chloride (1.24 g, 15.8 mmol) was carefully added to $H_2{}^{18}O$ (0.6 g, 31.6 mmol) of 50 % enrichment (Norsk Hydro) and refluxed for 20 h. With ice cooling sodium ethoxide, prepared from sodium (0.8 g) and ethanol (10 ml), was added dropwise through the same dropping funnel until the reaction mixture was just basic to phenolphthalein. The ethanol was distilled off and the residue dried (0.03 mmHg) over phosphorus pentoxide for 20 h. Acetyl chloride (2.8 g, 35.7 mmol) was added to the dry sodium acetate and the mixture heated at 140 °C (oil bath) for 6 h. The excess of acetyl chloride (1.6 g) was removed and subsequent distillation gave 0.54 g (34 %) of ^{18}O-labeled acetic anhydride (*14*) b.p. 139 °C.

Reaction of 2-cyclopropylpyridine N-*oxide in ^{18}O-labeled acetic anhydride.* The procedure was as described previously using 2-cyclopropylpyridine *N*-oxide (*1*) (0.61 g, 4.5 mmol) and ^{18}O-labeled acetic anhydride (*14*) (0.52 g, 5.1 mmol).

Acknowledgement. We wish to thank Norsk Hydro A/S for a generous gift of ^{18}O-labeled water.

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Heteroaromatic Boron Compounds. XIII. On the Bromination and Nitration of Some Thieno-fused 3,2-Borazaropyridines

SALO GRONOWITZ* and CURT ROOS**

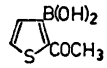
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Derivatives of 4,5-borazarothieno[2,3-*c*]pyridine and 7,6-borazarothieno[3,2-*c*]pyridine were prepared from 2-acetyl-3-thiopheneboronic acid and 3-acetyl-2-thiopheneboronic acid by reaction with hydrazines. The bromination of these systems with different reagents was investigated. Preparatively useful yields of 2,3-dibromo derivatives were obtained with bromine and silver sulfate in concentrated sulfuric acid and with *N,N*-dibromoisocyanuric acid in concentrated sulfuric acid. Isomer distributions in the nitration of the two *ortho* acetylthiopheneboronic acids with fuming nitric acid in concentrated sulfuric acid were determined and compared with those obtained in the same nitration of the borazarothienopyridine systems. It was found that 2-acetyl-4-nitro-3-thiopheneboronic acid and 3-acetyl-5-nitro-2-thiopheneboronic acid are rapidly deboronated at room temperature in alkaline medium.

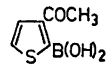
In previous papers, it has been demonstrated that *ortho*-formylthiopheneboronic acids react with hydrazines under hydrazone formation and cyclodehydration to give borazarothienopyridines.[1,2] It was found that these ring systems were stable towards hydrolysis[1,2] and that they under weakly acidic or non-acidic conditions could be brominated or nitrated on the remaining C–H position of the borazaropyridine ring.[3–5] It was also found that under strongly acidic conditions nitration occurs in the thiophene ring.[4]

Furan-fused 3,2-borazaropyridines have been prepared from *ortho*-formylfuranboronic acids.[6–8] However, these compounds were less stable towards hydrolysis than the thiophene analogues, and attempts to carry out electrophilic substitution were unsuccessful. Recently also selenophene-fused 3,2-borazaropyridines have been synthesized from *ortho*-formylselenopheneboronic acids, but no investigations of their hydrolytic stability and of their aromatic substitution have been carried out.[9]

In the present paper, the synthesis, bromination and nitration of primarily *C*-methyl substituted thieno-fused 3,2-borazaropyridines will be discussed.



I

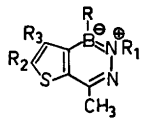


II

Starting from 2-acetyl-3-thiopheneboronic acid (I) and 3-acetyl-2-thiopheneboronic acid (II), for which several methods of synthesis have been described earlier,[10] 7-methyl-4,5-borazarothieno[2,3-*c*]-pyridines (III) and 4-methyl-7,6-borazarothieno[3,2-*c*]pyridines (IV) were prepared in high yield by reaction with hydrazine (IIIa and IVa) and methylhydrazine (IIIb and IVb). From IIIa, the B–CH_3 derivative (IIIc) was prepared by refluxing with butanol accompanied by water separation, and addition of methylmagnesium iodide after removal of the excess butanol. In the same way, the 4,7-dimethyl-(IVc) and 4,6,7-trimethyl-derivatives (IVd) were prepared from IVa and IVb, respectively.

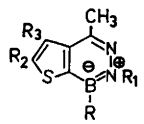
The structures followed from elemental analyses, NMR and mass spectra. The mass spectra

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** Taken in part from the Ph.D. thesis of C. Roos, University of Lund 1974.



III

a R=OH, $R_1=R_2=R_3=H$
b R=OH, $R_1=CH_3$, $R_2=R_3=H$
c R=CH_3, $R_1=R_2=R_3=H$
d R=OH, $R_1=H$, $R_2=R_3=Br$
e R=OH, $R_1=CH_3$, $R_2=R_3=Br$
f R=OH, $R_1=R_3=H$, $R_2=NO_2$
g R=OH, $R_1=R_2=H$, $R_3=NO_2$
h R=OH, $R_1=CH_3$, $R_2=NO_2$, $R_3=H$
i R=OH, $R_1=CH_3$, $R_2=H$, $R_3=NO_2$

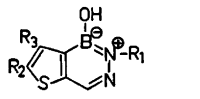


IV

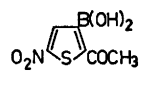
a R=OH, $R_1=R_2=R_3=H$
b R=OH, $R_1=CH_3$, $R_2=R_3=H$
c R=CH_3 $R_1=R_2=R_3=H$
d R=$R_1=CH_3$, $R_2=R_3=H$
e R=OH, $R_1=H$, $R_2=R_3=Br$
f R=OH, $R_1=CH_3$, $R_2=R_3=Br$
g R=OH, $R_1=R_3=H$, $R_2=NO_2$
h R=OH, $R_1=R_2=H$, $R_3=NO_2$
i R=OH, $R_1=CH_3$, $R_2=NO_2$, $R_3=H$
j R=OH, $R_1=CH_3$, $R_2=H$, $R_3=NO_2$

of the B–OH derivatives showed, besides the molecular ion, also peaks of mass numbers 2M–18 of varying intensity, possibly due to anhydride formation in the inlet system. In general, the facile anhydride formation of the B–OH derivatives caused trouble in their purification and made determination of melting points very difficult. Recrystallization from alcohols must be avoided, since esterification easily occurs. This can advantageously be used in some cases to prepare an ethoxy derivative in order to obtain satisfactory elemental analyses.

Qualitative experiments showed that the stability of the *C*-methyl derivatives towards alkaline and acid hydrolysis is approximately the same as that of the borazarothienopyridines studied earlier.[2] Thus 60 and 75 % of IIIa and IIIb, respectively, was recovered after refluxing for 2 h with 2 N aqueous sodium hydroxide. From similar treatment of IVa and IVb, 35 and 50 % of the starting material was recovered. Similar experiments with the 4-desmethyl derivatives corresponding to IVa and IVb only yielded the azines of 3-formylthiophene.[2] Refluxing the compounds IIIa, IIIb, IVa, and IVb with concentrated hydrochloric acid for 2 h led to a recovery of 75–80 % of starting material. The B–CH_3 derivatives IIIc and IVc showed approximately the same stability towards acid hydrolysis. After 2 h reflux, 75 and 80 %, respectively, of the starting material was recovered, including smaller amounts (5–10 %) of the corresponding B–OH derivatives. However, upon alkaline hydrolysis of IIIc and IVc (2 h in refluxing 2 N sodium hydroxide), complete cleavage of the B-methyl bond occurred and IIIa and IVa were isolated in 65 % and 45 % yields, respectively. These experiments were performed under the same conditions as described in Ref. 2.



V



VI

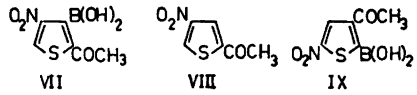
a $R_1=CH_3$, $R_2=R_3=H$
b $R_1=CH_3$, $R_2=H$, $R_3=Br$
c $R_1=CH_3$, $R_2=R_3=Br$
d $R_1=R_2=R_3=H$

Brominations. Six different methods of bromination were investigated. These employed bromine in acetic acid, bromine in hydrobromic acid, bromine in thionyl chloride,[18] bromine in carbon tetrachloride-pyridine, bromine in silver sulfate/sulfuric acid,[11] and dibromoisocyanuric acid (DBI) in concentrated sulfuric acid.[12,13] Only the two last methods were successful. Earlier experiments showed that the desmethyl derivatives of III and IV could be brominated by bromine in silver sulfate/concentrated sulfuric acid, yielding mixtures of 3-bromo and 2,3-dibromo derivatives.[14] We studied the bromination of 4-hydroxy-5-methyl-4,5-borazarothieno[2,3-*c*]pyridine (Va) with this reagent. Using one equivalent of bromine gave the 3-bromo derivative (Vb) in 16 % yield and the 2,3-dibromo derivative (Vc) in 31 % yield. Separation was achieved by precipitation at different pH values.

The structures of Vb and Vc followed from oxidation with permanganate, which gave 4-bromo-2-thiophenecarboxylic acid [15] and 4,5-dibromo-2-thiophenecarboxylic acid,[16] respectively. However, attempts to monobrominate IIIa, IIIb, IVa, and IVb with this reagent or with DBI were unsuccessful. Only the dibrominated derivatives were obtained besides starting material. Traces of the monosubstituted derivatives were perhaps present. When two equivalents of bromine or DBI in concentrated sulfuric acid were used, the dibromo derivatives IIId, IIIe, IVe, and IVf were obtained in preparatively useful yields of 63 to 75 %. The behaviour of IIIa, IIIb, IVa, and IVb is thus different from that of the parent thieno[2,3-*c*]-pyridine and thieno[3,2-*c*]pyridine, which with one equivalent of these brominating agents gives the 3-bromo derivatives in about 50 – 60 % yield.[17] With the latter compound, 10 % of 2,3-dibromothieno[3,2-*c*]pyridine was also obtained.[17] The high tendency for dibromination seems to be partly due to the substituents in the borazaropyridine ring part. It is clear that the introduction of the 4- and 7-methyl groups increases the tendency towards disubstitution. As mentioned earlier, it has been found that compounds with free C – H position in the borazaropyridine ring such as Va are brominated in this position by bromine in acetic acid [3] or by bromine in pyridine-carbon tetrachloride.[5] It was therefore of interest to find out what happens if this position is blocked by methyl groups. However, no substitution of the thiophene ring was observed under these conditions. We also tried to detect addition products by spectroscopic methods, as it is possible that the substitution of, for instance, V in the 7-position proceeds *via* an addition-substitution-elimination mechanism.[5] However, no addition products could be detected.

Attempts to use bromine in thionyl chloride[18] or hydrobromic acid were carried out, as these reagents selectively and in high yield brominated thieno[2,3-*b*]pyridine and thieno[2,3-*b*]-pyridine in the 3-position at 100 °C.[17] However, with the borazarothienopyridines only recovered starting material and tarry products were obtained under different conditions.

Nitrations. The systems IIIa, IIIb, IVa, and IVb were nitrated with fuming nitric acid in concentrated sulfuric acid to study the influence of the *C*-methyl group on isomer distribution. For comparison, the nitration of I and II was also investigated to obtain additional information about the effect of ring closure upon isomer distribution.



When the mixture from the nitration of I was poured onto ice, 2-acetyl-5-nitro-3-thiopheneboronic acid (VI) precipitated out in 23 % yield. From the mother liquor, by extraction with ether, 19 % of 2-acetyl-4-nitro-3-thiopheneboronic acid (VII) was obtained.

Extraction of the boronic acids with sodium carbonate had to be avoided, as complete deboronation of VII to 2-acetyl-4-nitrothiophene (VIII) occurred. In a first experiment carried out in this way, a 28 % yield of VI and 29 % of VIII was obtained. Pure VII could also be deboronated to the known VIII [19] (with the characteristic $J_{24} = 1.50$ Hz) by reflux in acetic acid, which proved the structure of VII. In the same way, VI was deboronated to the known 2-acetyl-5-nitrothiophene [19] ($J_{34} = 4.0$ Hz).

Upon nitration and acidic work-up, II gave only 3-acetyl-5-nitro-2-thiopheneboronic acid (IX) in 74 % yield. Extraction into sodium carbonate led to immediate deboronation to 4-acetyl-2-nitrothiophene (X) in 70 % yield ($J_{35} = 1.85$ Hz), which of course proves the structure of IX. Again IX could also be deboronated to X by refluxing in acetic acid.

Previously, it was found that in the nitration of 2-formyl-3-thiopheneboronic acid, 2-formyl-5-nitro-3-thiopheneboronic acid (22 %) and 4-nitro-2-formylthiophene (57 %) were formed.[4] It was believed that the latter compound was formed by acid-catalyzed deboronation of 2-formyl-4-nitro-3-thiopheneboronic acid, and the possibility that the facile deboronation was due to neighbouring participation of the nitro group was suggested. As the boronic acids in this case were extracted into sodium carbonate solution, it is obvious from the present results that the very facile alkaline deboronation was overlooked. Such neighbouring group participation is in any case

excluded for IX. It is very probable that we here have an example of the SE1 mechanism suggested by Kuivila *et al.*[20]

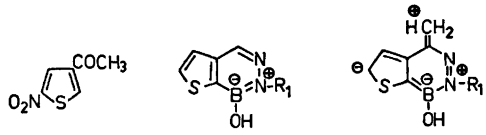
$$ArB(OH)_2 + OH^{\ominus} \rightleftharpoons ArB(OH)_3^{\ominus}$$
$$ArB(OH)_3^{\ominus} \longrightarrow Ar^{\ominus} + B(OH)_3$$
$$Ar^{\ominus} + H_2O \longrightarrow ArH + OH^{\ominus}$$

The formation of the aryl anion could be strongly facilitated by the two electron-withdrawing groups. The activating effect is evident from the fact that deboronation of VII and IX is complete after about 10 min at room temperature in 1 N sodium carbonate solution, while after treatment of I for 24 h at room temperature with 2 N sodium hydroxide, 65 % of this acid was recovered. After the same treatment of II, 10 % of the acid could still be recovered.

The formation of both VI and VII in the nitration of I is expected. In the nitration of 2-acetylthiophene, Gol'dfarb *et al.*[21] found that the 4- and 5-nitro derivatives were formed in the proportions 3:1. The known *meta* directing effect of the boronic acid group[22] under these conditions deactivates the 4-position more than the 5-position of I. As the total yield is only 60 %, and deboronation and some decomposition occurred, some care has to be exercised in drawing more quantitative conclusions. Somewhat surprisingly, the 4:5 ratio in nitration of 2-formylthiophene[21] and 2-formyl-3-thiopheneboronic acid[4] is the same (3:1). Nitration of 3-formylthiophene occurs exclusively in the 5-position,[23] and an introduction of the boronic acid function in the 2-position apparently does not overcome this selectivity.

Previous investigations have shown that 4-hydroxy-4,5-borazarothieno[2,3-*c*]pyridine (Vd) and its 5-methyl derivative (Va) are nitrated by fuming nitric acid in concentrated sulfuric acid in the thiophene ring, yielding the 3- and 2-nitro derivatives in the relative proportions 8:1 and 4:1, respectively.[4] The corresponding nitration of IIIa gave 71 % of a mononitro fraction, which according to NMR consisted of two components, which could be separated by preparative TLC (benzene as eluent) and which were isolated in the proportions 10:1. The major mononitro derivative showed signals at δ 10.57, 9.07, 6.98, and 2.45 in its NMR spectrum, while the minor component had peaks at δ 10.53, 8.53, 7.11, and 2.41. In both cases the relative intensities were 1:1:1:3. The signals are in both cases due to NH, thiophene hydrogen, OH and methyl group, but the NMR spectra do not allow definite structure assignments. Attempts to convert the two nitro derivatives by oxidation with alkaline permanganate to known nitrothiophenecarboxylic acids failed. Mainly tarry products were formed. The structures could, however, be proven by preparing authentic 4-hydroxy-7-methyl-2-nitro-4,5-borazarothieno[2,3-*c*]pyridine (IIIf) through the reaction of VI with hydrazine. This compound was identical (IR, NMR) with the minor nitro isomer formed in the nitration of IIIa. The major isomer therefore must be 4-hydroxy-7-methyl-3-nitro-4,5-borazarothieno-[2,3-*c*]pyridine (IIIg).

Nitration of IIIb under the same conditions gave a 58 % yield of two mononitro derivatives, which again could be separated by preparative TLC and were obtained in the proportions 6:1. The major nitro derivative had in the NMR signals at δ 9.12, 3.83, and 2.95 in CF_3COOH, while the minor derivative absorbed at δ 8.70, 3.87, and 3.00. The relative intensities were in both cases 1:3:3, the peaks being due to the thiophenic hydrogen, the $N-CH_3$ and the $C-CH_3$ group, respectively.



X XI XII

a $R_1 = H$
b $R_1 = CH_3$

Oxidation attempts were again unsuccessful and the minor component was therefore identified as 5,7-dimethyl-4-hydroxy-2-nitro-4,5-borazarothieno[2,3-*c*]pyridine (IIIh) by preparing an authentic sample through the reaction of VI with methylhydrazine. The major component therefore must be 5,7-dimethyl-4-hydroxy-3-nitro-4,5-borazarothieno[2,3-*c*]pyridine (IIIi).

Compared with the 7-desmethyl derivatives studied previously,[4] it is evident that the effect of the 7-methyl group on the isomer distribution in nitration is very small. A slight

increase in the amount of the 3-isomer might have occurred. From both series of compounds, it is also evident that the 5-*N*-CH_3 group on the other hand favours 2-substitution over 3-substitution when compared with the N–H derivatives.

Namtvedt[4] previously studied the nitration of 7-hydroxy-7,6-borazarothieno[3,2-*c*]pyridine (XIa) and 7-hydroxy-6-methyl-7,6-borazarothieno[3,2-*c*]pyridine (XIb). In these cases, only the 3-nitro derivatives were obtained.

Nitration of IVa yielded 62 % of a mixture, which according to spectroscopic data and elemental analyses consisted of two mononitro derivatives. Systematic efforts to separate these compounds by column chromatography or TLC failed.

The NMR spectrum in trifluoroacetic acid showed thiophenic signals of equal intensity at δ 8.95 and 8.65 and *C*-methyl signals at δ 3.07 and 3.04. Apparently the two nitro derivatives were formed in equal amounts. Reaction of IX with hydrazine gave 7-hydroxy-4-methyl-2-nitro-7,6-borazarothieno[3,2-*c*]pyridine (IVg) showing absorptions (CF_3COOH) at δ 8.64 and 3.04 with relative intensities of 3:1. These data, in conjunction with IR and mass spectra, prove that the component with peaks at δ 8.65 and 3.04 is IVg, and the other one is therefore 7-hydroxy-4-methyl-3-nitro-7,6-borazarothieno[3,2-*c*]pyridine (IVh). Also in the nitration of IVb two mononitro derivatives which could not be separated were obtained in an 1:1 ratio. The NMR spectrum in CF_3COOH showed peaks at δ 8.89, 8.59, 3.89, 3.85, 2.97, and 2.93. The relative intensities of the pairs of groups were 1:3:3. Synthesis of authentic 4,6-dimethyl-7-hydroxy-2-nitro-7,6-borazarothieno[3,2-*c*]pyridine (IVi) proved that the peaks at δ 8.59, 3.85, and 2.93 belonged to this isomer while the remaining peaks are those of 4,6-dimethyl-7-hydroxy-3-nitro-7,6-borazarothieno[3,2-*c*]pyridine (IVj).

It is thus evident that introduction of a methyl group in the 4-position increases the reactivity of the 2-position over that of the 3-position. This could partly be due to deactivation of the 3-position by steric hindrance from the methyl group (*peri* effect), but also to an activation of the 2-position by the hyperconjugative effect of the methyl group as illustrated by the resonance structure XII.

Comparing the nitration of the borazarothieno systems with that of the open models, the acetylthiopheneboronic acids, a trend towards increased substitution in the β-position is noticeable. Thus, while II only yields the α-isomer, equal amounts of the α- and β-isomers are obtained with IV. As discussed earlier by Namtvedt,[4] this provides evidence for the aromaticity of the borazarothienopyridines.

It was previously found that compounds Va and Vd are nitrated in the 7-position and XIa and XIb in the 4-position by nitric acid in acetic anhydride.[4] It was therefore of interest to find out if these systems could be nitrated in the thiophenic ring part when the 7- and 4-positions are blocked by methyl groups, or if addition products could be detected, which would shed light on the mechanism of this reaction. However, all attempts to nitrate IIIa, IIIb, or IVa, IVb with this reagent led either to recovery of starting material, or if higher temperatures and longer reaction times were used, to increasing tar formation.

EXPERIMENTAL

4-Hydroxy-7-methyl-4,5-borazarothieno[2,3-c]pyridine (IIIa). To 5.1 g (0.030 mol) of 2-acetyl-3-thiopheneboronic acid[10] dissolved in the minimum amount of ethanol (95 %), 1.50 g (0.030 mol) of hydrazine hydrate in 10 ml of ethanol was added with stirring. When the exothermic reaction subsided, crystals started to form and the mixture was stirred for 2 h. Evaporation of the ethanol gave 4.8 g of crude product, which was recrystallized from aqueous ethanol, yielding 4.4 g (88 %) of the title compound, m.p. 136–143 °C. NMR (CD_3SOCD_3): δ 10.07 (NH), 8.40 (OH), 7.90 (H-2), 7.81 (H-3), 2.54 (CH_3); J_{23} 4.8 Hz. [Found: C 43.02; H 4.29; N 17.08. Calc. for $C_6H_7BN_2OS$ (166.1): C 43.41; H 4.25; N 16.88.]

5,7-Dimethyl-4-hydroxy-4,5-borazarothieno[2,3-c]pyridine (IIIb). From 5.1 g (0.030 mol) of 2-acetyl-3-thiopheneboronic acid[10] in ethanol and 1.4 g (0.030 mol) of methylhydrazine, 4.7 g (87 %) of the title compound, m.p. 127–135 °C was obtained in the same way as described above. NMR (CD_3SOCD_3): δ 8.42 (OH), 7.85 (H-2), 7.72 (H-3), 3.61 (NCH_3), 2.48 (CH_3); J_{23} 5.0 Hz. [Found: C 46.49; H 4.66; N 15.98. Calc. for $C_7H_9BN_2OS$ (180.1): C 46.69; H 5.03; N 15.56.]

4,7-Dimethyl-4,5-borazarothieno[2,3-c]pyridine (IIIc). In an apparatus connected to a water-separator, 8.3 g (0.050 mol) of IIIa and 150 ml

of butanol were refluxed for 6 h. Excess butanol was evaporated *in vacuo*. The residual oil was dissolved in 150 ml of anhydrous ether, cooled to −10 °C, and 70 ml of 1.0 N (0.070 mol) of methylmagnesium iodide added with stirring under nitrogen at such a rate that the temperature did not rise above 0 °C. The mixture was refluxed for 30 min, and after cooling poured into 200 ml of 1 N hydrochloric acid. The ether phase was separated off and the pH of the water phase adjusted to 7 by addition of sodium bicarbonate, and the water phase was extracted with ether. The combined ether phases were dried over magnesium sulfate and the ether evaporated, yielding 5.9 g of crystalline product. Recrystallization from 50 % aqueous ethanol gave 5.1 g (62 %) of the title compound, m.p. 110 – 112 °C after sublimation. NMR (CD_3SOCD_3): δ 11.15 (NH), 7.86 (H-2), 7.62 (H-3), 2.57 (CCH_3), 0.90 (BCH_3); J_{23} 5.0 Hz. [Found: C 51.70; H 5.21; N 17.20. Calc. for $C_7H_9BN_2S$ (164.1): C 51.25; H 5.53; N 17.08.]

7-Hydroxy-4-methyl-7,6-borazarothieno[3,2-c]-pyridine (IVa). This compound was prepared in analogy with IIIa from 5.1 g (0.030 mol) of 3-acetyl-2-thiopheneboronic acid[10] and 1.5 g (0.030 mol) of hydrazine hydrate. After recrystallization from aqueous ethanol, 4.3 g (86 %) of the title compound, m.p. 147 – 155 °C, was obtained. NMR (CD_3SOCD_3): δ 9.82 (NH), 8.18 (OH), 8.03 (H-2), 7.55 (H-3), 2.49 (CCH_3); J_{23} 5.0 Hz. [Found: C 43.42; H 4.24; N 17.13. Calc for $C_6H_7BN_2OS$ (166.1): C 43.41; H 4.25; N 16.88.]

4,6-Dimethyl-7-hydroxy-7,6-borazarothieno-[3,2-c]pyridine (IVb). This compound was prepared in analogy with IIIa from 5.1 g (0.030 mol) of 3-acetyl-2-thiopheneboronic acid and 1.4 g (0.030 mol) of methylhydrazine, with ethanol as solvent. After recrystallization from aqueous ethanol, 4.7 g (87 %) of the title compound, m.p. 138 – 145 °C, was obtained. NMR (CD_3SOCD_3): δ 7.60 (OH), 8.06 (H-2), 7.56 (H-3), 3.61 (NCH_3), 2.53 (CCH_3); J_{23} 5.0 Hz. [Found: C 46.40; H 5.12; N 15.36. Calc. for $C_7H_9BN_2OS$ (180.1): C 46.70; H 5.04; N 15.56.]

4,7-Dimethyl-7,6-borazarothieno[3,2-c]pyridine (IVc). This compound was prepared in analogy with IIIc from 8.3 g (0.050 mol) of IVa, 150 ml of butanol, and 70 ml 1.0 N of methylmagnesium iodide. After recrystallization from 50 % aqueous ethanol, 4.8 g (59 %) of the title compound, m.p. 99 – 101 °C after sublimation, was obtained. NMR (CD_3SOCD_3): δ 11.15 (NH), 8.09 (H-2), 7.61 (H-3), 2.58 (CCH_3), 0.87 (BCH_3); J_{23} 4.9 Hz. [Found: C 51.72; H 5.45; N 17.85. Calc. for $C_7H_9BN_2S$ (164.1): C 51.25; H 5.53; N 17.08.]

4,6,7-Trimethyl-7,6-borazarothieno[3,2-c]pyridine (IVd). This compound was prepared in analogy with IIIc from 9.0 g (0.050 mol) of IVb, 150 ml of butanol, and 70 ml 1.0 N of methylmagnesium iodide. After recrystallization from 50 % aqueous ethanol, 5.9 g (66 %) of the title compound, m.p. 48 – 49 °C after sublimation, was obtained. NMR (CD_3SOCD_3): δ 8.04 (H-2), 7.57 (H-3), 3.66 (NCH_3), 2.51 (CCH_3), 0.86 (BCH_3); J_{23} 4.9 Hz. [Found: C 53.95; H 6.20; B 6.12; N 15.68. Calc. for $C_8H_{11}BN_2S$ (178.0): C 53.96; H 6.23; B 6.07; N 15.73.]

Bromination with bromine and silver sulfate in conc. sulfuric acid. General procedure. To a solution of 0.0050 mol of the borazarothienopyridine and 1.55 g (0.010 mol) of silver sulfate in 15 ml of conc. sulfuric acid at 0 °C, 0.53 ml (0.010 mol) of bromine was added dropwise. After stirring at 0 °C for 3 – 4 h, the reaction mixture was filtered through a glass filter onto 150 g of ice. The precipitate was washed twice with 5 ml of conc. sulfuric acid, and the filtrate combined with the water phase. The aqueous phase, in which crystals formed, was neutralized with solid sodium bicarbonate and the precipitate filtered off, washed with water and dried. The product was recrystallized from aqueous ethanol, which gave the pure 2,3-dibromo derivatives.

2,3-Dibromo-4-hydroxy-5-methyl-4,5-borazarothieno[2,3-c]pyridine (Vc) was obtained from 4-hydroxy-5-methyl-4,5-borazarothieno[2,3-c]-pyridine[2] in 63 % yield, m.p. 161 – 166 °C. NMR (CD_3SOCD_3): δ 8.13 (H-7), 7.32 (OH), 3.60 (NCH_3). [Found: C 22.55; H 1.41; Br 49.18; N 9.04. Calc. for $C_6H_5BBr_2N_2OS$ (323.8): C 22.26; H 1.56; Br 49.36; N 8.65.]

2,3-Dibromo-4-hydroxy-7-methyl-4,5-borazarothieno[2,3-c]pyridine (IIId) was obtained from IIIa in 66 % yield, m.p. 182 – 187 °C. NMR (CD_3SOCD_3): δ 9.83 (NH), 7.80 (OH), 2.33 (CCH_3). [Found: C 22.52; H 1.69; Br 49.34; N 8.80. Calc. for $C_6H_5BBr_2N_2OS$ (323.8): C 22.26; H 1.56; Br 49.39; N 8.65.]

2,3-Dibromo-5,7-dimethyl-4-hydroxy-4,5-borazarothieno[2,3-c]pyridine (IIIe) was obtained from IIIb in 69 % yield, m.p. 141 – 148 °C. NMR (CD_3SOCD_3): δ 7.53 (OH), 3.50 (NCH_3), 2.30 (CCH_3). [Found: C 25.34; H 2.01; Br 47.05; N 8.51. Calc. for $C_7H_7BBr_2N_2OS$ (337.8): C 24.89; H 2.09; Br 47.31; N 8.29.]

2,3-Dibromo-7-hydroxy-4-methyl-7,6-borazarothieno[3,2-c]pyridine (IVe) was obtained from IVa in 75 % yield, m.p. 195 – 200 °C. NMR (CD_3COCD_3): δ 10.10 (NH), 8.35 (OH), 2.67 (CCH_3). As a satisfactory elemental analysis could not be obtained, this compound was transformed to the ethyl ester by refluxing with ethanol; *2,3-dibromo-7-ethoxy-4-methyl-7,6-borazarothieno[3,2-c]-pyridine.* [Found: C 27.10; H 3.04; B 3.04; Br 45.72; N 7.95. Calc. for $C_8H_9BBr_2N_2OS$ (315.8): C 27.32; H 2.58; B 3.07; Br 45.43; N 7.96.]

2,3-Dibromo-4,6-dimethyl-7-hydroxy-7,6-borazarothieno[3,2-c]pyridine (IVf) was obtained from IVb in 62 % yield, m.p. 171 – 178 °C. NMR (CD_3SOCD_3): δ 8.41 (OH), 3.43 (NCH_3), 2.58 (CCH_3). [Found: C 24.84; H 2.39; B 3.20; Br 47.28; N 8.24. Calc. for $C_7H_7BBr_2N_2OS$ (337.8): C 24.89; H 2.09; B 3.20; Br 47.31; N 8.29.]

3-Bromo-4-hydroxy-5-methyl-4,5-borazarothie-

no[2,3-c]pyridine (Vb). Reacting 0.010 mol of 4-hydroxy-5-methyl-4,5-borazarothieno[2,3-*c*]-pyridine[2] with 1.5 g of silver sulfate in 25 ml of conc. sulfuric acid and 0.53 ml (0.01 mol) of bromine, according to the general method described above, led upon pouring on water to the precipitation of 1.2 g of Vc, which was filtered off. The filtrate was then neutralized with solid sodium bicarbonate, and the precipitate (0.70 g) was filtered off and recrystallized from aqueous ethanol, giving 0.40 g (16 %) of the title compound, m.p. 81–88 °C. NMR (CD_3SOCD_3): δ 8.18 (H-7), 7.85 (H-2), 7.58 (OH), 3.60 (NCH_3). [Found: C 29.74; H 2.53; Br 33.62; N 11.58. Calc. for $C_6H_6BBrN_2OS$ (244.9): C 29.42; H 2.47; Br 32.63; N 11.44.]

Brominations with N,N-dibromoisocyanuric acid in conc. sulfuric acid. General procedure. To a solution of 0.010 mol of borazarothieno-pyridine in 25 ml of conc. sulfuric acid at 0 °C, a solution of 2.90 g (0.010 mol) of *N,N*-dibromo-isocyanuric acid in 20 ml of conc. sulfuric acid was added dropwise with stirring. After 1/2–1 h at 0 °C the reaction mixture was poured onto 200 g of ice. The acidic solution was neutralized with solid sodium bicarbonate, the precipitate filtered off, washed with water and dried. After recrystallization from aqueous ethanol, the 2,3-dibromo derivatives were obtained, which had the same physical properties as the samples described above. The following yields were obtained: 2,3-dibromo-4-hydroxy-5-methyl-4,5-borazarothieno[2,3-*c*]pyridine 63 %; 2,3-dibromo-4-hydroxy-7-methyl-4,5-borazarothieno-[2,3-*c*]pyridine, 65 %; 2,3-dibromo-5,7-dimethyl-4-hydroxy-4,5-borazarothieno[2,3-*c*]pyridine, 70 %; 2,3-dibromo-7-hydroxy-4-methyl-7,6-borazarothieno[3,2-*c*]pyridine, 62 % and 2,3-dibromo-4,6-dimethyl-7-hydroxy-7,6-borazaro-thieno[3,2-*c*]pyridine, 69 %.

Nitration of 2-acetyl-3-thiopheneboronic acid. Work-up A. To 20 ml of conc. sulfuric acid and 0.85 ml of fuming nitric acid (*d* 1.52) cooled to –20 °C, 1.7 g (0.010 mol) of 2-acetyl-3-thio-pheneboronic acid[10] was added with vigorous stirring. After 2 1/2 h of stirring at –20 °C, the reaction mixture was poured onto 200 g of ice. The acidic water phase, which contained a precipitate, was extracted with three 100 ml portions of ether. The combined ether phases were extracted with three 50 ml portions of 1 N sodium carbonate solution, which immediately were acidified with 5 N hydrochloric acid. The precipitate was filtered off, washed with water and dried. Recrystallization from aqueous ethanol gave 0.60 g (28 %) of *2-acetyl-5-nitro-3-thiopheneboronic acid*, m.p. 134–139 °C. NMR (CD_3SOCD_3): δ 8.10 (H-4), 6.1 (OH), 2.12 (CH_3). [Found: C 33.85; H 3.07; N 6.82. Calc. for $C_6H_6BNO_5S$ (215.0): C 33.52; H 2.81; N 6.52.] The ether phase, which had been extracted with sodium carbonate solution, was washed with water, and dried over magnesium sulfate. Evaporation of the ether gave 0.6 g of a solid residue, which upon recrystallization from ethanol gave 0.50 g (29 %) of *2-acetyl-4-nitrothiophene*, m.p. 125–126 °C. NMR (CD_3SOCD_3): δ 9.07 and 8.47 (thioph.), 2.63 (CH_3); J_{35} 1.50 Hz. Lit. value,[19] m.p. 126–127 °C.

Work-up B. The experiment was carried out as described above until the mixture was poured onto ice. The crystals (0.7 g) which formed were filtered off after 15 min, washed with water and dried. Recrystallization from aqueous ethanol yielded 0.50 g (23 %) of 2-acetyl-5-nitro-3-thiopheneboronic acid, with the same IR and NMR spectrum as the sample described above. The acidic aqueous phase was extracted three times with 50 ml portions of ether. The combined ether fractions were dried and evaporated. The solid residue (0.50 g) was washed with a little chloroform and recrystallized from aqueous ethanol to give 0.40 g (19 %) of 2-acetyl-4-nitro-3-thiophene-boronic acid, m.p. 119–124 °C. NMR (CD_3SOCD_3): δ 8.99 (H-5), 6.20 (OH), 2.56 (CH_3). [Found: C 33.13; H 3.02; N 6.91. Calc. for $C_6H_6BNO_5S$ (215.0): C 33.52; H 2.81; N 6.52.]

Deboronation of 2-acetyl-5-nitro-3-thiophene-boronic acid. A solution of 0.43 g (0.0020 mol) of 2-acetyl-5-nitro-3-thiopheneboronic acid in 25 ml of acetic acid was refluxed for 3 h and the solution evaporated to dryness. The solid residue was dissolved in ether. The ether solution was extracted twice with 25 ml portions of sodium carbonate solution and dried over magnesium sulfate. After evaporation of the ether, the residue (0.25 g) was recrystallized from ethanol, yielding 0.20 g (59 %) of 2-acetyl-5-nitrothiophene, m.p. 106–108 °C. NMR (CD_3SOCD_3): δ 8.18 and 7.97 (thioph.), 2.65 (CH_3); J_{34} 4.40 Hz. Lit. value,[19] m.p. 108–109 °C.

Deboronation of 2-acetyl-4-nitro-3-thiophene-boronic acid. A solution of 0.127 g (0.0005 mol) of 2-acetyl-4-nitro-3-thiopheneboronic acid in 25 ml of acetic acid was refluxed for 2 h and worked up as described above. After recrystallization from ethanol, 0.040 g (47 %) of 2-acetyl-4-nitrothiophene, m.p. 125–126 °C, was obtained, with the same IR and NMR spectrum as the sample described above.

Nitration of 3-acetyl-2-thiopheneboronic acid. Work-up A. To a solution of 0.85 ml of fuming nitric acid (d = 1.52) in 20 ml of conc. sulfuric acid cooled to 0 °C, 1.70 g (0.010 mol) of 3-acetyl-2-thiopheneboronic acid[10] was added in portions with vigorous stirring. The reaction mixture was kept for 2 h at 0 °C and then poured onto 200 g of ice. The acidic solution, which contained a precipitate, was extracted three times with 100 ml of ether, and the combined ether phases were then extracted with three 50 ml portions of 1 N sodium carbonate solution. Acidification of the combined alkaline solutions precipitated 0.6 g of 4-acetyl-2-nitrothiophene. Evaporation of the ether solution gave an additional 0.7 g (total yield 1.3 g, 87 %) of the title compound, m.p. 62–63 °C after recrystallization from aqueous

ethanol. NMR (CD_3SOCD_3): δ 8.73 (thioph.), 8.31, 2.57 (CH_3); J_{24} 1.85 Hz. [Found: C 42.5; H 3.10; N 8.27. Calc. for $C_6H_5NO_3S$ (171.0): C 42.10; H 2.95; N 8.18.]

Work-up B. The experiment was carried out as described above until the mixture was poured onto ice. The precipitate (1.8 g) was filtered off and recrystallized from aqueous ethanol, giving 1.6 g (74 %) of 3-acetyl-5-nitro-2-thiopheneboronic acid, m.p. 149 – 154 °C. NMR (CD_3SOCD_3): δ 8.58 (H-4), 6.5 (OH), 2.64 (CH_3). [Found: C 33.84; H 3.05; N 6.99. Calc. for $C_6H_6BNO_5S$ (215.0): C 33.52; H 2.81; N 6.52.]

Deboronation of 3-acetyl-5-nitro-2-thiopheneboronic acid. A solution of 0.43 g (0.020 mol) of 3-acetyl-5-nitro-2-thiopheneboronic acid in 25 ml of acetic acid was refluxed for 3 h and worked up as described above. After recrystallization from ethanol, 0.20 g (59 %) of 4-acetyl-2-nitrothiophene, m.p. 62 – 64 °C, and with the same spectroscopic properties as the sample described above, was obtained.

Nitration of 4-hydroxy-7-methyl-4,5-borazarothieno[2,3-c]pyridine. To a solution of 1.66 g (0.010 mol) of IIIa in 20 ml of conc. sulfuric acid prepared at 0 °C, a solution of 0.5 ml of fuming nitric acid ($d = 1.52$) in 5 ml of conc. sulfuric acid was added dropwise with stirring. After 2 h at 0 °C, the reaction mixture was poured onto 200 g of ice and neutralized with solid sodium bicarbonate. The precipitate was filtered off, washed with water and dried. The crude product (1.5 g, 71 %), consisted of two components according to TLC (silica gel, benzene as eluent). The crude product (100 mg) was eluted five times on a silica gel plate (2 mm × 20 cm × 20 cm). Between each elution the plate was allowed to dry. Extraction of one of the two bands gave 70 mg of *4-hydroxy-7-methyl-3-nitro-4,5-borazarothieno[2,3-c]pyridine*, m.p. 155 – 170 °C. NMR (CD_3SOCD_3), δ 10.57 (NH), 9.07 (H-2), 6.98 (OH), 2.45 (CCH_3). [Found: C 34.78; H 3.09; N 19.20. Calc. for $C_6H_6BN_3O_3S$ (211.0): C 34.15; H 2.87; N 19.91.] The other band gave 7 mg of *4-hydroxy-7-methyl-2-nitro-4,5-borazarothieno[2,3-c]pyridine*, m.p. 175 – 180 °C. NMR (CD_3SOCD_3): δ 10.53 (NH), 8.53 (H-3), 7.11 (OH), 2.41 (CCH_3). [Found: C 34.68; H 3.16; N 20.08. Calc. for $C_6H_6BN_3O_3S$ (211.0): C 34.15; H 2.87; N 19.91.]

4-Hydroxy-7-methyl-2-nitro-4,5-borazarothieno[2,3-c]pyridine. To a solution of 0.43 g (0.0020 mol) of 2-acetyl-5-nitro-3-thiopheneboronic acid in 20 ml of ethanol, 0.10 g (0.0020 mol) of hydrazine hydrate in 20 ml of ethanol was added with stirring. After 4 h, the solution was evaporated to dryness. The residue (0.35 g) was washed with sodium bicarbonate solution and water. Recrystallization from aqueous ethanol gave 0.20 g (45 %) of the title compound, m.p. 175 – 180 °C, and with the same spectral properties as the sample described above.

Nitration of 5,7-dimethyl-4-hydroxy-4,5-borazarothieno[2,3-c]pyridine. In the same way as described above for the 5-desmethyl derivative, 1.80 g (0.010 mol) of IIIb in 20 ml of conc. sulfuric acid was nitrated with 0.5 ml of fuming nitric acid ($d = 1.52$) in 5 ml of conc. sulfuric acid, yielding 1.3 g (58 %) of an isomeric mixture. On TLC of 100 mg, 78 mg of *5,7-dimethyl-4-hydroxy-3-nitro-4,5-borazarothieno[2,3-c]pyridine*, m.p. 207 – 212 °C, was obtained. NMR (CF_3COOH): δ 9.12 (H-2), 3.83 (NCH_3), 2.95 (CCH_3). [Found: C 37.52; H 3.62; N 18.35. Calc. for $C_7H_8BN_3O_3S$ (225.0): C 37.36; H 3.58; N 18.67.] Furthermore, 13 mg of *5,7-dimethyl-4-hydroxy-2-nitro-4,5-borazarothieno[2,3-c]pyridine*, m.p. 236 – 244 °C, was also obtained. NMR (CF_3COOH): δ 8.70 (H-3), 3.87 (NCH_3), 3.00 (CCH_3). [Found: C 37.58; H 3.59; N 18.47. Calc. for $C_7H_8BN_3O_3S$ (225.0): C 37.36; H 3.58; N 18.67.]

5,7-Dimethyl-4-hydroxy-2-nitro-4,5-borazarothieno[2,3-c]pyridine. To a solution of 0.43 g (0.0020 mol) of 2-acetyl-5-nitro-3-thiopheneboronic acid in 25 ml of ethanol, 0.10 g (0.0020 mol) of methylhydrazine in 20 ml of ethanol was added with stirring. The precipitate (0.30 g) which formed was filtered off, washed with water and dried. Recrystallization from ethanol gave 0.20 g (44 %) of the title compound, m.p. 235 – 245 °C, with the same spectral properties as the sample described above.

Nitration of 7-hydroxy-4-methyl-7,6-borazarothieno[3,2-c]pyridine. In the same way as described above, 1.66 g (0.010 mol) of IVa in 20 ml of conc. sulfuric acid was nitrated with 0.5 ml of fuming nitric acid ($d = 1.52$) in 10 ml of conc. sulfuric acid, yielding 1.3 g (62 %) of a yellowish-brown precipitate. The NMR, IR, and mass spectra indicated that the crude product was a mixture of two mononitro derivatives in a 1:1 ratio. In spite of several attempts with thin-layer and column chromatography, no separation was achieved. Also after recrystallization the isomer composition was still 1:1. NMR (CF_3COOH): δ 8.95 and 8.65 (thioph.), 3.07 and 3.04 (CCH_3). [Found: C 34.41; H 3.06; N 19.69. Calc. for $C_6H_6BN_3O_3S$ (211.0): C 34.15; H 2.87; N 19.91.]

7-Hydroxy-4-methyl-2-nitro-7,6-borazarothieno[3,2-c]pyridine. To a solution of 0.43 g (0.0020 mol) of 3-acetyl-5-nitro-2-thiopheneboronic acid in 20 ml of ethanol, 0.10 g (0.0020 mol) of hydrazine hydrate in 20 ml of ethanol was added with stirring. After 4 h the alcohol was evaporated, yielding a solid residue. Recrystallization from aqueous ethanol gave 0.20 g (45 %) of the title compound, m.p. 190 – 205 °C. NMR (CF_3COOH): δ 8.65 (H-3), 3.04 (CCH_3). [Found: C 34.63; H 3.23; N 19.11. Calc. for $C_6H_6BN_3O_3S$ (211.0): C 34.15; H 2.87; N 19.91.]

Nitration of 4,6-dimethyl-7-hydroxy-7,6-borazarothieno[3,2-c]pyridine. In the same way as described above, 1.80 g (0.010 mol) of IVb in 20 ml of conc. sulfuric acid was nitrated with 0.5 ml of fuming nitric acid ($d = 1.52$) in

10 ml of conc. sulfuric acid, yielding 0.9 g (40 %) of a fine yellowish-brown precipitate. The NMR, IR, and mass spectra indicated that this product was a mixture of two mononitro derivatives in the proportions 1:1. In spite of several attempts with TLC and column chromatography, no separation was achieved. Also after recrystallization the isomer composition was still 1:1. NMR (CF_3COOH): δ 8.89 and 8.59 (thioph.), 3.89 and 3.85 (NCH_3), 2.97 and 2.93 (CCH_3). [Found: C 37.57; H 3.93; N 18.21. Calc. for $C_7H_8BN_3O_3S$ (225.0): C 37.36; H 3.62; N 18.35.]

4,6-Dimethyl-7-hydroxy-2-nitro-7,6-borazarothieno[3,2-c]pyridine. To a solution of 0.43 g (0.0020 mol) of 3-acetyl-5-nitro-2-thiopheneboronic acid in 20 ml of ethanol, 0.10 g (0.0020 mol) of methylhydrazine in 10 ml of ethanol was added with stirring. After a few minutes a precipitate formed, which was washed with water and dried. Recrystallization from aqueous ethanol yielded 0.20 g (44 %) of the title compound, m.p. 220–230 °C. NMR (CF_3COOH): δ 8.59 (H-3), 3.85 (NCH_3), 2.93 (CCH_3). [Found: C 37.92; H 4.06; N 17.94. Calc. for $C_7H_8BN_3O_3S$ (225.0): C 37.36; H 3.62; N 18.25.]

NMR spectra were recorded on a Varian A60 NMR spectrometer, mass spectra were obtained with an LKB A-9000 mass spectrometer and IR spectra with a Perkin Elmer 257 grating infrared spectrophotometer. Elemental analyses were carried out by the Analytical Department of the Chemical Center, by Miss Ilse Beetz, Mikroanalytisches Laboratorium, Kronach, Germany and by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mülheim/Ruhr, Germany.

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A New Sesquiterpene Alcohol of the Copane Series from Angelica Root Oil

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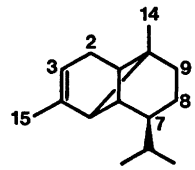
A new sesquiterpene alcohol was isolated from the volatile root oil of *Angelica archangelica* L. On the basis of spectral and chemical evidence structure *8*, *cis*-α-copaene-8-ol, was assigned to this compound.

The tricyclic sesquiterpene hydrocarbons, α-copaene (*1*) and β-copaene are reported to be present in a number of essential oils.[1-6] Two oxygenated copane derivatives have been identified, mustakone (*2*) from *Cyperus rotundus*[4] and α-copaene-11-ol (*3*) from *Parabenzoin preacox*.[5] Two sesquiterpenoid ketoaldehydes, called brachylaenalone A and B, have been isolated from the heartwood of *Brachylaena hutchinsii*.[7] The compounds (*4*) are thought to differ only in the configuration of the isopropyl group, so that one of them would have the configuration of copane.

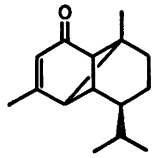
In a recent study the root oil of *Angelica archangelica* was found to contain α-copaene-11-ol and an unknown alcohol (about 0.5 % of each) as the main sesquiterpene alcohol components.[6] The present paper describes the structure determination of this unknown, for which the structure and relative configuration *8*, *cis*-α-copaene-8-ol, is proposed.

The mass spectrum of *8* displayed M^+ at *m/e* 220, which obviously corresponds to the molecular formula $C_{15}H_{24}O$. A rearrangement ion corresponding to loss of water appeared at *m/e* 202. The IR spectrum revealed the presence of a primary or secondary hydroxyl (ν_{max} 3420 and 1020 cm^{-1}), a *gem*-dimethyl group (ν_{max} 1383 and 1370 cm^{-1}) and a trisubstituted double bond (ν_{max} 3020 and 783 cm^{-1}). The ^1H NMR spectrum showed signals due to a methyl at a quaternary carbon (δ 0.83, 3 H, s), an isopropyl group (two 3 H doublets centered at δ 0.95 and 1.03, *J* 6.5 Hz), a methyl attached to an olefinic carbon (δ 1.66, 3 H, m), one olefinic proton (δ 5.19, 1 H, m) and a methine proton adjacent to oxygen (δ 4.21, 1 H, m).

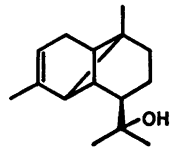
Hydrogenation of *8* with PtO_2 in glacial acetic acid yielded a saturated alcohol with a molecular weight of 222 (MS, IR). This confirms that alcohol *8* has a tricyclic structure. The only tricyclic skeleton present in the sesquiterpenoids found in angelica root oil is that of copane.[6] The structure derived from α-copaene (*1*) with hydroxyl at the 2-, 8- or 9-position would be compatible with the spectral data including the unusual position of the C—H deformation band of the double bond. The lowered frequency



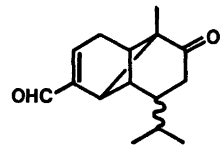
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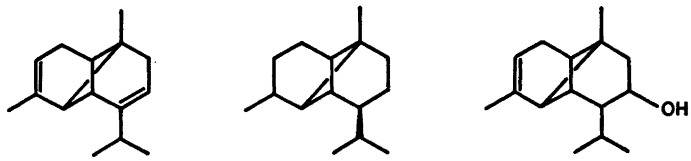
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3



4



5 6 7

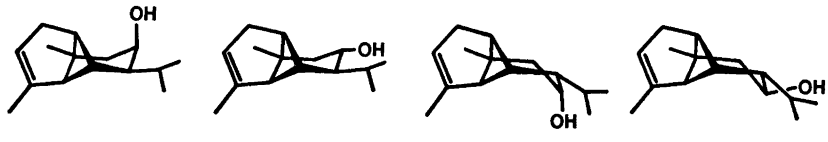
(about 780 cm^{-1}) is typical for the strained bicyclo[3,1,1]heptene system (*e.g.* α-pinene, α-copaene).[8]

Dehydration of *8* with thionyl chloride in pyridine afforded an unsaturated hydrocarbon, the mass spectrum of which displayed the molecular peak at *m/e* 202. The IR spectrum of this hydrocarbon showed notable absorptions only at 780 cm^{-1} in the region of C–H out-of plane bending vibrations of double bonds. This could be expected if the new double bond is located at the 7,8-position as in *5*.

On catalytic hydrogenation of the diene *5*, two saturated hydrocarbons with molecular weights of 206 were obtained in a ratio of 2:3. The minor compound was identified as copane (*6*) by comparing its IR and mass spectra with those of the hydrogenation product of α-copaene from angelica root oil. The other, with very similar spectra, was evidently the isopropyl epimer, ylangane. Formation of both is expected if the diene has the structure *5*.

This evidence suggests the structure *7* for the unknown alcohol, leaving the stereochemical details open. The four possible isomers differing in their configuration at C-7 and C-8 can be represented by the perspective figures *8–11*. Structures *8* and *9* are favoured by the fact that all the other cadalene-type sesquiterpenes found in angelica root oil belong to the muurolane group with related stereochemistry. Considering the smooth dehydration with thionyl chloride in pyridine, structure *8* is preferred as it has the C-7 hydrogen more favourably located for E2 elimination.

Further support for structure *8* was obtained from the relative lanthanide induced chemical shifts. ^1H NMR spectra were recorded after successive additions of $Eu(dpm)_3$ to the alcohol sample in $CDCl_3$. The relative europium induced shifts (DEu values) of signals that could be correctly assigned were calculated as the slopes of the lines when the shifts were plotted against the $Eu(dpm)_3$ addition. The shift effect on the isopropyl methyl signals were found to be 2–3 times greater than on the other methyl signals. This is in agreement with the assignment of the hydroxyl at C-8. The average structure for the complex of *8–11* and the shift reagent was determined by iterative computer fitting of the relative shift effect values to the pseudo-contact equation of McConnel and Robertson.[9,10] The observed shift values of the C-3, C-8, C-14, C-15 and one of the C-9 proton signals were used. The Eu–O distances and α–C–O–Eu angles obtained were 2.4 Å, 165° for the complex of *8*, 3.7 Å, 128° for the complex of *9*, 3.0 Å, 129° for the complex of *10*, and 3.9 Å, 130° for the complex of *11*. The corresponding data reported for *cis*-4-*t*-butylcyclohexanol, involving an axial hydroxyl group, are 2.2 Å, 160° and for *trans*-4-*t*-butylcyclohexanol, with an equatorial hydroxyl group, 2.5 Å, 139°.[11] Only the values for *8* are in reasonable agreement with the data for the 4-*t*-butylcyclohexanol with the corresponding configuration. Another conformation involving an axial isopropyl group is also possible for the compounds represented by structures *8–11*. In this conformation compound *11* would have given iden-



8 9 10 11

tical results to those calculated for *8*. This conformation, however, seems to be unfavorable. Moreover, the large shift effect observed in the signals due to the methyls of the isopropyl group seems to exclude this possibility. Accordingly, the relative configuration *8* was assigned to the sesquiterpene alcohol.

EXPERIMENTAL

Gas chromatography was performed on a Hewlett-Packard 7620 A instrument with 6 mm × 3 m stainless steel columns packed with 10 % FFAP or 5 % SE-30 on Chromosorb W AW. IR spectra were recorded on a Perkin-Elmer 521 instrument. Mass spectra were run on JMS-D 100 mass spectrometer at the Technical Research Centre of Finland. ^{1}H NMR spectra were determined in $CDCl_3$ on a JEOL JMM PFT 100 spectrometer in the Department of Organic Chemistry, University of Helsinki. The average structures of the Eu-complexes were computed in the Department of Chemistry, University of Jyväskylä. The isolation of angelica root oil and separation of the fraction containing the oxygenated compounds have been described before.[6]

cis-α-*Copaene-8-ol* was separated from the fraction of oxygen containing compounds by preparative GLC first on the FFAP column. After rechromatography on SE-30 column about 10 mg of pure *8* was collected. MS: *m/e* (%) 220 (M^+, 1), 202 (10), 187 (6), 177 (5), 160 (45), 159 (85), 146 (12), 145 (59), 134 (14), 133 (31), 132 (36), 121 (15), 120 (25), 119 (100), 117 (18), 109 (15), 107 (19), 106 (14), 105 (50), 93 (23), 92 (13), 91 (33), 81 (10), 79 (15), 77 (16), 55 (14), 43 (12), 41 (22). IR: ν_{max} 3420, 3020, 1383, 1370, 1020, 783 cm^{-1}. NMR: δ ($CDCl_3$) 0.83 (3 H, s), 0.95 (3 H, d, *J* 6.5 Hz), 1.03 (3 H, d, *J* 6.5 Hz), 1.66 (3 H, m), 4.21 (1 H, m), 5.19 (1 H, m). DEu values (% from the value of α-proton): 100.0 (observed)/100.22 (calculated) (C-8 H); 65.5/65.0 (C-9 H_{eq}); 9.9/11.2 (C-14 3 H); 9.9/5.7 (C-3 H); 7.5/10.0 (C-15 3 H).

Hydrogenation of 8. 1 mg of *8* in glacial acetic acid (5 ml) containing PtO_2 (5 mg) was shaken in a 20 ml ampule filled with hydrogen for 2 h at room temperature. The catalyst was filtered off and the filtrate neutralized with NaOH solution. The product was extracted into pentane and a saturated alcohol was isolated by GLC. MS: *m/e* (%) 222 (1), 204 (35), 189 (33), 161 (100), 149(28), 133 (24), 119 (30), 105 (70), 95 (48), 93 (42), 81 (54), 69 (40), 55 (45), 43 (40), 41 (65). IR: ν_{max} 3420, 1380, 1370, 1360, 1015 cm^{-1}.

Dehydration of 8. 10 μl of thionyl chloride in 200 μl of pyridine was added to 2 mg of *8* in 200 μl of pyridine at 0 °C. The solution was allowed to warm up to room temperature, neutralized and extracted with ether. Diene *5* was isolated as the main product by GLC. MS: *m/e* (%) 202 (38), 187 (23), 159 (100), 145 (36), 131 (31), 119 (43), 106 (21), 105 (22), 91 (28), 77 (14), 41 (20). IR: ν_{max} 3020, 1377, 1369, 780 cm^{-1}.

Hydrogenation of diene 5. 0.5 mg of *5* was hydrogenated in glacial acetic acid with PtO_2 as above. The product consisted of two saturated hydrocarbons (3:2), which were separated by GLC. The mass spectra of both were almost identical with that published for copane. [12] The minor component was identified as the hydrogenation product of α-copaene by comparing the IR spectra.

Copane from α-copaene. 2 mg of α-copaene was isolated from angelica root oil [6] and hydrogenated as above. The saturated hydrocarbon was identified as copane (*6*) by its mass spectrum.[12]

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Studies on Orchidaceae Glycosides. 2.* The Structures of Loroglossine and Militarine, two Glucosides from *Orchis militaris* L.

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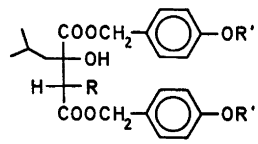
Two glucosides, loroglossine (I) and militarine (II), have been isolated from *Orchis militaris* L. Loroglossine (I) is shown to be bis[4-(β-D-glucopyranosyloxy)benzyl] *erythro*-isobutyltartrate and militarine (II) bis[4-(β-D-glucopyranosyloxy)benzyl] (*R*)-2-isobutylmalate. Syntheses of the(±)-*erythro* and (±)-*threo* isomers of dimethyl isobutyltartrate are described.

In 1919 Bourquelot and Bridel [2] isolated a glucoside from *Loroglossum hircinum* (L.) L.C. Rich. for which they proposed the name loroglossine. This glucoside was later found in *Orchis militaris* L. and twenty-one other Orchidaceae species.[3–7]

In this communication we report the structure of loroglossine (I) and another glucoside, for which we propose the name militarine (II), both of which were isolated from *O. militaris*.

The two glucosides, which differ by only one hydroxyl group, have the molecular weights 742 and 726, respectively. In spite of the small differences in size and structure, I and II were separated by gel permeation on Sephadex G-15.

Loroglossine (I). On the basis of elemental analysis and molecular weight determinations, Delauney[8] suggested the molecular formula $C_{30}H_{42}O_{18}$ for loroglossine (I). Later Karrer and Matter [9] prepared some derivatives of I and from the results of the elemental analysis of these, they considered loroglossine (I) to be a mono-glucoside with the composition $C_{14}H_{20}O_8$.



I : R=OH, R'=β-D-glucopyranosyl
II: R=H, R'=β-D-glucopyranosyl

In the present study, spectrochemical and elemental analyses indicated that loroglossine (I) has the molecular formula $C_{34}H_{46}O_{18}$. Sugar and methylation analyses [10] showed I to be a glucopyranoside. Catalytic hydrogenation of I produced *p*-cresyl β-D-glucopyranoside [11] and isobutyltartaric acid. As there are no signals due to aromatic methyl groups present in the NMR spectrum of I, loroglossine (I) must have suffered hydrogenolysis during the reduction. This evidence together with the NMR spectrum of I, which shows *inter alia* eight aromatic protons, indicates the presence of two 4-(β-D-glucopyranosyloxy)benzyl residues in loroglossine (I). Since loroglossine (I) is a neutral compound it must be a diester and hence have the structure I.

The relative configuration of the isobutyltartaric acid was established by synthesis. Hydroxylation of isobutylfumaric acid [12] and isobutylmaleic anhydride [12] with osmium tetroxide gave, after methylation (±)-*threo* and (±)-*erythro* dimethyl isobutyltartrate, respectively. The *erythro* isomer was found to be identical, except for the optical rotation, with the dimethyl ester of the natural acid.

* For paper 1 in this series, see Ref. 1.

Attempts to determine the absolute configuration of the isobutyltartaric acid are in progress.

Militarine (II). The molecular formula for militarine (II) was found to be $C_{34}H_{46}O_{17}$, *i.e.* one oxygen atom less than loroglossine (I). Catalytic hydrogenation of II gave *p*-cresyl β-D-glucopyranoside[11] and (*R*)-2-isobutylmalic acid.[13,14] From the spectral similarities of loroglossine (I) and militarine, it is evident that the latter has the structure II.

p-Hydroxybenzyl alcohol has been isolated from *O. militaris* infected by the fungus *Rhizoctonia repens* Bern.[15–17] Since this alcohol is normally absent from the plant,[15–17] it seems probable that it has been released from loroglossine (I) and/or militarine (II) by enzymatic hydrolysis during the period of infection.

EXPERIMENTAL

All melting points are corrected. Mass spectra were measured on a Perkin-Elmer 270 or a Varian MAT 311 spectrometer, optical rotations on a Perkin-Elmer 141 polarimeter, IR spectra on a Perkin-Elmer 257 instrument, UV spectra on a Beckman DK2 instrument, and NMR spectra on a Varian XL 100 spectrometer. Concentrations were performed under diminished pressure, at bath temperatures not exceeding 40 °C. Elemental analyses were carried out at Alfred Bernhardt, Mikroanalytisches Laboratorium, Elbach über Engelskirchen, Germany and Mikroanalyslaboratoriet Lantbrukshögskolan, Uppsala, Sweden.

Isolation and characterisation of I and II. Fresh plants of *O. militaris* (5.5 kg) were extracted with methanol (15 l). The filtrate was concentrated to 0.7 l, washed with chloroform (3 × 0.25 l) and extracted with butanol (13 × 0.15 l). The butanolic phase was washed with water (0.15 l) and evaporated to dryness. Half of the residue (14 g) was filtered through a column of Sephadex LH 20 (8 × 60 cm) with water-ethanol (1:1, 1 ml/min) as eluent. The fraction (4.6 g) containing the glucosides was chromatographed on the same column giving a mixture of I and II (3.4 g). Crystallisation from water gave I (0.9 g) as needles, m.p. 153–157 °C (decomp). $[\alpha]_D^{25}$ −34° (*c* 0.36, methanol). (Lit.[8] m.p. 149–150 °C, $[\alpha]_D$ −36.28°, methanol. Lit.[9] m.p. 149–151 °C). (Found: C 55.1; H 6.36; O 38.7. Calc. for $C_{34}H_{46}O_{18}$: C 55.0; H 6.24; O 38.8). IR: ν_{max} (KBr) 3600–3000(s), 1735(s), 1615(m), 1515(s) cm^{-1}. NMR (pyridine-d_5): δ 0.85 (d, 3 H, *J* 6 Hz), 0.97 (d, 3 H, *J* 6 Hz), 1.8–2.2 (m, 2 H), 2.2–2.5 (m, 1 H), 3.8–4.5 (m, 12 H), 4.98–5.16 (m, 4 H), 5.21 (m, 2 H), 5.6–6.8 (broad s, 10 H), 7.25 (m, 8 H). UV, nm (ε): λ_{max} (methanol) 277 (1340), 270.5 (1590), 223.5 (25 400).

Part of the mother liquor (100 mg) from the crystallisation of I was chromatographed on Sephadex G 15 (1.6 × 70 cm) with water as eluent, giving I (50 mg) and a mixture of I and II (20 mg). Further chromatography in the same system, followed by evaporation gave pure II as a colourless amorphous solid, $[\alpha]_{578}^{25}$ −47° (*c* 0.78, methanol). (Found: C 56.2; H 6.60; O 37.4. Calc. for $C_{34}H_{46}O_{17}$: C 56.2; H 6.38; O 37.4). IR: ν_{max} (KBr) 3700–3000(s), 1735(s), 1615(m), 1515(s) cm^{-1}. NMR (pyridine-d_5: δ 0.90 (d. 3 H, *J* 6 Hz), 0.97 (d, 3 H, *J* 6 Hz), 1.7–1.94 (m, 2 H), 1.94–2.3 (m, 1 H), 2.99 (d, 1 H, *J* 15 Hz) and 3.19 (d, 1 H, *J* 15 Hz, AB system), 3.9–4.6 (m, 12 H), 5.08 (s. 2 H), 5.17 (s, 2 H), 5.38–5.64 (m, 2 H), 5.8–7.2 (broad s, 9 H), 7.22–7.45 (m, 8 H). UV, nm (ε): λ_{max} (methanol) 277 (1430), 270.5 (1730), 223 (24 900).

Hydrogenation of I and II. A solution of I (140 mg) in methanol (10 ml) was hydrogenated over palladium (20 mg, 10 % on carbon) at room temperature and atmospheric pressure. After 2.5 h the catalyst was filtered off and the solution was evaporated to dryness. The residue was purified by preparative TLC on silica gel using chloroform-methanol (1:1) as eluent. Crystallisation from a mixture of propan-2-ol and water (3:1) gave *p*-cresyl β-D-glucoside (67 mg) m.p. 178–181 °C (Lit.[11] m.p. 178–179.5 °C), $[\alpha]_D^{23}$ −66.5° (*c* 2.26, water) (Lit.[11] $[\alpha]_D^{20}$ −67.7°, water). IR: ν_{max} (KBr) 3490(s), 3420(s), 3380(s), 1610(m), 1510(s) cm^{-1}. NMR (pyridine-d_5): δ 2.14 (s, 3 H), 3.9–4.6 (m, 6 H), 5.4–5.6 (m, 1 H), 6.6–7.3 (m, 8 H). UV, nm (ε): λ_{max} (methanol) 281 (1090), 275 (1370), 220 (11 200). The same compound (m.p., specific rotation, IR, NMR, and UV) was obtained when II was hydrogenated under the same conditions.

Dimethyl isobutyltartrate. I (620 mg) was hydrogenated as described above. Evaporation of the solvent gave a residue which was purified by preparative TLC on silica gel using methanol-butanol-chloroform (1:3:6) as eluent. The eluate from the plates was filtered through Dowex 50 W-X8 (H^+, 1 × 5 cm) using water as eluent. The filtrate was evaporated to dryness giving isobutyltartaric acid, which was esterified with diazomethane. The crude ester was purified by sublimation (0.1 Torr, 45 °C) giving dimethyl isobutyltartrate (67 mg) as white needles, m.p. 57–59 °C, $[\alpha]_{578}^{25}$ +31° (*c* 0.43, methanol). (Found: C 51.4; H 7.88; O 41.1. Calc. for $C_{10}H_{18}O_6$: C 51.3; H 7.75; O 41.0.) IR: ν_{max} ($CHCl_3$) 3530(m), 1740(s) cm^{-1}. NMR ($CDCl_3 + D_2O$): δ 0.85 (d, 3 H, *J* 6.5 Hz), 0.95 (d, 3 H, *J* 6.5 Hz) 1.33–2.14 (m, 3 H), 3.73 (s, 3 H), 3.80 (s, 3 H), 4.33 (s, 1 H). MS, *m/e* (rel. intensity): M^+ 234 (absent), 175 (7), 157 (4), 145 (4), 129 (4), 115 (7), 103 (5), 101 (18), 90 (100), 85 (38), 73 (9), 59 (10), 57 (42), 43(33).

Dimethyl (R)-*2-isobutylmalate.* II (150 mg) was hydrogenated as described above for I.

The catalyst was filtered off and an excess of diazomethane in ether was added. After 1 h, the reaction mixture was evaporated to dryness and the residue was filtered through a column of silica gel (2.5 × 7 cm) using chloroform-methanol (9:1) as eluent. The crude dimethyl ester was purified by preparative GLC (5 % SE-52 on Chromosorb W, 3 mm × 1.8 m, 130 °C, flow rate 40 ml/min, retention time 4 min) giving dimethyl (*R*)-2-isobutylmalate (20 mg) as an oil $[\alpha]_{578}^{25}$ −0.55°, $[\alpha]_{436}^{25}$ +0.30°, $[\alpha]_{365}^{25}$ +5.3° (*c* 2.0, ethanol) [Lit.[13,14] $[\alpha]_{578}^{24}$ −0.64°, $[\alpha]_{436}^{24}$ +0.16°, $[\alpha]_{365}^{24}$ +3.9° (*c* 1.0, ethanol)]. IR: ν_{max} ($CHCl_3$) 1740 cm^{-1}. NMR ($CDCl_3$): δ 0.85 (d, 3 H, *J* 6 Hz), 0.95 (d, 3 H, *J* 6 Hz), 1.55–1.75 (m, 3 H), 2.73 (d, 1 H, *J* 16 Hz) and 2.89 (d, 1 H, *J* 16 Hz, AB spectrum), 3.67 (s, 3 H); 3.79 (s, 3 H), 3.2–4.0 (1 H, exchangeable in D_2O).

Dimethyl (±)-erythro-*isobutyltartrate*. Isobutylmaleic anhydride[12] (191 mg) was dissolved in pyridine (15 ml) and osmium tetroxide (333 mg) was added. The mixture was stirred at room temperature for 35 min after which sodium hydrogen sulfite (0.6 g), pyridine (7 ml), and water (10 ml) were added. After 30 min at room temperature the solution was heated to 60 °C for 15 min. The reaction mixture was evaporated to dryness and the residue was filtered through Dowex 50W–X8 (H^+, 2.5 × 8 cm) using water as eluent. The dark eluate (180 ml) was extracted with ethyl acetate (6 × 50 ml) and the organic phase was dried and evaporated to dryness. The residue (175 mg) was treated with diazomethane giving the crude ester which was purified by chromatography on silica gel (2.5 × 5.5 cm) using chloroform as eluent. The fraction containing the dimethyl ester was purified by sublimation (0.02 Torr, 45 °C) giving dimethyl (±)-*erythro*-isobutyltartrate (114 mg), m.p. 80–82 °C, indistinguishable (IR, NMR, MS, and GLC) from the natural product.

Dimethyl (±)-threo-*isobutyltartrate*. Isobutylfumaric acid[12] (220 mg) was reacted as described above for isobutylmaleic anhydride, except that the heating was omitted. Sublimation (0.02 Torr, 50 °C) gave dimethyl (±)-*threo*-isobutyltartrate (112 mg) m.p. 50–52 °C. IR: ν_{max} ($CHCl_3$) 3530(m), 1740(s) cm^{-1}. NMR ($CDCl_3$): δ 0.83 (d, 3 H, *J* 6 Hz), 0.94 (d, 3 H, *J* 6 Hz), 1.63–1.92 (m, 3 H), 3.83 (s, 6 H), 4.28 (s, 1 H).

Acknowledgements. We are indebted to Dr. Björn Lüning for supplying the plant material and for his interest in this work. We thank the *Swedish Natural Science Research Council* for support.

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Light-induced Oxidation of Lignin. The Behaviour of Structural Units Containing a Ring-conjugated Double Bond*

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The methyl ethers of isoeugenol, coniferyl alcohol and coniferaldehyde, model compounds for end group units in spruce lignin, have been irradiated with near UV-light in the presence of oxygen. Veratric aldehyde and products arising from addition of solvent to intermediate peroxirane structures were isolated from the reaction mixtures indicating the participation of singlet oxygen.

This mechanism was verified (a) by retarding the reaction with specific singlet oxygen quenchers and (b) by reacting the same compounds with chemically generated singlet oxygen.

The results show that conjugated double bonds in lignin are susceptible to light-induced oxidation giving rise to carbonyl structures which are known to participate in the light-induced oxidation of phenolic units in lignin.

Due to a shortage in the supply of wood, the production of high yield pulps including mechanical pulp has become increasingly attractive. Recent technological improvements have made it possible to bleach mechanical pulp to a high brightness level and to maintain a high yield. Such pulps can substitute low yield, bleached chemical pulps in certain paper grades. One disadvantage of mechanical pulp, whether bleached or unbleached, is its rapid rate of yellowing on exposure to daylight. If a way could be found to improve the stability of such pulps towards the influence of light, the field of application of the pulps could probably be extended considerably. Great efforts have been made both technically and in the laboratory to find a solution to this problem, but these efforts have so far been unsuccessful.

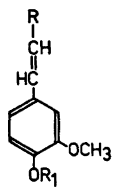
In a recent series of papers much of our present understanding of the light-induced degradation of lignocellulosic materials was elucidated.[1–6] In that work it was shown that among the numerous structural elements present in lignin only a few were able to absorb ultraviolet light present in daylight, *viz.* arylpropane units containing α,β-double bonds, carbonyl groups in conjugation with aromatic rings and biphenyl structures. Furthermore, it was shown that conjugated carbonyl groups excited by light are capable of abstracting hydrogen from phenolic units thus generating phenoxy radicals. In subsequent reaction steps, these phenoxy radicals may react with oxygen to give coloured polymeric structures.

Double bonds in conjugation with aromatic rings are present in lignin end groups of the cinnamyl alcohol and cinnamaldehyde types.[7] In the present paper, studies are described which deal with the behaviour of non-phenolic model compounds of these types under the influence of UV-light with wavelengths between 300 – 400 nm, *i.e.* the UV-part of daylight radiation.

RESULTS

Coniferyl alcohol and coniferaldehyde structures are linked to the lignin polymer through their phenolic hydroxyl groups and/or the aromatic 5-positions.[7] The corresponding aryl methyl ethers (III, IV) were chosen as model compounds. These were irradiated in the presence of oxygen both in solution and absorbed on filter paper thus imitating the conditions

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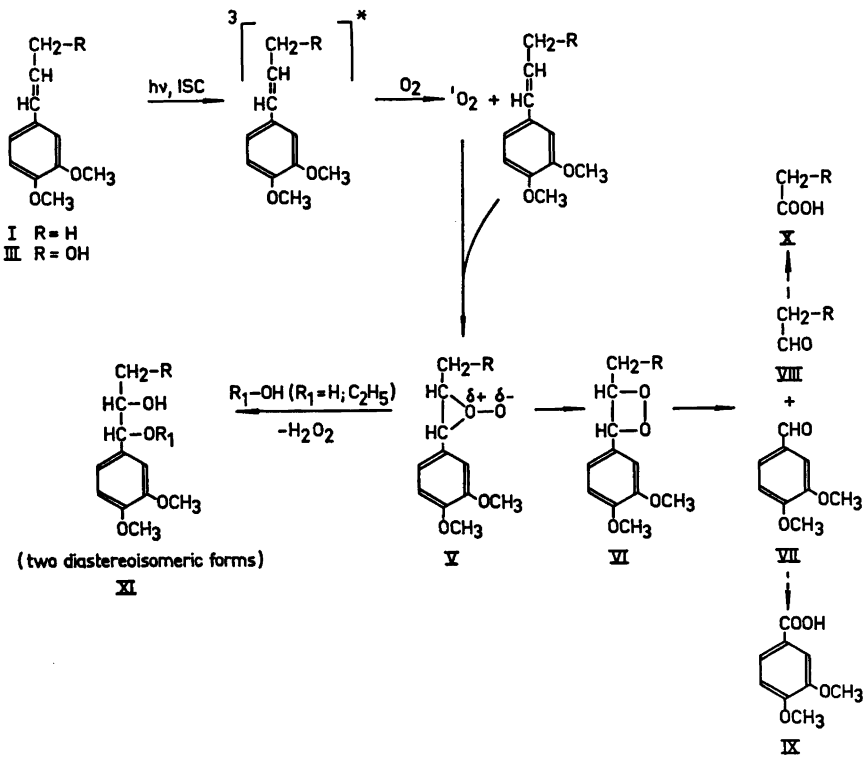


I R = R_1 = CH_3
II R = CH_3, R_1 = H
III R = CH_2OH, R_1 = CH_3
IV R = CHO, R_1 = CH_3

prevailing in a "mechanical pulp". Under these two sets of conditions, coniferyl alcohol methyl ether (III) and the simpler lignin model isoeugenol methyl ether (I) both afforded the same types of reaction products (see Scheme 1).

The reaction sequence may be rationalized in the following way. The model compound absorbs light and enters an excited singlet state. *Via* intersystem crossing (ISC), an excited triplet state is generated, oxygen quenching of which leads to the formation of singlet oxygen. (For comprehensive reviews of the physical and chemical properties of singlet oxygen see Refs. 8 and 9.) Singlet oxygen, which is known to react specifically with olefins, then adds to the double bond giving rise to a peroxirane intermediate (V) (*cf.* Ref. 10). This structure partly rearranges to a dioxetane (VI) which in turn cleaves to yield the two aldehydic fragments VII and VIII. A small amount of veratric acid (IX), formed by further oxidation of veratric aldehyde (VII), was also obtained. (The formation of VIII and its further oxidation to X have been described before [11,12]). At the same time, the peroxirane (V) is attacked directly by solvent with the formation of the glycol structures (XI). In addition, trace amounts of products arising by direct addition of solvent across the double bonds of III and I, respectively, were detected (see Experimental).

Comparative experiments carried out in the absence of oxygen also afforded products formed by addition of solvent across the double bond. In addition, various dimeric products were obtained (see Experimental).



Scheme 1.

In order to confirm the participation of singlet oxygen in the degradative reactions, quenching experiments were carried out. When solutions of isoeugenol methyl ether (I) were irradiated in the presence of β-carotene [13] or DABCO (1,4-diazabicyclo[2.2.2]octane) [14] strong retarding effects were observed, as shown by thin layer chromatography.

Singlet oxygen may also be generated chemically.[9] The simplest and most frequently used method is the oxidation of hydrogen peroxide with sodium hypochlorite. This method was used to oxidize isoeugenol methyl ether (I). As shown by thin layer chromatography, the reaction products were in all respects identical with those obtained from the photochemical oxidation.

In contrast to the methyl ethers of isoeugenol and coniferyl alcohol, coniferaldehyde methyl ether (IV) was remarkably stable when irradiated in solution or in the solid state. Thus, after several days of irradiation only a small amount of veratric aldehyde could be detected. The low degree of reactivity is obviously due to the presence of the aldehyde group which decreases the electron density at the double bond. This is in accordance with literature data. It has been repeatedly demonstrated that 1,2-cycloaddition between singlet oxygen and olefins requires the presence of an electron-rich double bond.[8,9]

In order to elucidate the reactions of phenolic units of the styrene type present in lignin, the light-induced oxidation of isoeugenol (II) was investigated. Contrary to the behaviour of the non-phenolic model compounds I and III, the sidechain double bond of II was attacked to only a minor extent. When the irradiation was carried out in solution only oligomeric coupling products were obtained. In the solid state, polymeric material was formed together with a small amount of vanillin (thin layer chromatography).

DISCUSSION

The results of these model studies indicate that daylight irradiation of lignin causes the degradation of non-phenolic end groups of the coniferyl alcohol type into an aromatic and aliphatic aldehyde fragment, as well as the conversion of such groups into arylglycerol structures. Previous investigations have shown that excited aromatic carbonyl structures exert a dominant influence upon the light-induced yellowing reaction of lignin.[2–6,15] The results here obtained show that arylpropane units containing α,β-double bonds may induce the same type of reaction after their partial conversion into aromatic aldehydes.

Most of the present knowledge about photooxidation of various model compounds for lignin is based upon results obtained in studies in which homogeneous solutions were irradiated. In a polymer matrix such as wood, functional groups, due to their restricted mobility may be widely separated in space. For this reason it is likely that, in addition to a direct hydrogen abstraction mechanism,[4] excited carbonyl structures may transfer energy to oxygen giving rise to the formation of singlet oxygen. Singlet oxygen is reasonably stable [9] and has the mobility required to transfer energy within a polymer matrix (*cf.* Ref. 16). Support for this view can be found in previous studies of dye-sensitised photochemical degradation of textile fibres [17] and in studies of the autoxidation of aromatics in polymer films [18,19] (*cf.* also Ref. 20). It is furthermore known that singlet oxygen may abstract hydrogen from phenolic hydroxyl groups and thus generate phenoxy radicals.[21,22] It thus seems justified to assume that singlet oxygen is an important participant in the lignin-yellowing reactions. As mentioned above, the major colour-forming reactions start with the addition of ground state oxygen to generated phenoxy radicals. The further reactions of the resulting hypothetical hydroperoxy intermediates and whether or not it is possible to generate radicals from structures in wood other than phenols under the influence of light are, however, still unknown and are important subjects for furture work in this field.

EXPERIMENTAL

Chromatography. TLC on silica gel HF_{254}. Preparative separations were carried out in a liquid chromatograph (Chromatronix Inc. Berkeley, Cal. USA) using silicic acid (Bio-Sil A 20–44 μm, Bio Rad Lab. Richmond, Cal. USA) as the stationary phase.

NMR. Perkin-Elmer R-12 (60 MHz, $CDCl_3$). Internal standard: Tetramethylsilane. Chemical shifts. δ-values.

MS. Perkin-Elmer 270. Electron energy: 20 eV. Temperature of probe heater: 50 °C. Relative intensities of the main peaks are given within parenthesis.

Model compounds: trans-Isoeugenol methyl ether (I) was prepared by methylation of *trans*-isoeugenol acetate (recrystallized twice from ethanol) with dimethyl sulfate in alkaline solution using water-dioxane (4:1) as solvent. Distillation under vacuum afforded the pure *trans* isomer.[23] $B.p._{0.05} = 79.5$ °C.

Coniferyl alcohol methyl ether (III). Reduction of 3,4-dimethoxycinnamic acid methyl ester[24] with $LiAlH_4$. M.p. 78–79 °C (benzene–hexane) (lit.[25] m.p. 78 °C) *trans*-3,4-dimethoxy-cinnamaldehyde (IV). Oxidation of eugenol methyl ether with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) for 3 days at room temperature in dioxane containing 0.5 % water;[26] (*cf.* also Ref. 27). M.p. 83–84 °C (benzene). (lit.[28] M.p. 83–84 °C.)

Irradiation. 1 g of the model compound concerned was dissolved in 100 ml ethanol-water (1:1) in a quartz flask. Air (nitrogen was used in some comparative experiments) was bubbled through the solution during irradiation in a photochemical reactor equipped with eight light tubes emitting light centered around 350 nm (Rayonet RPR-208, Southern New England Ultraviolet Co. Middletown, Conn. USA). After irradiation the solution was neutralized with aqueous sodium bicarbonate and evaporated to a small volume. Extraction with ethyl acetate afforded the neutral products. To the remaining aqueous solution, 2 ml 0.5 M tetrabutylammonium sulfate was added. Acidic reaction products were extracted as ion pairs with methylene chloride and reacted with methyl iodide yielding the corresponding methyl esters.[29]

Comparative experiments in the solid state were carried out by absorption of methylene chloride solutions of the model compounds on Whatman No. 1 filter paper sheets. After irradiation, the papers were extracted with acetone and the products investigated by TLC.

Products from isoeugenol methyl ether (I).

a) *Irradiation in O_2 for 48 h.* Neutral fraction: 920 mg. Acidic fraction: 60 mg of veratric acid methyl ester (identified by comparison with an authentic sample). The neutral fraction, after acetylation, was preparatively separated in light petroleum (60–70 °C)/ethyl acetate (gradient from 5:1 to 3:1).

Fraction 1 (fastest moving). Mixture of four components; 35 mg. Analysis by GLC-MS (10 % OV-1 on Chromosorb 100–200 mesh. 30 ml N_2/min): 1-(3,4-Dimethoxyphenyl)-1-ethoxypropane; MS, $m/e = 224(6)$, 195(55), 178(100), 167(24), 163(46), 139(36). 3,4-Dimethoxypropiophenone (comparison with an authentic sample); MS, $m/e = 194(8)$, 165(100), 137(12). 1-(3,4-Dimethoxyphenyl)-1,2-diethoxypropane (first form); MS, $m/e = 268(1)$, 222(20), 195(100), 167(34), 165(20), 151(16), 139(47). 1-(3,4-Dimethoxyphenyl)-1,2-diethoxypropane (second form); MS, see above.

Fraction 2. 1-(3,4-Dimethoxyphenyl)-1-ethoxy-2-acetoxypropane (first form); 70 mg. NMR, 1.17 (3 H, t), 1.21 (3 H, d), 1.91 (3 H, s), 3.41 (2 H, q), 3.84 (6 H, s), 4.21 (1 H, d), 5.0 (1 H, m), 6.81 (3 H, broad s); MS, $m/e = 282(3)$, 222(6), 195(100), 167(10), 165(5), 151(3), 139(5), 143(metastable). (Found: C 63.72; H 7.63; O 28.59. $C_{15}H_{22}O_5$ requires C 63.80; H 7.85; O 28.35).

Fraction 3. 1-(3,4-Dimethoxyphenyl)-1-ethoxy-2-acetoxypropane (second form); 170 mg. NMR, 1.06 (3 H, d), 1.14 (3 H, t), 2.00 (3 H, s), 3.38 (2 H, q), 3.84 (6 H, s), 4.14(1 H, d), 5.08 (1 H), 6.80 (3 H, broad s); MS, see fraction 2.

Fraction 4. Veratric aldehyde. 380 mg (comparison with an authentic sample).

Fraction 5. 1-(3,4-Dimethoxyphenyl)-1,2-diacetoxypropane (first form); 85 mg. NMR, 1.06 (3 H, d), 2.02 (3 H, s), 2.03 (3 H, s), 3.84 (3 H, s), 3.86 (3 H, s), 5.23 (1 H), 5.68 (1 H, d), 6.84 (3 H, broad s); MS, $m/e = 296(5)$, 236(11), 209(6), 195(10), 194(24), 178(11), 167(100), 151(19), 139(30). (Found: C 60.92; H 6.71; O 32.29. $C_{15}H_{20}O_6$ requires C 60.80; H 6.81; O 32.39).

Fraction 6. 1-(3,4-Dimethoxyphenyl)-1,2-diacetoxypropane (second form); 60 mg. NMR, 1.17 (3 H, d), 2.00 (3 H, s), 2.08 (3 H, s), 3.81 (3 H, s), 3.84 (3 H, s), 5.20 (1 H, m). 5.83 (1 H, d), 6.85 (3 H, broad s); MS, see fraction 5.

(b) Irradiation in N_2 for 72 h. Neutral fraction: 950 mg. No acids were found. Preparative separation (after acetylation) in light petroleum (60–70 °C)/ethyl acetate (gradient from 4:1 to 3:1).

Fraction 1 (fastest moving). Starting material. 580 mg.

Fraction 2. Dimer.C_2H_5OH; 15 mg. No detailed structure can be given. Mass peaks at $m/e = 402(1)$, 356(16), 178(100).

Fraction 3. Dimer.C_2H_5OH; 25 mg. No detailed structure can be given. Mass peaks at $m/e = 402(5)$, 356(35), 221(31), 195(22), 178(67), 149(100).

Fraction 4. Bis-(3,4-dimethoxyphenyl)-dimethylcyclobutane; 25 mg. NMR: 0.38 (3 H, d), 1.29 (3 H, d), 2.41 (2 H, m), 3.56 (3 H, s), 3.63 (3 H, s), 3.77 (6 H, s), 6.6(6 H) (signals of benzylic protons hidden by signals of OCH_3). MS: m/e = 356(1), 178(100).

Fraction 5. 1-*cis*-2-Bis(3,4-dimethoxyphenyl)-*trans*-3,*trans*-4-dimethylcyclobutane. 100 mg. M.p. 62.5–63.5 °C. NMR (*cf.* Ref. 30): 1.18 (6 H, d), 2.79 (2 H, m), 3.43 (2 H, d), 3.60 (6 H, s), 3.74 (6 H, s), 6.4 (6 H). MS: $m/e = 356(2)$, 178(100). (Found: C 74.88;

H 7.57; O 17.60. $C_{22}H_{28}O_4$ requires C 74.13; H 7.91; O 17.96).

Fraction 6. Dimer.C_2H_5OH; 30 mg. No detailed structure can be given. Mass peaks at $m/e = 402(2)$, 356(1), 196(42), 178(33), 168(100), 139(54), 115(metastable).

Fraction 7. 1-(3,4-Dimethoxyphenyl)-1-acetoxypropane 110 mg. NMR: 0.87 (3 H, t), 1.80 (2 H, m), 2.04 (3 H, s), 3.86 (3 H, s), 3.88 (3 H, s), 5.62 (1 H, t), 6.87 (3 H, s). MS: $m/e = 194(34)$, 178(23), 167(100), 139(71). 142, 116(metastable ions). (Found: C 65.64; H 7.72; O 26.65. $C_{13}H_{18}O_4$ requires C 65.53; H 7.61; O 26.86).

(c) Irradiation in O_2 for 48 h in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO). Irradiation was performed in the presence of 100 mg DABCO. Only small amounts of reaction products were formed (TLC).

(d) Reaction with chemically generated singlet oxygen ($^1O_2$). Isoeugenol methyl ether (I), dissolved in ethanol, was reacted with $^1O_2$ generated by oxidation of H_2O_2 with NaOCl following a procedure described by Foote *et al.*[31] The product mixture (TLC) consisted of starting material and the same reaction products as in experiment (a) (see above).

Irradiation of coniferyl alcohol methyl ether (III).

(a) In O_2 for 96 h. Neutral fraction: 1.36 g (after acetylation). Acidic fraction: 50 mg of veratric acid methyl ester. Preparative separation in light petroleum (60 – 70 °C)/ethyl acetate (gradient from 7:3 to 3:2).

Fraction 1 (fastest moving). 1-(3,4-Dimethoxypenyl)-1-ethoxy-3-acetoxypropane (impure material); 15 mg. Structure based on MS only. $m/e = 282(15)$, 195(100), 167(20), 139(30).

Fraction 2. Veratric aldehyde (comparison with an authentic sample); 210 mg.

Fraction 3. 1-(3,4-Dimethoxyphenyl)-1-acetoxy-2-propene. [Allylic rearrangement of the starting material (*cf.* Refs. 32, and 33)]; 85 mg. NMR, 2.03 (3 H, s), 3.82 (6 H, s), 4.81 (2 H, broad d), 5.7 (1 H, m), 6.57 (1 H, broad d), 6.78 (3 H, s); MS. $m/e = 236(100)$, 194(27), 193(43), 177(47), 176(33), 165(27), 151(18).

Fraction 4. 1-(3,4-Dimethoxyphenyl)-1-ethoxy-2,3-diacetoxypropane (first form); 185 mg. NMR, 1.14 (3 H, t), 1.89 (3 H, s), 2.01 (3 H, s), 3.39 (2 H, q), 3.85 (6 H, s), 4.3 (3 H), 5.2 (1 H), 6.81 (3 H, broad s); MS $m/e = 340(4)$, 280(1), 236(3), 221(3), 195(100), 167(29), 165(14), 151(5), 139(31). (Found: C 59.88; H 7.02; O 33.01. $C_{17}H_{24}O_7$ requires C 59.99; H 7.10; O 32.91).

Fraction 5. 1-(3,4-Dimethoxyphenyl)-1-ethoxy-2,3-diacetoxypropane (second form); 65 mg. NMR, 1.16 (3 H, t), 2.02 (6 H, s), 3.38 (2 H, q), 3.84 (6 H, s), 4.2(3 H), 5.2(1 H), 6.81 (broad s); MS, see fraction 4.

Fraction 6. 1-(3,4-Dimethoxyphenyl)-1,2,3-triacetoxy-propane (first form); 260 mg. NMR, 1.97 (3 H, s), 2.03 (3 H, s), 2.09 (3 H, s), 3.84 (3 H, s), 3.86 (3 H, s), 4.22 (2 H, d), 5.4 (1 H), 5.91 (1 H, d), 6.86 (3 H, broad s); MS, $m/e = 354(16)$, 294(20), 252(4), 234(4), 209(16), 192(46), 167(100), 165(12), 243(metastable). (Found: C 58.24; H 6.61; O 35.14. $C_{17}H_{22}O_8$ requires C 57.62; H 6.26; O 36.12).

Fraction 7. 1-(3,4-Dimethoxyphenyl)-1,2,3-triacetoxy-propane (second form); 370 mg. NMR, 2.02 (3 H, s), 2.04 (6 H, s), 3.83 (3 H, s), 3.86 (3 H, s), 4.14 (2 H, d), 5.4 (1 H), 5.89(1 H, d), 6.87 (3 H, broad s); MS, see fraction 6.

(b) In N_2 for 96 h. Neutral fraction 1.41 g (after acetylation). No acidic products. Preparative separation in light petroleum (60 – 70 °)/ethyl acetate (gradient from 7:3 to 3:2).

Fraction 1 (fastest moving). 1-(3,4-Dimethoxyphenyl)-3-ethoxy-1-propene (*cf.* Ref. 33); 40 mg. NMR, 1.24 (3 H, t), 3.52 (2 H, q), 3.84 (6 H, s), 4.09 (2 H, d), 5.88 – 6.31 (1 H, m), 6.53 (1 H, d, J 15.2 Hz), 6.9 (3 H, m); MS, $m/e = 222(100)$, 193(9), 177(15), 176(14), 165(16).

Fraction 2. 1-(3,4-Dimethoxyphenyl)-1-acetoxy-2-propene; 105 mg. NMR and MS data, see above. (Found: C 65.96; H 6.87; O 27.17. $C_{13}H_{16}O_4$ requires C 66.08; H 6.84; O 27.08).

Fraction 3. 1-(3,4-Dimethoxyphenyl)-1-ethoxy-3-acetoxypropane; 45 mg. NMR, 1.11 (3 H, t), 2.02 (3 H, s), 1.8 – 2.2 (2 H), 3.32 (2 H, q), 3.86 (6 H, s), 4.0 – 4.4 (3 H), 6.80 (3 H, broad s); MS, $m/e = 282(16)$, 236(1), 222(1), 195(100), 177(6), 176(9), 167(18), 139(17). Metastable ions at $m/e = 143$, 116. (Found: C 64.03; H 8.01; O 27.96. $C_{15}H_{22}O_5$ requires C 63.80; H 7.85; O 28.33).

Fraction 4. 1-(3,4-Dimethoxy-α-ethoxybenzyl)-2-(3,4-dimethoxyphenyl)-3-ethoxymethyl-cyclobutane; 30 mg. Tentative structure based mainly on MS. NMR, signals from two C_2H_5O-, four CH_3O-groups and six arom. H were clearly discernible; MS, $m/e = 444(9)$, 398(17), 386(11), 340(23), 339(33), 221(6), 195(100), 177(12), 167(16), 151(19), 139(14).

Fraction 5. 1,6-di-(3,4-Dimethoxyphenyl)-1,5-hexadiene; 45 mg. Tentative structure based mainly on NMR. NMR, 3.84 (12 H, s), 4.05 – 4.35 (4 H), 5.50 – 6.70 (4 H), 6.80 (6 H, broad s); MS $m/e = 354(1)$, 300(9), 178(100), 177(53). Six further fractions were obtained in a total amount of 800 mg. NMR and MS indicated dimers and possibly trimers.

Irradiation of coniferaldehyde methyl ether (IV) in O_2 for 150 h. Starting material and a small amount of veratric aldehyde (TLC).

Irradiation of isoeugenol (II) in O_2 for 48 h (*cf.* Refs. 34 and 35) Neutral fraction: 1000 mg. No acids were formed. Preparative separation (after acetylation) in light petroleum (60 – 70 °C)/ethyl acetate (gradient from 4:1 to 3:1).

Fraction 1 (fastest moving). Starting material. 560 mg.

Fraction 2. 4-(2,3-Dihydro-7-methoxy-3-methyl-5-propenyl-2-benzofuranyl)-2-methoxyphenyl acetate. (Dehydrodiisoeugenol acetate).

Identified by comparison with an authentic sample; 180 mg.

Fraction 3. 1-Ethoxy-1-(4-acetoxy-3-methoxyphenyl)-2-(4-propenyl-2-methoxyphenoxy)-propane; 110 mg (For NMR and MS data see Ref. 34). (Found: C 69.32; H 7.28; O 23.18. $C_{24}H_{30}O_6$ requires C 69.57; H 7.24; O 23.19.

Fraction 4. Trimer.C_3H_5OH (as diacetate); 60 mg. No detailed structure can be given. MS, $m/e = 618(1)$, 574(3), 532(5), 490(5), 414(3), 368(8), 326(5), 250(4), 223(54), 181(100), 164(24), 153(32), 152(84).

Irradiation of veratric aldehyde. When irradiated for 48 h in the presence of oxygen, veratric aldehyde was partially converted into veratric acid. From the aqueous solution, 160 mg of veratric acid methyl ester was obtained.

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Structural Studies on a Water-soluble Arabinan Isolated from Rapeseed (*Brassica napus*)

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The structure of an arabinan, isolated from rapeseed flour, has been investigated. Methylation analysis on the original polysaccharide and on products obtained after mild acid hydrolysis and after Smith degradation of the polysaccharide, have provided the essential information in this study. A tentative structure for the polysaccharide is presented

RESULTS AND DISCUSSION

Oil-free, heat-treated and coat-free rapeseed flour from *Brassica napus* var. *Sinus* was extracted first with ethanol and then with hot water. The aqueous fraction was precipitated with ethanol. The precipitate was removed and from the concentrated ethanol phase an arabinogalactan (A) $[\alpha]_{578}$ −111° and an arabinan (B) $[\alpha]_{578}$ −83° were isolated by gel chromatography (Fig. 1). The structural investigation of the arabinan will be presented in this paper.

The arabinan showed a symmetrical elution curve on both Sepharose 2B and Sephadex G-200. IR spectroscopy, paper chromatography and paper electrophoresis of a hydrolysate of the polymer revealed that it was a pure arabinan devoid of uronic acid residues. A GLC analysis showed that arabinose accounted for 95 % of the polysaccharide. As the optical rotation of the polymer changed from a large negative value to a large positive value on acid hydrolysis under mild conditions, it is assumed that the polysaccharide is composed of α-L-arabinofuranose units.[1]

The arabinan was fully methylated and hydrolysed, and the methylated sugars were converted into their alditol acetates and analysed by GLC-MS.[2] (Table 1, column A). From the methylation analysis, it was concluded that the arabinan consisted of (1→5)-linked arabinofuranoside residues and that about 33 % of the sugar residues were further substituted at O-3. A small amount of L-arabinose, substituted in the 2,3 and 5 positions was also found. It is reasonable to assume that part of the volatile 2,3,5-tri-*O*-methylarabinose and its derivatives was lost during the concentrations. This explains the poor agreement between terminal and branching L-arabinose units in the methylation analysis. No 3-*O*-methylarabinose was detected.

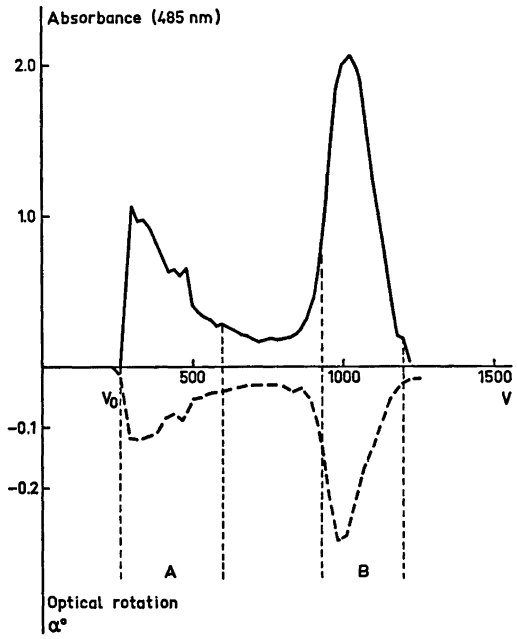


Fig. 1. Purification of a water-soluble arabinan on Sepharose 2 B. Absorbance at 485 nm in phenol-sulfuric acid. Elution volume (V) in ml.

Table 1. Hydrolysis products of (A) methylated arabinan, (B) partially hydrolysed, deuterium reduced and methylated arabinan, (C) periodate oxidised, reduced and methylated arabinan, (D) periodate oxidised, Smith degraded (20 h), and methylated arabinan, and (E) periodate oxidised, Smith degraded (69 h) and methylated arabinan.

Sugars	r^a	Mol % A	B	C	D	E
1,2,3,4,5-Penta-*O*-methyl-L-arabinitol [b]	0.05		13			
1,2,3,4-Tetra-*O*-methyl-L-arabinitol [b]	0.17		5			
1,2,4,5-Tetra-*O*-methyl-L-arabinitol [b]	0.17					
1,2,4-Tri-*O*-methyl-L-arabinitol	0.29		4			
2,3,5-Tri-*O*-methyl-L-arabinose	0.40	32	33		67	68
3,5-Di-*O*-methyl-L-arabinose	0.75		2			
2,5-Di-*O*-methyl-L-arabinose	0.84		5			
2,3-Di-*O*-methyl-L-arabinose	1.01	32	24		33	32
2-Mono-*O*-methyl-L-arabinose	1.79	28	11	77		
L-arabinose	2.52	8	3	23		

[a] Retention times of the corresponding alditol acetate on the 3 % OV-225 column, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b] The quantitation of these volatile sugar derivatives is uncertain.

The 100 MHz ^{1}H NMR spectrum of the original arabinan, dissolved in pyridine showed *inter alia* three signals of similar intensity at δ 5.45, 5.64 and 5.77. Each of these signals integrated for one proton and they were assigned to the anomeric protons from the terminal, the chain and the branched arabinose residues.

In order to verify that the L-arabinose units are furanosidic, the polysaccharide was subjected to a mild acid treatment, generating new terminal units. The partially hydrolysed arabinan was reduced with sodium borodeuteride. Methylation analysis (Table 1, column B) yielded *inter alia* 1,2,4-tri-*O*-methyl-L-arabinitol and 2,3,5-tri-*O*-methyl-L-arabinose but no 2,3,4-tri-*O*-methyl-L-arabinose. The 1,2,4-tri-*O*-methyl-L-arabinitol was deuterated at C-1, and derived from the reducing end of the degraded polysaccharide. The tetra- and penta-*O*-methyl ethers were also deuterated which facilitated their identification on MS. The absence of 2,3,4-tri-*O*-methyl-L-arabinose ($r = 0.48$) demonstrates that all the terminal units in the degraded polysaccharide are furanosidic, indicating that all the arabinose units in the arabinan are furanosidic. In order to investigate the distribution of the branched arabinose units, the polysaccharide was subjected to a Smith degradation,[3] that is periodate oxidation, borohydride reduction and acid hydrolysis under mild conditions. Only the branched arabinose units should remain intact and their glycosidic linkages should not be degraded by this treatment. The products of the Smith degradation were examined by methylation analysis (Table 1, columns C, D, and E).[4] If every second residue in a chain was branched, only 2,3,5-tri-*O*-methyl arabinose should have been obtained in the methylation analysis of the degraded polymer. If the branching residues were concentrated in block structures, 2,3-di-*O*-methyl arabinose would have predominated. As neither of these alternatives were observed, it is evident that the arabinan has both isolated and adjacent branching residues. The structural features observed are summarized in Fig. 2, which does not, however, represent the exact structure of the polysaccharide. It is unlikely that the polysaccharide has an ordered structure and the length of the side chains has not been deduced.

Genuine arabinans of plant origin, generally associated with pectin in the cell wall, have been studied earlier.[4-8] The structural features of these arabinans were discussed mainly on the basis of methylation analysis data on the original arabinan. Rees and Richardson,[4] however, used Smith degradation together with methylation analysis in their investigation of arabinan from white mustard. The mode of linkage is the same in all arabinans of plant origin, but the detection of only traces of 3-*O*-

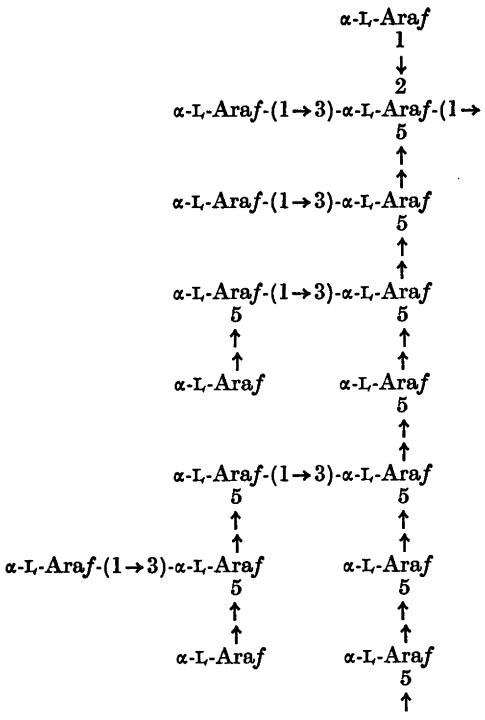


Fig. 2. Structural features of the arabinan.

methyl-arabinose in the methylation analysis of arabinans from the mustard/rape-family seems to be characteristic. The degree of branching, the molecular weight and the optical rotation differs between arabinans from different sources. However, these differences are small between arabinans isolated from the mustard and the rape family.[4,6]

EXPERIMENTAL

Concentrations were performed at reduced pressure at bath temperature not exceeding 40 °C. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on a Varian HA-100 D spectrometer and IR spectra with a Perkin-Elmer 337 spectrometer. GLC was conducted with a Varian model 2700 instrument fitted with a column containing 3 % OV-225 on Gas-Chrom Q. For GLC-MS, a Varian CH7 gaschromatograph-mass spectrometer was used. Paper chromatograms were run on Whatman No. 1 papers, using the following systems (v/v): (a) ethyl acetate-acetic acid-water, 3:1:1, (b) pyridine-ethyl acetate-water, 2:8:1, (c) butanol-acetic acid-water, 6:2:1. The components were detected with *p*-anisidine hydrochloride or silver nitrate-sodium hydroxide.

Preparation of the polysaccharide. Oil-free, coat-free, heat-treated and milled rapeseed from *Brassica napus* var. *Sinus* (1.9 kg dry weight) was extracted (6 × 20 min) with 80 % ethanol (6 × 5 l) on a boiling waterbath under reflux. The ethanol insoluble fraction was extracted (5 × 2 h) with hot water (5 × 5 l) and the water extract was concentrated to 3 l, centrifuged and precipitated with 4 volumes of ethanol. The precipitate was dissolved in water (3 l) and reprecipitated with 2 volumes of ethanol. The ethanol phases from the precipitations were concentrated and deproteinised by the hot phenol-water procedure of Westhpal *et al.*[9] The aqueous phase was dialysed against distilled water, concentrated and precipitated with 6 volumes of ethanol. The precipitate (1.8 g), on acid hydrolysis yielded arabinose and galactose in the relative proportions 24:1; traces of mannose were also found in the hydrolysate. The material (0.9 g) was dissolved in water (20 ml) and applied on a Sepharose 2 B column (3.5 × 100 cm) which was irrigated with water. 10 ml fractions were collected and the separation was monitored by the phenol sulfuric acid method[10] and with optical rotation. The polysaccharide fractions, A and B (Fig. 1) were concentrated, dialysed and freeze-dried. A, (243 mg), $[\alpha]_{578} - 111°$, (*c* 0.54, water), on hydrolysis yielded arabinose and galactose in the relative proportions 10:1. B, (423 mg), $[\alpha]_{578} - 83°$ (*c* 0.88, water), yielded on hydrolysis only arabinose. Molecular weight determinations using calibrated Sepharose columns[11] gave $\overline{M}_w$-values of 7×10^6 and of 15×10^3 for A and B, respectively.

Sugar analysis of the arabinan. A mixture of the arabinan (B) (5 mg) and *myo*-inositol (5 mg) was treated with 0.25 M sulfuric acid, for 16 h at 100 °C. The hydrolysate was neutralised ($BaCO_3$), reduced (KBH_4), acetylated, and analysed by GLC-MS. The sugar analysis revealed that arabinose accounted for 95 % of the material.[12] The identity of the sugars was confirmed by paper chromatography, system a, b, and c.

Methylation analysis of the arabinan. The polysaccharide (10 mg) was methylated in dimethyl sulfoxide by treatment with sodium methylsulfinyl carbanion and methyl iodide.[13] The resulting solution was dialysed against distilled water, concentrated, and the product was hydrolysed, reduced, converted into partially methylated alditol acetates, and analysed by GLC-MS.[2] The results are summarised in Table 1, column A.

Analysis of partially hydrolysed arabinan. The arabinan (25 mg) was hydrolysed in 10 ml sulfuric acid (0.045 M) for 2.5 h at 82 °C. The hydrolysate was neutralized ($BaCO_3$) and reduced ($NaBD_4$). Methylation analysis of the material was performed as described above with

the exception that the methylated products were isolated by partition between chloroform and water. The results are summarised in Table 1, column B.

Methylation analysis of periodate oxidized, reduced and Smith degraded arabinan.[4] The arabinan (38 mg) was dissolved in 0.0400 M sodium metaperiodate (13.3 ml), propanol (0.5 ml) was added and the mixture was kept in the dark at room temperature. The reaction was followed by measurement of the optical rotation. When the oxidation was complete (36 h), ethylene glycol and then an excess of potassium borohydride were added and the solution was kept at room temperature for 24 h. The reaction mixture was neutralised by the addition of Dowex 50 (H^+ form), filtered, dialysed against distilled water and concentrated. The product was dissolved in 0.5 M sulfuric acid (20 ml) and left at room temperature for 70 h. Samples were withdrawn after 0, 20, and 70 h, neutralised ($BaCO_3$) and subjected to methylation analyses. The methylation analyses were performed as described above. The results are presented in Table 1, columns C, D, and E.

Acid hydrolysis of the arabinan. A solution of the arabinan (26 mg) in water (5 ml) was diluted with the same volume of cold 0.09 M sulfuric acid, transferrred to a 1 dm polarimeter tube, thermostated at 82 °C and the optical rotation was determined at intervals. An increase of $[\alpha]_{578}$ from -111 to $+83°$ in 16.5 h was observed.

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Bacterial Carotenoids. XLVIII.* Total Syntheses of Carotenes of the 1,2-Dihydro Series

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The total syntheses of 1,2,7,8,1′,2′,7′,8′-octahydro-ψ,ψ-carotene (*1*), 1,2,7,8-tetrahydro-ψ,ψ-carotene (*2*), 1,2,1′,2′-tetradehydro-ψ,ψ-carotene (*3*), 1,2-dihydro-ψ,ψ-carotene (*4*), 1,2-dihydro-3,4-didehydro-ψ,ψ-carotene (*5*), and 1,2,1′,2′-tetrahydro-3,4,3′,4′-tetrahydro-ψ,ψ-carotene (*6*) are described. The properties of products and intermediates, including the three new apocarotenals 1,2,7,8-tetrahydro-12′-apo-ψ-caroten-12′-al (*20*), 1,2-dihydro-8′-apo-ψ-caroten-8′-al (*25*), and 1,2-dihydro-3,4-didehydro-8′-apo-ψ-caroten-8′-al (*32*), are reported.

A fragment ion at M−68 on electron impact appears to be characteristic for carotenoids with a 1,2,7,8-tetrahydro end-group.

A photosynthetic bacterium isolated by Eimhjellen [1] and subsequently named *Rhodopseudomonas viridis* [2] was initially [3] considered to contain common aliphatic carotenes, mainly neurosporene. However, Malothra *et al.*[4,5] demonstrated that common aliphatic carotenes were only minor constituents, the major carotenes were of a novel 1,2-dihydro (1,2,1′,2′-tetrahydro) aliphatic series, both in normal cells and cells grown in the presence of diphenylamine.

Due to the restricted quantities available some of these carotenes were identified on the basis of their visible light absorptions and fragmentation patterns on electron impact alone. Total synthesis have now been performed in order to allow full physical characterization.

The synthesis of the lycopene derivatives *3* and *4* has previously been reported in a priority note.[6] We now describe in full the preparations of the series *1*—*6*.

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RESULTS AND DISCUSSION

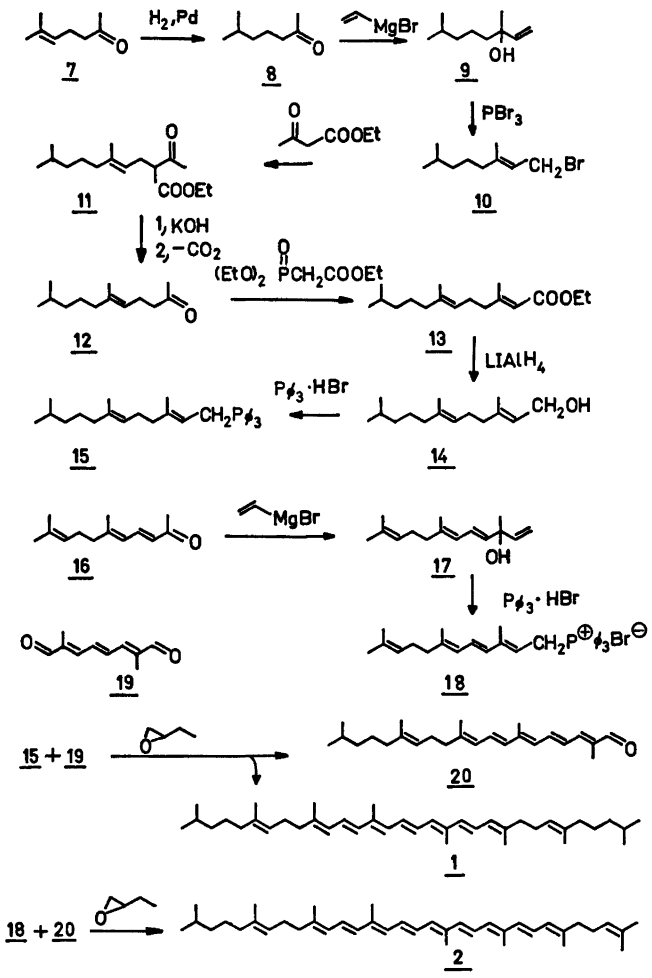
The synthesis of 1,2,7,8,1′,2′,7′,8′-octahydro-ψ,ψ-carotene (*1*) and 1,2,7,8-tetrahydro-ψ-ψ-carotene (*2*) is summarized in Scheme 1.

Methylheptenone (*7*) was hydrogenated in the presence of palladium catalyst to give the saturated ketone *8*. Reaction of *8* with vinyl magnesium bromide gave the tertiary allylic alcohol *9* which was converted to the primary allylic bromide *10* with phosphorus tribromide. Condensation of *10* with acetoacetic ester gave *11* which was hydrolyzed and decarboxylated directly to give dihydrogeranylacetone (*12*). Horner condensation of *12* with ethyl diethylphosphonoacetate gave the conjugated ester *13* which was reduced to the allylic alcohol *14* with $LiAlH_4$ and subsequently converted to the phosphonium salt *15* with triphenylphosphonium bromide.

Similarly pseudoionone (*16*) was converted to the phosphonium salt *18 via* the tertiary alcohol *17*.

Wittig condensation of the ylid of *15* with the C_{10}-dialdehyde *19* gave a mixture of 1,2,7,8,1′,2′,7′,8′-octahydro-ψ,ψ-carotene (*1*, with heptaene chromophore) and the C_{25}-aldehyde *20* which were separated by chromatography. The C_{25}-aldehyde *20* was subsequently condensed with the ylid of the phosphonium salt *18* in a second Wittig reaction to 1,2,7,8-tetrahydro-ψ,ψ-carotene (*2*, with nonaene chromophore).

1,2,1′,2′-Tetrahydro-ψ,ψ-carotene (*3*) and 1,2-dihydro-ψ,ψ-carotene (*4*) were synthesized as depicted in Scheme 2:

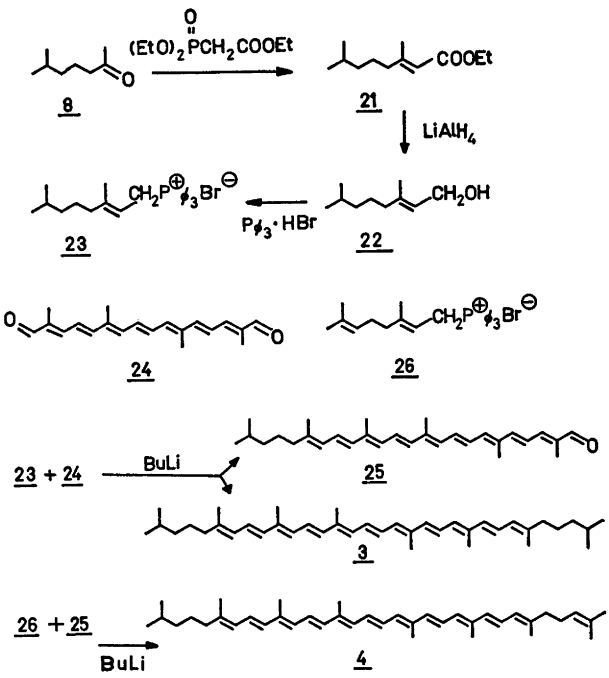


Scheme 1.

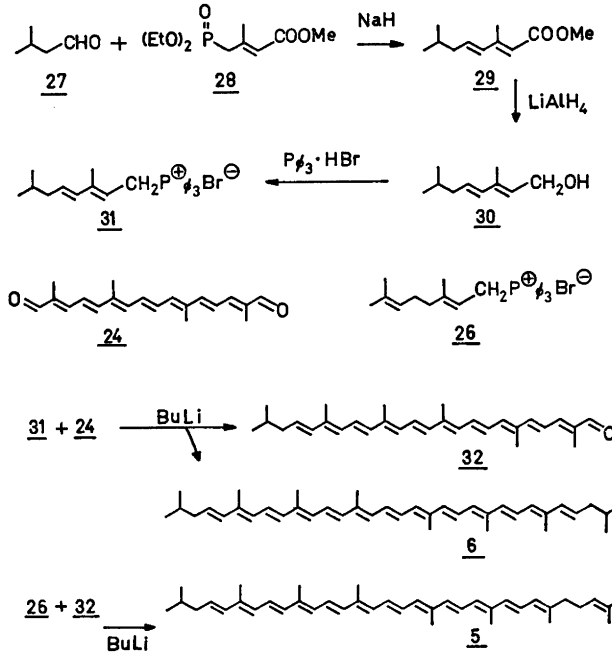
The saturated ketone *8* was condensed with ethyl diethylphosphonoacetate in a Horner reaction to give the unsaturated ester *21* which was reduced with $LiAlH_4$ to dihydrogeraniol (*22*), in turn converted to the phosphonium salt *23* with triphenylphosphonium bromide. Wittig condensation of the ylid of *23* with crocetindial (*24*) gave a mixture of 1,2,1′,2′-tetrahydro-ψ,ψ-carotene (*3*, with undecaene chromophore) and the C_{30}-carotenal *25* which were separated by chromatography. A second Wittig condensation of *25* with geranyltriphenylphosphonium bromide (*26*) provided the lycopene derivative 1,2-dihydro-ψ,ψ-carotene (*4*). The physical properties of *3*, *4*, and *25* are compiled in Tables 1—3.

The preparation of 1,2-dihydro-3,4-didehydro-ψ,ψ-carotene (*5*, with dodecaene chromophore) and 1,2,1′,2′-tetrahydro-3,4,3′,4′-tetradehydro-ψ,ψ-carotene (*6*, with tridecaene chromophore) was accomplished as depicted in Scheme 3:

Horner condensation of isovaleraldehyde (*27*) and the phosphonate *28* gave the conjugated ester *29* which gave the allylic alcohol *30* on reduction with $LiAlH_4$. Treatment of *30* with triphenylphosphonium bromide resulted in the phosphonium salt *31*, the ylid of which was condensed with crocetindial (*24*) in a Wittig reaction to give a mixture of *6* and the apocarotenal *32*. Separation of these products by chromatography and subsequent Wittig condensation of *32* with the ylid of geranyltri-



Scheme 2.



Scheme 3.

phenylphosphonium bromide (*26*) gave *5*.

The IR spectra of the hydrocarbons *1*—*6*, as well as of the apo-carotenals *20*, *25* and *32* were not informative and only normal carotenoid CH absorptions as well as carbonyl frequencies for the apo-carotenals were observed.

The visible light properties were all consistent with those observed for the analogues of the common aliphatic series.[7] Certain deviations in extinction coefficients and spectral fine-structure may be due to the purity of the samples.

In the ^{1}H NMR spectra the only characteristic feature is a six proton doublet at *ca.* δ 0.88 caused by the isopropyl end group coupled to the C-1 methine proton. All other signals were in close agreement with those observed for the common analogues.[7]

The mass spectra (Table 1) all showed fragments derived by in-chain eliminations (Table

Table 1. Fragmentations induced by electron impact of carotenoids of the 1,2-dihydro series.

A. In-chain eliminations.

Compound	Molecular ion *m/e* (rel.int.)	Relative intensity of fragments M−68	M−79	M−92	M−106	M−158	Base peak
1	544 (100)	15.3	–	0.7	–	–	544
2	540 (100)	12.2	0.4	1.8	1.4	–	540
3	540 (57)	–	0.4	6.7	5.6	1.4	43
4	538 (100)	–	0.9	7.4	15.4	1.5	538
5	536 (25)	–	0.5	1.5	26.6	9.8	91
6	536 (55.5)	–	–	2.1	55.5	–	69
20	354 (100)	21	–	–	–	–	354
25	418 (100)	–	0.8	1.8	0.8	–	418
32	416 (34.5)	–	–	0.8	17.1	–	91

B. In-chain cleavages.

Compound	Losses from the molecular ion [x, (rel. int.)] derived by cleavage of bond 3,4	7,8	11,12	15,15′	11′,12′	7′,8′	3′,4′
1	–	139(5.1)	207(0.9)	–	–	–	–
2	–	139(1.8)	207(0.5)	–	203(0.4)	137 (0.6)	69(1.4)
3	71(0.6)	139(1.0)	205(1.5)	–	–	–	–
4	71(0.5)	139(0.7)	205(1.3)	–	203(1.6)	137(0.9)	69(2.9)
5	–	137(9.8)	–	–	–	137(9.8)	–
6	–	–	–	–	–	–	–
20	71(0.9)	139(82)	207(24.6)	–	–	–	–
25	71(1.1)	139(1.3)	205(2.6)	149(2.3)	–	–	–
32	–	–	–	149(1.7)	–	–	–

C. Other losses.

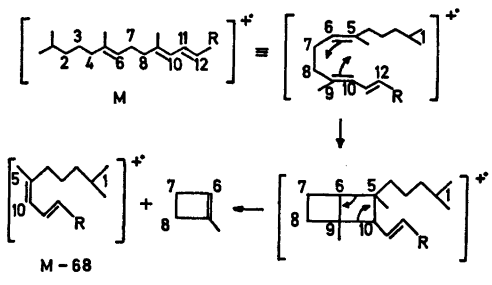
Compound	Expelled fragments [M−x, (rel.int.)]
5	M−80(0.5)
6	M−24(1.2), M−26(0.4), M−40(1.8), M−66(1.9), M−68(0.9), M−132(0.8), M−175(2.9)
20	M−15(0.6), M−29(0.5), M−83(1.0), M−85(0.7), M−113(2.0), M−126(12.3)
25	M−16(0.7), M−28(0.4), M−29(0.4), M−83(0.7), M−85(0.7), M−113(0.7), M−121(0.7, M−135(1.0), M−137(0.8)
32	M−40(0.7), M−54(0.9), M−66(1.4), M−120(1.2)

1A). For *1* only the loss of 92 (toluene) mass units (m.u.) was observed in agreement with its structure and the accepted mechanism for these fragmentations.[7] The other C_{40}-compounds all exhibited losses of 79 (methylcyclopentadienyl),[8] 92 (toluene), 106 (xylene), and 158 (dimethylcyclodecapentaene)[8] m.u. except for *6* which only exhibited losses of 92 and 106 m.u. The C_{30}-carotenals *25* and *32* exhibited losses of 92 and 106 m.u. but no loss of 158 m.u. was observed.

Diagnostic important fragments derived by in-chain cleavages of the 3,4; 7,8; 11,12; 15,15'; 11',12'; 7',8' and 3',4' bonds were also observed (Table 1 B). For compounds *1*—*4*, *20*, and *25* these fragments are observed at 2 m.u. higher than the corresponding fragments of the common aliphatic (1,2-ene) analogues.

For the carotenes *1* and *2* and the apocarotenal *20* a relatively strong fragment ion M−68 (Table 1), previously not encountered in carotenoid mass spectra, was observed. This fragmentation, supported by a metastable peak, seems restricted to the 1,2,7,8-tetrahydro end group, since it has not been observed for carotenoids with the 1,2- or 7,8-dihydro end groups alone.[6,8,9] The fragmentation may be explained by an in-chain elimination of methylcyclobutene (Scheme 4). This thermally forbidden rearrangement[10] could take place under electron impact. When a 1,2-double bond is present, this type of reaction appears to be suppressed by the favoured simple cleavage of the 3,4-bond (M−69). Malhotra *et al.*[5] reported no M−68 fragment for their natural 1,2,7,8-tetrahydrocarotenes, possibly due to different recording conditions (200−240 °C, 70 eV? *vs.* 150−200 °C, 70 eV in our work).

A number of other fragments were also observed, but have no obvious diagnostic value (Table 1C).



Scheme 4.

In view of the very similar adsorptive and visible light properties of the carotenoids of this 1,2-dihydro series with those of the common (1,2-ene) series[7] it is imperative that full spectral characterization, including MS and advisably ^{1}H NMR, be employed even for the identification of carotenes.

So far the occurrence of these 1,2-dihydrocarotenes is restricted to *Rhsp. viridis*. However, this restricted occurrence is not considered definite, since misidentification with common carotenes is possible.

EXPERIMENTAL

Materials and methods were as normally employed in our laboratory and are summarized elsewhere.[12] If of satisfactory purity, as judged by ^{1}H NMR spectrometry or other criteria, crude intermediates were used directly without further purifications. Otherwise purifications by distillation or chromatography on columns or thin layer plates were resorted to.

6-Methylheptan-2-one (8). Methylheptenone (*7*, 12.6 g) in methanol (100 ml) was hydrogenated in the presence of palladium (10 % on $BaSO_4$) catalyst (0.5 g) until hydrogen uptake had ceased, *ca.* 30 h. Filtration and removal of the solvent afforded 12.1 g (95 %) crude *8*; $n_D^{19}=1.4283$; ν_{max} (liq) 3000−2820 (CH), 1720 (C=O), 1670 (CH_2), 1390, 1370 (CH_3), 1172, 793, 767 and 683 cm^{-1}; δ ($CDCl_3$) 0.86 (d, 6 H, *J* 5 Hz, isopropyl CH_3), 1.0−1.9 (m, 5 H, $2\times CH_2+H$), 2.00 (s, 3 H, methyl ketone), 2.36 (t, 2 H, *J* 7 Hz, α-CH_2).

3,7-Dimethyloct-1-ene-3-ol (dihydrolinalool, 9). Vinylmagnesium bromide, prepared from magnesium (5.6 g) and vinyl bromide (25 g) in dry ether (20 ml), was reacted with *8* (15 g) in dry ether (20 ml) at room temperature for 3 h. The reaction mixture was decomposed with saturated aqueous NH_4Cl and the reaction products extracted into ether. The extract was washed with water, dried over $MgSO_4$ and evaporated to give 13 g (70 %) crude *9*; δ ($CDCl_3$) 0.88 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.25 (s, 3 H, CH_3 at C-3), 1.15−1.6 (m, ~7 H, $3\times CH_2+H$) 2.19 (1 H, OH), 5.0 (dd, 1 H, $J_{1,2}$ 10.5 Hz, $J_{1,1'}$ 2 Hz, H-1, *cis*), 5.14 (dd, 1 H, $J_{1',2}$ 17.5 Hz, $J_{1,1'}$ 2 Hz, H-1, *trans*), 5.94 (dd, 1 H, $J_{1,2}$ 10.5 Hz, $J_{1',2}$ 17.5 Hz, H-2).

3,7-Dimethyloct-2-enyl bromide (dihydrogeranyl bromide, 10). Dihydrolinalool (*9*, 13 g) in petroleum ether (25 ml) and pyridine (2.5 ml) were cooled to −10 °C and phosphorus tribromide (10.5 g) in petroleum ether (15 ml) was added dropwise. The reaction mixture was stirred at −10 °C for a further 20 min and then decomposed with ice and water. The reaction

products were extracted into petroleum ether, the extract washed with water, $NaHCO_3$ solution and dried over $MgSO_4$. Evaporation of the solvent afforded 14 g (71 %) crude *10*; δ ($CDCl_3$) 0.89 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.1–1.7 (5 H, $2\times CH_2$+H), 1.71 (s, 3 H, CH_3 at C-3), 2.02 (t, 2 H, *J* 6 Hz, H-4), 3.99 (d, 2 H, *J* 8 Hz, H-1).

6,10-Dimethylundeca-5-ene-2-one (dihydrogeranyl acetone, 12). Acetoacetic ester (11.5 g) and *10* (14 g) were cooled to −15 °C and sodium ethoxide solution (prepared from sodium (4.5 g) and ethanol (100 ml), 35 ml) was added dropwise while the temperature was kept at *ca.* −10 °C. The reaction was continued at room temperature overnight. The solution was heated to 80 °C and 10 % aqueous sodium hydroxide (110 ml) was added with vigorous stirring and the reaction mixture kept at 80 °C for 4 h. The mixture was cooled, the products extracted into petroleum ether, the extract washed with water and dried over $MgSO_4$. Evaporation of the solvent and distillation of the residue gave 10 g (85 %) *12* of b.p. 152 °C/15 Torr; δ ($CDCl_3$) 0.88 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.1–1.7 (5 H, $2\times CH_2$+H), 1.61 (s, 3 H, CH_3 at C-6), 1.75–2.5 (2×2 H, allylic, CH_2), 2.10 (s, 3 H, CH_3-1), 2.39 (t, 2 H, *J* 5 Hz, CH_2-3), 5.08 (t, 1 H, *J* 6.5 Hz, H-5).

Ethyl 3,7,11-trimethyldodeca-2,6-dienoate (ethyl dihydrogeranylacetonylidene acetate, 13). Ethyl diethylphosphonoacetate (60 g) was added to a suspension of sodium hydride (50 % in mineral oil, 1.35 g, washed repeatedly with petroleum ether before use) and the mixture stirred until hydrogen evolution had ceased (*ca.* 1 h). *12* (4.1 g) was added and the mixture was reacted overnight and then decomposed with water. The products were extracted into ether, the extract washed with water and dried over Na_2SO_4. Evaporation of the solvent gave 4.54 g (81 %) crude *13*; ν_{max} (liq) 2960–2870 (CH), 1720 (C=O), 1650 (C=C), 1455 (CH), 1385, 1369 (CH_3, isopropyl), 1220, 1157, 1142, 1055, and 862 cm^{-1}; δ ($CDCl_3$) 0.87 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.26 (t, 3 H, *J* 7 Hz, ethyl CH_2), 1.60 (s, 3 H, CH_3 at C-7), 1.89 and 2.16 (d + d, 3 H, *J* 1 Hz, CH_3 at C-3, *cis*+*trans*), 4.14 (q, 2 H, *J* 7 Hz, ethyl CH_2), 5.10 (m, 1 H, H-6), 5.65 (broad s, 1 H, H-2).

3,7,11-Trimethyldodeca-2,6-dien-1-ol (dihydrogeranylacetonylidene ethanol, 14). 13 (4.07 g) in dry ether (25 ml) was reduced with $LiAlH_4$ (1 g) at 0 °C for 4 h. Saturated aqueous NH_4Cl solution was added, the mixture filtered and the filtrate extracted with ether and dried over Na_2SO_4. Evaporation of the solvent gave 3.33 g (97.5 %) crude *14*; ν_{max} (liq) 3320 (OH), 2950–2865 (CH), 1470 (CH), 1385, 1367 (CH_3, isopropyl) and 1005 cm^{-1}; δ ($CDCl_3$) 0.86 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.51 (s, 3 H, CH_3 at C-7), 1.68 (s, 3 H, CH_3 at C-3), 4.10 and 4.14 (d+d, 2 H, *J* 7 Hz, CH_2-1, *cis*+*trans*), 5.10 (m, 1 H at C-6), 5.32 and 5.42 (t+t, 1 H, *J* 7 Hz, H at C-7, *cis*+*trans*).

(3,7,11-Trimethyldodeca-2,6-dienyl)triphenylphosphonium bromide (15). 14 (2.78 g) in dry $CHCl_3$ (70 ml) was reacted with triphenylphosphonium bromide (4.43 g) at room temperature for 60 h. Crystallization from ethyl acetate/ether gave 2.71 g (40 %) colourless crystals of *15*, m.p. 121 °C; ν_{max} (KBr) 3045–2780 (CH), 1587 (C=C), 1435 (CH), 1383, 1366 (CH_3, isopropyl). 1112, 996, 887, 856, 745, 725, and 692 cm^{-1}; δ ($CDCl_3$) 0.87 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.33 and 1.41 (s+s, 3 H, CH_3 at C-3), 1.52 (s, 3 H, CH_3 at C-7), 4.2–5.3 (m, 4 H, CH_2 at C-1 and olefinic H), 7.73 (s, *ca.* 15 H, phenyl-H).

3,7,11-Trimethyldodeca-1,4,6,10-tetraene-3-ol (17). The Grignard reagent, prepared by treating vinyl bromide (8.25 g) in tetrahydrofuran (100 ml) with magnesium (1.9 g), was cooled to 0 °C and ψ-ionone (*16*, 2.5 g) in tetrahydrofuran (50 ml) was added dropwise. The mixture was stirred for a further 30 min at 20 °C followed by addition of saturated aqueous ammonium chloride (*ca.* 50 ml). The organic phase was extracted with ether, the extracts washed several times with water and dried over Na_2SO_4 and K_2CO_3. Evaporation of the solvent and distillation (76 °C, 0.1 Torr) gave 2.46 g (85 %) of the alcohol *17* as a colourless oil; λ_{max} (methanol) 243 nm (ε 21 600); ν_{max} (liq) 3370 (OH), 3082–2930 (CH), 1450 (CH), 1377, 1105, 1090, 970, 920, 830, and 690 cm^{-1}; δ ($CDCl_3$) 1.28 (s, 3 H, CH_3 at C-3), 1.52 and 1.58 (s+s, 6 H, 2 CH_3 at C-11), 1.67 (s, 3 H, CH_3 at C-7), 2.0–2.2 (m, 4 H, 2 CH_2 allylic), 4.8–6.7 (7 H, olefinic H).

(3,7,11-Trimethyldodeca-2,4,6,10-tetraene-1-yl)triphenylphosphonium bromide (18). 17 (1.98 g) in abs. methanol (45 ml) was reacted with triphenylphosphonium bromide (3.16 g) at room temperature for 24 h. The solvent was evaporated, the residue dissolved in hot ethyl acetate and the resulting solid precipitate recrystallized in ethyl acetate giving 380 mg colourless crystals of *18*, m.p. 148 °C (decomp.); λ_{max} (methanol) 295.5 nm (ε 28 800); ν_{max} (KBr) 3050–2780 (CH), 1587 (C=C), 1437 (CH), 1112, 995, 955, 745, 725, and 694 cm^{-1}; δ ($CDCl_3$) 1.60 and 1.66 (s+s, 2 CH_3 at C-11), 1.77 (s, CH_3 at C-3 and C-7), 2.0–2.3 (m, CH_2-8 and -9), 4.5–6.3 (m, CH_2-1 and olefinic H), 7.72 (s, phenyl-H). The combined mother liquors after evaporation of the solvent provided further 4.36 g *18* as a viscous oil.

1,2,7,8-Tetrahydro-12'-apo-ψ-carotene-12'-al (20). Butylene oxide (4 ml), $CHCl_3$ (4 ml), C_{10}-dial (*19*, 482 mg) and *18* (801 mg) were reacted in a sealed tube under N_2-atmosphere at 90 °C for 1 h. The reaction mixture was cooled and suspended in benzene/ether, washed several times with water and dried over Na_2SO_4. Chromatography on alumina grade 2 [13] using benzene and collection of the main fraction gave 304 mg *20* (58.5 %) as an oil; λ_{max} (acetone) 392.5 nm (ε 53 700); ν_{max} (liq) 3035 (olefinic CH), 2950–2800 (CH), 2705 (−CHO), 1673

(C=O), 1610, 1560 (C=C), 1470–1430 (CH), 1405, 1380, 1355 (CH_3), 1265, 1203, 1185, 1008, 990, 960 (*trans* –CH=CH–), 883, 835 (>C=CH–) and 690 cm^{-1}; δ ($CDCl_3$) 0.87 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.1–1.4 (m, 4 H, CH_2-2 and -3), 1.60 (s, 3 H, CH_3 at C-5), 1.64 (d, 1 H, *J* 6 Hz, *tert.* H), 1.84 (s, 6 H, CH_3 at C-9 and C-13′), 2.00 (s, 3 H, CH_3 at C-13), 1.8–2.3 (m, 6 H, CH_2-4,7,8), 4.95–5.3 (H at C-6), 5.7–7.2 (m, 7 H, olefinic), 9.42 (1 H, aldehydic); *m/e* (Table 1).

1,2,7,8,1′,2′,7′,8′-Octahydro-ψ,ψ-carotene (1,2,-1′,2′-tetrahydro-ξ-carotene, 1). Butylene oxide (1 ml), C_{10}-dial (*19*, 20 mg) and *15* (200 mg) were reacted in a sealed tube under N_2 at 90 °C for 30 min. The reaction mixture was cooled and suspended in benzene. Triphenylphosphinoxide was removed by filtration and the product purified by TLC on silica using petroleum ether as developer; yield 20 mg (30 %) *1* as an oil; λ_{max} (light petroleum) 378.5, 399 and 424 nm % III/II [11] = 70; ν_{max} (liq 3040–2820 (CH), 1740, 1635 (C=C), 1480–1440 (CH_2), 1390, 1370 (CH_3), 970 (*trans* –CH=CH–), 890 and 790 cm^{-1}; δ $CDCl_3$) 0.87 (d, 12 H, *J* 6 Hz, 4×CH_3 at C-1,1′), 1.60 (s, 6 H, CH_3 at C-5,5′), 1.81 (s, 6 H, CH_3 at C-9,9′), 1.94 (s, 6 H, CH_3 at C-13,13′), 5.12 (m, 2 H, H-6,6′) and 5.7–6.8 (10 H, olefinic); *m/e* (Table 1).

1,2,7,8-Tetrahydro-ψ,ψ-carotene (1,2-dihydro-neurosporene, 2). *18* (2 g) and *20* (334 mg) in 12 ml butylene oxide/$CHCl_3$ (1:1) were reacted in a sealed tube under N_2-atmosphere at 90 °C for 1 h. The reaction mixture was cooled, suspended in benzene/ether, washed with water and dried over Na_2SO_4. The residue was chromatographed twice on alumina (Woelm, grade 3 [13]) gradually eluted with increasing amounts of benzene in hexane (1:1–2:1) for the first column and increasing amounts of ether (5–10 %) in hexane for the second column, separating the main fraction into two zones. From the more polar zone *2* (108 mg) was isolated as a *cis-trans* mixture. The less polar zone gave 79 mg mainly *all-trans 2* (total yield 37 %). Further purification of the *all-trans* product by TLC on silica using 3 % acetone in hexane and crystallization from acetone/methanol gave 14.4 mg crystalline *all-trans 2*, m.p. 96 °C; λ_{max} (hexane) 468.5, 439 (ε 138 200) and 415 nm; % III/II = 102; ν_{max} (KBr) 3040 (olefinic CH), 2950–2860 (CH), 1627 (C=C), 1480–1420 (CH_2), 1383, 1367 (CH_3), 960 (*trans* –CH=CH–), 885, and 825 cm^{-1}. δ ($CDCl_3$) 0.89 (d, 6 H, *J* 6 Hz, CH_3 isopropyl), 1.1–1.4 (m, CH_2-2,3), 1.61 (s, 6 H, CH_3 at C-5 and C-1′), 1.70 (s, 3 H, CH_3 at C-1′), 1.83 (s, 6 H, CH_3 at C-5′ and 9), 1.97 (s, 9 H, CH_3 at C-13, 13′ and 9′), 1.8–2.3 (m, CH_2-4,7,8), 5.0–5.35 (m, 2 H at C-6 and C-2′), 5.7–6.9 (13 H olefinic); *m/e* (Table 1).

Ethyl 3,7-dimethyloct-2-enoate (21). To sodium hydride (50 %) in mineral oil, 3.12 g) in dry dimethoxyethane (40 ml) was added ethyl diethylphosphonoacetate (11.5 g). The mixture was stirred until hydrogen evolution had ceased (*ca.* 1 h). *8* (6.4 g) in dry dimethoxyethane (50 ml) was added and the mixture stirred at room temperature for 24 h. Water (1.5 l) was added and the product extracted with ether. The combined ether extracts were washed with water until neutral and dried over calcium chloride. Evaporation of the solvent afforded 9.9 g (100 %) crude *21*; $n_D^{19} = 1.4550$; ν_{max} (liq) 3000–2820 (CH), 1720 (C=O), 1650 (conj. C=C), 1465 (CH), 1388, 1360 (CH_3, isopropyl), 1227, 1151 (C–O–) and 1050 cm^{-1}; δ ($CDCl_3$) 6.14 and 5.60 (q+q, 1 H, *J ca.* 1 Hz, *cis*+*trans* α-H), 4.10 (q, 2 H, *J* 7 Hz, CH_2CH_3), 2.13 d and 1.88 (d+d, 3 H, *J ca.* 1 Hz, *trans*+*cis* β-CH_3), 1.25 (t, 3 H, *J* 7 Hz, CH_2CH_3), 2.1–1.0 (m, 7 H, 3×CH_2+*tert.* H), and 0.90 (d, 6 H, *J* 5 Hz, isopropyl CH_3).

3,7-Dimethyloct-2-en-1-ol (22). *21* (5.0 g) in dry ether (30 ml) was added to a cooled (0 °C) suspension of lithium aluminium hydride (0.7 g) in dry ether (20 ml) and the mixture was stirred at room temperature for 5 h. Ammonium chloride (0.9 g) in water (3 ml) was added and stirring continued for ½ h. The mixture was filtered, the filter washed with ether and the combined filtrates evaporated to dryness; yield 3.9 g (99 %) crude *22*; ν_{max} (liq) 3600–3100 (bonded OH), 3000–2820 (CH), 1470 (CH), 1388, 1370 (CH_3, isopropyl), 1010 (OH), and 762 cm^{-1}; δ ($CDCl_3$) 5.83 and 5.42 (t, 1 H, *J* 7 Hz, *cis*+*trans* α-H), 4.13 (d, 2 H, *J* 7 Hz, CH_2O-), 3.05 (s, 1 H, OH), *ca.* 2.0 (m, allylic CH_2), 1.69 (s, 3 H, β-CH_3), 2.0–1.0 (m, 5 H, 2×CH_2+*tert.* H), 0.90 (d, 6 H, *J* 6 Hz, isopropyl CH_3).

(3,7-Dimethyloct-2-enyl)triphenylphosphonium bromide (23). *22* (3.12 g) and triphenylphosphonium bromide (7.2 g) in methanol (50 ml) were stirred under nitrogen at room temperature for 24 h and finally refluxed for 4 h. The reaction mixture was poured into water and extracted with chloroform. The combined extracts were dried over anhydrous sodium sulfate, the solvent evaporated and the resultant oil triturated with ethyl acetate. Filtration and recrystallization from chloroform-ethyl acetate afforded 4.94 g (50.5 %) *23* of m.p. 168–170 °C; δ ($CDCl_3$) *ca.* 7.8 (m, 15 H, aromatic), 5.13 (t, 1 H, *J* 7 Hz, α-H), 4.57 (dd, 2 H, J_{H-P} 15 Hz, J_{H-H} 7 Hz, CH_2P-), *ca.* 1.9 (m, 2 H, allylic CH_2), 1.40 and 1.34 (s+s, 3 H, CH_3), 1.5–1.0 (m, 5 H, 2×CH_2+*tert.* H), and 0.80 (d, *J* 6 Hz, isopropyl CH_3).

1,2,1′,2′-Tetrahydro-ψ,ψ-carotene (3) and 1,2-dihydro-8′-apo-ψ-caroten-8′-al (25). To a suspension of *23* (1000 mg) in dry ether (30 ml) was added 0.6 N butyl lithium (8 ml) and the resultant deep red solution stirred for *ca.* 5 min. Crocetindial (*24*, 600 mg) in methylene chloride (30 ml) was added and the mixture refluxed under nitrogen for 70 min. The reaction mixture was poured into water and the products extracted with ether. The combined extracts were washed with water and evaporated with benzene to dryness.

The individual pigments were separated by chromatography on alumina grade 2.[13] Rechromatography afforded the homogeneous pigments. Crocentindial (*24*, 323 mg) was recovered.

1,2-Dihydro-8'-apo-ψ-caroten-8'-al (25, 232 mg) was eluted with 10 % ether in petroleum ether from alumina grade 2. Crystallization from acetone-methanol afforded crystalline *25* (69.4 mg) of m.p. 128–130 °C; λ_{max} (acetone) 467 (ε = 107 000) and (490) nm; ν_{max} (KBr) 3040 (olefinic CH), 3000–2800 (CH), 2720 (–CH=O), 1682 (C=O), 1630, 1625 (conj. C=C), 1590, 1560, 1520 (C=C), 1470–1440 (CH), 1405, 1388, 1370, 1360 (CH_3), 1320, 1280, 1185, 1160, 1030, 1005, 990, 960 (*trans* –CH=CH–), 888, 835 (>C=CH–), 740, 696, and 660 cm^{-1}; δ ($CDCl_3$) 9.50 (s, 1 H, aldehydic), 7.0–5.75 (m, 13 H, olefinic), 2.3–2.0 (m, 2 H, allylic CH_2), 2.00 (s, 3 × 3 H, in-chain CH_3), 1.91 (s, 3H, α-CH_3), 1.83 (s, 3 H, end-of-chain CH_3) 1.8–1.0 (5 H, 2 × CH_2 + *tert.* H), 0.90 (d, 6 H, *J* 6 Hz, isopropyl CH_3); *m/e* (Table 1).

1,2,1',2'-Tetrahydro-ψ,ψ-carotene (3, 104 mg) was eluted with 15–25 % ether in petroleum ether from alumina grade 1.[13] Crystallization from methylene chloride-methanol afforded crystalline *3* (59.4 mg) of m.p. 150–155 °C; λ_{max} (petroleum ether) 442, 468.5 (ε = 171 000), and 499 nm; % III/II = 67; ν_{max} (KBr) 3040 (olefinic CH), 3000–2820 (CH), 1630, 1550 (C=C), 1470–1430 (CH), 1386, 1369 (CH_3), 961 (*trans* –CH=CH–) and 828 (>C=CH–) cm^{-1}; δ ($CDCl_3$) 6.90–5.80 (16 H, olefinic), 3.03 (2 × 2 H, allylic CH_2), 1.87 (s, 4 × 3 H, in-chain CH_3), 1.82 (s, 2 × 3 H, end-of-chain CH_3), 1.7–1.1 (6 H, 2 × CH_2 + 2 × *tert.* H), and 0.87 (d, 4 × 3 H, *J* 6 Hz, isopropyl CH_3); *m/e* (Table 1).

1,2-Dihydro-ψ,ψ-carotene (4). To a suspension of geranyltriphenyl phosphonium bromide (*26*, 240 mg) in dry ether (10 ml) was added 0.6 N butyl lithium (2 ml) and the resultant dark red solution stirred for *ca.* 1 min. 1,2-Dihydro-apo-8'-lycopenal (*25*, 104 mg) in dry ether (40 ml) was added and the mixture refluxed under nitrogen for 90 min. Geranyltriphenylphosphonium bromide (*28*, 240 mg) was treated with 0.6 N butyl lithium (2 ml) and this was added to the solution. The reaction was stopped after a further 15 min by the addition of water. The products were extracted with ether and the combined extracts washed with water, evaporated with benzene to dryness and the pigments were purified by chromatography twice on alumina grade 2. *4* (83.5 mg) was eluted with 5 % ether in petroleum ether. Crystallization from acetone-methanol afforded crystalline *4* (18 mg) of m.p. 162–165 °C; λ_{max} (light petroleum) 443, 469 (ε = 164 000) and 499.5 nm % III/II = 63; ν_{max} (KBr) 3040 (olefinic CH), 3000–2820 (CH), 1720, 1675, 1630, 1550 (C=C), 1470–1430 (CH), 1385, 1370, (CH_3), 961 (*trans* –CH=CH–), 888 and 828 (>C=CH–) cm^{-1}; δ ($CDCl_3$) 7.0–5.8 (16 H, olefinic), 5.15 (m, 1 H, H-2'), 2.22 (3 × 2 H, allylic CH_2), 1.98 (s, 4 × 3 H, in-chain CH_3), 1.83 (s, 2 × 3 H, end-of-chain CH_3), 1.72 s and 1.63 (s+s, 3+3 H, isopropenyl CH_3), 1.6–1.0 (m, 3 H, non-allylic CH_2 + *tert.* H), and 0.88 (d, 2 × 3 H, *J* 6 Hz, isopropyl CH_3); *m/e* (Table 1).

Methyl 3,7-dimethylocta-2,4-dienoate (29). To a suspension of sodium hydride (50 % in mineral oil, 3.1 g, washed repeatedly with petroleum ether) was added methyl 3-methyl-4-diethylphosphonocrotonate (*30*, 16 g) in dry ether (20 ml) and the mixture stirred until H_2-evolution had ceased. Isovaleraldehyde (*27*, 7.9 g) was added and the reaction run overnight with stirring. The reaction mixture was poured into water, the products extracted into ether, the extract washed with water, dried over $MgSO_4$ and evaporated to give 8.4 g (92 %) crude *29*; ν_{max} (liq) 3040 (olefinic CH), 3000–2820 (CH), 1720 (>C=O), 1640, 1610, 1430–1470 (CH), 1385, 1370 (CH_3), 1240, 1150, 1040, 960–980, 850 cm^{-1}; δ ($CDCl_3$) 0.92 (d, 6 H, *J* 6 Hz, CH_3 isopropyl), 1.5–2.0 (m, H-6), 2.0 and 2.3 (d+d, 3 H, *J* 1 Hz, CH_3 vinylic *trans* and *cis*), 3.70 (s, 3 H, $-OCH_3$), 5.67 and 5.77 (m+m, 1 H at C-2, *trans* and *cis*), 6.19 (dt, 1 H, $J_{4,5}$ 16 Hz, $J_{5,6}$ 7 Hz, H at C-5), 7.67 (d, 1 H, *J* 16 Hz, H at C-4).

3,7-Dimethylocta-2,4-diene-1-ol (30). *29* (4.6 g) in dry ether (30 ml) was added to a cooled (0 °C) suspension of $LiAlH_4$ (1 g) in dry ether (30 ml) and the reaction continued at room temperature for 1 h. Excess $LiAlH_4$ was destroyed by addition of ethyl acetate, the reaction mixture was poured into ice–1 N H_2SO_4, and the products extracted into ether. The ether extract was washed with water and dried over $MgSO_4$. Evaporation of the solvent gave 2 g (52 %) crude *30*; n_D^{19} = 1.4350; ν_{max} (liq) 3600–3100 (OH), 3000–2820 (CH), 1640, 1610, 1470–1430 (CH), 1370 (CH_3), 1240, 1150, 1040, and 980 cm^{-1}; δ ($CDCl_3$) 0.91 (d, 6 H, *J* 6 Hz, CH_3 isopropyl), 1.5–2.0 (m, H-6), 1.76 and 1.83 (d+d, 3 H, *J* 1 Hz, CH_3 vinylic *trans* and *cis*), 1.7–2.2 (m, CH_2), 3.92 (s, 1 H, OH), 4.22 (d, 2 H, *J* 7 Hz, $-CH_2O-$), 5.3–6.6 (m, 3 H, olefinic *cis* and *trans*).

(3,7-Dimethylocta-2,4-dienyl)triphenylphosphonium bromide (31). Triphenylphosphonium bromide (4.5 g) and *30* (2 g) in dry methanol (25 ml) were stirred at room temperature for 56 h. Removal of the solvent gave an oil which was triturated with ether. The resultant viscous oily product was dried in high vacuum and gave a colourless semi-solid (2 g, 44 %) which was used without further purification.

1,2-Dihydro-3,4-didehydro-8'-apo-ψ-caroten-8'-al (32). To *31* (250 mg) and crocetindial (*24*, 100 mg) in dimethylformamide (30 ml) was added 1.1 N $NaOCH_3$ (0.5 ml) dropwise with stirring, the reaction mixture stirred at room temperature for 1.5 h and then poured into water. The pigments were extracted into benzene, the extract washed with water, dried over Na_2SO_4 and evaporated to dryness. The products were chromatographed on alumina

grade 2.[13] *32* was eluted with benzene. Evaporation of the solvent and crystallization twice from petroleum ether gave 50 mg (36 %) *32* of m.p. 110–112 °C. λ_{max} (acetone) 473 (ε = 99 800) nm; ν_{max} (KBr) 3000–2820 (CH) 1750, 1675 (C=O), 1610 (C=C), 1470–1440 (CH_2), 1385, 1369 (CH_3), 961 (*trans* –CH=CH–) cm^{-1}. δ ($CDCl_3$), 0.87 (d, 6 H, *J* 7 Hz, isopropyl CH_3), 1.82 (s, 6 H, CH_3 at C-5 and C-9′), 1.97 (s, 9 H, CH_3 at C-9,13 and 13′), 5.8–7.0 (15 H, olefinic), 9.50 (s, 1 H, aldehydic); *m/e* (Table 1).

1,2,1′,2′-Tetrahydro-3,4,3′,4′-tetradehydro-ψ,ψ-carotene (6). To a suspension of *31* (250 mg) in dry ether (25 ml) was added 1 N butyl lithium (0.55 ml) and the mixture stirred until the phosphonium salt had dissolved. Methylene chloride (3 ml) followed by crocetindial (*24*, 140 mg) in methylene chloride (25 ml) were added. The reaction mixture was refluxed under N_2 for 2 h, cooled and poured into water. The pigments were extracted with ether, the extract washed with water, dried over $MgSO_4$, evaporated and the residue chromatographed on alumina grade 2. *6* was eluted with 10 % ether in petroleum ether. The solvent was evaporated and the residue redissolved in petroleum ether, isomerized with iodine in light and rechromatographed. Evaporation of the solvent and crystallization from acetone-methanol afforded 60 mg (25 %) *6* of m.p. 197–199 °C; λ_{max} (pet. ether) 460, 488 (ε = 168 100) and 520 nm; % III/II = 43; ν_{max} (KBr) 3040–2800 (CH), 1470–1440 (CH_2), 1373, 1368 (CH_3), 970 (*trans* –CH=CH–) and 830 (>C=CH–) cm^{-1}; δ ($CDCl_3$) 0.87 (d, 12 H, *J* 6 Hz, isopropyl CH_3), 1.91 (s, 6 H, CH_3 at C-5,5′), 1.96 (s, 12 H, CH_3 at C-9,13,13′ and 9′), 5.8–7.0 (20 H, olefinic); *m/e* (Table 1).

1,2-Dihydro-3,4-didehydro-ψ,ψ-carotene (5). To a suspension of geranyltriphenylphosphonium bromide (*26*, 100 mg) in ether (20 ml) was added 1 N butyl lithium in ether (0.25 ml) and the resultant deep red ylid solution stirred until all phosphonium salt had gone into solution. Methylene chloride (3 ml) followed by *32* (40 mg) in methylene chloride (10 ml) were added and the reaction mixture refluxed for 1 h. Another portion of ylid, prepared from *26* (50 mg) and butyl lithium (0.12 ml), was added and the reaction continued for another 30 min. The reaction mixture was poured into water and the pigments extracted into ether. The extract was washed with water, dried over $MgSO_4$ and evaporated. The crude product was chromatographed on alumina, grade 2, and eluted with 10 % ether in light petroleum. The pigment was isomerized with iodine in light and rechromatographed. Evaporation of the solvent and crystallization from acetone-methanol afforded 13 mg (25 %) *5* of m.p. 180–185 °C; λ_{max} (light petroleum) 453, 480 (ε = 163 400) and 513 nm; % III/II = *64*; ν_{max} (KBr) 3040 (=CH–), 3000–2820 (CH), 1570, 1540 (C=C), 1470–1440 (CH_2), 1378, 1369 (CH_3), 960 (*trans* –CH=CH–), and 830 (>C=CH–) cm^{-1}; δ ($CDCl_3$) 0.87 (d, 6 H, *J* 6 Hz, isopropyl CH_3), 1.62 and 1.69 (s+s, 3+3 H, 2×CH_3 at C-1), 1.77 (s, 3 H, CH_3 at C-5), 1.87 (s, 3 H, CH_3 at C-5′), 1.97 (s, 12 H, CH_3 at C-9,13,13′,9′), 2.12 (6 H, allylic CH_2), 5.15 (m, 1 H, H-2), 5.8–7.0 (18 H, olefinic), *m/e* (Table 1).

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Action of Propranolol on Mitochondrial Proton Fluxes

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The action of propranolol, a β-blocking adrenergic agent, on mitochondrial proton fluxes in non-energized and energized conditions has been studied. 1. The drug inhibited the actions of valinomycin, nigericin and FCCP on the inner mitochondrial membrane. 2. It decreased the rate and extent of active energized proton expulsion and the passive collapse of the proton gradient so formed. 3. Propranolol was able to increase the permeation of chloride ion through the inner mitochondrial membrane in non-energized and energized conditions. 4. The drug inhibited mitochondrial contraction but stimulated swelling in various conditions.

It is suggested that propranolol is able to change the proton and chloride permeabilities of mitochondria by perturbing the structure of inner membrane phospholipids, thus enlarging the water-lipid interface.

Propranolol (1-isopropylamino-3-(1-naphthol-oxy)-propan-2-ol hydrochloride) is a widely used β-blocking adrenergic drug that is also known as a potent local anaesthetic. Its effects on membranes have been related to its lipophilicity.[1] The actions of local anaesthetics on liposome and mitochondrial membranes are rather controversial. It has been shown that they inhibit ion transport processes,[2-5] but stimulatory effects have also been reported.[4-8] Quite recently we have found that there are apparent differences in the action of propranolol on the inner mitochondrial membrane depending on the energetic state of the mitochondria.[5,8] In non-energized conditions the permeation of potassium and chloride into mitochondria was stimulated as judged by swelling techniques. In energized oscillatory conditions in the presence of potassium phosphate plus valinomycin the rates of ion fluxes were decreased by propranolol. However, the extent of the ion uptake was increased by the drug if the external potassium phosphate was more than *ca.* 15 mM at pH 7.2. The inhibition of the rates of ion fluxes was abolished by raised pH or nigericin suggesting that propranolol decreased the passive permeation of protons through the inner mitochondrial membrane.

In order to elucidate the reason for these apparent differences in the action of propranolol we began this work on the effects of propranolol on mitochondrial proton fluxes coupled to potassium fluxes in non-energized and energized conditions. To record the simultaneous pH changes in the membrane phase we used bromothymol blue as a probe. Even if energization [9] and local anaesthetics [10] change the binding of BTB * on mitochondria, the absorbance changes of BTB were mainly in line with the observed ion movement.

MATERIAL AND METHODS

Rat liver mitochondria were prepared, and pH changes recorded mainly as described earlier.[8,11] Mitochondrial swelling was followed by an Aminco DW 2 spectrophotometer at 630 nm.[5,8] Mitochondrial protein was determined by a biuret method.[12] When the energized proton translocation was studied anaerobic mitochondria were energized with hydrogen peroxide in the presence of succinate, mainly as described by Mitchell and Moyle.[13] In these experiments a Beckman pH electrode (39045) and a calomel electrode with an agar-KCl bridge were used, and malonate was added to decrease the rate of the respiration-induced pH

* Abbreviations used: BTB, bromothymol blue; FCCP, carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone; HOQNO, 2-heptyl-4-hydroxy-quinone *N*-oxide; Hepes, *N*-2-hydroxymethylpiperazine-*N'*-2-ethane-sulfonic acid; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

changes. Oxygen consumption was simultaneously followed with a Clark type electrode connected through an Aminco amplifier to a recorder.[8] The changes of the absorbance of BTB were monitored at 618 nm using 700 nm as the reference wavelength with an Aminco DW 2 spectrophotometer.

In order to minimize the movements of divalent cations, 1 mM EDTA was present in all the media used. Propranolol was a gift from I.C.I., Macclesfield, England. It was dissolved in ethanol and an equal amount of ethanol was added in the control experiments. BTB was obtained from British Drug Houses, Ltd, Poole, England, and recrystallized.[14] The source of other reagents was as described elsewhere.[5]

RESULTS

Effects on non-energized ion fluxes. The ion conductors valinomycin, nigericin, and FCCP all increase the penetration of potassium through the inner mitochondrial membrane.[15,16] Valinomycin induces an electrogenic uptake of potassium, nigericin mediates an electroneutral exchange of protons and potassium, and FCCP increases the permeability of the membrane to protons, thus decreasing the resistance to H^+/K^+-exchange.

When fresh non-energized mitochondria are incubated in sucrose, an exchange of internal potassium for external protons can be induced by the above mentioned lipophilic ion carriers.

The action of propranolol on the uptake of protons was studied in these conditions. The drug inhibited the rate of the uptake induced by each of the mentioned ion conductors (Fig. 1). The parallel contraction induced by the ion efflux was also inhibited by propranolol (Fig. 1C).

When the external medium was changed to potassium chloride, the three ion carriers again caused an uptake of protons (Fig. 2). This uptake was also inhibited by propranolol, but when nigericin was added in the presence of propranolol, the proton uptake seen in the control experiment was reversed, and a proton release occurred (Fig. 2A). This was seen over a wide range of nigericin concentrations.

In potassium chloride propranolol further stimulated the swelling of mitochondria that was induced by valinomycin, nigericin, or FCCP (Fig. 3). The stimulation was most conspicuous when nigericin was used (3A). Since in these conditions the swelling is thought to be limited

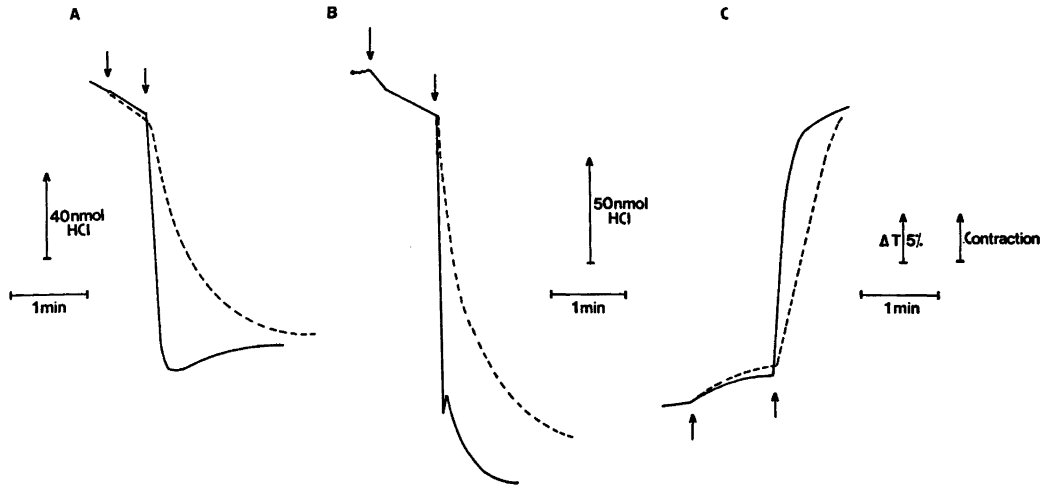


Fig. 1. The effect of propranolol on mitochondrial proton uptake and contraction in sucrose. The mitochondria were incubated in 0.15 M sucrose, 1 mM EDTA, then 7 μM rotenone and 10 μg oligomycin were added. The first arrow indicates the addition of 4 μM HOQNO, (A) mitochondrial pH change, the second arrow indicates the addition of 0.2 μg/ml valinomycin, the concentration of propranolol 260 μM, mitochondrial protein 3.7 mg/ml. (B) as in (A) except 1.7 μM FCCP and not valinomycin was added. (C) mitochondrial contraction, the second arrow indicates the addition of 0.18 μg/ml valinomycin. The concentration of mitochondrial protein was 2.6 mg/ml. ——— indicates the control and – – – propranolol added before HOQNO. The pH was 7.2 before each addition of the ion conductors. The final volume in (A) and (B) was 1.5 ml.

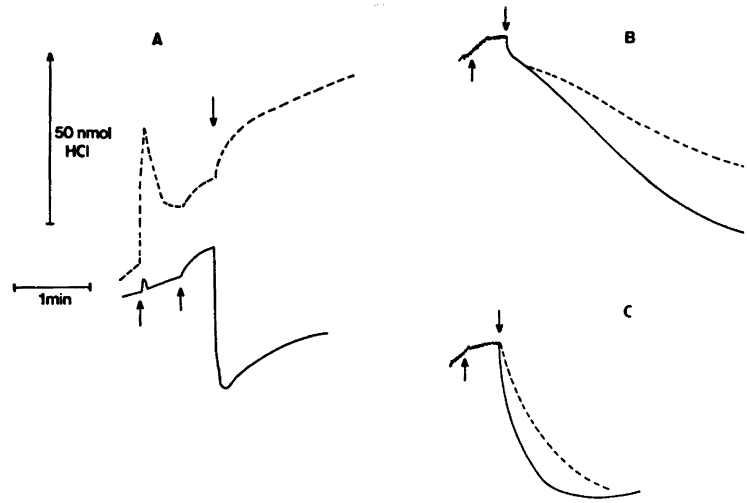


Fig. 2. The effect of propranolol on proton fluxes in potassium chloride. The mitochondria were incubated in 0.1 M KCl, 1 mM EDTA, then 10 μM rotenone and 10 μg oligomycin were added. Propranolol when added was 200 μM, HOQNO 4 μM; mitochondrial protein was 4.8 mg/ml. (A) the first arrow indicates the addition of propranolol, the second that of HOQNO, and the third that of nigericin 0.3 μg/ml. (B) and (C) the first arrows indicate the additions of HOQNO and the second those of 0.2 μg/ml valinomycin (B) or 1.7 μM FCCP. ——— is control and – – – propranolol present. The pH was 7.2 before each addition of the ion conductors. The final volume was 1.5 ml.

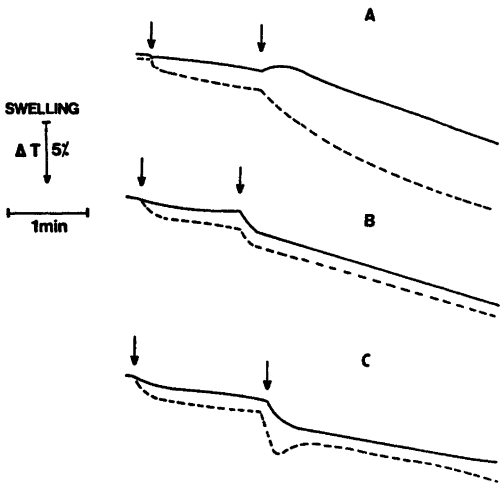


Fig. 3. The effect of propranolol on non-energized mitochondrial swelling in potassium chloride. The mitochondria were incubated in the presence of 0.1 M KCl, 1 mM EDTA, then 10 μM rotenone, 10 μg oligomycin and 4 μM HOQNO were added. The first arrow indicates the addition of 160 μM propranolol, the second that of 0.2 μg/ml nigericin in (A) or that of 0.1 μg/ml valinomycin in (B), or that of 1.2 μM FCCP in (C) in 10 μl of ethanol. Mitochondrial protein was 5.7 mg/ml.

by anion entry it is evident that chloride permeation was stimulated. The slight swelling seen in the control was preceded by an initial contraction, and this contraction phase was totally abolished by propranolol.

Propranolol clearly decreased the functioning of all the ion conductors. However, although propranolol decreased the rate of respiration in the presence of small concentrations of FCCP the inhibition was abolished by increasing amounts of FCCP, when the respiration was limited by the capacity of respiratory chains and not by the proton permeability (Fig. 4). We also found earlier that propranolol inhibited the rate of swelling of energized mitochondria in the presence of potassium acetate plus valinomycin.[8]

Effects on active ion fluxes. Mitochondria eject protons when energized in the absence of ADP. Propranolol decreased the rate of proton release induced by energization (H_2O_2 plus catalase).[11] It also depressed the extent of this release and the rate of passive uptake of protons that followed exhaustion of the oxygen (Fig. 5A). Valinomycin could not abolish the inhibition caused by propranolol (Fig. 5B), even at very high concentrations. This suggests, in

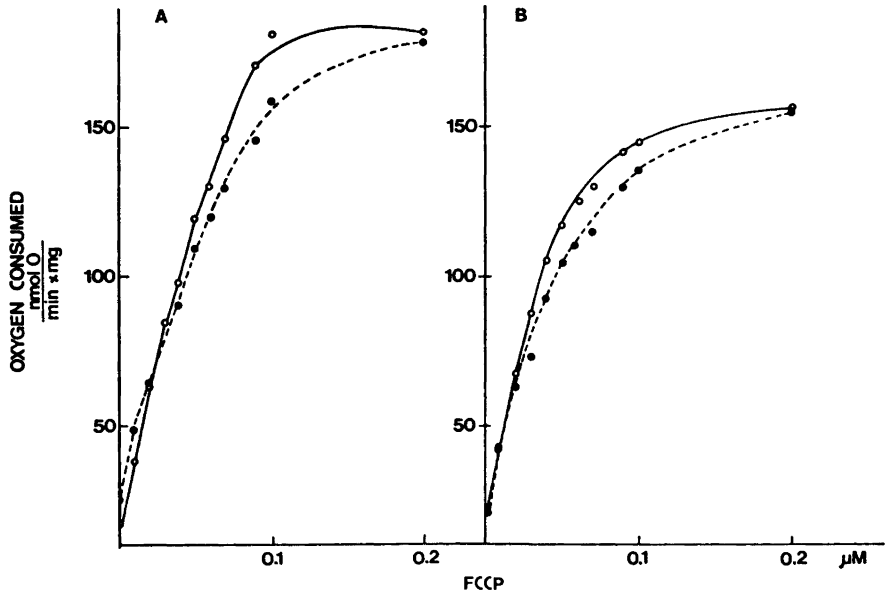


Fig. 4. The effect of propranolol on the uncoupling of mitochondrial respiration by FCCP. The mitochondria were incubated in the presence of 10 μM rotenone. The respiration was started by the addition of 10 mM Tris-succinate. The effect of FCCP was titrated by addition of small concentrations of FCCP. The points are means of two determinations. The medium in (A) 0.1 M KCl, 0.02 M Hepes, 1 mM EDTA, pH to 7.2 with Tris, and in (B) 0.2 M sucrose, 0.02 M Hepes, 1 mM EDTA, pH to 7.2 with Tris. ——— the control, — — — 150 μM propranolol.

agreement with our earlier findings,[8] that the drug indeed inhibits proton translocation *per se*.

Proton pulses induced by small amounts of ATP were similarly inhibited by propranolol, but the translocation of ATP and ADP at small concentrations was also inhibited by propranolol [not shown (*cf.* Refs. 17, 18].

Using beef heart submitochondrial particles we could not find any oligomycin-like coupling effect [19] with propranolol (not shown).

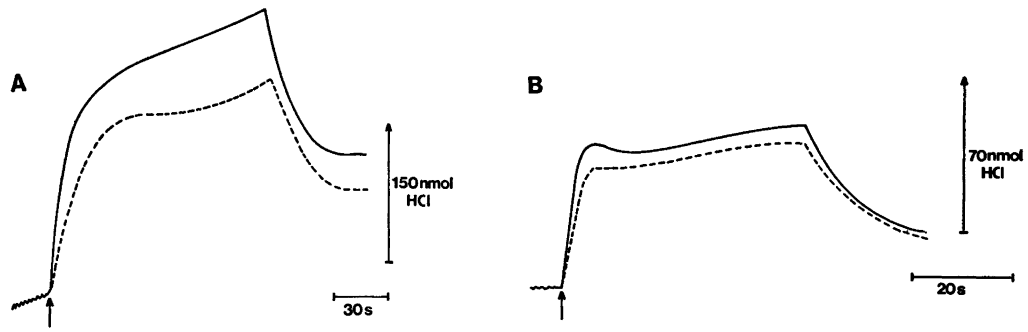


Fig. 5. The effect of propranolol on the proton ejection of energized mitochondria (A) in the absence of valinomycin and (B) in the presence of valinomycin. The mitochondria were incubated in the presence of 0.1 M KCl, 1 mM EDTA, 15 μM rotenone, 12 mM K-succinate, 2 mM K-malonate and catalase at 28 °C. The respiration was initiated with H_2O_2 (300 μM) see also the method). (A) 280 μM propranolol, mitochondrial protein 5.4 mg/ml. (B) 0.17 μg/ml valinomycin, 200 μM propranolol, mitochondrial protein 4.0 mg/ml. ——— the control, — — — propranolol. The pH was 7.2 before each addition of H_2O_2; the final volume was 2.5 ml.

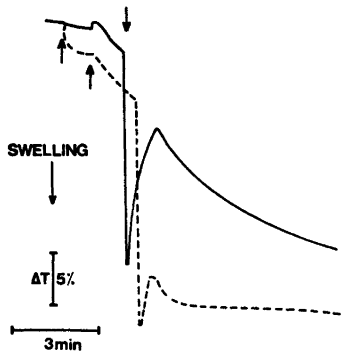


Fig. 6. The effect of propranolol on energized mitochondrial swelling in the presence of potassium chloride and valinomycin. The mitochondria were incubated in the presence of 7 μM rotenone, 0.1 M KCl, 1 mM EDTA, 0.02 M Hepes, pH 7.2 with Tris. The first arrow indicates the addition of 160 μM propranolol, the second that of 8 mM K-succinate, the third that of 0.1 μg/ml valinomycin. ——— the control, – – – propranolol.

In the absence of valinomycin propranolol did not affect the swelling of energized mitochondria. In the presence of valinomycin the extent of swelling was increased and contraction inhibited (Fig. 6). The increase of swelling was not caused by succinate uptake, because we found not difference in the swelling if succinate was changed to ascorbate plus TMPD. It is concluded that propranolol increased the uptake of chloride in these conditions.

Changes in the absorbance of BTB. BTB has been used in the study of the pH changes occurring during ion uptake by mitochondria.[7] Yet propranolol and local anaesthetics are known to change the binding of BTB.[8]

When mitochondria were incubated in the presence of BTB in KCl, the addition of propranolol caused a drastic change in the BTB absorbance ("alkalinization") (Fig. 7, the first arrow). The change was partially abolished by valinomycin (7A) and totally by nigericin (7B)

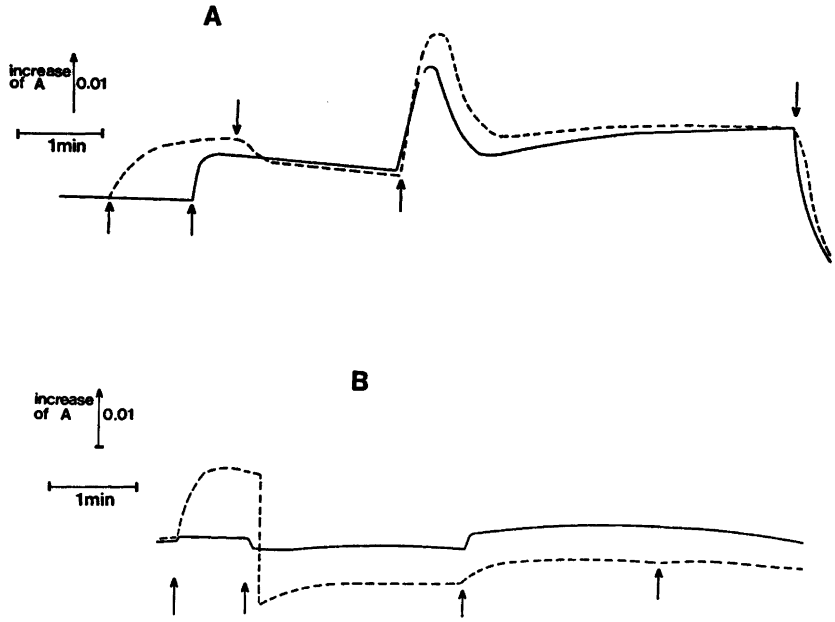


Fig. 7. The effect of propranolol on the extinction of bromothymol blue. The mitochondria were incubated in 0.1 M KCl, 0.02 M Hepes, 1 mM EDTA, pH 7.2, in the presence of 2 μM BTB and 7 μM rotenone. Mitochondrial protein was 2.0 mg/ml. The first arrow indicates the addition of 160 μM propranolol, the second in (A) 0.1 μg/ml valinomycin and in (B) 0.2 μg/ml nigericin. The third arrow indicates the addition of 8 mM succinate and the fourth that of 4 μM HOQNO.

and FCCP (not shown). If nigericin was added before propranolol the addition of propranolol did not change the absorbance of BTB. This suggests that the changes in BTB absorbance at least partly followed the pH changes in the membrane environment of BTB. The data with non-energized mitochondria were identical.

When the mitochondria were energized in the presence of BTB and valinomycin the changes in the absorbance were greater in the presence of propranolol than in the control (Fig. 7A), suggesting an increased alkalinity in the membrane. This is in line with the swelling experiment results (Fig. 6) where propranolol increased the ion uptake. When nigericin was present energization had no effect on the absorbance of BTB (Fig. 7B).

DISCUSSION

Propranolol inhibited all the proton fluxes that followed the addition of the ionophores nigericin or valinomycin or the uncoupler FCCP (see Ref. 4). The inhibition is probably related to the binding of propranolol to mitochondrial phospholipids.[9] It is clear that propranolol changes the surface potential of the membrane when bound, but its lipophilicity may also change the solubility of the lipophilic ion conductors or increase the viscosity of the membrane *per se*.

In contrast to the effects of dibucaine reported by Papa *et al.*[4] propranolol decreased the rates and extent of mitochondrial proton fluxes (Fig. 5). Earlier we have found that propranolol only slightly affected the mitochondrial ADP/O-ratio. Thus propranolol does not act as an uncoupler in mitochondria.

However, it is not likely that propranolol is a specific inhibitor of proton transfer. The inhibition is more likely to result from a retardation of proton transfer from one polarizible group (phospholipid) to another.[20] This retardation might be related to the increase of mitochondrial buffering groups that propranolol generates in non-energized conditions.[11] Because the concentrations of propranolol used are of the same order as the changes of proton concentrations we have studied, it is even possible that movements of propranolol in or through the inner mitochondrial membrane may change the proton gradients involved. Even if the concentration of uncharged propranolol is low at pH 7.2, the affinity of the lipid membrane may be higher for the uncharged form of the drug.[21]

It is obvious that propranolol does not increase the permeability of the inner mitochondrial membrane to potassium in energized conditions. This suggests that the re-ordering that occurs in the inner mitochondrial membrane [22] on energization changes the mechanism of the permeation of potassium through the inner membrane, although no definite evidence for the existence of a monovalent cation pump in mitochondria has been reported so far.[23–27]

We found earlier that propranolol stimulated mitochondrial swelling in 0.1 M NH_4Cl at pH 7.2 in the presence of FCCP.[5] In these conditions the rate of mitochondrial swelling depends on the rate of anion permeability only, because NH_3 and protons easily penetrate mitochondrial membranes.

In line with the earlier findings propranolol increased the uptake of chloride into mitochondria (Fig. 3A and 6). Yet, immediately after the addition of nigericin, the anion permeability did not seem to be rate-limiting, since an increase of pH and a contraction were needed before the mitochondria began to swell (Figs. 2A and 3A). Propranolol seemed to abolish both the pH increase and the contraction, and the mitochondria were able to take up potassium and chloride immediately after the addition of nigericin.

Propranolol may increase the permeability of mitochondria to chloride in at least two ways. The drug has one positive charge and, because it is bound to the phospholipids of mitochondria,[10] it could act as a carrier of chloride either into or through the inner membrane of mitochondria (*cf.* Ref. 28). Another possibility is a novel suggestion [29] that chloride might consume protons of the membrane water, creating a localized intramembrane pH difference. Local anaesthetics and propranolol expand biological membranes increasing the membrane water (for a review, see Ref. 30). As a result of this the accessibility of chloride to the membrane water would be easier, and the penetration could be stimulated. Whatever the mechanism is, this stimulation of chloride permeation can partly explain the rapid consumption of acid

that follows the acidification caused by the addition of propranolol to a mitochondrial suspension (Fig. 2A), and the reverse action of nigericin (seen in the same figure).

Energization seems to change the structure of mitochondria so that propranolol is no longer able to increase the permeability of the inner membrane to potassium. Yet, the drug inhibits the translocation of protons and stimulates that of chloride, stressing the important role of phospholipids in mitochondrial ion transport.

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Synthesis of *p*-Nitrophenyl 4-*O*-(α-D-Mannopyranosyl)-α-L-rhamnoside and *p*-Acetamidophenyl 4-*O*-(β-D-Mannopyranosyl)-α-L-rhamnoside

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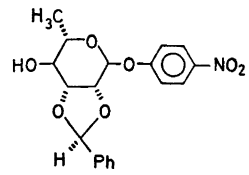
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p-Nitrophenyl 4-*O*-(α-D-mannopyranosyl)-α-L-rhamnoside has been synthesized by condensation of 3,4,6-tri-*O*-acetyl-1,2-*O*-methylorthoacetyl-β-D-mannose and *p*-nitrophenyl 2,3-*O*-benzylidene-(H-*endo*)-α-L-rhamnoside followed by removal of protecting groups. *p*-Acetamidophenyl 4-*O*-(β-D-mannopyranosyl)-α-L-rhamnoside has been synthesized *via* condensation of 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-α-D-glucose and *p*-nitrophenyl 2,3-*O*-benzylidene-(H-*endo*)-α-L-rhamnoside, deblocking, C-2-oxidation of the D-glucose moiety, stereoselective reduction to the corresponding D-mannose derivative and removal of protecting groups.

The structural element α-D-Man-(1→4)-α-L-Rha- occurs in the O-specific side chains of *Salmonella* serogroup A lipopolysaccharides,[1] and the structural element β-D-Man-(1→4)-α-L-Rha- occurs in the serogroups D_2 and E lipopolysaccharides.[2,3] For immunochemical studies the corresponding disaccharides, linked to aglycones containing functional groups through which they could be attached to proteins, thus giving artificial antigens, were needed. The syntheses of two such glycosides are reported in the present communication. During the course of this work the corresponding free disaccharides have been prepared by Bebault and Dutton.[4,5]

RESULTS AND DISCUSSION

The aglycone used in both syntheses was *p*-nitrophenyl 2,3-*O*-benzylidene-(H-*endo*)-α-L-rhamnopyranoside (II, see below). Treatment of *p*-nitrophenyl α-L-rhamnopyranoside [6] with α,α-dibromotoluene in pyridine [7] yielded a mixture of the 2,3-benzylidene-(H-*endo*)- and (H-*exo*)-derivatives which, after acetylation, were separated by column chromatography on silica gel. The structures of these *O*-acetates were assigned from the chemical shifts of the benzylidene protons in NMR,[8] δ 6.28 and 5.98, respectively. In the subsequent glycoside syntheses, fair to good yields were obtained with the H-*endo* (II) but not with the H-*exo* isomer (II a). In molecular models, the hydroxyl group at C-4 in the latter seems to be less accessible because of the bulky phenyl group.

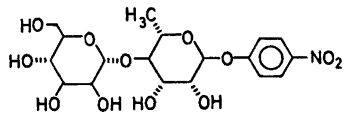


II

Reaction between 3,4,6-tri-*O*-acetyl-1,2-*O*-methylorthoacetyl-β-D-mannose [9] and II in nitromethane, as devised by Kochetkov *et. al.*,[10] followed by deacetylation and hydrolysis under mild conditions, yielded *p*-nitrophenyl 4-*O*-(α-D-mannopyranosyl)-α-L-rhamnoside (V). The yield, based upon the amount of orthoester used, was 35 %.

β-D-Mannopyranosides are less accessible than the corresponding α-D-mannopyranosides. For the synthesis of the other disaccharide,

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V

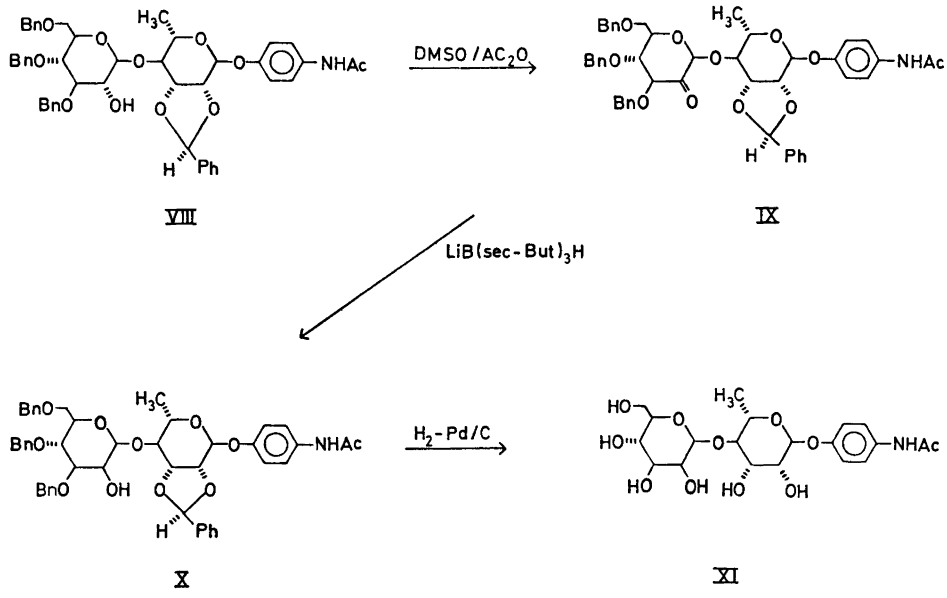
the corresponding β-D-glucopyranoside with a free hydroxyl group at C-2 was first prepared and then transferred into the β-D-mannopyranoside by oxidation to the keto derivative followed by stereoselective reduction.[11] The orthoester glycoside synthesis using benzyl ether protecting groups in the orthoester has proved suitable for preparing such β-D-glucopyranosides.[12] The method has recently been used in the synthesis of a trisaccharide containing a β-D-mannopyranose residue.[13]

Reaction of the aglycone (II) and 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-α-D-glucose [10,12] in nitromethane using mercuric bromide as catalyst gave the expected disaccharide derivative VI in 16 % yield. Since the presence of the *p*-nitrophenyl group in this derivative was expected to cause complications in later steps, this group was transferred into a *p*-acetamidophenyl group by selective reduction followed by *N*-acetylation. The *O*-deacetylated derivative (VIII) was oxidized to the hexulose derivative (IX) by dimethyl sulfoxide-acetic anhydride.[14] As IX was labile it was not purified, but directly reduced, using lithium tri-*sec*-butylborohydride [15] in tetrahydrofuran. The pure β-D-mannopyranoside (X) was obtained in a yield of 78 %, based on VIII. Removal of the protecting groups by catalytic hydrogenation yielded the disaccharide derivative XI.

The disaccharide derivatives V and XI both yielded equimolecular parts of D-mannose and L-rhamnose on acid hydrolysis. That V is a α- and XI a β-D-mannopyranoside is evident from their modes of syntheses and from the chemical shifts of the anomeric protons assigned to these residues, δ 4.98 and 4.90 respectively. The rather small difference in optical rotation between the compounds, $[\alpha]_{578}$ −90° for the α-mannoside and $[\alpha]_{578}$ −105° for the β-mannoside, may be explained by the larger negative contribution to the optical rotation by the *p*-nitrophenyl-α-L-rhamnoside moiety in V than by the *p*-acetamidophenyl-α-L-rhamnoside moiety in XI.

EXPERIMENTAL

General methods. Elemental analyses were performed by Dr. A. Bernhardt's laboratory.



VIII IX X XI

Scheme 1.

Melting points are corrected. Concentrations were performed at reduced pressure at temperatures not exceeding 40 °C. NMR spectra were recorded on Varian A 60 A, HA-100 and XL-100 instruments. Interpretations were made on a first order basis. For deuteriochloroform and tetradeuteriomethanol solutions TMS was used as internal standard and for deuterium oxide solutions 3-(trimethylsilyl)-1,1,2,2,3,3-hexadeuteriopropanesulfonic acid sodium salt. TLC was performed on silica gel F_{254} plates (Merck). For visualization of the compounds UV-light or spraying with sulfuric acid followed by heating at 120 °C was used. Column chromatography was performed on silica gel (< 0.08 mm, Merck). The following solvent systems were used; A (ethyl acetate-light petroleum, 1:2), B (diethyl ether-hexane, 3:1), C (methanol-ethyl acetate, 1:9), D (ethyl acetate-toluene, 1:1), E (ethyl acetate-toluene, 1:4), F (ethyl acetate-toluene, 2:1). For GLC a Perkin-Elmer 990 instrument was used. Separations were performed on an ECNSS-M column (180 × 0.15 cm, 3 % on Gas Chrom Q) at 190 °C. Optical rotations were recorded on a Perkin-Elmer 141 instrument and IR in a Perkin-Elmer 257 instrument.

p-*Nitrophenyl-4*-O-*acetyl-2,3*-O-*benzylidene-(H*-endo-*and H*-exo-*)*-α-L-*rhamnoside (I and I a)*. *p*-Nitrophenyl-α-L-rhamnopyranoside [6] (20 g) was dissolved in pyridine (400 ml). α,α-Dibromotoluene (24 ml) was added and the mixture was refluxed for 75 min. Acetic anhydride (160 ml) was added and the solution was left for 1 h at room temperature. The solution was poured into water (3 l), and the mixture was extracted with chloroform (5 × 600 ml). The organic layer was washed with water (1000 ml), aqueous sodium hydrogen carbonate (4 × 600 ml) and water (3 × 1000 ml), dried over magnesium sulfate and evaporated. The crude product was chromatographed on a silica gel column (solvent system A) to yield the H-*endo*-isomer (I) (12.4 g, 42 %) and the H-*exo*-isomer (I a) (9.1 g, 31 %), both crystalline after evaporation. After crystallization from methanol the H-*endo*-isomer showed m.p. 84 – 87 °C, $[\alpha]_{578}^{23}$ −74° (*c* 1.0, chloroform). (Found: C 60.6; H 5.08; N 3.50. Calc. for $C_{21}H_{21}O_8N$: C 60.7; H 5.11; N 3.37). NMR ($CDCl_3$, 60 MHz): δ 8.21 and 7.17 (2 H each, both d, $J_{H,H}$ 9.5 Hz, *p*-$NO_2C_6H_4O$), 7.3 – 7.7 (5 H, m, other aromatics), 6.28 (1 H, s, benzylidene proton), 5.90 (1 H, s, $J_{1,2}$ < 1 Hz, H-1), 5.14 (1 H, q, $J_{3,4}$ 8.0 Hz, $J_{4,5}$ 9.5 Hz, H-4), 4.69 (1 H, q, $J_{2,3}$ 5.6 Hz, $J_{3,4}$ 8.0 Hz, H-3), 4.39 (1 H, $J_{2,3}$ 5.0 Hz, H-2), 3.88 (1 H, oct, $J_{4,5}$ 9.5 Hz, $J_{5,6}$ 6.5, H-5), 2.13 (3 H, s, $OCOCH_3$), 1.19 (3 H, d, $J_{5,6}$ 6.5 Hz, H-6).

The H-*exo*-isomer was crystallized from chloroform-hexane and showed m.p. 153 – 154 °C. $[\alpha]_{578}$ −65° (*c* 1.1, chloroform). (Found: C 60.6; H 5.12; N 3.49. Calc. for $C_{21}H_{21}O_8N$: C 60.7; H 5.11; N 3.37). NMR ($CDCl_3$, 60 MHz): δ 8.23 and 7.18 (2 H each, both d, $J_{H,H}$ 9.5 Hz, *p*-$NO_2C_6H_4O$), 7.3 – 7.7 (5 H, m, other aromatics), 5.98 (1 H, s, benzylidene proton), 5.93 (1 H, s, $J_{1,2}$ < 1 Hz, H-1), 4.85-5.20 (1 H, m, H-4), 3.87 (1 H, oct, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.5 Hz, H-5), 2.10 (3 H, s, $OCOCH_3$), 1.14 (3 H, d, $J_{5,6}$ 6.5 Hz, H-6).

I a in the presence of 0.17 mol Eu $(DPM)_3$/mol I a ($CDCl_3$, 60 MHz): δ 8.25 and 7.23 (2 H each, both d, $J_{H,H}$ 9.5 Hz, *p*-$NO_2C_6H_4O$), 7. 65 – 7.90 (2 H, m, other aromatics), 7.20 – 7.40 (3 H, m, other aromatics), 6.13 (1 H, s, benzylidene proton), 6.04 (1 H, s $J_{1,2}$ < 1 Hz, H-1), 5.89 (1 H, q, $J_{3,4}$ 6.5 Hz, $J_{4,5}$ 10.0 Hz, H-4), 4.92 (1 H, trip, $J_{2,3}$ 6.5 Hz, $J_{3,4}$ 6.5 Hz, H-3), 4.66 (1 H, d, $J_{2,3}$ 6.5 Hz, H-2), 4.17 (1 H, oct, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.5 Hz, H-5), 2.52 (3 H, s, $OCOCH_3$), 1.38 (3 H, d, $J_{5,6}$ 6.5 Hz, H-6).

p-*Nitrophenyl 2,3*-O-*benzylidene-H*-endo)-α-L-*rhamnopyranoside (II)*. I (12.2 g) was dissolved in methanol (100 ml) and 3 % methanolic ammonia (50 ml) was added. The solution was left at room temperature for three days. Evaporation yielded II (10.9 g, 99 %) as a syrup which slowly crystallized. Crystallization from diethyl ether-hexane yielded the pure substance, m.p. 78 – 82 °C. $[\alpha]_{578}^{23}$ −65.0° (*c* 1.0, chloroform). A satisfactory elemental analysis could not be obtained for this compound. NMR ($CDCl_3$, 100 MHz): δ 8.16 and δ 7.10 (2 H each, both d, $J_{H,H}$ 9.0 Hz, *p*-$NO_2C_6H_4O$), 7.2 – 7.6 (5 H, m, other aromatics), 6.18 (1 H, s, benzal proton), 5.84 (1 H, s, $J_{1,2}$ < 1 Hz, H-1), 4.54 (1 H, q, $J_{2,3}$ 5.0 Hz, $J_{3,4}$ 7.0 Hz, H-3), 4.32 (1 H, d, $J_{2,3}$ 5.0 Hz, H-2), 1.28 (3 H, d, $J_{5,6}$ 5.0 Hz, H-6).

p-*Nitrophenyl 2,3*-O-*benzylidene-(H*-exo)-α-L-*rhamnopyranoside (II a)*. I a (1.0 g) was dissolved in methanol (30 ml) and dichloromethane (10 ml). Barium oxide (0.20 g) was added and the mixture was refluxed for 1 h. The solvents were evaporated and the residue was suspended in ethyl acetate. After filtration and evaporation II a was obtained as a syrup (0.90 g, 100 %) which slowly crystallized. Crystallization from ethanol yielded the pure substance, m.p. 128 – 130 °C. $[\alpha]_{578}^{23}$ −143° (*c* 1.7, chloroform). (Found: C 61.2; H 5.19; N 3.88. Calc. for $C_{19}H_{17}NO_7$: C 61.1; H 5.13; N 3.75). NMR ($CDCl_3$, 60 MHz): δ 8.21 and 7.16 (2 H each, both d, $J_{H,H}$ 9.5 Hz, *p*-$NO_2C_6H_4O$), 7.3 – 7.7 (5 H, m, other aromatics), 5.98 (1 H, s, benzylidene proton), 5.93 (1 H, s, $J_{1,2}$ < 1 Hz, H-1), 1.17 (3 H, d, $J_{5,6}$ 5.5 Hz, H-6).

p-*Nitrophenyl 2,3*-O-*benzylidene-H*-endo)-*4*-O-(α-D-*mannopyranosyl*)-α-L-*rhamnoside* (IV). II (4.72 g) and 3,4,6-tri-*O*-acetyl-1,2-*O*-methylorthoacetyl-β-D-mannose [9] (4.0 g) were dissolved in nitromethane (100 ml), mercuric bromide (0.36 g) was added and the mixture was distilled. The volume was kept constant by addition of nitromethane. After 1 h, when examination by TLC (solvent system B) indicated a complete reaction, yielding the acetylated disaccharide derivative III, the solvent was evaporated. The residue was suspended in

methanolic ammonia (100 ml, 1.67 %) and left at room temperature overnight. After filtration and evaporation a crude syrup (6.32 g) was obtained. Chromatography on a silica gel column (solvent system C) yielded IV (2.07 g, 35 %) as a slowly crystallizing syrup. Crystallization from 2-propanol yielded the pure substance, m.p. 108–114 °C. $[\alpha]_{578}^{23}$ −19° (*c* 0.7, methanol). (Found: C 55.9; H 5.49; N 2.67. Calc. for $C_{25}H_{29}NO_{13}$: C 56.1; H 5.45; N 2.67). NMR (CD_3OD, 100 MHz) δ 8.20 and 7.24 (2 H each, both d, $J_{H,H}$ 9.0 Hz, *p*-$NO_2C_6H_4O$), 7.3–7.6 (5 H, m, other aromatics), 6.21 (1 H, s, benzal proton), 5.93 (1 H, s, $J_{1,2} < 1$ Hz, H-1 L-rhamnosyl residue), 4.97 (1 H, s, $J_{1,2} < 1$ Hz, H-1 D-mannosyl residue), 4.37 (1 H, d, $J_{2,3}$ 5.0 Hz, H-2 L-rhamnosyl residue), 1.26 (3 H, d, $J_{5,6}$ 6.5 Hz, H-6 L-rhamnosyl residue).

p-*Nitrophenyl 4*-O-*(α*-D-*mannopyranosyl)*-α-L-*rhamnoside (V)*. IV (1.0 g) was dissolved in 50 % aqueous acetic acid (50 ml) and the solution was kept at 100 °C for 30 min and evaporated. The residue was suspended in water (50 ml) and extracted with diethyl ether (3 × 30 ml). The water phase was evaporated to yield crude V (0.78 g). After acetylation with acetic anhydride (10 ml) and pyridine (12 ml) at room temperature overnight, a syrup (1·21 g) was obtained, which was chromatographed on a silica gel column (solvent system D) to yield a chromatographically homogeneous hexaacetate (0.93 g, 76 %) that did not crystallize. After deacetylation with 5 % methanolic ammonia pure V (0.60 g, 100 %) was obtained as a slowly crystallizing syrup. Crystallization from a small volume of water afforded the pure compound with 1 mol of water, m.p. 126–128 °C. $[\alpha]_{578}^{23}$ −90° (*c* 0.5, water). (Found: C 46.4; H 5.45; N 3.09. Calc. for $C_{18}H_{25}NO_{12}$·H_2O: C 46.5; H 5.84; N 3.01). NMR (D_2O, 100 MHz): δ 8.21 and δ 7.20 (2 H each, both d, $J_{H,H}$ 9.0 Hz, *p*-$NO_2C_6H_4O$); δ 5.67 (1 H, d, $J_{1,2}$ 2 Hz, H-1 L-rhamnosyl residue), 4.98 (1 H, d, $J_{1,2}$ 1 Hz, H-1 D-mannosyl residue), 1.25 (3 H, d, $J_{5,6}$ 6.0 Hz, H-6 L-rhamnosyl residue).

p-*Nitrophenyl 4*-O-*(2*-O-*acetyl-3,4,6-tri*-O-*benzyl-β*-D-*glucosyl)-2,3*-O-*benzylidene-(H*-endo)-α-L-*rhamnoside (VI)*. II (7.72 g) and 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-α-D-glucose [12] were dissolved in nitromethane (125 ml). Solvent was distilled off and the volume was kept constant by addition of nitromethane. The reaction was monitored by TLC (solvent system E). After 6 h when no further reesterification appeared to take place, mercuric bromide (0.23 g) was added and the mixture was refluxed for 15 h. After filtration and evaporation a crude syrup was obtained, which after chromatography on a silica gel column yielded VI (2.16 g, 16 %) as a syrup, which slowly crystallized. Crystallization from methanol yielded the pure substance, m.p. 137–139 °C. $[\alpha]_{578}^{23}$ −51° (*c* 0.4, chloroform). (Found: C 68.0; H 5.86; N 1.81. Calc. for $C_{48}H_{49}NO_{13}$: C 68.0, H 5.83; N 1.65). NMR ($CDCl_3$, 100 MHz): δ 8.16 and 7.10 (2 H each, both d, $J_{H,H}$ 9.0 Hz, *p*-$NO_2C_6H_4O$), 7.2–7.5 (20 H, m, other aromatics), 6.13 (1 H, s, benzylidene proton), 5.80 (1 H, s, $J_{1,2} < 1$ Hz, H-1 L-rhamnosyl residue), 4.28 (1 H, d, $J_{2,3}$ 6 Hz, H-2 L-rhamnosyl residue), 1.98 (3 H, s, $OCOCH_3$), 1.30 (3 H, d, $J_{5,6}$ 5.0 Hz, H-6 L-rhamnosyl residue).

p-*Acetamidophenyl 4*-O-*(2*-O-*acetyl-3,4,6-tri*-O-*benzyl-β*-D-*glucosyl)-2,3*-O-*benzylidene-(H*-endo)-α-L-*rhamnoside (VII)*. VI (1.4 g) was hydrogenated at room temperature and atmospheric pressure in the presence of Adams' catalyst (0.15 g) using tetrahydrofuran (30 ml) as solvent. When hydrogen consumption had ceased, acetic anhydride (5 ml) and pyridine (8 ml) were added. After 1 h at room temperature, the solution was filtered and evaporated. VII was obtained as a chromatographically homogeneous syrup (1.42 g, 99 %), which slowly crystallized. Crystallization from methanol yielded the pure substance, m.p. 193–195 °C. $[\alpha]_{578}^{23}$ −37° (*c* 0.7, chloroform). (Found: C 70.0; H 6.31; N 1.74. Calc. for $C_{50}H_{53}NO_{12}$: C 69.8; H 6.21; N 1.62). NMR ($CDCl_3$, 100 MHz): δ 6.97 (2 H, d, $J_{H,H}$ 9.0 Hz, *p*-$AcNHC_6H_4O$, other 2 H in other aromatics), 7.1–7.6 (22 H, m, other aromatics), 6.11 (1 H, s, benzylidene proton), 5.65 (1 H, s, $J_{1,2} < 1$, H-1 L-rhamnosyl residue), 4.26 (1 H, d, $J_{2,3}$ 5.0 Hz, H-2 L-rhamnosyl residue), 2.13 (3 H, s, *N*-$COCH_3$), 1.98 (3 H, s, $OCOCH_3$), 1.31 (3 H, d, $J_{5,6}$ 6.0 Hz, H-6 L-rhamnosyl residue).

p-*Acetamidophenyl 4*-O-*(3,4,6-tri*-O-*benzyl-β*-D-*glucosyl)-2,3*-O-*benzylidene-(H*-endo)-α-L-*rhamnoside (VIII)*. VII (1.3 g) was dissolved in a mixture of methanol and dichloromethane (1:1, 40 ml), barium oxide (0.20 g) was added. The mixture was refluxed for 1 h, cooled and the solvent was evaporated. The residue was suspended in ethyl acetate (30 ml) and filtered. After evaporation to dryness, IX was obtained as a crude syrup. After purification on silica gel (solvent system F) a homogeneous syrup (1.0 g, 79 %) which slowly crystallized was obtained. Crystallization from methanol afforded the pure compound, m.p. 175–178 °C. $[\alpha]_{578}^{23}$ −53° (*c* 0.5, chloroform). (Found: C 70.3; H 6.24; N 1.85. Calc. for $C_{43}H_{51}NO_{11}$: C 70.5; H 6.28; N 1.71). NMR ($CDCl_3$, 100 MHz): δ 6.96 (2 H, d, $J_{H,H}$ 9.0 Hz, *p*-$AcNHC_6H_4O$, other 2 H in other aromatics), 7.1–7.6 (22 H, m, other aromatics), 6.14 (1 H, s, benzylidene proton), 5.66 (1 H, s, $J_{1,2} < 1$ Hz, H-1 L-rhamnosyl residue), 4.28 (1 H, d, $J_{2,3}$ 5.0 Hz, H-2 L-rhamnosyl residue), 2.12 (3 H, s, *N*-$COCH_3$), 1.33 (3 H, d, $J_{5,6}$ 5.0 Hz, H-6 L-rhamnosyl residue).

p-*Acetamidophenyl 4*-O-*(3,4,6-tri*-O-*benzyl-β*-D-arabino-*hexoside-2-ulose)-2,3*-O-*benzylidene-(H*-endo)-α-L-*rhamnoside (IX)*. VIII (0.80 g) was dissolved in dimethyl sulfoxide (80 ml) and acetic anhydride (4 ml). The solution was kept at room temperature under nitrogen for two days and the reagents were removed by

lyophilization. The crude product was used without purification in the following step. IR (KBr) showed, *inter alia*, a strong absorption at 1750 cm^{-1} (C=O, ketone).

p-*Acetamidophenyl 4-O-(3,4,6-tri-O-benzyl-β-D-mannosyl)-2,3-O-benzylidene-(H-endo)-α-L-rhamnoside(X)*. A solution of IX (from 0.80 g VIII) in tetrahydrofuran (10 ml) was added, under nitrogen, to a stirred M solution of lithium tri-*sec*-butylborohydride in tetrahydrofuran (5 ml) at −75 °C. The solution was kept for 3 h at −75 °C. Excess reagent was decomposed by the addition of methanol, after which the alkylboranes were oxidized with 35 % aqueous hydrogen peroxide. The solution was removed from the salts by decantation and concentrated. The crude syrup was chromatographed on a silica gel column (solvent system F), to yield X as a chromatographically homogeneous syrup (0.62 g, 78 %) which crystallized from 95 % ethanol to yield the pure substance, m.p. 162−165 °C. $[\alpha]_{578}^{23}$ −62° (*c* 0.7, chloroform). (Found: C 70.6; H 6.33; N 1.72. Calc. for $C_{48}H_{51}NO_{11}$: C 70.5; H 6.28; N 1.71). NMR ($CDCl_3$, 100 MHz): δ 6.94 (2 H, d, $J_{H,H}$ 9.0 Hz, *p*-$AcNHC_6H_4O$, other 2 H in other aromatics); δ 7.1−7.6 (22 H, m, other aromatics), 6.10 (1 H, s, benzylidene proton), 5.66 (1 H, s, $J_{1,2}<1$ Hz, H-1 L-rhamnosyl residue), 4.93 (1 H, s, $J_{1,2}<1$ Hz, H-1 D-mannosyl residue), 4.29 (1 H, d, $J_{2,3}$ 6.0 Hz, H-2 L-rhamnosyl residue), 2.11 (3 H, s, *N*-$COCH_3$), 1.33 (3 H, d, $J_{5,6}$ 6.0 Hz, H-6 L-rhamnosyl residue).

p-*Acetamidophenyl 4-O-(β-D-mannopyranosyl)-α-L-rhamnoside (XI)*. X (0.32 g) was hydrogenated at room temperature and atmospheric pressure in the presence of 10 % palladium on charcoal (0.35 g) using 95 % ethanol (100 ml) as solvent. After processing, XII (0.17 g) was obtained. Crystallization from ethanol afforded the pure substance, m.p. 153−155 °C. $[\alpha]_{578}^{23}$ −105° (*c* 0.5, water). (Found: C 52.2; H 6.44; N 3.22. Calc. for $C_{20}H_{29}NO_{11}$: C 52.3; H 6.36; N 3.05). NMR (D_2O, 100 MHz): δ 7.31 and 7.06 (2 H each, both d, $J_{H,H}$ 9.0 Hz, *p*-$AcNHC_6H_4O$), 5.48 (1 H, s, $J_{1,2}<1$ Hz, H-1 L-rhamnosyl residue), 4.90 (1 H, s, $J_{1,2}<1$ Hz, H-1 D-mannosyl residue), 2.15 (3 H, s, *N*-$COCH_3$), 1.30 (3 H, d, $J_{5,6}$ 6.0 Hz, H-6 L-rhamnosyl residue).

Sugar analysis of compounds V and XI. Samples (≃5 mg) were hydrolysed (0.25 M sulfuric acid, 100 °C, 14 h), neutralized ($BaCO_3$), and the sugars were transformed into their alditol acetates by reduction (sodium borohydride) and acetylation (acetic anhydridepyridine, 1:1). The mixtures were analysed on GLC.[16]

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Heteroaromatic Boron Compounds. XIV. Halogen—Lithium Interconversion of Some Bromo- and Iodo-substituted 3,2-Borazaropyridines

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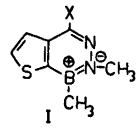
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Halogen-metal exchange has successfully been carried out between some iodo- and bromo-substituted 3,2-borazaropyridines and butyllithium, which opens the route to many substituted derivatives of this heteroaromatic boron heterocycle. In 4,6-dibromo-2,3-dimethyl-5-ethyl-3,2-borazaropyridine, it is the 6-bromine which undergoes halogen-metal exchange.

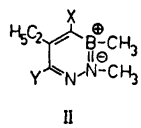
It has been found[1] that 6,7-dimethyl-7,6-borazarothieno[3,2-*c*]pyridine can be iodinated to the 4-iodo derivative (Ia), and that 2,3-dimethyl-5-ethyl-3,2-borazaropyridine can be iodinated to the 4-iodo derivative (IIa) and brominated to the 4,6-dibromo derivative (IIb).

We were interested in finding out if such halogen derivatives undergo halogen-metal exchange with alkyllithium derivatives, which would open the possibility to introduce various substituents into the borazaropyridine system.

It is known from the classical work of Gilman and Spatz[2,3] that 2-bromopyridine, 3-bromo- and 3-iodopyridine give halogen-metal exchange with butyllithium at −35 °C, and Wibaut *et al.*[4] found that this could also be achieved with 4-bromopyridine at −75 °C. Under these conditions, complications due to addition over the azomethine bond were not serious. Gronowitz and Röe[5] found that such addition was the preferred reaction with 5-bromopyrimidine at −70 °C, and halogen-metal exchange could only be obtained as the preferred reaction when the temperature was decreased to −100 °C. It was therefore not *a priori* possible to predict how the 3,2-borazaropyridine system would react even though it is isoelectronic with pyridine.



I



II

a X=I
b X=CHO
c X=Br
d X=OCH_3

a X=I, Y=H
b X=Y=Br
c X=CHO, Y=H
d X=Br, Y=H
e X=Br, Y=CHO

We found that both Ia and IIa rapidly give halogen-metal exchange at −70 °C with butyllithium, yielding upon reaction with *N,N*-dimethylformamide, 6,7-dimethyl-4-formyl-7,6-borazarothieno[3,2-*c*]pyridine (Ib) and 2,3-dimethyl-5-ethyl-4-formyl-3,2-borazaropyridine (IIc) in good yields. Reaction of IIb with one equivalent of butyllithium at −70 °C showed that the 6-bromine was the most reactive. Hydrolysis of the lithium derivative with methanol gave only one product according to GLC. Its NMR spectrum proved it to be 4-bromo-2,3-dimethyl-5-ethyl-3,2-borazaropyridine (IId). As mentioned earlier,[1] bromination of 2,3-dimethyl-5-ethyl-3,2-borazaropyridine gave a mixture of the 4- and 6-bromo derivatives, which could not be separated. The preparation of pure IId in the above-mentioned way was of importance

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** Taken in part from the Ph.D. thesis of A. Maltesson, University of Lund, 1974.

for the analyses of the bromination products.[1] Reaction of the 4-bromo-6-lithio derivative with *N,N*-dimethylformamide gave 4-bromo-2,3-dimethyl-5-ethyl-6-formyl-3,2-borazaropyridine (IIe) in good yield.

Attempts to obtain a dilithium derivative by the use of excess butyllithium failed. The reason for this became evident when the reaction between IIb and butyllithium was studied. After 45 min at −70 °C, no reaction had occurred. At −30 °C the starting material disappeared, and several new compounds were formed after hydrolysis, according to GLC analysis. However, none was the expected 2,3-dimethyl-5-ethyl-3,2-borazaropyridine. It is thus evident that with the 4-bromo derivative (in contrast to the 4-iodo derivative) halogen-metal exchange is so slow that other reactions compete. However, it is evident from our results that 6-bromo and 4-iodo derivatives of 3,2-borazaropyridines behave similarly to the isoelectronic pyridines, and the halogen-metal exchange reaction opens the route to many derivatives.

We have also made some preliminary attempts to carry out nucleophilic aromatic substitution with some halo derivatives of 3,2-borazaropyridines, a well-known reaction of pyridines. Reacting 4-bromo-6,7-dimethyl-7,6-borazarothieno[3,2-*c*]pyridine [1] (Ic) with sodium methoxide at room temperature gave 4-methoxy-6,7-dimethyl-7,6-borazarothieno-[3,2-*c*]pyridine (Id) in only 19 % yield. The reason for this was, as found by GLC analysis of the reaction mixture, ring-opening reactions of Id.

In contrast to the pyridines, all attempts to carry out the Tschitchibabin reaction with 6,7-dimethyl-7,6-borazarothieno[3,2-*c*]pyridine or 2,3-dimethyl-4-ethyl-3,2-borazaropyridine failed. Only starting material was recovered.

EXPERIMENTAL

6,7-Dimethyl-4-formyl-6,7-borazarothieno[3,2-c]-pyridine (Ib). To a solution of 1.17 g (0.0041 mol) of 6,7-dimethyl-4-iodo-7,6-borazarothieno-[3,2-*c*]pyridine [1] in 150 ml of anhydrous ether cooled to −70 °C, 5.0 ml of 1.50 N (0.0075 mol) butyllithium in hexane diluted with 20 ml of anhydrous ether was added dropwise with stirring under nitrogen. After the addition was complete (10 min), the mixture was stirred for 10 min, and 0.68 g of *N,N*-dimethylformamide in 15 ml of anhydrous ether was added. The cooling bath was removed, and when the temperature had risen to −20 °C, the mixture was poured onto ice-water and acidified with 5 N hydrochloric acid. The ether phase was separated and the aqueous phase neutralized with solid sodium bicarbonate and extracted three times with ether. The combined ether phases were washed with water, dried over magnesium sulfate and evaporated. The solid residue was recrystallized from 60 % aqueous ethanol, yielding 0.59 g (76 %) of the title compound, m.p. 96.5–97.5 °C. NMR (CD_3COCD_3)$_2$: δ 9.82 (CHO), 8.35 and 8.13 (thioph.), 3.95 (NCH_3), 1.05 (BCH_3); J_{23} 4.8 Hz. [Found: C 49.91; H 4.72; B 5.61; N 14.53. Calc. for $C_8H_8BN_2OS$ (192.1): C 50.03; H 4.72; B 5.63; N 14.59.]

2,3-Dimethyl-5-ethyl-4-formyl-3,2-borazaro-pyridine (IIc). From 0.20 g (0.765 mmol) of 2,3-dimethyl-5-ethyl-4-iodo-3,2-borazaropyridine,[1] 2.0 ml of 1.42 N butyllithium and 2.5 g of *N,N*-dimethylformamide in the appropriate amounts of anhydrous ether, following the description given above, 73 mg (58 %) of the title compound was obtained as a liquid after distillation *in vacuo*. NMR ($CDCl_3$): δ 10.57 (CHO), 7.83 (H-6), 3.77 (NCH_3), 2.82 (CH_3), 1.22 (CH_3), 0.87 (BCH_3). [Found: C 58.49; H 7.88; B 6.47. Calc. for $C_8H_{13}BN_2O$ (164.0): C 58.59; H 7.99; B 6.59.]

4-Bromo-2,3-dimethyl-5-ethyl-6-formyl-3,2-bor-azaropyridine (IIe). From 1.20 g (0.0041 mol) of 4,6-dibromo-2,3-dimethyl-5-ethyl-3,2-borazaropyridine [1] in 60 ml of anhydrous ether, 7.0 ml of 1.50 N of butyllithium in hexane, diluted with 50 ml of dry ether and 3.0 g of anhydrous *N,N*-dimethylformamide in 50 ml of ether, 0.51 g (51 %) of the title compound, b.p. 75–76 °C/0.25 mmHg, was obtained following the procedure given above. Recrystallization from 60 % aqueous ethanol gave white needles, m.p. 68.5–69.5 °C. NMR ($CDCl_3$): δ 9.73 (CHO), 3.90 (NCH_3), 3.22 (CH_2), 1.13 (CH_3), 0.98 (BCH_3). [Found: C 39.71; H 5.05; B 4.57; Br 33.05; N 11.41. Calc. for $C_8H_{12}BBrN_2O$ (242.9): C 39.56; H 4.98; B 4.45; Br 32.89; N 11.53.]

4-Bromo-2,3-dimethyl-5-ethyl-3,2-borazaropyri-dine (IId). To a solution of 0.80 g (2.73 mmol) of 4,6-dibromo-2,3-dimethyl-5-ethyl-3,2-borazaropyridine [1] in 50 ml of anhydrous ether, cooled to −70 °C, 5.2 ml of 1.50 N butyllithium in hexane diluted with ether was added dropwise under nitrogen. After 10 min, 5 ml of methanol was added to the reaction mixture and the cooling-bath removed. When the temperature had risen to −20 °C, the reaction mixture was worked up as described above. Distillation *in vacuo* gave 175 mg (59 %) of the title compound, b.p. 104–106 °C/10 mmHg. NMR ($CDCl_3$): δ 7.70 (H-6), 3.73 (NCH_3), 2.68 (CH_2), 1.18 (CH_3), 0.85 (BCH_3). [Found: C 39.19; H 5.65; B 4.99; Br 37.23; N 13.00. Calc. for $C_7H_{12}BBrN_2$ (214.9): C 39.12; H 5.63; B 5.03; Br 37.18; N 13.04.]

6,7-Dimethyl-4-methoxy-7,6-borazarothieno-[3,2-c]pyridine (Id). A solution of 4.86 g (0.020 mol) of 4-bromo-6,7-dimethyl-7,6-borazarothieno[3,2-c]pyridine in 200 ml of 0.2 N sodium methoxide in methanol was stirred for 10 h. The solution was neutralized with dilute hydrochloric acid and extracted with ether. The solvent was evaporated and the residual oil purified by preparative TLC on silica gel using hexane-ether (4:1) as eluent. The band corresponding to the title compound was identified by GLC. In order to obtain good separation, the plate was dried and re-eluated twice. After recrystallization from 50 % aqueous ethanol, 0.58 g (15 %) of the title compound, m.p. 38.0–39.5 °C, was obtained. NMR ($CDCl_3$): δ 7.73 and 7.75 (thioph.), 3.97 (OCH_3), 3.67 (NCH_3), 0.88 (BCH_3); J_{23} 4.8 Hz. [Found: C 49.93; H 5.58; B 5.66; N 14.68. Calc. for $C_8H_{11}BN_2OS$ (194.0): C 49.51; H 5.71; B 5.57; N 14.43.]

GLC analyses were carried out on a Varian model 1400 gas chromatograph, NMR spectra were recorded on a Varian A-60 NMR spectrometer and mass spectra were recorded on an LKB A-9000 mass spectrometer. Elemental analyses were carried out by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mülheim/Ruhr.

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An Interpretation of Kinetic Data for Cyclopropyl-assisted Solvolysis in a 3-Bicyclo[3.1.0]hexyl System Using Spectroscopic Information

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Analysis of the kinetics of cyclopropyl-assisted solvolysis in a 3-bicyclo[3.1.0]hexyl systems using far-IR and microwave spectroscopic information indicates that the reaction occurs in a highly excited vibrational state in which the molecule briefly has a chair-like conformation.

The cyclopropyl-assisted reaction may go through a possible intermediate trishomocyclopropenyl cation. The spectroscopic data give an estimated value for the relative rate of reaction for the chair conformation (k_{chair}/k_{obs}). The calculated k_{chair} agrees well with observed rates of cyclopropyl-assisted solvolysis in rigid 3-bicyclo[3.1.0]hexyl systems.

Extensive NMR,[1–13] UV,[14] far-IR,[15,16] Raman,[17] and microwave [18–23] spectroscopic investigations have shown that in bicyclo[3.1.0]hexane systems the most stable conformations are boat-like forms. X-Ray crystallographic structure determinations of a few bicyclo[3.1.0]hexane derivatives also show boat-like conformations [24–26] and additional support is given by dipole moment studies.[27] A knowledge of the conformations of bicyclo[3.1.0]hexane derivatives is important for the interpretation of kinetic data on rearrangement reactions [4,7,28] and of chiro-optical data provided by conjugated cyclopropyl chromophores in these systems.[14]

In this communication we wish to discuss the significance of conformational changes for the kinetics of cyclopropyl-assisted solvolysis in 3-bicyclo[3.1.0]hexyl systems. This discussion is based on spectroscopic results and especially on recent far-IR and microwave spectroscopic studies on bicyclo[3.1.0]hexane [16,21] and some related compounds.[15–20,22,23]

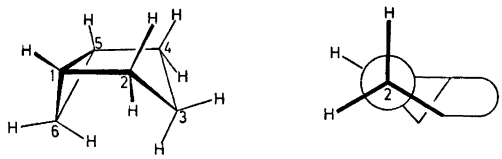
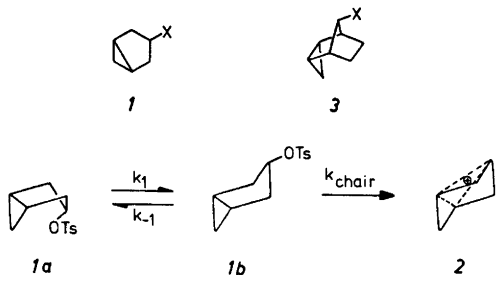


Fig. 1. (a) Boat-like conformation of bicyclo-[3.1.0]hexane and (b) Newman-projection of the C(1)–C(2) bond.

In bicyclo[3.1.0]hexane systems, in contrast to cyclohexane derivatives, the boat conformation leads to the preferred staggered arrangement of C(1)–C(2) and C(4)–C(5) bonds (*cf.* Fig. 1). In the chair conformation these bonds possess an eclipsed arrangement.

The rate of the cyclopropyl-assisted solvolysis of *cis*-3-bicyclo[3.1.0]hexyl tosylate [4,29] (*1*, X = *p*-toluenesulfonate, OTs) is very low compared with the rates of other cyclopropyl-assisted reactions, *e.g.* the solvolysis of the tricyclic homologue (*3*, X=*p*-nitrobenzoate).[30–32] In order to explain the low rate of solvolysis of *1* Winstein *et al.*[4,7,33] suggested that *cis*-3-bicyclo[3.1.0]hexyl tosylate exists mainly in a boat-like conformation (*1a*) while the reaction takes place primarily from a chair-like conformation (*1b*) by a cyclopropyl-assisted ionization to form a trishomocyclopropenyl cation (*2*).



1 *3*

1a *1b* *2*

Winstein *et al.*[4,7] (*cf.* also Refs. 34, 35, and 36) have assumed an equilibrium between the boat and the chair conformations with a low value of the equilibrium constant $K = k_1/k_{-1}$. Furthermore, they assumed that the equilibrium between *1a* and *1b* is maintained during the process. From these assumptions an expression for the over-all reaction rate was derived in terms of the equilibrium constant and k_{chair},

$$k_{obs} = K(1+K)^{-1}k_{chair} \approx Kk_{chair}$$

The second assumption would by necessity demand that k_{chair} is small compared to k_{-1}. However, k_{chair} should be relatively large and should correspond to the rate constants of cyclopropyl-assisted solvolysis in related rigid systems.[35,36] The use of a steady-state approximation for the concentration of the chair conformation (*1b*) would give the more correct result

$$k_{obs} = K(1+k_{chair}/k_{-1})^{-1}k_{chair}$$

The angle $180° - \angle(C_1C_2C_4C_5) - (C_2C_3C_4)$ is denoted by ϕ. Positive values of ϕ correspond to the boat conformation and negative values to the chair conformation. In the systems hitherto investigated, the angle ϕ at equilibrium varies from approximately 20° [22] up to 40°.[18–21] A bulky substituent at the *cis*-3-position might possibly cause a smaller equilibrium angle. It is also of interest to note that the vibrational potential curves obtained from far-IR [15,16,22] and Raman [17] spectra of bicyclo[3.1.0]hexane and some related compounds give indications of a stable chair conformation only in the case of 6-oxabicyclo-[3.1.0]hexane. In this molecule a stable chair conformation might possibly occur at approximately 13 kJ mol^{-1} above the ground-state of the boat conformation.

The ring-bending motion usually has a large amplitude even in the ground vibrational state. Amplitudes of vibration in different states of 3-bicyclo[3.1.0]hexanone have been estimated in terms of the angle ϕ.[22] In higher vibrational states the large amplitude of the ring-bending motion will cause the molecule to pass through a chair-like conformation, with ϕ less than approximately $-20°$, for a short period in the vibrational cycle.

It is well-known that a vibration associated with a heavy group (large reduced mass) will result in a closer spacing of the vibrational levels than in one with a lighter group. Far-IR results [16,22] are in agreement with this conclusion. A heavy substituent on C(3), such as a *p*-toluenesulfonate group, will accordingly result in very low-lying vibrational states.

In the light of the discussion above we prefer to consider the ionization process of *cis*-3-bicyclo[3.1.0]hexyl tosylate (*1*) as occurring in a highly excited vibrational state in which the molecule for a short moment has a chair-like conformation. From these assumptions we can make a qualitative estimate of the rate of reaction, k_{obs}, in terms of k_{chair}.

The assisted solvolysis is possible only if the molecule is in or above a vibrational state n, which is the lowest state in which the molecule for a short moment can go over to a chair conformation. In order to simplify the calculation of the probability for a molecule to be in a state $j \geq n$, the potential function is approximated as a harmonic one. The probability is then

$$P(j \geq n) = \exp[-nh\nu(RT)^{-1}]$$

in which ν is the fundamental ring-bending vibration frequency.

The product $nh\nu$ $(= E_n - (1/2)h\nu)$ for several bicyclohexanes,* calculated with n as the first vibrational state above the inflection or the second minimum point of the potential curve (*cf.* Fig. 2), is relatively constant and varies only between 13 and 19 kJ mol^{-1}. This energy, ΔE, can be considered as the energy above ground state of the lowest vibrational state in which a chair conformation occurs. The prob

* Bicyclo[3.1.0]hexane,[16] 3-oxabicyclo[3.1.0]-hexane,[16] 6-oxabicyclo[3.1.0]hexane,[15,16] 3,6-dioxabicyclo[3.1.0]hexane,[16] and 3-bicyclo[3.1.0]hexanone.[22]

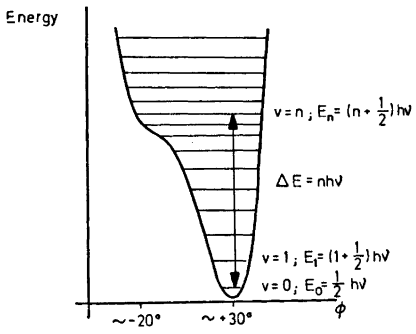


Fig. 2. A typical ring-bending potential function for a bicyclo[3.1.0]hexyl system.

ability for a molecule to be in a state $j \geq n$ can then be calculated to be somewhere between 4×10^{-4} and 5×10^{-3} at 25 °C.

When the molecule is in an excited state, $j \geq n$, the ring-bending vibration is of large amplitude between boat and chair forms as previously discussed. The probability of a chair conformation, P(chair), will thus be smaller than $P(j \geq n)$. Since the vibrational amplitude is not well established at higher vibrational levels, P(chair) is difficult to determine. However, any reasonable assumption will reduce the derived value of $P(j \geq n)$ by at least a factor of 5 to 10. Accordingly, we find the total probability of the molecule being in a chair-like conformation to lie between 4×10^{-5} and 1×10^{-3}. These values can be regarded as limits for the "equilibrium constant" K.

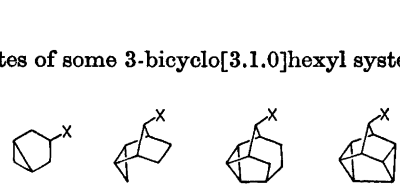
The ratio k_{obs}/k_{chair} is equal to $[1b]/([1a] + [1b]) = [1b]/[1a]$. The chair conformation (*1b*) will react faster than equilibrium can be attained. As long as k_{-1} is unknown it is, however, reasonable to adopt the approximation of Winstein *et al.*[4,7] that the ratio $[1b]/[1a]$ does not differ very much from that at equilibrium. From the known values [29] $\Delta H^{\ddagger} = 101$ kJ mol^{-1} and $\Delta S^{\ddagger} = -21$ J K^{-1} mol^{-1} we may calculate $k_{obs} = 1.1 \times 10^{-6}$ s^{-1} at 25 °C. Finally, from the value of P(chair) calculated above, we get $k_{chair} = 0.001$ to 0.03 s^{-1}.

The value 0.001 s^{-1} of k_{chair} can probably be regarded as a lower limit. A vibration of larger amplitude in a state of higher energy than that assumed, n, might be required for successful trapping of the molecule. In such a case the calculations above would give a higher value of k_{chair}.

Some rigid systems with quite large negative values of the angle ϕ (*e.g. 3*, *4* and *5*) show high values for the rate of solvolysis.[29–32,34,35,37,38] The solvolytic reactivities of these rigid systems can now be compared with the calculated rate of solvolysis from a chair conformation of *1* (k_{chair}). The relative rate for the chair conformation is of the same order of magnitude as those of the rigid systems *3*, *4* and *5* as shown in Table 1. This interpretation is in agreement with the higher rate of solvolysis of the compound *3*, which has a large negative value of the angle ϕ.

This discussion has been concerned with the solvolysis of *cis*-3-bicyclo[3.1.0]hexyl tosylate.

Table 1. Relative solvolysis rates of some 3-bicyclo[3.1.0]hexyl systems.[a]



1 *3* *4* *5*

Temp. of solvolysis (°C)	$k_{1b,\,calc}/k_1 \times 10^{-4}$	$k_3/k_1 \times 10^{-4}$	$k_4/k_1 \times 10^{-4}$	$k_5/k_1 \times 10^{-4}$
25[b]	0.1 – 2.5	–	0.11	–
100[c]	0.03 – 0.5	36	–	1.2

[a] In this table k_1 refers to the observed rate constant of solvolysis for compound *1*, k_3 is the observed rate constant for compound *3*, *etc.* $k_{1b,\,calc}$ is equal to the calculated value of k_{chair}. [b] In AcOH. [c] In 70 % aqueous dioxane solvent.

It has been based on potential functions for the ring-bending of some bicyclo[3.1.0]hexane derivatives. The potential curve of the tosylate will, of course, be slightly different from those here. However, the same effects, ring-strain and H,H-interaction, will undoubtedly determine the vibrational potential of the tosylate as well. The spectroscopic results have been obtained from measurements in the gas-phase while the kinetic studies have been performed in the liquid phase. In spite of these shortcomings the agreement of the kinetic data calculated on the basis of spectroscopic results with data obtained from solvolysis experiments is surprisingly good.

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Mass Spectrometry of Onium Compounds. XIX.[1] Ionisation Potentials of Some Pyridines and Pyridinium Betaines. Structure Analysis

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Ionisation potentials for series of *N*-vinylpyrid-2-thiones, 2-vinylthiopyridines and pyridinium betaine analogues have been determined. The values have, together with characteristic fragmentation modes, been used in structure analysis of the volatile components from pyridinium betaines.

Dihydrothiazolo[3,2-*a*]pyridinium derivatives have been studied in the mass spectrometer and structures assigned to the volatile species on the basis of their fragmentation modes.[2,3] Recently it has been found that ionisation potentials (IP) are useful in structure elucidation in the gas phase.[4-6] A very recent article reviews general application of ionisation and appearance potentials (AP) in structure analysis.[7]

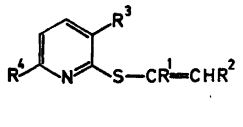
In this work we have determined IP data for certain pyridines as references for our studies [2,3] of the gaseous products formed on evaporation of pyridinium betaines (Table 1).

The first ionisation potential of pyridine corresponds to the removal of an electron from the most energetic occupied aromatic orbital and not from the nitrogen lone pair.[8] Substituent effects on the π-electrons of the nitrogen heterocyclic ring parallel those on the π-electrons of a normal carbo-aromatic ring with decrease in IP for electron releasing substituents and *vice versa*.[9] IP for pyridine is reported as 9.85 eV [9] and for 3-hydroxypyridine as 9.55 eV.[5] The 2-vinylthiogroup in (*1a*) has a large lowering effect on IP (7.95 eV). IP is further decreased (7.52 eV) with a β-carboxy group (*1c*). The allylthio group in (*3*) exerts a similar effect. Table 1 also shows that a 3-hydroxy group in the pyridine reduces the IP with 0.2 – 0.3 eV and a methyl group acts in the same way (0.1 – 0.2 eV). Extension of the conjugative system with a β-phenyl group (*1g*) decreases IP further (7.35 eV). A phenyl group on the α-carbon (*1e*) has less effect (7.8 eV). The latter is formed from the β-carbon acid (*1e*, $R^2 = CO_2H$) which is decarboxylated before evaporation in the instrument. Decarboxylation is also the dominating pyrolytic reaction for the other β-acid analogues which give rise to isomers (*1d*) and (*1f*). The primary site of ionisation is presumably in the pyridine ring since a comparison between (*1h*) and (*1i*) shows that the *para*-nitro group has little effect on the IP.

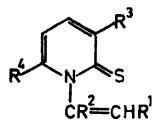
In the *N*-vinyl series (*2*) similar substituent variations in IP are seen (Table 1). The *N*-ethyl-(*4*) and the *N*-vinyl-(*2c*) groups affect to a similar extent the ease of ionisation. Simple *N*-alkylated 2-thiones of pyridine have lower IP's than their isomeric thioethers.[5,10] A comparison of IP's for the vinylthio pyridine (*1a*) and its *N*-vinyl analogue (*2b*) shows that the thioether also in this case has the higher (about 0.4 eV) IP.

Based on the above information IP data for the betaines (Table 2) can be used in structure analysis. Thus the IP's for the *N*-vinyl derivatives (*2b*) and (*2c*) (Table 1) are about 0.3 eV higher than the values for the isomeric betaines (*5a*) and (*5b*) (Table 2). The low IP's for the betaines (*5a* – *5d*) are therefore explained by these molecules being evaporated without structural rearrangement to non-charged molecules. The same conclusion was reached from studies

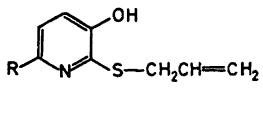
Table 1. Ionisation potentials of pyridines.



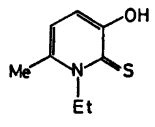
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2



3



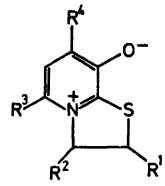
4

Comp.	R^1	R^2	R^3	R^4	IP ± 0.05 eV
1a	H	H	OH	H	7.95
1b	H	CO_2H	H	H	7.80
1c	H	CO_2H	OH	H	7.52
1d	Ph	H	H	H	(8.0)[a]
1e	Ph	H	OH	H	(7.8)[a]
1f	Ph	H	OH	Me	(7.5)[a]
1g	H	Ph	OH	H	7.35
1h	H	Ph	OH	Me	7.25
1i	H	*p*-NO_2-Ph	OH	He	7.28
2a	H	H	H	H	7.75
2b	H	H	OH	H	7.57
2c	H	H	OH	Me	7.35
2d	Me	H	H	H	7.49
2e	Me	H	OH	H	7.31
2f	Me	H	OH	Me	7.07
2g	H	Ph	OH	H	7.60
2h	H	Ph	OH	Me	7.38
3a	R = H				7.72
3b	R = Me				7.50
4					7.37

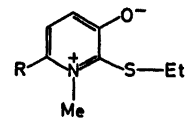
[a] Formed by pyrolysis in the instrument from the respective β-carboxylic acids ($R^2 = CO_2H$).

of the fragmentation patterns.[2] The substituent effect of the 7-bromine in (*5c*) is as expected of little importance since pyridine and 4-bromopyridine have almost the same IP's.[9] The weak effect of the 7-*p*-nitrophenyl group in (*5d*) indicates primary ionisation in the hetero-aromatic ring. Similarly the 3-phenyl derivatives (*5h*) (7.02 eV) and (*5i*) (6.73 eV) have IP's 0.6 eV below those of the respective isomeric *N*-vinyl analogues (*2g*) and (*2h*). Direct evaporation without structural change is therefore again implied. The conclusion is further supported by differences in the fragmentation patterns of the

Table 2. Ionisation potential of pyridinium betaines.



5

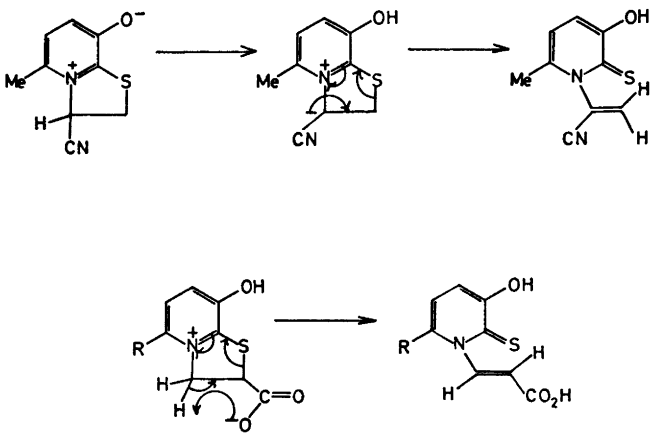


6

Comp.	R^1	R^2	R^3	R^4	IP ± 0.05 eV
5a	H	H	H	H	7.32
5b	H	H	Me	H	7.02
5c	H	H	Me	Br	7.05
5d	H	H	Me	*p*-NO_2-Ph	7.15
5e	H	$CONH_2$	Me	H	(7.4)[a]
5f	H	CN	Me	H	(7.51)[b]
5g	Ph	H	Me	H	7.04
5h	H	Ph	H	H	7.02
5i	H	Ph	Me	H	6.73
5j	CO_2H	H	H	H	(7.64)[b]
5k	CO_2H	H	Me	H	(7.43)[b]
6a	R = H				7.16
6b	R = Me				6.92

[a] Mixture of the *N*-vinyl isomer and the betaine in the gas phase. [b] The IP values refer to the *N*-vinyl isomers in the gas phase.

vinyl derivatives and the betaines. The *N*-vinylpyrid-2-thiones and their vinylthio analogues have very similar spectra. The principal primary fragmentation is rationalised by expulsion of a substituent on the β-carbon of the side-chain to a common thiazolo[3,2-*a*]pyridinium ion with low tendency for further fragmentation.[3] Accordingly the simple vinylthio derivative (*1a*) expels a hydrogen radical and the vinylthio acids (*1b*) and (*1c*) expel a formyloxy radical; the same ions are formed by hydrogen radical expulsion from the *N*-vinyl derivatives (*2a*) and (*2b*). The *N*-vinyl derivatives with terminal methyl group (*2d* – *2f*) have [M – 15] as base peak, the relative intensities for the molecular ion and the [M – 1] peaks being of the order 30 and 10 %, respectively. Similarly the fragmentation of the α-phenyl derivatives (*1d* – *1f*) and (*2g*, *2h*) are characterised by [M – 1] being the base peak. For the vinylthio-β-phenyl derivatives (*1g* – *1i*) other fragmentations are also important. Typical percentages for relative intensities being [M] 10, [M – H] 10, [M – Ph] 50



and [M − PhC≡CH] 50 with the base peak of *m/e* 39. The 3-phenyl betaine isomers (5h, 5i) have the base peak at [M − PhC≡CH]. Other characteristic peak intensities are due to [M] 60 − 90, [M − H] 40, [M − SH] 10, [M − Ph] 35 − 50 %. This mode of fragmentation is significantly different from that of vinyl analogues. The same conclusion is reached for the 2-phenyl derivative (*5g*) which is characterised by [M − PhC≡CH] being the base peak.

IP for the 3-nitrile (*5f*) is found to be 0.5 eV higher than for the parent compound (*5b*). The relatively large difference cannot be ascribed to the nitrile substituent alone. In fact the observed IP is comparable to the values for the *N*-vinyl analogue (*2c*) (7.35 eV) and the vinylthio acid (*1c*) (7.52 eV). It is therefore concluded that the betaine has suffered ring-opening before evaporation. This conclusion is supported by its spectrum which is characterised by the [M − H] species. Ring-opening to its *N*-vinyl isomer is explained by strong activation of the methine carbon by the quaternary nitrogen atom and the cyano group. The phenolate ion in a neighbour molecule is the base.

3-Carboxy derivatives of the betaine (*5*) are decarboxylated before evaporation in the mass spectrometer. The 2-carboxy derivatives, however, give a molecular ion corresponding to the mass number for the betaine.[2] The fragmentation patterns suggest *N*-vinyl formation.[2] The same conclusion is arrived at from the IP data (Table 2) for (*5j*; 7.64 eV) and (*5k* 7.43 eV). The values are about 0.4 eV higher than for the betaines (*5a*, *5b*) and comparable to those of the parent *N*-vinyl compounds (*2b*, *2c*) in Table 1. *N*-Vinyl rather than *S*-vinyl formation is also supported by the fragmentation patterns which is characterised by formyloxy radical expulsion.

The proton on the methine carbon in the 3-carboxamide (*5e*) is less acidic than in the nitrile (*5f*). The compound is sensitive to recording conditions which indicates a mixture of the amide and its ring-opened analogue in the gas phase.[2] The intermediate IP value (7.4 eV) is in accordance with this conclusion.

The IP's for the monocyclic pyridinium betaines (*6*) are of the same order as found for the bicyclic analogues (*5a*, *5b*). It is therefore concluded that these are also evaporated directly without any structural rearrangement.

EXPERIMENTAL

The mass spectra were recorded on an AEI MS-902 mass spectrometer attached to an AEI DS-30 data system. The compounds were introduced by direct insertion with source temperature 200 − 225 °C. Low resolution spectra were recorded at 70 eV electron energy and 100 μA trap current. During recording of the ionisation efficiency curves, the repeller was a cage potential and the ionising current was 20 μA. Xenon (12.127 eV) and iodobenzene (8.91 eV) were the reference compounds. The IE-curves were interpreted by the semilog plot method.[11] The recorded values, with the exception for (*1d* − *1f*) and (*5e*), are the average of three determinations, the deviation being ± 0.05 eV.

The IP values for (*2c*) and (*4*) (7.35 eV) differ from that originally published (7.75 eV).[5] This is due to a systemic error of some 0.3 − 0.4 eV for the low IP substances in the originally published report. The relative differences in IP's

in the series of compounds investigated, however, is the same and confirms previous conclusions.

The compounds used in these investigations were available from other work: (*1b*, *1c*),[3] (*2a*),[3] (*5a*–*5g*),[2] (*5j*, *5k*),[2] (*6*),[2] (*1a*),[12] (*1d*–*1f*),[12] (*2b*–*2h*),[12] (*3*),[12] and (*5h*, *5i*).[12]

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Carbon-13 Nuclear Magnetic Resonance Spectra of Some Podocarpane Derivatives

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^{13}C NMR studies of a series of ring C substituted podocarpane and podocarpene derivatives using proton-noise and single frequency off resonance decoupling, partially relaxed Fourier transform techniques, shift reagents and specifically deuterium labelled derivatives have permitted unequivocal assignment of nearly all signals. The shielding effects caused by introduction of hydroxyl and carbonyl groups are discussed as is the pronounced deshielding of the δ-carbon caused by hydroxyl and methyl groups in close steric proximity to this carbon. Some effects of deuterium substitution on the carbon resonances are presented.

^{13}C NMR has become a very useful analytical technique in natural product chemistry and has been applied in structural and stereochemical studies of various terpenoids.[1–10]

The common occurrence of diterpenoids and nor-terpenoids in tobacco [11] and the utility of ^{13}C NMR in the structural determination of these compounds have encouraged further studies.[12] The present paper is devoted to a systematic investigation of ring C oxygenated podocarpanes and podocarpenes, which are relatively rigid and conformationally well defined and therefore suitable models for the evaluation of substituent shielding effects.

RESULTS

The compounds examined in this study comprise besides the parent hydrocarbon podocarpane (*1*), ring C oxygenated podocarpanes (*2* – *12*) and podocarpenes (*13* – *23*), as well as deuterated analogues (*24* – *43*; Table 1). The methyl carbon signals were assigned using single frequency off resonance (SFOR) decoupling, while the partially relaxed Fourier transform (PRFT) technique was employed to facilitate recognition of methylene, methine, and quaternary carbon signals, thereby making use of the well-known difference in spin lattice relaxation rates, $1/T_1$, between such carbons.[9,13,14] The PRFT technique was found particularly useful in this work because of the proximity of some of the sp^3 carbon signals, which prevented unambiguous assignment by SFOR decoupling. Further assistance in the assignment of the non-trivial resonances was obtained by comparison of published data for

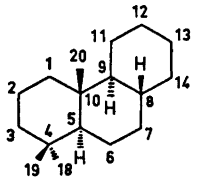
Table 1. Deuterium labelled compounds examined.

No.	Compound
24	8β,13,13,14,14-d_5-Podocarpane
25	8β-d_1-Podocarpan-12α-ol
26	8β-d_1-Podocarpan-12β-ol
27	9α-d_1-Podocarpan-12α-ol
28	9α-d_1-Podocarpan-12β-ol
29	11,11,13,13-d_4-Podocarpan-12α-ol
30	11,11,13,13-d_4-Podocarpan-12β-ol
31	9α-d_1-Podocarpan-14α-ol
32	9α-d_1-Podocarpan-14β-ol
33	8β,13,13-d_3-Podocarpan-14α-ol
34	8β,13,13-d_3-Podocarpan-14β-ol
35	8β-d_1-Podocarpan-12-one
36	9α-d_1-Podocarpan-12-one
37	11,11,13,13-d_4-Podocarpan-12-one
38	8β,13,13-d_3-Podocarpan-14-one
39	8β,11,13,13-d_4-Podocarp-9(11)-en-12-one
40	8β-d_1-Podocarp-13-en-12β-ol
41	9α-d_1-Podocarp-13-en-12β-ol
42	8β-d_1-Podocarp-13-en-12-one
43	9α-d_1-Podocarp-13-en-12-one

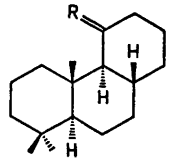
structurally related compounds and especially by examination of the selectively deuterated analogues (*24*–*43*), since deuterium labelling, as has been pointed out previously,[15–18] allows unambiguous identification of the signals due to the labelled carbons and frequently also of those due to adjacent carbons. The assignments made on these bases are given in Tables 2 and 3.

Podocarpane (*1*). The shift analysis of the spectrum of podocarpane (*1*) was made possible by comparison with published ^{13}C NMR data for pimaradienes and with the spectrum of 8β,13,13,14,14-d_5-podocarpane (*24*). Thus, nine peaks in the spectrum of podocarpane, having chemical shift values virtually identical to those found for the C-1 to C-6 and C-18 to C-20 signals in the spectrum of pimara-8(14),-15-diene [6] and being of appropriate multiplicity, were assigned on this basis alone. The remaining singlet at δ 36.9 in the off-resonance decoupled spectrum of *1* was then ascribed to C-10.

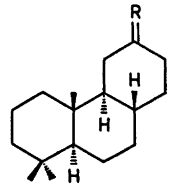
The two methine carbon signals at δ 36.8 and 56.3 were attributed to C-8 and C-9, respectively, since the former signal was virtually absent and the latter experienced an upfield shift of 0.08 ppm in the spectrum of 8β, 13, 13, 14, 14-d_5-podocarpane (*24*). Similarly, isotope induced upfield shifts of 0.15, 0.24, and 0.08 ppm for the signals at δ 35.9, 25.1, and 27.1 are only compatible with their assignments as C-7, C-11, and C-12, respectively. The remaining two signals at δ 26.4 and 35.5, hardly visible in the spectrum of the labelled derivative, were ascribed to C-13 and C-14, respectively; the differentiation was based on the fact that C-14 being adjacent to a branched center should be deshielded. Hence we consider the assignment of the spectrum of podocarpane



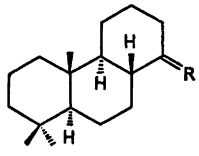
1



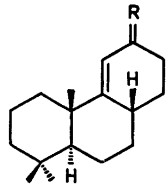
2 R = H, α-OH
3 R = H, β-OH
10 R = O



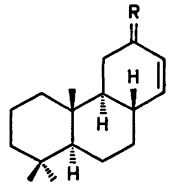
4 R = H, α-OH
5 R = H, β-OH
8 R = H, α-OAc
9 R = H, β-OAc
11 R = O



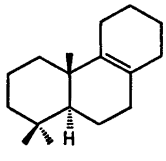
6 R = H, α-OH
7 R = H, β-OH
12 R = O



13 R = H_2
14 R = H, α-OH
15 R = H, β-OH
16 R = H, α-OAc
17 R = H, β-OAc
18 R = O



19 R = H, α-OH
20 R = H, β-OH
21 R = H, β-OAc
22 R = O



23

Table 2. Carbon-13 chemical shifts and assignments for the podocarpane derivatives *1*–*12*[a]

Compound	Carbon																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	18	19	20
Podocarpane (*1*)	39.1	19.0	42.4	33.3	55.5	21.8	35.9	36.8	56.3	36.9	25.1	27.1	26.4	35.5	33.6	22.0	14.3
Podocarpan-11α-ol (*2*)	41.7	19.1	42.0	33.6	55.3	21.9	35.7	36.4	62.0	39.0	71.9	38.4	23.9	34.9	33.9	22.1	15.0
Podocarpan-11β-ol (*3*)	39.6	18.7	42.2	33.2	55.7	21.7	36.0	30.1	58.9	37.5	66.4	35.4	19.1	35.6	34.0	22.1	16.7
Podocarpan-12α-ol (*4*)	38.8	18.9	42.2	33.3	55.4	21.7	35.4	36.4	48.5	36.4	31.5	67.0	32.3	28.6	33.6	21.7	14.1
Podocarpan-12β-ol (*5*)	39.0	18.9	42.2	33.2	55.2	21.6	34.9	35.6	53.9	36.7	34.6	71.4	35.2	32.9	33.6	21.9	14.3
Podocarpan-14α-ol (*6*)	39.3	18.9	42.2	33.3	54.9	21.5	30.6	40.7	48.2	36.7	24.8	20.0	33.6	71.0	33.6	22.0	14.1
Podocarpan-14β-ol (*7*)	39.4	19.0	42.2	33.2	55.1	21.1	31.0	44.1	53.9	36.7	24.4	24.0	35.4	76.8	33.6	21.9	14.3
Podocarpan-12α-yl acetate (*8*)[c]	38.9	18.8	42.2	33.3	55.4	21.6	35.3	36.1	49.4	36.4	29.2[b]	70.7	29.6[b]	28.8	33.6	21.9	14.0
Podocarpan-12β-yl acetate (*9*)[d]	39.0	18.8	42.1	33.2	55.1	21.6	34.7	35.7	53.6	36.8	30.9[b]	74.4	31.5[b]	32.4	33.6	21.9	14.2
Podocarpan-11-one (*10*)	38.4	18.7	42.3	33.3	54.9	21.2	35.8	40.0	67.4	36.7	211.8	44.3	26.7	34.5	33.7	22.0	14.6
Podocarpan-12-one (*11*)	38.7	18.7	42.0	33.2	54.8	21.4	34.3[b]	35.6	56.1	37.3	41.0	213.4	41.1	34.2[b]	33.6	21.9	13.8
Podocarpan-14-one (*12*)	39.4	19.0	42.0	33.3	54.6	20.6	26.3[b]	49.7	57.3	37.6	24.4	26.5[b]	41.8	213.7	33.5	21.9	13.9

[a] δ-values relative to TMS. [b] Assignment may be reversed. [c] $-\overset{\overset{O}{\|}}{C}-CH_3$ 170.7 and 21.7. [d] $-\overset{\overset{O}{\|}}{C}-CH_3$ 170.6 and 21.6.

Table 3. Carbon-13 chemical shifts and assignments for the podocarpene derivatives *13*–*23*[a]

Compound	Carbon																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	18	19	20
Podocarp-9(11)-ene (*13*)	37.6	19.2	42.5	33.8	54.0	21.2	36.4	33.4	151.5	39.6	114.3	26.0	22.2	32.0	33.4	21.9	21.1
Podocarp-9(11)-en-12α-ol(*14*)	37.3	19.0	42.3	33.8	53.5	22.0	35.7	33.3	155.2	39.6	117.4	66.2	29.7	26.6	33.3	21.9	21.0
Podocarp-9(11)-en-12β-ol (*15*)	37.4	19.0	42.3	33.7	53.8	21.9	36.2	33.4	153.1	39.3	119.3	67.4	31.1	28.6	33.3	21.9	19.0
Podocarp-9(11)-en-12α-yl acetate (*16*)[c]	37.3	19.0	42.2	33.8	53.3	21.9	35.5	33.4	157.4	39.8	113.4	69.5	26.8[b]	26.6[b]	33.3	21.9	21.1
Podocarp-9(11)-en-12β-yl acetate (*17*)[d]	37.3	19.0	42.2	33.8	53.8	21.9	35.9	33.3	155.7	39.7	114.8	70.8	27.0	27.9	33.3	21.9	20.9
Podocarp-9(11)-en-12-one (*18*)	36.7	18.7	41.7	33.9	53.1	21.4	35.2	34.2	175.9	40.9	119.5	200.4	35.8	29.4	33.3	22.0	21.1
Podocarp-13-en-12α-ol (*19*)	38.4	18.8	42.3	33.3	55.7	21.9	33.7	36.5	47.4	36.2	30.6	65.0	126.7	137.8	33.5	21.7	14.6
Podocarp-13-en-12β-ol (*20*)	38.4	18.8	42.3	33.3	55.4	21.9	33.9	36.3	52.6	36.4	32.3	69.6	129.9	135.0	33.4	21.6	14.3
Podocarp-13-en-12β-yl acetate (*21*)[e]	38.4	18.8	42.2	33.3	55.3	21.9	33.8	36.2	52.3	36.5	28.1	72.5	125.6	137.0	33.4	21.6	14.2
Podocarp-13-en-12-one (*22*)	38.0	18.6	42.0	33.2	54.8	21.7	32.9	36.6	54.0	36.6	37.8	201.1	128.1	155.9	33.4	21.7	14.4
Podocarp-8-ene (*23*)	36.5	19.0[b]	42.0	33.3	51.9	19.1[b]	32.6	138.4	125.9	37.8	23.0[b]	23.9[b]	23.6[b]	30.8	33.3	21.7	19.3

[a] δ-values relative to TMS. [b] Assignment may be reversed. [c] $-\overset{\overset{O}{\|}}{C}-CH_3$ 171.0 and 21.6. [d] $-\overset{\overset{O}{\|}}{C}-CH_3$ 170.9 and 21.5. [e] $-\overset{\overset{O}{\|}}{C}-CH_3$ 170.8 and 21.4.

(*1*) the parent hydrocarbon of the compounds included in the present study, firmly established and reliable for extraction of substituent effects when compared with the spectra of the substituted podocarpane derivatives.

Podocarpanols (*2*–*7*). Previous studies have demonstrated that a hydroxyl substituent incorporated in rigid molecules such as steroids influences only the chemical shifts of the α-, β-, γ-, and δ-carbons.[17] Consonant with this, the signals due to the three methyl groups, ring A carbons – except for the C-1 resonance derived from podocarpan-11α-ol (*2*) – and the methylene carbons of ring B – except for the C-7 signals in the spectra of the two podocarpan-14-ols (*6*–*7*) – were found at fields almost identical to those observed for the parent hydrocarbon (*1*). The resonances associated with the hydroxyl substituted carbon atoms (α-carbons) were readily identified, since they invariably appeared in the lowest field regions of the spectra (δ 66.4–76.8), while the remaining shift assignment were delineated as follows.

Selective deuterium labelling of C-8 (*25*–*26*), C-9 (*27*–*28*), and C-11, C-13 (*29*–*30*) of the podocarpan-12-ols (*4*–*5*) served to identify not only the resonances due to the carbon atoms carrying the label but also those due to the adjacent carbon atoms, *i.e.* C-7, C-10, and C-14.

Similarly, incorporation of deuterium at C-9 (*31*–*32*) and C-8, C-13 (*33*–*34*) solved the shift assignments of C-7 to C-13 for the two podocarpan-14-ols (*6*–*7*).

Use of the shift reagent Eu(dpm)$_3$ facilitated the identification of the remaining carbon signals in the spectra of the podocarpan-11-ols (*2*–*3*), *e.g.* differentiation between the C-7, C-12, and C-14 signals, which in the case of the β-epimer (*3*) are positioned at δ 36.0, 35.4, and 35.6, respectively (Table 4).

Table 4. Lanthanide-induced shifts (LIS) for podocarpan-11α- and -11β-ol (*2*–*3*).

Carbon	Compound			
	2		*3*	
	δ	LIS[a]	δ	LIS
7	35.7	0.08	36.0	0.13
11	71.9	1.00	66.4	1.00
12	38.4	0.49	35.4	0.45
14	34.9	0.14	35.6	0.17

[a] Relative shifts using Eu(dpm)$_3$.

Podocarpan-12-yl acetates (*8*–*9*). Acetylation of podocarpan-12α- and -12β-ol (*4*–*5*) caused virtually no shift of the C-1 to C-10 and C-18 to C-20 signals, while the signal due to C-12 showed an expected downfield shift.[18] A clear differentiation between the β-carbon (C-11, C-13) resonances, shifted upfield on account of acetylation,[18] was not possible since these peaks were too closely spaced in the spectra of both acetates (*8*–*9*). The remaining peaks present at δ 28.8 and 32.4 in the spectra of podocarpan-12α-yl acetate (*8*) and -12β-yl acetate (*9*), respectively, whose positions are only slightly altered relative to the positions of the C-14 resonances for the corresponding alcohols (*4*–*5*), were ascribed to C-14.

Podocarpanones (*10*–*12*). In agreement with previous findings for keto steroids,[16] introduction of an oxo group at C-11 (*10*), C-12 (*11*) or C-14 (*12*) in podocarpane (*1*) affects only the shieldings of the α-, β-, γ-, and δ-carbons and leaves the signals due to C-1 to C-6 and the three methyl groups at invariant positions. The carbonyl carbon singlets (α-carbons), located above δ 200, were readely recognised, whereas the identification of the remaining resonances for podocarpan-12-one (*11*) and -14-one (*12*) was aided by deuterium labelling. Thus, examination of the spectra of 8β-d_1-, 9α-d_1-, and 11,11,13,13-d_4-podocarpan-12-one (*35*–*37*) enabled the assignments of the C-8 to C-11 and C-13 signals, but did not allow a distinction between the C-7 and C-14 resonances, which are positioned at δ 34.2 and 34.3. Similarly, the peaks corresponding to C-7, C-8, C-12, and C-13 were unequivocally identified from the spectrum of 8β, 13, 13-d_3-podocarpan-14-one (*38*).

The assignments of the remaining signals in the spectrum of podocarpan-11-one (*10*) were guided by the substituent parameters extracted from the results for podocarpan-12- and -14-one (*11,12*).

Podocarp-9(11)-enes (*13*–*18*). Comparison of the spectra of the podocarp-9(11)-enes (*13*–*18*) with that of podocarpane (*1*) and application of simple chemical shift theory, allowed ready recognition of the signals corresponding to the functionalised carbons and

also assignment of the other signals, except those due to C-1, C-7, C-13, and C-14 – and in the case of podocarp-9(11)-ene (*13*) also that due to C-12.

The shift analysis for podocarp-9(11)-en-12-one (*18*) was completed with the aid of deuterium labelling. Hence, observations of appropriate geminal and vicinal isotope effects for the corresponding 8β,11,13,13-d_4 derivative (*39*), confirmed the shift assignments of C-8 and C-11 and identified the signals due to C-7, C-13, and C-14. The remaining signal at δ 36.7, being about 1.5 ppm downfield of the C-7 resonance, must then be ascribed to C-1. Since the chemical environments of C-1 and C-7 of podocarp-9(11)-en-12-one (*18*) are relatively similar to those of C-1 and C-7 of the other podocarp-9(11)-enes (*13*–*17*), this result was applied to differentiate the C-1 and C-7 signals in the spectra of the latter compounds, *i.e.* of the two possible signals that at lower field was attributed to C-1.

Distinction between the C-13 and C-14 signals for the podocarp-9(11)-en-12-ols (*14*, *15*) was achieved by spectral comparison with their corresponding acetates (*16*, *17*). The signal at δ 29.7 in the spectrum of the 12α-alcohol (*14*) and the signal at δ 31.1 in that of the 12β-alcohol (*15*) displayed considerable upfield shifts (3–4 ppm) as a result of acetylation.[18] These signals were therefore identified as being due to C-13, while the peaks at δ 26.6 and 28.6 for *14* and *15*, respectively, undergoing expected small upfield shifts on acetylation, were ascribed to C-14.

Having established the peak assignment of C-14 for the two allylic acetates (*16*, *17*), the identification of the corresponding signal for podocarp-9(11)-ene (*13*) follows from a spectral comparison. Thus, since previous studies have demonstrated that the introduction of an allylic acetoxy group leads to shielding of the γ-sp^3 carbon,[19,20] it is evident that the signal at δ 32.0 in the spectrum of *13*, which is downfield of the C-14 resonances for the acetates *16* and *17*, must be ascribed to C-14. Introduction of an allylic acetoxy group has also been found to cause deshielding of the γ-olefinic carbon, a result which accords with our findings for the C-9 resonances.[19,20]

Of the remaining two peaks in the spectrum of podocarp-9(11)-ene (*13*), that at δ 26.0, having a chemical shift value close to the value observed for the C-3 signal for cyclohexene (δ 25.5),[21] was ascribed to C-12, thereby leaving C-13 to account for the signal at δ 22.2.

Podocarp-13-enes (19–22). Comparison of the spectra of the podocarp-13-enes (*19*–*22*) with that of podocarpane (*1*) left the resonances due to C-1, C-7, C-11 to C-14 to be accounted for. Of these, the signals due to C-12, the carbon atom carrying the hydroxyl (*19*, *20*), acetoxyl (*21*) or the oxo (*22*) substituents were readily recognized, while some of the remaining assignments were aided by deuterium labelling. Hence, the C-7 and C-11 signals for podocarp-13-en-12β-ol (*20*) and -12-one (*22*) were identified and the peak assignments of C-8, C-9, and C-10 confirmed by an examination of the ^{13}C NMR data of the corresponding derivatives labelled at C-8 (*40*, *42*) and C-9 (*41*, *43*). The signals at δ 38.4 and 38.0 for the 12β-alcohol (*20*) and the 12-ketone (*22*), respectively, were then ascribed to C-1.

With these results for podocarp-13-en-12β-ol (*20*) in hand, the assignments of C-1, C-7, and C-11 for the corresponding acetate (*21*) followed from a spectral comparison. While the shift values of the C-1 and C-7 signals were virtually unaffected, the signal due to C-11 showed an expected upfield shift of 4 ppm as a result of acetylation.[18]

Moreover, comparison of the ^{13}C NMR data of podocarp-13-en-12β-ol (*20*) and the corresponding acetate (*21*) distinguished the C-13 and C-14 resonances. Thus, in accordance with the allyl acetate effect [19] the peak at δ 129.9 in the spectrum of the allylic alcohol (*20*), undergoing an upfield shift of 4 ppm on acetylation, was attributed to C-13, whereas the peak at 135.0, shifted downfield (2 ppm) in the spectrum of the acetate (*21*), must be due to C-14.

Similarly, the peak assignments for podocarp-13-en-12α-ol (*19*) were facilitated by a spectral comparison with the corresponding 12β-alcohol (*20*). Hence, four peaks in the spectrum of *19*, which had chemical shift values close to those found for the C-1, C-7, C-13, and C-14 resonances of the 12β-alcohol (*20*), were assigned on this basis. The remaining peak at 30.6 ppm in the spectrum of the 12α-alcohol (*19*) was then attributed to C-11.

The C-13 and C-14 resonances for podocarp-13-en-12-one (*22*), positioned at δ 128.1 and

155.9, respectively, were differentiated using the ^{13}C NMR data of cyclohex-2-enone as a guide line.[22] Thus, the chemical shift value of the C-13 resonance was almost identical to that of the C-2 resonance for cyclohexenone, while the C-14 resonance was 6.1 ppm downfield of that due to C-3 in the model compound, a result which is in harmony with the fact that C-14 is adjacent to a methine carbon.

Podocarp-8-ene (*23*). Comparison of the spectrum of podocarp-8-ene (*23*) with ^{13}C NMR data for pimara-8,15-diene [6] allowed assignment of all peaks except for a differentiation of the C-2 and C-6 signals, positioned at δ 19.0 and 19.1, and of the C-11, C-12 and C-13 signals, having chemical shift values of δ 23.0, 23.6 and 23.9.

DISCUSSION

Previous studies on ^{13}C NMR spectra of various alicyclic alcohols such as cyclohexanols,[23] *trans*-decalols,[24] and sterols [17,18] have established that a hydroxyl substituent deshields both the α- and β-carbons, whereas the effect on the γ-carbon is shielding. The α- and β-effects produced by an equatorial hydroxyl group are usually larger than those observed for an axial substituent. The γ-effect, on the other hand, is usually more pronounced for compounds incorporating an axial hydroxyl group as this orientation generally leads to a *gauche* arrangement of the substituent and the γ-carbon, while an equatorial orientation normally corresponds to an *anti* arrangement and generates less steric interaction with and less polarization of proximate hydrogen atoms. In the case of δ-effects this theoretical model seems to fail since the *syn*axial arrangement, which involves interactions between non-bonded atoms similar to those of the *γ-gauche* configurations, causes downfield shifts of 2.0 to 3.5 ppm.[24]

Our results for the podocarpan-11-, -12-, and -14-ols (*2*–*3*, *4*–*5*, *6*–*7*), summarized in Table 5, conform to these findings. Thus the α-carbon signals for podocarpan-12β-ol (*5*) and -14β-ol (*7*) are found 4.4 and 5.8 ppm downfield of the α-carbon signals displayed by their axial counterparts (*4*, *6*). The difference between the α-carbon chemical shifts for the two podocarpan-11-ols (*2*, *3*) is also of the usual magnitude (5.5 ppm) which is in agreement with the fact that the hydroxyl group in each isomer experiences roughly the same steric interaction. In this context it may be noted that the difference in chemical shift between the α-carbon signals for 11α- and 11β-hydroxyprogesterone,[17] where the hydroxyl group of the axial epimer is subjected to a further δ-interaction from the 19-methyl group, is much smaller (0.9 ppm). This is also the case when the hydroxyl group in the axial, but not in the equatorial isomer, is subjected to a δ-interaction as exemplified by the chemical shift differences for the two epimeric pairs 10β-methyl-5α-decal-2α- and -2β-ol (−0.7 ppm) and 10β-methyl-5α-decal-4α-and-4β-ol (−1.8 ppm.[24]

The β-effects are consistently larger for podocarpan-11α-ol (*2*), -12β-ol (*5*) and -14β-ol (*7*) than for the corresponding axial epimers (*3*, *4*, *6*). In agreement with earlier observations,[25] the methylene β-carbons are affected to a higher degree than the methine β-carbons, *e.g.* the C-12 signal undergoes a downfield shift of 11.3 ppm, while the C-9 signal is shifted 5.7 ppm downfield on introduction of an equatorial hydroxyl group at C-11 in podocarpane.

The shielding effects on the γ-carbon atoms are in all but one case considerably larger for the axial epimers (*3*, *4*, *6*) than for their equatorial counterparts (*2*, *5*, *7*; *i.e.* 6.7–8.1 ppm *versus* 0.4–3.1 ppm); the exception being the γ-(*peri*-) effects on C-7 produced by the axial and equatorial hydroxyl groups in the two podocarpan-14-ols (*6*–*7*). These *peri*-effects are of approximately the same magnitude for the two epimers, 5.3 (14α-OH) and 4.9 (14β-OH) ppm, a result which may be rationalized by the fact that the 1,3 non-bonded proton-hydroxyl interactions are almost the same in the two cases.

In contrast to these shielding effects, the hydroxyl substituents in the podocarpan-11-ols (*2*, *3*) cause deshielding on the quaternary C-10. Although the reason for this is not fully understood, it is worth mentioning that similar results have been obtained for the C-10 signals in the spectra of 11α- and 11β-hydroxyprogesterone [17] and 10β-methyl-5α-decal-2α- and 4α-ol.[24]

The δ-effects caused by introduction of a hydroxyl substituent at C-12 or C-14 in podo-

carpane are all small and directed towards higher fields. In contrast, introduction of an equatorial hydroxyl group at C-11 leads to a substantial deshielding of C-1 (2.6 ppm) and insertion of an axial hydroxyl group at C-11 moves the signal due to C-20, the methyl substituent at C-10, 2.4 ppm downfield. These downfield shifts are in agreement with recent findings for other compounds comprising *syn*axial hydroxyl-methyl interactions such as 11α- and 11β-hydroxyprogesterone [17] and 10β-methyl-5α-decal-2β-ol and -4β-ol.[24]

Since the general equivalence of hydroxyl and methyl groups has been recognised and *syn*axial methyl-methyl interactions are of common occurrence in terpenoids, it was of interest to estimate the influence of the C-19 methyl group on the shielding of the C-20 methyl group in podocarpane (*1*; 14.3 ppm). Starting from the chemical shift value of the methyl group in 10β-methyl-5α-decalin (δ 15.8) and accounting for the *γ-gauche* interaction from C-11 on introduction of ring C [26] gives a value of δ 11.8, which differs from that observed by 2.5 ppm. This differential value, taken as the δ *syn*axial effect from the C-19 methyl group, is in good agreement with the values found for corresponding hydroxyl group interactions and emphasizes the importance of the δ-effect on methyl groups in terpenoids.

Introduction of an equatorial or axial hydroxyl group at C-12 in podocarp-9(11)-ene, *14*, *15*, results in deshielding of the α- and β-carbon atoms (Table 5). In comparison with

Table 5. Substituent effects [a] of the hydroxyl group in the spectra of the podocarpanols *2*–*7* and the podocarp-9(11)-en-12-ols *14*–*15*.

Compound		α		β		γ		δ	
2	(eq.-OH)	C-11	46.8	C-9	5.7	C-8	−0.4	C-1	2.6
				C-12	11.3	C-10	2.1	C-5	−0.2
						C-13	−2.5	C-7	−0.2
								C-14	−0.6
								C-20	0.7
3	(ax.-OH)	C-11	41.3	C-9	2.6	C-8	−6.7	C-1	0.5
				C-12	8.3	C-10	0.6	C-5	0.2
						C-13	−7.3	C-7	0.1
								C-14	0.1
								C-20	2.4
4	(ax.-OH)	C-12	39.9	C-11	6.4	C-9	−7.8	C-8	−0.4
				C-13	5.9	C-14	−6.9	C-10	−0.5
5	(eq.-OH)	C-12	44.3	C-11	9.5	C-9	−2.4	C-8	−1.2
				C-13	8.8	C-14	−2.6	C-10	−0.2
6	(ax.-OH)	C-14	35.5	C-8	3.9	C-7	−5.3	C-6	−0.3
				C-13	7.2	C-9	−8.1	C-10	−0.2
						C-12	−7.1	C-11	−0.3
7	(eq.-OH)	C-14	41.3	C-8	7.3	C-7	−4.9	C-6	−0.7
				C-13	9.0	C-9	−2.4	C-10	−0.2
						C-12	−3.1	C-11	−0.7
14	(ax.-OH)	C-12	40.2	C-11	3.1	C-9	3.7	C-8	−0.1
				C-13	7.5	C-14	−5.4	C-10	0
15	(eq.-OH)	C-12	41.4	C-11	5.0	C-9	1.6	C-8	0
				C-13	8.9	C-14	−3.4	C-10	−0.3

[a] Values represent $\delta_C(ROH) - \delta_C(RH)$ for corresponding carbon signals in each case. A negative sign designates an upfield shift on substitution.

the situation for the saturated alcohols the shifts are somewhat less dependent on the orientation of the hydroxyl group and the magnitudes of the β-effects on the sp^2 carbon atoms, C-11, are only half of those found on the sp^3 carbon atoms, C-13. The γ-sp^3 carbons, C-14, suffer expected upfield shifts, whereas the γ-sp^2 carbons, C-9, carrying no hydrogens behave like quaternary carbons and undergo downfield shifts. In both cases the effects are more pronounced for podocarp-9(11)-en-12α-ol (*14*) than for the equatorial epimer (*15*).

The carbonyl carbon signals for the saturated oxo derivatives podocarpan-11-, 12-, and 14-one (*10* – *12*) appear at δ 211.8 – 213.7 (Table 1), while, as expected, the corresponding signals for the α,β-unsaturated oxo derivatives podocarp-9(11)-en-12-one (*18*) and podocarp-13-en-12-one (*22*) occur at somewhat higher fields, δ 200.4 and 201.1, respectively (Table 2).

The observed downfield shifts of the β-methylene (14.7 – 17.2 ppm) and β-methine carbon signals (11.1 – 12.9 ppm) for the podocarpanones *10* – *12* (Table 6) are consistent with the known deshielding effect of an oxo group on β-sp^3 carbon atoms and its dependence on the branching at this carbon.[16,25,27]

According to previous studies, γ-carbons are either shielded or deshielded by an oxo group. The effect is normally fairly small except for cases involving eclipsing of the carbonyl group and the γ-carbon or involving a bridgehead γ-carbon, when appreciable shifts are found.[16,25,27] The shifts observed for the γ-carbons in the podocarpanones *10* – *12* conform well to these results, and range within $\pm$1.3 ppm in all but two cases. Thus, the signal due to the bridgehead carbon C-8 of podocarpan-11-one (*10*) is shifted downfield by 3.2 ppm, and that due to the eclipsed carbon C-7 of podocarpan-14-one (*12*) is shifted upfield by 9.6 ppm. In contrast, C-10 in podocarpan-11-one (*10*), also eclipsed with the carbonyl group, is only slightly affected, which might be due to the fact that C-10 is quaternary and accordingly lacks a distortable carbon hydrogen bond.

The effects at the δ-carbon atoms produced by the oxo substituents in the podocarpanones *10* – *12* are found to be small and range within $\pm$1.2 ppm.

Introduction of an oxo group at C-12 in podocarp-9(11)-ene (*13*) causes deshielding of the β-sp^3, β-sp^2, and γ-sp^2 carbon atoms, whereas the γ-sp^3 carbon, C-14, undergoes an upfield shift. The effect on C-11 is of reduced magnitude compared with the influence of the oxo group on the β-carbon in the saturated analogues *10* – *12*. On the other hand, the downfield shift at the γ-sp^2 carbon, C-9, is substantial, observations which are readily rationalised in terms of the

Table 6. Effects[a] of the carbonyl group in the spectra of the podocarpanones *10* – *12* and podocarp-9(11)-en-12-one (*18*).

Compound	β		γ		δ	
10	C-9	11.1	C-8	3.2	C-1	−0.7
	C-12	17.2	C-10	−0.2	C-5	−0.6
			C-13	0.3	C-7	−0.1
					C-14	−1.0
					C-20	0.3
11	C-11	15.9	C-9	−0.2	C-8	−1.2
	C-13	14.7	C-14	−1.3	C-10	0.4
12	C-8	12.9	C-7	−9.6	C-6	−1.2
	C-13	15.4	C-9	1.0	C-10	0.7
			C-12	−0.6	C-11	−0.7
18	C-11	5.2	C-9	24.4	C-8	0.8
	C-13	13.6	C-14	−2.6	C-10	1.3

[a] Values represent $\delta_C(R{=}O) - \delta_C(RH_2)$ for corresponding carbon signals in each case. A negative sign designates an upfield shift on substitution.

electron withdrawing power of the carbonyl group.

Deuterium isotope effects. In agreement with previous results,[15–18] replacement of a hydrogen by a deuterium atom at various positions in the podocarpane derivatives (*24–43*, Table 1) was found to cause a drastic reduction of the intensity of the signal due to the labelled carbon atom. This reduction, which has been ascribed to a combination of quadrupolar broadening, spin-spin splitting and a decreased nuclear Overhauser enhancement,[18] made a direct measurement of the geminal isotope shift possible in only a few cases. Thus, the spectrum of 9α-d_1-podocarp-13-en12-one (*43*) displayed a triplet at δ 53.5 (J 19.5 Hz), shifted 0.5 ppm upfield of the C-9 signal for the corresponding non-labelled compound (*22*).

The resonances due to the carbon atoms adjacent to the labelled carbon atoms showed isotope shifts, whose magnitudes were proportional to the number of deuterium atoms. Thus, a single deuterium atom was found to cause an upfield shift of 0.1 ppm at a vicinal sp^3 carbon, whereas two deuterium atoms gave rise to a vicinal shift of 0.2 ppm. Very small isotope shifts, 0.05 ppm, were detected at carbons three bonds distant from the deuterium atom. Resonances due to carbonyl carbons and quarternary carbons proved to undergo a considerable reduction of intensity when the neighbouring carbon atom was labelled, an observation which conforms with previous results for labelled keto steroids.[16]

EXPERIMENTAL

The Fourier transform ^{13}C NMR spectra were obtained on a Varian XL-100-12 WG spectrometer operating at 25.16 MHz and equipped with an S-124 XL FT accessory. The instrument was controlled *via* a Varian 620 L 16 K computer.

The samples were examined as 0.1–0.5 M solutions in $CDCl_3$ using TMS as internal standard. To examine the influence of concentration on chemical shifts the ^{13}C NMR spectra of 1.0 and 0.1 M solutions of the podocarpan-11- and -12-ols (*2–3*, *4–5*) were recorded. All signals, except those due to the α- and β-carbon atoms of the two podocarpan-12-ols (*4–5*), which exhibited downfield shifts of 0.4 and 0.2 ppm, respectively, in the more dilute solutions, remained at virtually invariant positions. The observed dilution shifts are best interpreted by molecular association through hydrogen bonding. The proximity of the equatorial hydroxyl group to C-1 in podocarpan-11α-ol (*2*) and the 1,3-diaxial interaction between the hydroxyl group and the methyl group at C-10 in podocarpan-11β-ol (*3*) probably diminish the importance of the hydrogen bonding for these compounds thus explaining the absence of dilution shifts.

An RF flip-angle (45°, 21 μs) was usually used to achieve optimum sensitivity enhancements in survey spectra. Attempts were made to separate all peaks by the use of 8 K data points for 2.5 or 2.0 kHz spectrum widths. The precision of the peak positions in the proton noise decoupled (PND) spectra was ±0.03 for sp^3 and ±0.05 ppm for sp^2 carbon signals.

Single frequency off-resonance (SFOR) decoupled spectra were obtained by irradiation 300 Hz upfield from TMS in the proton spectrum. Partially relaxed Fourier transform (PRFT) spectra were obtained by the inversion-recovery method in which a pulse sequence of (180°-t-90°-T) was employed.

^1H NMR spectra were recorded at 100 MHz in $CDCl_3$ solutions. Melting points were determined on a Leitz Wetzlar instrument and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 spectrometer and low resolution mass spectra on an LKB 2091 instrument at 70 eV with an ion source temperature at 200 °C. The high resolution mass measurements were performed on an Atlas SM 1 instrument. Microanalyses were performed by A. Bernhardt, Elbach über Engelskirchen, West Germany.

Podocarpane (*1*). To a solution of 0.45 g of KOH in 5 ml of diethylene glycol was added 0.2 ml of hydrazine hydrate and 100 mg of podocarpan-12-one (*11*). The reaction mixture was refluxed under nitrogen for 2 h. Water was distilled off, and the reaction mixture was refluxed for an additional 3 h, now at a slightly higher temperature. Acidification, dilution with water, extraction with ether and chromatography over silica gel gave 45 mg of podocarpane (*1*) as a gum. (Found: C 87.31; H 12.75; M^+ 234. Calc for $C_{17}H_{30}$: C 87.10; H 12.90; mol. wt 234); ^1H NMR peaks at δ 0.82 (6 H, s) and 0.85 (3 H, s).

Podocarp-9(11)-en-12α-ol (*14*) *and podocarp-9(11)-en-12β-ol* (*15*). To a solution of 1.4 g of podocarp-9(11)-en-12-one (*18*) in 30 ml of methanol was added 1.0 g of $NaBH_4$. The reaction mixture was stirred at 0 °C for 0.5 h. Acidification, dilution with water and extraction with ether afforded a mixture of alcohols. Part of this mixture (0.4 g) was separated by chromatography over silica gel into 80 mg of podocarp-9(11)-en-12α-ol (*14*) as a gum (Found: M^+ 248. Calc for $C_{17}H_{28}O$: mol. wt. 248); IR bands at 3340 and 1655 cm^{-1}, ^1H NMR peaks at δ 0.87 (6 H, s), 1.03 (3 H, s), 4.17 (1 H, m, $W_{\frac{1}{2}} \sim 14$), and 5.49 (1 H, m, $W_{\frac{1}{2}} \sim 10$) and 230 mg of podocarp-9(11)-en-12β-ol (*15*), m.p. 105–107 °C. (Found: C 82.02; H 11.49; M^+

248.2136. Calc. for $C_{17}H_{28}O$: C 82.20; H 11.36; mol. wt. 248.2140); IR bands at 3330 and 1645 cm^{-1}; ^{1}H NMR peaks at δ 0.87 (6 H, s), 1.04 (3 H, s), 4.17 (1 H, m, $W_{\frac{1}{2}} \sim 14$) and 5.35 (1 H, m, $W_{\frac{1}{2}} \sim 12$).

Podocarp-9(11)-ene (13). A solution of 0.9 g of a mixture consisting of (*14*) and (*15*) in 10 ml of pyridine and 10 ml of acetic anhydride was kept at room temperature for 6 h. Work up in the usual manner gave a mixture of acetates. Without further purification this mixture and 0.7 g of lithium were added to 30 ml of ethylamine kept in a flask fitted with a dry ice-acetone condenser.[28] The reaction mixture was stirred for 1.5 h, then 60 ml of 2-methylbutan-2-ol was added. When all lithium had been consumed the ethyl amine was driven off with the aid of a stream of nitrogen. The residue was dissolved in water, acidified with ammonium chloride and 10 % acetic acid, and extracted with ether. Evaporation afforded a crude product which was purified by chromatography over silica gel to give 0.45 g of podocarp-9(11)-ene (*13*) as a gum (Found: C 87.88; H 12.12; M$^+$ 232. Calc. for $C_{17}H_{28}$: C 87.86; H 12.14; mol.wt. 232); IR band at 1645 cm^{-1}, ^{1}H NMR peaks at δ 0.84 (6 H, s), 1.03 (3 H, s) and 5.32 (1 H, m, $W_{\frac{1}{2}} \sim 9$).

Podocarpan-11-one (10). To a solution of 0.4 g of podocarp-9(11)-ene (*13*) in 10 ml of ether was added 1.8 g of BF_3-etherate and 0.38 g of LAH in 20 ml of ether. The reaction mixture was stirred for 1 h at room temperature. A saturated aqueous solution of Na_2SO_4 was then added followed by solid Na_2SO_4. The mixture was stirred for 1 h, filtered and evaporated. The residue was dissolved in 80 ml of ethanol containing 0.4 g of NaOH and stirred with 4 ml of 30 % H_2O_2 for 30 min.[28] Work up gave a gum, which was dissolved in 15 ml of acetone and stirred with 7 ml of Jones' reagent for 30 min. Work up in the usual manner afforded a mixture of podocarpan-9α-H-11-one (*10*) and podocarpan-9β-H-11-one. A solution of this mixture in 8 ml of methanol and 1 ml of 40 % NaOH was subsequently refluxed under nitrogen for 1 h. Acidification, dilution with water, extraction with ether and chromatography over silica gel furnished 0.12 g of podocarpan-9α-H-11-one (*10*), m.p. 48 – 51 °C. (Found: M$^+$ 248.2132. Calc. for $C_{17}H_{28}O$; mol. wt. 248.2140); IR band at 1710 cm^{-1}, ^{1}H NMR peaks at δ 0.86 (6 H, s) and 1.11 (3 H, s).

Podocarpan-11α-ol (2) and podocarpan-11β-ol (3). To a solution of 90 mg of podocarpan-11-one (*10*) in 10 ml of tetrahydrofuran and 0.8 ml of 5 % NaOH was added 190 mg of $NaBH_4$. The reaction mixture was refluxed for 17 h. Acidification, dilution with water and extraction with ether afforded a mixture, which was separated by chromatography over silica gel into 41 mg of podocarpan-11β-ol (*3*) as a gum. (Found: M$^+$ 250. Calc. for $C_{17}H_{30}O$: mol. wt. 250). IR band at 3450 cm^{-1}, ^{1}H NMR peaks at δ 0.85 (6 H, s), 1.11 (3 H, s) and 4.26 (1 H, m, $W_{\frac{1}{2}} \sim 10$); and 33 mg of podocarpan-11α-ol (*2*); m.p. 86 – 88 °C. (Found: M$^+$ 250. Calc. for $C_{17}H_{30}O$: mol. wt. 250); IR band at 3380 cm^{-1}, ^{1}H NMR peaks at δ 0.83 (6 H, s), 0.97 (3 H, s) and 3.61 (1 H, m, $W_{\frac{1}{2}} \sim 22$).

Podocarpan-12α-yl acetate (8), podocarpan-12β-yl acetate (9) podocarp-9(11)-en-12α-yl acetate (16), podocarp-9(11)-en-12β-yl acetate (17) and podocarp-13-en-12β-yl acetate (21). A solution of 30 mg of podocarpan-12α-ol (*4*) in 5 ml of pyridine and 5 ml of acetic anhydride was refluxed for 5 h. Work up in the usual manner gave 32 mg of podocarpan-12α-yl acetate (*8*), m.p. 62 – 65 °C. (Found: M$^+$ 292. Calc. for $C_{19}H_{32}O_2$: mol. wt. 292); IR bands at 1740, 1240, and 1255 cm^{-1}, ^{1}H NMR peaks at δ 0.81 (3 H, s), 0.84 (3 H, s), 0.86 (3 H, s), 2.05 (3 H, s) and 5.12 (1 H, m, $W_{\frac{1}{2}} \sim 7$).

Podocarpan-12β-yl acetate (*9*) m.p. 104 – 107 °C. (Found: M$^+$ 292. Calc. for $C_{19}H_{32}O_2$; mol. wt. 292), podocarp-9(11)-en12α-yl acetate (*16*) gum. (Found: M$^+$ 290. Calc. for $C_{19}H_{30}O_2$: mol. wt. 290), podocarp-9(11)-en-12β-yl acetate (*17*) gum (Found: M$^+$ 290. Calc. for $C_{19}H_{30}O_2$; mol. wt. 290) and podocarp-13-en-12β-yl acetate (*21*), m.p. 139 – 140 °C. (Found: M$^+$ 290. Calc. for $C_{19}H_{30}O_2$; mol. wt. 290) were prepared by treatment of podocarpan-12β-ol (*5*), podocarp-9(11)-en-12α-ol (*14*), podocarp-9(11)-en-12β-ol (*15*), and podocarp-13-en-12β-ol (*20*), respectively, with a mixture of pyridine and acetic anhydride (1:1) at 0 °C for 12 h. *9* had IR bands at 1740 and 1250 cm^{-1}, ^{1}H NMR peaks at δ 0.85 (9 H, s), 2.02 (3 H, s) and 4.66 (1 H, m, $W_{\frac{1}{2}} \sim 21$). *16* had IR bands at 1730 and 1645 cm^{-1}, ^{1}H NMR peaks at δ 0.86 (6 H, s), 1.03 (3 H, s), 2.04 (3 H, s), 5.14 (1 H, m, $W_{\frac{1}{2}} \sim 9$) and 5.21 (1 H, dd, *J* 1.5 and 4.5). *17* had IR bands at 1745 and 1650 cm^{-1}, ^{1}H NMR peaks at δ 0.85 (6 H, s), 1.05 (3 H, s), 2.03 (3 H, s), and 5.27 (2 H, m). *21* had IR bands at 1735 and 1650 cm^{-1}, ^{1}H NMR peaks at δ 0.84 (3 H, s), 0.86 (6 H, s), 2.05 (3 H, s), and 5.5 (3, H, m).

Podocarp-8-ene (23). Reduction of 12-methoxypodocarpa-8,11,13-triene using lithium in ammonia[29] afforded as a minor component podocarp-8-ene *(23)* m.p. 24 – 26 °C. (Found: C 87.95; H 12.10; M$^+$ 232; Calc. for $C_{17}H_{28}$: C 87.86; H 12.14; mol. wt. 232); ^{1}H NMR peaks at δ 0.93 (3 H, s), 0.95 (3 H, s) and 0.98 (3 H, s).

8β,13,13,14,14-d_5*-Podocarpane (24).* To a solution of 20 mg of podocarpan-14-one (*12*) in 0.5 ml deuterioacetic acid was added dry amalgamated zinc filings and a mixture obtained by treating 1 ml of acetyl chloride with 0.5 ml of deuterium oxide. The reaction mixture was refluxed for 4 h. Work up and chromatography over silica gel afforded 5 mg of *8β*,13,13,-14,14-d_5-podocarpane (*24*), whose retention time on GLC was identical to that of the corresponding nondeuterated derivative and whose ^{1}H NMR spectrum displayed the three methyl resonances at unaltered positions.

Isotopic composition according to MS: 7 % d_3, 29 % d_4, 62 % d_5, and 2 % d_6.

The syntheses of the other compounds examined here have been or will be described elsewhere.[29,30]

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The Crystal Structure of a 1,4-Etheno-2,8-diacetoxy-2,4,6,8-tetramethyloctahydronaphthal-5-ene-3,7-dione

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The Diels-Alder product 1,4-etheno-2,8-diacetoxy-2,4,6,8-tetramethyloctahydronaphthal-5-ene-3,7-dione is a dimer of 6-acetoxy-2,6-dimethyl-2,4-cyclohexadienone which is formed upon lead tetraacetate oxidation of 2,6-dimethylphenol. A determination of the crystal structure of the dimer shows that it has *endo* configuration and that two enantiomeric molecules have dimerised. The structure is rather strained with $C(sp^3)-C(sp^3)$ bond lengths of 1.591 and 1.553 Å for the bonds C(4) – C(4a) and C(4a) – C(8a), respectively.

The structure was determined by direct methods from counter intensities. The crystals are monoclinic, space group $P2_1/c$, with $a=11.442$, $b=10.841$, $c=14.718$ Å, $\beta=95.11°$ and $Z=4$. The final R value was 0.045 for 2454 reflexions.

Oxidation of methyl homologues of *o*-cresol with sodium periodate results in the formation of *o*-quinols which generally undergo rapid Diels-Alder dimerisation to give substituted 1,4-ethenonaphthalenes.[1,2] Oxidation with lead tetraacetate gives *o*-quinol acetates which similarly dimerise at 120 °C.[3]

Oxidation of 2,6-dimethylphenol with lead tetraacetate gives as the main product 6-acetoxy-2,6-dimethyl-2,4-cyclohexadienone. Dimeric *o*-quinol acetates are formed at 120 °C and 165 °C, respectively. The title compound is the stereoisomer formed at 165 °C.[4]

This X-ray investigation, within a program of crystal-structure studies of Diels-Alder adducts of *o*-quinols, has been undertaken primarily to determine whether the configuration of the dimer is *endo* or *exo* and to establish the steric arrangement at the asymmetric carbon atoms.

EXPERIMENTAL

Cell dimensions were obtained from a least-squares treatment of the angular positions of 25 accurately centered reflexions as measured on the computer-controlled Philips PW 1100 diffractometer. Crystal data are: space group $P2_1/c$, $a=11.442(1)$, $b=10.841(2)$, $c=14.718(2)$ Å, $\beta=95.11(2)°$ $V=1818.4$ Å^3, $Z=4$, $D_c=1.316$ g cm^{-3}.

Intensity data were collected to a 2θ value of 130° by the $\omega-2\theta$ scan technique with graphite monochromatized Cu$K\alpha$ radiation. The intensities of 3102 independent reflexions were measured. Backgrounds were estimated by stationary counting at $2\theta \pm 0.75°$ from the peak maxima. The scan speed was 0.015° s^{-1}. Three standard reflexions were measured every 90 min. No systematic variation was detected. All reflexions with net intensity $I<4\sigma(I)$, where $\sigma(I)$ is standard deviation estimated from counter statistics, were ignored. The remaining 2454 independent reflexions were used in the refinement of the structure. Lorentz and polarization factors were applied, but not absorption corrections.

STRUCTURE DETERMINATION AND REFINEMENT

The structure factor phases were determined by direct methods. Normalized $|E|$'s were calculated from structure amplitudes on approximately absolute scale and from the Wilson[5] average temperature factor. Phase determination by "variance-weighted" $\sum_2$-relationships[6] was restricted to the 245 reflexions with $|E|>1.75$. Eight trial phase sets were generated from starting sets consisting of three origin reflexions and three reflexions as variables. An E map computed from 204 well-phased

Table 1. Positional and anisotropic thermal parameters of the non-hydrogen atoms. The β-values refer to the temperature factor expression $\exp[-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+hk\beta_{12}+hl\beta_{13}+kl\beta_{23})]$. Estimated standard deviations are given in parentheses. Values are $\times 10^4$.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	1476(2)	3293(2)	2752(2)	49(2)	56(2)	45(1)	−4(3)	20(2)	−7(2)
C(2)	831(2)	4538(2)	2580(2)	51(2)	63(2)	44(1)	7(3)	24(2)	5(2)
C(3)	1151(2)	5346(2)	3424(2)	70(2)	61(2)	43(1)	25(3)	40(2)	4(2)
C(4)	1957(2)	4725(2)	4171(2)	76(2)	63(2)	38(1)	26(3)	37(2)	−3(2)
C(4a)	3127(2)	4328(2)	3740(2)	61(2)	51(2)	36(1)	−7(3)	22(2)	−7(2)
C(5)	3906(2)	3674(2)	4462(2)	66(2)	82(2)	35(1)	3(3)	10(2)	−17(3)
C(6)	4140(2)	2470(2)	4479(2)	68(2)	84(2)	33(1)	25(3)	21(2)	5(2)
C(7)	3656(2)	1718(2)	3696(2)	57(2)	57(2)	42(1)	19(3)	34(2)	8(2)
C(8)	3580(2)	2410(2)	2785(2)	47(2)	55(2)	35(1)	−1(3)	22(2)	−5(2)
C(8a)	2809(2)	3571(2)	2857(2)	49(2)	50(2)	33(1)	−1(3)	21(2)	0(2)
C(9)	1095(2)	2815(2)	3644(2)	63(2)	59(2)	62(2)	0(3)	50(3)	24(3)
C(10)	1321(2)	3541(2)	4354(2)	80(2)	76(2)	48(2)	32(3)	61(3)	27(3)
C(11)	−491(2)	4389(3)	2391(2)	51(2)	99(3)	69(2)	15(4)	24(3)	16(4)
O(12)	1286(1)	5286(2)	1863(1)	61(1)	66(2)	40(1)	4(2)	14(2)	5(2)
C(13)	1295(2)	4846(2)	1005(2)	60(2)	89(2)	40(1)	13(3)	4(2)	−12(3)
O(14)	968(2)	3835(2)	778(2)	141(2)	107(2)	55(1)	−33(3)	18(3)	−39(2)
C(15)	1763(3)	5788(3)	399(2)	93(3)	125(3)	42(2)	−8(5)	22(3)	10(3)
O(16)	764(2)	6370(2)	3488(2)	149(2)	81(2)	60(1)	103(3)	13(3)	−10(2)
C(17)	2181(3)	5568(3)	4996(2)	131(3)	87(3)	40(1)	60(5)	27(3)	−22(3)
C(18)	4839(3)	1818(3)	5248(2)	111(3)	136(4)	40(2)	97(5)	13(3)	18(4)
O(19)	3301(2)	672(2)	3763(1)	100(2)	66(2)	61(1)	−1(3)	54(2)	21(2)
C(20)	3202(2)	1592(2)	1980(2)	63(2)	71(2)	42(1)	−3(3)	23(2)	−25(3)
O(21)	4722(1)	2966(1)	2644(1)	50(1)	64(2)	38(1)	1(2)	24(2)	1(2)
C(22)	5689(2)	2261(2)	2766(2)	53(2)	66(2)	40(1)	14(3)	16(2)	−10(2)
O(23)	5681(2)	1209(2)	3020(2)	67(2)	79(2)	89(2)	29(2)	35(2)	22(2)
C(24)	6750(2)	2955(3)	2558(2)	52(2)	94(3)	65(2)	0(4)	18(3)	9(3)

Table 2. Positional parameters ($\times 10^3$) of the hydrogen atoms. Estimated standard deviations are given in parentheses.

Atom	x	y	z	B Å^2
H(1)	127(2)	276(2)	228(2)	2827
H(4a)	353(2)	504(2)	357(2)	3025
H(5)	426(2)	422(2)	498(2)	3299
H(8a)	301(2)	408(2)	234(2)	2478
H(9)	69(2)	209(2)	365(2)	3457
H(10)	109(2)	337(2)	494(2)	3711
H1(11)	−67(2)	388(3)	185(2)	4236
H2(11)	−74(3)	403(3)	291(2)	4236
H3(11)	−85(3)	516(3)	226(2)	4236
H1(15)	200(3)	544(3)	−9(2)	4689
H2(15)	242(3)	618(3)	66(2)	4689
H3(15)	121(3)	636(3)	29(2)	4689
H1(17)	267(3)	627(3)	483(2)	4525
H2(17)	147(3)	591(3)	521(2)	4525
H3(17)	266(3)	518(3)	550(2)	4525
H1(18)	516(3)	246(3)	567(2)	4998
H2(18)	544(3)	139(3)	504(2)	4998
H3(18)	434(3)	130(3)	555(2)	4998
H1(20)	307(2)	206(3)	148(2)	3466
H2(20)	249(2)	119(2)	207(2)	3466
H3(20)	379(2)	100(2)	190(2)	3466
H1(24)	703(2)	334(3)	309(2)	3926
H2(24)	659(3)	349(3)	218(2)	3926
H3(24)	737(3)	247(3)	249(2)	3926

reflexions displayed all the non-hydrogen atoms as the prominent peaks. The structure was refined by the full-matrix least-squares method. When the R value reached 0.084 with anisotropic temperature factors, a difference synthesis was calculated. This revealed all the hydrogen atoms. The hydrogen atoms were given the final isotropic thermal parameters of the atoms to which they are attached and only the positional parameters were included in further refinement. The R value reduced to 0.045. Scattering factors for C and O were those of Freeman [7] and for H, those of Stewart, Davidson and Simpson.[8] Hughes [9] weighting procedure with $F_{o,min}=3.0$ was applied. The final positional and thermal parameters are given in Tables 1 and 2. A list of the observed and calculated structure factors is available from the authors on request.

RESULTS AND DISCUSSION

A view of the crystal structure in Fig. 1 shows that the dimerisation has followed the

Table 3. Bond angles (°) involving non-hydrogen atoms, with estimated standard deviations in parentheses.

C(2) – C(1) – C(8a)	107.3(2)
C(2) – C(1) – C(9)	105.7(2)
C(8a) – C(1) – C(9)	109.8(2)
C(1) – C(2) – C(3)	106.8(2)
C(1) – C(2) – C(11)	112.9(2)
C(1) – C(2) – O(12)	114.0(2)
C(3) – C(2) – C(11)	111.7(2)
C(3) – C(2) – O(12)	101.1(2)
C(11) – C(2) – O(12)	109.8(2)
C(2) – C(3) – C(4)	114.6(2)
C(2) – C(3) – O(16)	121.8(2)
C(4) – C(3) – O(16)	123.6(2)
C(3) – C(4) – C(4a)	107.9(2)
C(3) – C(4) – C(10)	103.4(2)
C(3) – C(4) – C(17)	111.0(2)
C(4a) – C(4) – C(10)	106.1(2)
C(4a) – C(4) – C(17)	113.1(2)
C(10) – C(4) – C(17)	114.7(2)
C(4) – C(4a) – C(5)	108.2(2)
C(4) – C(4a) – C(8a)	109.5(2)
C(5) – C(4a) – C(8a)	115.0(2)
C(4a) – C(5) – C(6)	125.5(2)
C(5) – C(6) – C(7)	117.7(2)
C(5) – C(6) – C(18)	124.5(3)
C(7) – C(6) – C(18)	117.7(2)
C(6) – C(7) – C(8)	113.6(2)
C(6) – C(7) – O(19)	123.9(2)
C(8) – C(7) – O(19)	122.4(2)
C(7) – C(8) – C(8a)	109.1(2)
C(7) – C(8) – C(20)	112.8(2)
C(7) – C(8) – O(21)	110.0(2)
C(8a) – C(8) – C(20)	114.0(2)
C(8a) – C(8) – O(21)	101.3(2)
C(20) – C(8) – O(21)	109.0(2)
C(1) – C(8a) – C(4a)	110.0(2)
C(1) – C(8a) – C(8)	113.4(2)
C(4a) – C(8a) – C(8)	113.3(2)
C(1) – C(9) – C(10)	115.6(2)
C(4) – C(10) – C(9)	115.5(2)
C(2) – O(12) – C(13)	120.8(2)
O(12) – C(13) – O(14)	123.7(2)
O(12) – C(13) – C(15)	110.6(2)
O(14) – C(13) – C(15)	125.7(3)
C(8) – O(21) – C(22)	118.7(2)
O(21) – C(22) – O(23)	123.5(2)
O(21) – C(22) – C(24)	111.4(2)
O(23) – C(22) – C(24)	125.1(2)

endo addition rule. The configuration at carbon atom C(8) is different from that found in X-ray investigations of related compounds.[10–12] This is probably due to steric requirements since the rather bulky acetoxy group has replaced a hydroxyl group [10,11] or a chlorine.[12] Here again, the bulkiest group on carbon atom C(8) is oriented away from the reaction center.

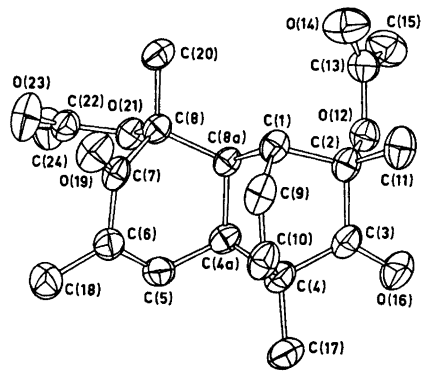


Fig. 1. A perspective view of the molecule.

Intramolecular bond distances are given in Fig. 2 and bond angles involving non-hydrogen atoms in Table 3. No corrections for thermal motion have been applied. Most bond lengths in this structure agree with those observed in similar structure;[10–12] the bond C(4) – C(4a) (1.591 Å) is again significantly longer than the mean value, 1.536 Å, of the other C(sp^3) – C(sp^3) bonds in the structure.

The four atoms C(4a), C(5), C(6), and C(7) in the cyclohexene ring are coplanar within 0.017 Å. Atoms C(8) and C(8a) deviate 0.832 and 0.260 Å from the plane, respectively. The three rings containing the etheno moiety are all boat-shaped.

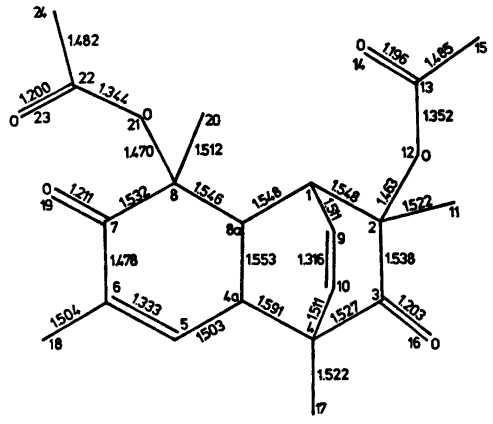


Fig. 2. Bond distances in the molecule. Standard deviations are estimated to be 0.003 Å.

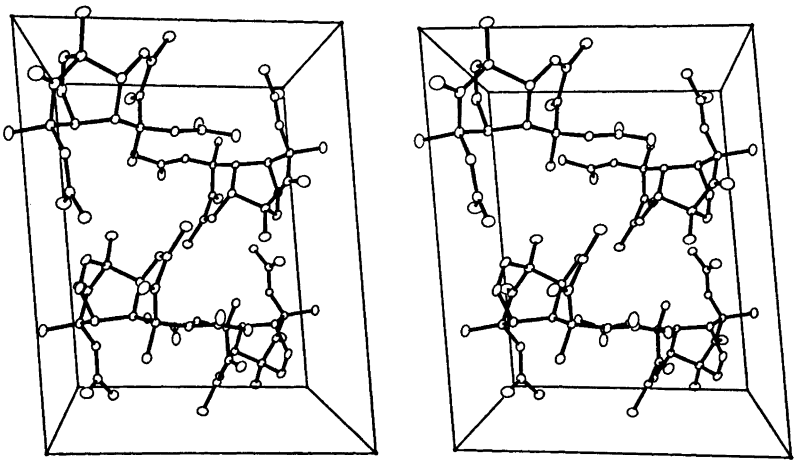


Fig. 3. A stereoscopic view of the molecular packing.

The arrangement of molecules in the unit cell is shown in Fig. 3. All intermolecular contacts are normal van der Waals distances.

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Reactions of 1,2-Dinitrophenylethanols. Intramolecular Participation by the Nitro Group

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The reaction of 1,2-di(*o*-nitrophenyl)ethanol (*1*) under acidic conditions gave *o,o'*-dinitrostilbene (7 %), 2-(*o*-nitrophenyl)isatogen (*2*) (8 %), and 3-(*o*-nitrobenzoyl)anthranil (*3*) (6 %). 1-(*o*-Nitrophenyl)-2-(*p*-nitrophenyl)ethanol under the same conditions gave *o,p'*-dinitrostilbene (2 %), 2-(*p*-nitrophenyl)isatogen (21 %) and 3-(*p*-nitrobenzoyl)anthranil (7 %). 2-(*o*-Nitrophenyl)-1-(*p*-nitrophenyl)ethanol gave its tosylate (trace) and *o,p'*-dinitrostilbene. *o*-Nitrotolan provided 3-benzoylanthranil (27 %) and 2-phenylisatogen (16 %) as products.

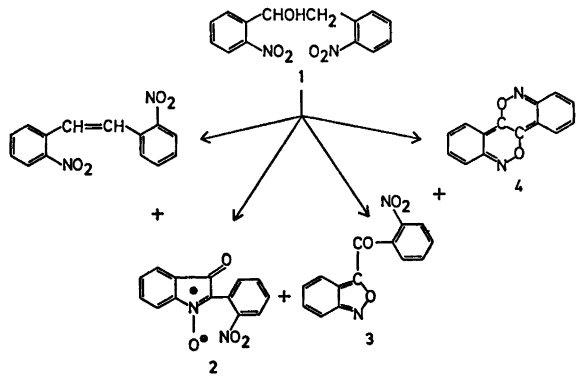
Some time ago, we reported the synthesis of 1,2-di(*o*-nitrophenyl)ethanol (*1*) from *o*-nitrotoluene.[1] In order to verify the structure of *1*, dehydration with *p*-toluenesulfonic acid was attempted. However, a rather complex reaction mixture was obtained, which contained only traces of *o,o'*-dinitrostilbene.

We recently reported the reactions of *o*-nitrobenzyl alcohol [2] and of 1-(*o*-nitrophenyl)-2-phenylethanol [3] under acidic conditions. The relationship between one of the nitro groups and the hydroxyl group in 1,2-di(*o*-nitrophenyl)-ethanol (*1*) is the same as in the former two substances, and *1* might be expected to react analogously. The dehydration of *1* was therefore reinvestigated and the results obtained, together with those for some related subtances, are reported here.

The reaction of 1,2-di(*o*-nitrophenyl)ethanol was slow, and gave four major products, in addition to low yields of a number of unidentified substances.

The structure of one of the isolated substances, ($C_{14}H_8N_2O_2$ by M.S.), (yield 5 %), has not yet been conclusively demonstrated. The spectral data are in accord with the compound *4*, but not with the isomeric 6*H*,12*H*-indazolo[2,1-*a*]indazole-6,12-dione. Non-identity with the latter compound was conclusively demonstrated by direct comparison of the present product with an authentic sample.[4]

The other isolated compounds were 2-(*o*-nitrophenyl)isatogen (*2*) (8 %), 3-(*o*-nitro-



benzoyl)anthranil (*3*) (6 %), and *o,o'*-dinitrostilbene (7 %). Compounds *2* and *3* were not interconverted on treatment with *p*-toluenesulfonic acid and were recovered unchanged.

The ratio of the yields of *2* to *3* probably reflects the influence of the nitro group in the 2-aryl ring of 1,2-di(*o*-nitrophenyl)ethanol. When 1-(*o*-nitrophenyl)-2-phenylethanol was reacted under the same conditions, only traces of 2-phenylisatogen were found.[3] A mechanism was discussed for that reaction where the enol form of the postulated intermediate *o*-nitrosophenyl benzyl ketone provided precursors for 2-phenylisatogen and 3-benzoylanthranil. It was suggested that the anthranil might have been formed by attack of the hydroxyl group of the enol compound on the nitroso nitrogen, and the isatogen by addition of the π-electrons to the nitroso group. According to that mechanism, the introduction of a nitro group into the 2-phenyl ring could be reflected in the ratio of the yield of isatogen to anthranil. The nitro group would increase the electron density around the carbon atom 2 in the chain, and also lower the electron density at the hydroxyl oxygen of the enol. Both these effects would increase the ratio of the yields of the isatogen to the anthranil, as observed in the present reaction of *1*

To test this point further, and to confirm that only the nitro group of the 1-aryl ring of *1* directly participated in the reaction, two other compounds, 1-(*o*-nitrophenyl)-2-(*p*-nitrophenyl)ethanol and 2-(*o*-nitrophenyl)-1-(*p*-nitrophenyl)ethanol were subjected to the same reaction conditions.

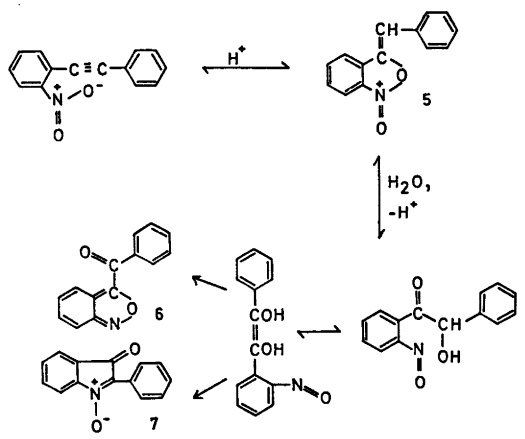
The reaction of 1-(*o*-nitrophenyl)-2-(*p*-nitrophenyl)ethanol under acidic conditions proved to be analogous to that of 1,2-di(*o*-nitrophenyl)-ethanol: both 2-(*p*-nitrophenyl)isatogen (21 %) and 3-(*p*-nitrobenzoyl)anthranil (7 %) were formed. The ratio of isatogen to anthranil was higher than for the reaction of *1*, in agreement with the known greater influence of a *para* as compared with an *ortho* nitro group. Further, this showed that both 2-(*o*-nitrophenyl)isatogen (*2*) and 3-(*o*-nitrobenzoyl)anthranil (*3*) were formed by reactions between the hydroxyl group and the nitro group of the 1-aryl ring, and that the nitro group of the 2-aryl ring was not directly involved in the reaction.

From these results, and those reported earlier,[3] 2-(*o*-nitrophenyl)-1-(*p*-nitrophenyl)ethanol was expected to yield *o,p'*-dinitrostilbene on reaction with *p*-toluenesulfonic acid. This indeed proved to be the case. Further, a low yield of 2-(*o*-nitrophenyl)-1-(*p*-nitrophenyl)ethyl *p*-toluenesulfonate was isolated, but neither 2-(*p*-nitrophenyl)isatogen nor 3-(*p*-nitrobenzoyl)-anthranil were produced. This is further evidence that only the nitro group of the 1-aryl ring of 1,2-dinitrophenylethanol (*1*) participated in the reaction of *1*, just as indicated by the reaction of 1-(*o*-nitrophenyl)-2-(*p*-nitrophenyl)ethanol.

In an attempt to obtain information about the mechanism discussed above, especially at which stage the oxidation took place, *o*-nitrotolan was treated with *p*-toluenesulfonic acid. Acetylenic bonds are susceptible to nucleophilic attack, a capacity increased by electronegative substituents.[6] In *o*-nitrotolan this increase, combined with the proximity of the nitro group, would give opportunities for intramolecular reactions similar to those discussed above. Reactions of nitro groups with acetylenic bonds, both under acidic conditions[7,8] and by photochemical stimulation,[9–11] have been reported.

The main products from the present reaction of *o*-nitrotolan were 3-benzoylanthranil (*6*) (27 %) and 2-phenylisatogen (*7*) (16 %). The reactions of *o*-nitrotolan have previously been reported to yield 2-phenylisatogen,[7–11] and 3-benzoylanthranil was not then mentioned even as a by-product. This difference may stem from variation in the reaction conditions. Bayer[7,8] ran his reactions in aqueous sulfuric acid, and the photochemical reactions were mainly carried out in nucleophilic solvents, *e.g.* pyridine.[9,11] Due to the electrophilic nature of the acetylenic bond, the reactive species in these reactions may have been a complex between the solvent and *o*-nitrotolan. The addition of amines to acetylenes is well known.[6,12]

The formation of *6* and *7* may be explained by a mechanism similar to the one discussed above. An attack of the nitro group oxygen atoms on the acetylenic bond, together with proton addition would give the intermediate *5*. From this, the anthranil *6* and the isatogen *7* could be obtained, *e.g.* by the reactions depicted below.



The resemblance in the product composition of the reactions of 1-(*o*-nitrophenyl)-2-phenylethanol [3] and of *o*-nitrotolan may indicate a common intermediate. In the reaction of *o*-nitrotolan, no oxidation step is necessary to obtain the anthranil (*6*) and isatogen (*7*). The results from this reaction may thus indicate the oxidation steps in the reactions of 2-aryl-1-(*o*-nitrophenyl)ethanols to take place earlier than discussed in Ref. 3, and before the ring closure steps. Nevertheless, the higher ratio of isatogen (*7*) to anthranil (*6*) in the reaction of *o*-nitrotolan than in the case of 1-(*o*-nitrophenyl)-2-phenylethanol also indicates the reactions not to proceed entirely by common intermediates.

It seems unlikely that *o*-nitrotolan itself was an intermediate in the reaction of 1-(*o*-nitrophenyl)-2-phenylethanol, especially as both *o*-nitrostilbene and benzyl *o*-nitrophenyl ketone were stable under the reaction conditions.[3] Neither did *o*-nitrobenzyl *o*-nitrophenyl ketone react under these conditions.

The results of the present study and of previous work [2,3] indicate alchohols of the *o*-nitrobenzyl-type to react *via* analogous intermediates, originating from intramolecular expulsion of the hydroxyl group. The reaction of *o*-nitrotolan seems to proceed *via* similar intermediates.

EXPERIMENTAL

The general instrumentation used in the experiments has been described earlier.[3]

Reactions of 1,2-di(o-nitrophenyl)ethanol (1). (*a*) *In* p-*xylene.* 1,2-Di(*o*-nitrophenyl)ethanol (2 g), *p*-toluene sulfonic acid (4 g) and *p*-xylene (200 ml) were refluxed for 2.5 h. The solution, after neutralisation, gave 1.2 g of a mixture which yielded 63 mg of a crystalline substance (m.p. 215–218 °C) from ethyl acetate solution. Recrystallisation gave 10 mg with m.p. 220–221 °C, IR (KBr): 1630, 1515, 1450, 1370, 1165, 1155, 1145 cm^{-1}. NMR: δ 8.30 (1 H), 8.15 (1 H), 7.85–7.0 (6 H, m). Mass spectrum: *m/e* 336 (100 %, $C_{14}H_8N_2O_2$), 208 (13 %, $C_{13}H_8NO$), 179 (64 %, $C_{12}H_7N_2$). UV: (96 % EtOH) λ_{max} 330 nm (ε_{max} = 686 m^2 mol^{-1}), 229 (1275). A tentative structure for the crystalline compound is *4*.

(*b*) *In toluene.* The analogous reaction in toluene gave three products in addition to *4* (5 %), *o,o'*-dinitrostilbene (7 %, identified by IR and NMR); 3-(*o*-nitrobenzoyl)anthranil (6 %) m.p. 230–236 °C. IR (KBr): 3100, 1670, 1630, 1560, 1530, 1450, 1445, 1360, 1300, 1230, 940 cm^{-1}. NMR: δ 7.25–8.4 m. Mass spectrum: *m/e* 268 (10 %, $C_{14}H_8N_2O_4$), 150 (15 %, $C_7H_4NO_3$), 134 (100 %, $C_7H_4NO_2$); 2-(*o*-nitrophenyl) isatogen (8 %), m.p. 201–203 °C (lit.[9] 202–203 °C). IR (KBr): 3080, 1710, 1610, 1570, 1520, 1470, 1385, 1350, 1290, 1190, 1180 cm^{-1}. NMR: δ 8.05–8.3 (1 H, m), δ 7.5–8.0 (7 H, m). Mass spectrum: *m/e* 268 (4 %, $C_{14}H_8N_2O_4$), 134 (100 %, $C_7H_4NO_2$). The experiment was repeated several times with essentially the same result.

(*c*) *In benzene.* The reaction was run analogously with the preceding one, and gave similar results, except that only 54 % of the 1,2-di-(*o*-nitrophenyl)ethanol had reacted.

Several attempts were made to improve the yields. The reaction was run with the addition of water or with a water eliminator attached, without any noticeable difference. Further, the reaction was run in the exclusion of air (five freeze-thaw cycles under vacuum) or with air bubbled through the reaction mixture without affecting the result. The addition of nitrobenzene as an oxidant (24 mol nitrobenzene per mol of *1*) produced no noticeable effect.

Stability of 2-(o-nitrophenyl)isatogen (2) and 3-(o-nitrobenzoyl)anthranil (3). Both these compounds were stable to the reaction conditions applied to 1,2-di(*o*-nitrophenyl)ethanol (see above).

3-(o-Nitrobenzyl)anthranil (3). 2-(*o*-Nitrophenyl)isatogen (*2*) (75 mg) was dissolved in ethanol (15 ml) and conc. sulfuric acid (0.8 ml) added.[5] After reflux for 20 h, the reaction mixture was neutralised with sodium bicarbonate, filtered, and evaporated. 50 mg of a substance identical to 3-(*o*-nitrobenzoyl)anthranil from the reaction of *1* was isolated.

1-(o-Nitrophenyl)-2-(p-nitrophenyl)ethanol. *p*-Nitrotoluene (16 g), *o*-nitrobenzaldehyde (18 g), dimethylsulfoxide (100 ml), and KOH (0.2 g) were stirred overnight at 22 °C. After work-up and removal of excess reactants by vacuum distillation, the residue was chromatographed on a silica gel column. Recrystal-

lisation gave 3.2 g of 1-(*o*-nitrophenyl)-2-(*p*-nitrophenyl)ethanol, m.p. 145–145.5 °C. IR (KBr): 3510, 3110, 2940, 1610, 1600, 1520, 1350, 1110, 1040 cm^{-1}. NMR (acetone-d_6): δ 7.4–8.3 (8 H, m, signals due to AA'XX' from *p*-nitrophenyl could be recognized), 5.35–5.75 (1 H, X part of ABX), 4.75 (1 H, *OH*, disappeared on addition of D_2O), 2.9–3.3 (2 H, AB part of ABX). Mass spectrum: *m/e* 152 (23 %), 137 (100 %) 120 (8 %), 104 (19 %).

2-(o-Nitrophenyl)-1-(p-nitrophenyl)ethanol. *o*-Nitrotoluene (40 g), *p*-nitrobenzaldehyde (12 g), dimethylsulfoxide (100 ml) and KOH (2 g) were stirred at 22 °C overnight. After work-up, 2-(*o*-nitrophenyl)-1-(*p*-nitrophenyl)ethanol (1.1g) was obtained, m.p. 118–121 °C. IR (KBr): 3560, 3190, 2950, 1610, 1520, 1350, 1060 cm^{-1}. NMR ($CDCl_3$): δ7.1–8.4 (8 H, m), 5.0–5.4 (1 H), 3.1–3.5 (2 H), 2.7–3.0 (1 H, disappeared on addition of D_2O). Mass spectrum: *m/e* 152 (9 %), 137 (78 %), 120 (100 %), 107 (12 %), 92 (31 %).

Reaction of 1-(o-nitrophenyl)-2-(p-nitrophenyl)ethanol. *p*-Toluenesulfonic acid (3.0 g) and 1-(*o*-nitrophenyl)2-(*p*-nitrophenyl)ethanol (1.5 g) in toluene (150 ml) were refluxed for 25 h. The toluene solution contained 1.25 g of a mixture which was chromatographed (silica gel, chloroform), giving four fractions: F_3 (0.81 g) consisted mainly of the starting material and gave 0.45 g crystals (30 %) identical with this. F_1 (0.51 g) was rechromatographed (silica gel, toluene) giving four fractions: F_{1-1} (0.024 g) consisted of *o,p'*-dinitrostilbene (2 %). F_{1-2} (0.056 g) gave 30 mg of 3-(*p*-nitrobenzoyl)-anthranil, m.p. 188.5–189.5 °C. IR (KBr): 3140, 1650, 1600, 1550, 1520, 1450, 1350, 1290, 1220 cm^{-1}. Mass spectrum: *m/e* 268 (100 %), 224 (13 %). F_{1-4} (0.262 g) gave 0.18 g of 2-(*p*-nitrophenyl)isatogen m.p. 253–255 °C, (lit[13] for 2-(*p*-nitrophenyl)isatogen: 250–254 °C). The crystals had IR (KBr): 3140,1720,1600, 1520, 1490, 1390, 1450 cm^{-1}. Mass spectrum: *m/e* 268 (100 %), 251 (38 %), 76 (26 %), 59 (20 %). Because of low solubility, no NMR spectrum was obtained. F_{1-3} was a mixture of these two compounds. By inspection of TLC of F_{1-2} to F_{1-4}, the total yield of 3-(*p*-nitrobenzoyl)anthranil was estimated to be 0.1 g (7 %) and of 2-(*p*-nitrophenyl)isatogen 0.3 g (21 %). Reactions were also run in benzene and *p*-xylene with the same result, the only difference being the required reaction time. The presence or absence of oxygen was without effect on the reaction. The isatogen was transformed to the anthranil when refluxed with sulfuric acid in ethanol.[5]

Reaction of 2-(o-nitrophenyl)-1-(p-nitrophenyl)ethanol. *p*-Toluenesulfonic acid (0.5 g) and 2-(*o*-nitrophenyl)-1-(*p*-nitrophenyl) ethanol (0.25 g) in toluene (25 ml) were refluxed for 2 h. TLC then indicated complete reaction, and a single product to have been formed. However, TLC at earlier stages indicated the presence of a substance which slowly disappeared from the reaction mixture. The product (0.25 g) gave crystals (0.12 g), m.p. 141–142 °C. IR (KBr) 3130, 1600, 1515, 1345, 1110, 970, 960 cm^{-1}. NMR ($CDCl_3$): δ 6.85–8.3 (m). The AA'XX' signal of the *p*-nitrophenyl system, and a doublet (1 H) at δ 7.10, J = 15 Hz, probably due to *trans* protons of the double bond, were recognized.

The reaction was repeated in benzene: *p*-Toluenesulfonic acid (40 mg) and 2-(*o*-nitrophenyl)-1-(*p*-nitrophenyl)ethanol (40 mg) in benzene (4 ml) were refluxed for 3 h. Yield: 30 mg which was chromatographed (silica gel, chloroform). One of the fractions contained a substance with R_F on TLC identical to the one that disappeared in the toluene reaction above. Recrystallisation of this fraction gave a substance (12 mg) with IR (KBr): 3110, 1610, 1520, 1370, 1345, 1180, 1170 cm^{-1}. NMR ($CDCl_3$): δ 6.9–8.3 (12 H, m), 5.8–6.0 (1 H), 3.1–3.6 (2 H), 2.35 (3 H). Mass spectrum: *m/e* 306 (17 %, $C_{14}H_{12}NO_5S$), 291 (8 %, $C_{14}H_{13}NO_4S$), 155 (100 %, $C_7H_7O_2S$), 136 (62 %, $C_7H_6NO_2$), 91 (70 %).

Reaction of o-nitrotolan (2 g) and *p*-toluenesulfonic acid (4 g) in toluene (200 ml) at reflux for 3 h gave 2.0 g of product. Chromatographic separation (silica gel, chloroform) of this gave 3-benzoylanthranil (0.51 g, 27 %) and 2-phenylisatogen (0.30 g, 16 %), in addition to recovered *o*-nitrotolan (0.16 g, 8 %).

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Intermolecular Hydride Transfer Reactions. IV. Acid-induced Redox Reactions of 4-Substituted Flav-2-enes

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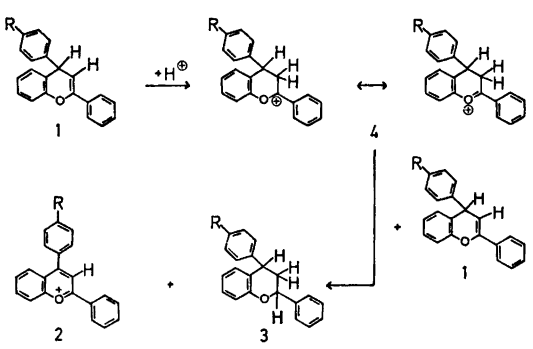
Treatment of 4-phenyl- or 4-(*p*-dimethylaminophenyl)-flav-2-ene with acid resulted in formation of the corresponding flavylium cations and flavane derivatives. The carbonium ion formed by protonation at C-3 of the flavene oxidized the unprotonated flavene through a hydride transfer reaction. The *cis*- and *trans*-4-phenyl flavanes were formed in 10:1 ratio. The stereospecificity of the hydride transfer reaction is interpreted to mean preference for a quasi axial phenyl group at C-4 of the hydride acceptor.

From treatment of 4-phenylflav-2-ene (*1*, R = H) with perchloric acid, Kröhnke and Dickoré isolated two products.[1] One of these was identified as 4-phenylflavylium perchlorate (*2*, R = H). The other compound was not identified, only melting point (141 – 142 °C) and yield were given. For this reason Kröhnke and Dickoré were unable to explain the observed reaction.

Barnes *et al.*[2] have reported that catalytic hydrogenation of 4-phenylflav-2-ene (*1*, R = H) yielded 4-phenylflavane (*3*, R = H) (m.p. 145 – 147 °C).

This work deals with the oxidation of flavenes to flavylium cations in acidic solutions. Re-examination of the work of Kröhnke and Dickoré resulted in the isolation of the flavylium salt *2* (R = H), and the unidentified compound in approximately the reported yields. Melting point, ^{1}H NMR spectrum and the mass spectrum of the latter compound were in agreement with the values of 4-phenylflavane (*3*, R = H) reported by Barnes *et al.*[2]

The acid induced intermolecular hydride transfer reaction depicted in Scheme 1 explains the formation of the flavylium salt *2* (R = H)



Scheme 1.

and the flavane derivative *3* (R = H). The yields of these products were in agreement with the equation:

$2(1) + HClO_4 = (2)\ ClO_4^- + (3)$

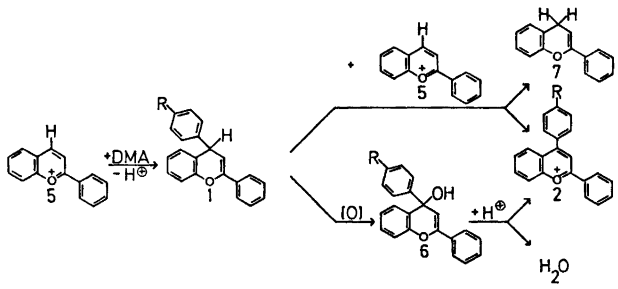
The reaction was repeated in trifluoroacetic acid (TFA) solution. The flavane derivative *3* (R = H) precipitated from the reaction mixture in 80 % yield, according to the equation above. The flavylium perchlorate (*2*, R = H) was isolated after addition of a mixture of acetic and perchloric acids to the concentrated TFA solution.

The flavene derivative *1* (R = H) used in these experiments was synthesized from flavylium perchlorate and the Grignard reagent of bromobenzene.[3,4] Consequently *1* (R = H) was a racemic mixture. ^{1}H NMR analysis of the racemic reduction product *3* (R = H) showed that the phenyl substituents at C-2 and C-4 were *cis* oriented. The yield of *3* (R = H) suggested a highly stereospecific hydride

transfer reaction. The *trans*-isomers were obtained from hydrogenolysis of 4-hydroxy-4-phenylflavane, synthesized from racemic flavanone and the Grignard reagent of bromobenzene.[4] The hydrogenolysis of the carbinol yielded a mixture of *cis*- and *trans*-4-phenylflavanes (*3*, R=H), which were separated by fractional crystallisation. A reaction mixture from treatment of 4-phenylflav-2-ene (*1*, R=H) with TFA was analyzed by GLC and this showed that the *cis*- and *trans*-flavanes were formed in 10:1 ratio, respectively.

A reasonable explanation for the stereochemical control of the reaction is as follows. The initial step is formation of a resonance stabilized carbonium ion *4* by protonation (Scheme 1). A twist conformation of this heterocyclic system is expected due to the Pitzer strain of the 3,4-bond in the planar conformation. The next step is a hydride transfer from C-4 of the unprotonated flavene *1*, to the created sextet at C-2 of *4*. This transfer is almost certainly dependent on the sterical environment at the C-2 reaction center of *4*. From models the environment at C-2 will be different whether the phenyl group at C-4 is quasi equatorial or quasi axial. In the quasi equatorial conformer, the rotation of the phenyl group at C-4 will be restricted due to interaction with the hydrogen at C-5. In the quasi axial conformer there are no serious interactions of the phenyl group at C-4 with other parts of the molecule. Consequently the latter conformer is assumed to be the preferred one. Approach of the hydride donor *1* (R=H) towards C-2 of *4* at the same side as the quasi axial phenyl group will strongly be hindered. No such interaction will interfere on approach from the opposite side. From this consideration a reduction product with the phenyl group at C-4 and the transferred hydrogen at C-2 in a *trans* configuration is expected. Mutual interaction of the axial phenyl groups at C-4 and C-2 (*cis* configuration) in the product which is formed primarily, interconverts the heterocyclic ring into the more stable conformation with the phenyl groups in a quasi diequatorial position.

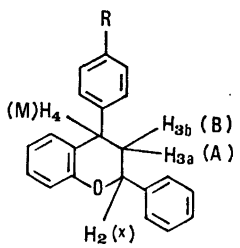
Shriner and Shotton have studied the reaction of flavylium perchlorate (*5*) with dimethylaniline in the presence of air to yield the 4-(*p*-dimethylaminophenyl)flavylium perchlorate (*2*, R=$N(CH_3)_2$).[5] The reaction was proposed to proceed through electrophilic substitution with formation of 4-(*p*-dimethylaminophenyl)flav-2-ene [*1*, R=$N(CH_3)_2$] which subsequently was oxidized by air to the 4-hydroxy-4-(*p*-dimethylaminophenyl)flav-2-ene [*6*, R=$N(CH_3)_2$]. The carbinol *6* [R=$N(CH_3)_2$] and the perchloric acid liberated in the substitution step, yielded the flavylium salt *2* [R=$N(CH_3)_2$]. Recently this reaction was reinvestigated and an alternative explanation of the formation of *2* [R=$N(CH_3)_2$] was suggested on the basis of the parent flav-2-ene (*7*) which was shown to be formed in the reaction.[6] An intermolecular hydride transfer reaction between the substitution product, [*1*, R=$N(CH_3)_2$] and unreacted flavylium cation was proposed to account for the observed products. These alternative reaction sequences are summarized in Scheme 2. The air oxidation sequence could not be excluded since Shriner and Shotton obtained the flavylium salt *2* [R=$N(CH_3)_2$] from a solution consisting of only the flavene derivative *1* [R=$N(CH_3)_2$] which had been exposed to air and then treated with strong acid.[5] However, the conditions used in the air oxidation experiment were not defined, and the postulated



Scheme 2.

intermediate carbinol *6* [R=N(CH$_3$)$_2$] was not reported to be identified prior to addition of acid to the solution of *1* [R=N(CH$_3$)$_2$]. As demonstrated with the 4-phenylflav-2-ene (*1*, R=H), protonated flavenes are potential oxidants in transformation of flavenes to flavylium cations. A reaction path corresponding to the one depicted in Scheme 1 was considered as an alternative explanation of the results from the oxidation experiment reported by Shriner and Shotton. The feasibility of the acid induced redox reaction of the flavene derivative *1* [R=N(CH$_3$)$_2$] was demonstrated by examining the ^{1}H NMR spectrum of this compound in TFA solution. The ^{1}H NMR spectrum showed signals due to a 1:1 mixture of the flavylium cation of *2* [R=N(CH$_3$)$_2$] and the flavane derivative *3* [R=N(CH$_3$)$_2$]. Thus acid treatment of the flavene derivative *1* [R=N(CH$_3$)$_2$] can lead directly to the corresponding flavylium cation without passing through a carbinol stage.

Table 1. ^{1}H NMR spectrum of *3* in $CDCl_3$ (δ)



Configuration	*cis*	*trans*	*cis*
Compound *3*; R	H	H	N(CH$_3$)$_2$
H$_2$(X)	5.19	5.07	5.18
H$_4$(M)	4.33	4.20	4.25
H$_{3a}$(A)	2.27	2.2–	2.0–
H$_{3b}$(B)	2.39	2.6	2.5
Arom H	6.6–	6.7–	6.5–
	7.6	7.5	7.6
R			2.90
$J_{2,3a}=J_{AX}$	10.6	13.0[a]	13.5[a]
$J_{2,3b}=J_{BX}$	1.8		
$J_{4,3a}=J_{AM}$	11.8	9.5[b]	18.5[b]
$J_{4,3b}=J_{BM}$	5.8		
$J_{3a\,3b}=J_{AB}$	−13.5		

[a] $J_{AX}+J_{BX}$. [b] $J_{AM}+J_{BM}$.

^{1}H NMR analysis of the 4-substituted flavanes *3*[7]

cis-4-Phenylflavane (m.p. 145 °C). The protons H$_2$, H$_3$a, H$_3$b, and H$_4$ form an ABMX pattern which on irradiation at either ν_M (δ 4.33) or at ν_X (δ 5.19) collapses to an ABX pattern. Calculation of the spectral parameters gave the results quoted in Table 1. The large coupling constants J_{AX}=10.6 Hz and J_{AM}=11.8 Hz reveal the quasi axial orientation of the protons X (H$_2$) and M (H$_4$). Consequently the two phenyl groups at C-2 and C-4 must be quasi equatorially and *cis* oriented.

trans-*4-Phenylflavane (m.p. 71 °C).* Protons H$_2$, H$_3$a, H$_3$b, and H$_4$ again form an ABMX pattern. A full analysis including spin decoupling was not carried out. However, $J_{AM}+J_{BM}$ is 9.5 Hz and much smaller than the corresponding value of the *cis*-isomers discussed above (16.6 Hz). This indicates that there is no axial-axial vicinal coupling involved and consequently M(H$_4$) must be quasi equatorially oriented and thus the phenyl group at C-4 is quasi axial. Furthermore $J_{AX}+J_{BX}$ is 13.0 Hz and compared to the value of the *cis*-isomers (12.4 Hz) this indicates that the proton X(H$_2$) is quasi axially oriented. Consequently the phenyl group at C-2 is quasi equatorial and *trans* oriented to the phenyl group at C-4.

cis-*4-(p-Dimethylaminophenyl)flavane.* The ^{1}H NMR spectrum was nearly identical to the spectrum of the *cis*-4-phenylflavane with respect to signals due to the alicyclic protons. This indicates *cis*-configuration of the C-2 and C-4 substituents.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Varian A-60A and on a Varian HA100 instrument with TMS as internal standard. A Perkin Elmer F11 instrument equipped with an OV-17 column was used for the GLC analysis. The mass spectra were run on a AEI MS902 instrument.

4-Phenylflavane (3, R=H) and 4-phenylflavylium perchlorate (2, R=H). From treatment of 4-phenylflav-2-ene (*1*, R=H);[4]

(a) *with perchloric acid/acetic acid.* The results of Kröhnke and Dickoré were obtained.[1]

(b) *with trifluoroacetic acid.* 4-Phenylflav-2-ene (*1*, R=H) (2.84 g, 0.01 mol) was added to trifluoroacetic acid (25 ml) with stirring. The solution was left at 20 °C overnight and the

precipitated *cis*-4-phenylflavane was collected by filtration in 80 % (1.23 g) yield with m.p. 143 – 145 °C.[2] ^{1}H NMR spectrum see Table 1. The remaining solution after removal of *3* (R = H) was concentrated and the residual oil was redissolved in a mixture of acetic acid (10 ml) and 70 % perchloric acid (2 ml). Addition of ether precipitated the 4-phenyl-flavylium perchlorate (*2*, R = H) in 80 % (1.5 g) yield with m.p. 218 °C (AcOH).[1]

The ^{1}H NMR spectrum of a solution prepared from addition of *1* (R = H) to TFA with subsequent removal of the precipitated *3* (R = H), confirmed that the flavylium cation was formed prior to the work up with the perchloric and acetic acid mixture.

cis- *and* trans-*4-Phenylflavanes (3, R = H) from treatment of 4-phenylflav-2-ene (1, R = H) with TFA*. The conditions of experiment (b) were used. However, instead of removing the precipitated *cis*-4-phenylflavane, the solvent was evaporated. The residual oil was completely dissolved in chloroform. The homogeneous solution was analyzed by GLC and the *cis*:*trans* ratio was determined to 10:1.

trans-*4-Phenylflavane (3, R = H)*. 4-Hydroxy-4-phenylflavane (1.0 g), prepared from flavanon and phenylmagnesium bromide,[4] was hydrogenated over Pd/C (10 %) (1.0 g) catalyst in ethyl acetate (100 ml) in a Parr apparatus with an initial pressure of 250 kPa for 24 h at 20 °C. The hydrogenolysis was quantitative as shown by the ^{1}H NMR spectrum of the concentrated solution after removal of the catalyst, and furthermore the *cis*- and *trans*-4-phenylflavanes were formed in approximately 1:1 ratio. The separation of the diastereomers was achieved by fractional crystallization. The *cis*-isomers were less soluble in ethanol than the *trans*-isomers. *trans*-4-Phenylflavane: m.p. 70 – 71 °C (EtOH). (Found: C 88.27; H 6.42. Calc. for $C_{21}H_{18}O$: C 88.10; H 6.29). ^{1}H NMR spectrum, see Table 1.

A solution of the *trans*-isomers in chloroform was mixed with TFA and left standing for 24 h at 20 °C. Isomerization to the *cis*-isomers could not be detected as shown by GLC analysis of the solution.

4-(p-Dimethylaminophenyl)flavane [*3*, *R* = *N*(CH_3)$_2$]. 4-(*p*-Dimethylaminophenyl)flav-2-ene [*1*, R = N(CH_3)$_2$][5,6] (1.63 g, 0.005 mol) was dissolved in trifluoroacetic acid (10 ml) with stirring. After 2 h at 20 °C the solvent was evaporated and the residual oil was dissolved in dimethyl sulfoxide (25 ml). A saturated solution of sodium bicarbonate (25 ml) was added with stirring and after 1 h the mixture was diluted with water.

The precipitated material was collected by filtration, washed with water and crystallized from dilute ethanol. M.p. 137 °C. Molecular weight by mass spectrometry: Found: 329.1779. Calc. for $C_{23}H_{23}NO$: 329.1780. ^{1}H NMR spectrum, see Table 1.

The ^{1}H NMR spectrum of a solution prepared from addition of 4-(*p*-dimethylaminophenyl)-flav-2-ene [*1*, R = N(CH_3)$_2$] to TFA showed signals due to the flavylium cation of *2* [R = N(CH_3)$_2$][6] and the flavane derivative *3* [R = N(CH_3)$_2$] in 1:1 ratio.

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The Crystal Structure of *trans*-2,5-Dibromo-7-thiabicyclo-[4.2.0]-1(6)-octene 7,7-Dioxide

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The structure of *trans*-2,5-dibromo-7-thiabicyclo[4.2.0]-1(6)-octene 7,7-dioxide has been determined by single crystal X-ray methods. The unit cell is monoclinic, space group No. 14 $P2_1/c$. The structure was solved by means of Patterson and electron density calculations and refined by least squares methods to a final R-value of 0.08. The bromine atoms are in a *trans* position and the cyclohexene ring takes a half-chair conformation.

In connection with the synthesis of the four-membered ring sulfone 2*H*-benzo[*b*]thiete 1,1-dioxide,[1,2] the photobromination of 7-thiabicyclo[4.2.0]-1(6)-octene 7,7-dioxide was studied. The main product in this reaction is *trans*-2,5-dibromo-7-thiabicyclo[4.2.0]-1(6)-octene 7,7-dioxide obtained as colour-less crystals from a benzene-ether solvent mixture. The stereochemistry of this compound has been discussed in a previous paper [2] where arguments favouring the *cis* configuration were given. A single crystal diffraction investigation has now been completed, the result of which shows that the configuration is *trans*. It may also be noted that the crystallized compound contains benzene of crystallization.

EXPERIMENTAL

The compound was prepared according to Ref. 2 and the crystals were isolated as transparent needles. In order to obtain accurate cell dimensions powder photographs were taken with a Guinier focusing camera using Cu$K\alpha_1$ radiation ($\lambda = 1.54050$ Å) and lead nitrate as internal standard. Refinement of the cell dimensions using the program POWDER [3] and 33 indexed reflexions yielded the following values:

$a = 10.8140 \pm 0.0009$ Å, $b = 7.9623 \pm 0.0007$ Å, $c = 17.298 \pm 0.002$ Å $\beta = 99.233 \pm 0.009°$, $V = 1470.1$ Å^3 ($t = 23°$ C).

The experimental density of 1.76 g/cm^3, determined by flotation in thallium formate, agrees well with the theoretical value of 1.783 g/cm^3, assuming four formula units and four benzene molecules of crystallization per unit cell (F.W. = 316.6).

Weissenberg photographs were taken for layers 0—6 about the b-axis using the equi-inclination multiple film technique and Ni-filtered Cu radiation. The intensities were estimated visually by comparison with a standard scale prepared from one of the strong reflexions from the crystal. The intensities from the different films in each set were scaled together and corrected for Lorentz and polarisation effects (SCALE and DATAP2).[4] The intensities from the different layers were scaled together approximately, assuming a linear relationship between intensity and time of exposure. A total of 1333 independent reflexions was measured. The linear absorption coefficient is 89.8 cm^{-1} and an absorption correction was applied.

The presence of benzene of crystallization was indicated early by loss of weight of the compound when stored [2] and a strong smell of benzene even after prolonged drying.

The crystal used in the investigation was of size $0.18 \times 0.26 \times 0.35$ mm and was mounted in a 0.3 mm glass capillary to prevent efflorescence due to loss of benzene of crystallization.

STRUCTURE DETERMINATION

From the unobserved reflexions $l = 2n + 1$ for $h0l$ and $k = 2n + 1$ for $0k0$ the space group was found to be $P2_1/c$.

A three dimensional Patterson synthesis was calculated (DFR)[4] from which the positions

of the two bromine atoms were easily found. Successive electron density calculations revealed the rest of the atoms including the benzene carbon atoms. After three cycles of block diagonal least squares refinement (BLOCK)[4] in which atomic coordinates, isotropic temperature factors and individual scale factors for each layer were allowed to vary, the weighting scheme of Cruickshank [5] $[w=(a+F_o+cF^2{}_o+dF_o{}^3)^{-1}]$ with $a=18$, $c=0.008$ and $d=0$ and the X-ray scattering factor tables of Doyle and Turner [6] being used an R-value of 0.13 was obtained. At this stage a difference synthesis was calculated which did not show any new atoms but indicated anisotropic thermal motion of the bromine atoms. Consequently allowance for anisotropic thermal motion of the bromine atoms was made in the final refinement. Six cycles of full matrix least squares refinement (LALS)[4] in which atomic coordinates, thermal parameters and individual scale factors for each layer were allowed to vary (in all 89 parameters) gave a final R-value of 0.08. All the last shifts in the parameters were less than 5 % of the estimated standard deviation of the corresponding parameter. A difference synthesis calculated after the last refinement showed no peaks higher than about 0.8 e/Å^3. No attempts were made to locate the hydrogen atoms.

A list of measured and calculated structure factors can be obtained from the author on request.

RESULTS

The final atomic parameters are given in Table 1. A list of interatomic distances and

Table 1. Atomic coordinates as fractions of the cell edges (standard deviations within parentheses). The anisotropic temperature coefficient is $\exp-[\frac{1}{4}(a^{*2}U_{11}h^2+b^{*2}U_{22}k^2+c^{*2}U_{33}l^2+a^*b^*U_{12}hk+ +a^*c^*U_{13}hl+b^*c^*U_{23}kl]$. The primed atoms are benzene carbon atoms.

Atom	x	y	z	B(Å^2)
Br(1)	0.4364(2)	0.0944(2)	0.2595(1)	
Br(2)	0.1551(2)	−0.0526(3)	−0.0468(1)	
S	0.3929(3)	0.3130(5)	0.0020(2)	3.35
C(1)	0.427(1)	−0.011(2)	0.155(1)	3.60
C(2)	0.311(1)	−0.125(2)	0.140(1)	3.67
C(3)	0.189(1)	−0.023(2)	0.119(1)	4.08
C(4)	0.189(1)	0.088(2)	0.048(1)	3.23
C(5)	0.315(1)	0.159(2)	0.050(1)	2.89
C(6)	0.417(1)	0.124(2)	0.099(1)	2.84
C(7)	0.518(1)	0.245(2)	0.077(1)	3.63
O(1)	0.411(1)	0.264(1)	−0.074(1)	5.06
O(2)	0.352(1)	0.481(1)	0.012(1)	4.64
C(1)′	−0.002(2)	0.351(3)	0.230(3)	7.51
C(2)′	0.118(2)	0.370(3)	0.233(1)	8.11
C(3)′	0.171(2)	0.455(3)	0.174(1)	6.51
C(4)′	0.098(2)	0.517(3)	0.113(1)	6.16
C(5)′	−0.032(2)	0.501(3)	0.107(1)	8.09
C(6)′	−0.087(3)	0.413(3)	0.170(2)	8.24

Atom	Br(1)	Br(2)
U_{11}	0.0846	0.0520
U_{22}	0.0534	0.0887
U_{33}	0.0404	0.0583
U_{12}	−0.0107	−0.0042
U_{13}	0.0002	−0.0039
U_{23}	−0.0023	−0.0479

Table 2. Interatomic distances with standard deviations. The primed atoms are benzene carbon atoms. The atomic numbering is in accordance with Fig. 1.

Bond	Distance, Å	Bond	Distance, Å
Br(1) – C(1)	1.98(1)	C(7) – S	1.80(2)
Br(2) – C(4)	1.98(1)	O(1) – S	1.41(1)
C(1) – C(2)	1.54(2)	O(2) – S	1.43(1)
C(2) – C(3)	1.54(2)	C(1)′ – C(2)′	1.30(4)
C(3) – C(4)	1.51(2)	C(2)′ – C(3)′	1.42(3)
C(4) – C(5)	1.47(2)	C(3)′ – C(4)′	1.30(3)
C(5) – C(6)	1.32(2)	C(4)′ – C(5)′	1.39(3)
C(6) – C(1)	1.44(2)	C(5)′ – C(6)′	1.49(4)
C(6) – C(7)	1.54(2)	C(6)′ – C(1)′	1.36(4)
C(5) – S	1.76(1)		

Table 3. Bond angles with standard deviations. The primed atoms are benzene carbon atoms. The atomic numbering is in accordance with Fig. 1.

Angle	A, °	Angle	A, °
Br(1) – C(1) – C(2)	109(1)	C(6) – C(7) – S	84(1)
Br(1) – C(1) – C(6)	106(1)	C(5) – S – C(7)	79(1)
Br(2) – C(4) – C(3)	109(1)	C(5) – S – O(1)	113(1)
Br(2) – C(4) – C(5)	106(1)	C(5) – S – O(2)	114(1)
C(6) – C(1) – C(2)	111(1)	C(7) – S – O(1)	113(1)
C(1) – C(2) – C(3)	112(1)	C(7) – S – O(2)	114(1)
C(2) – C(3) – C(4)	113(1)	O(1) – S – O(2)	118(1)
C(3) – C(4) – C(5)	110(1)	C(1)′ – C(2)′ – C(3)′	123(2)
C(4) – C(5) – C(6)	127(1)	C(2)′ – C(3)′ – C(4)′	119(2)
C(5) – C(6) – C(1)	124(1)	C(3)′ – C(4)′ – C(5)′	120(2)
C(4) – C(5) – S	141(1)	C(4)′ – C(5)′ – C(6)′	120(2)
C(6) – C(5) – S	92(1)	C(5)′ – C(6)′ – C(1)′	115(2)
C(5) – C(6) – C(7)	105(1)	C(6)′ – C(1)′ – C(2)′	123(2)
C(1) – C(6) – C(7)	131(1)		

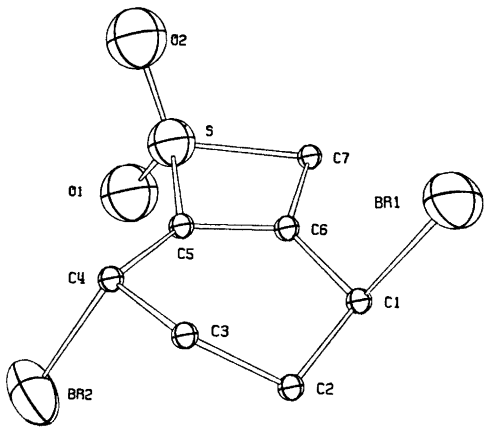


Fig. 1. Spatial drawing of the *trans*-2,5-dibromo-7-thiabicyclo[4.2.0]-1(6)-octene 7,7-dioxide molecule.

angles is given in Tables 2 and 3, respectively. A spatial drawing of the molecule is shown in Fig. 1 (ORTEP).[4] Note that a different numbering system for the atoms is used than that dictated by organic nomenclature, *cf.* Ref. 2. As shown in Fig. 1 the bromine atoms are in a *trans* position and the cyclohexene ring takes a half-chair conformation.

The bond lengths found in the cyclohexene part of this molecule are in good agreement with those found in the cyclohexene molecule except for the C(4) – C(5) and C(6) – C(1) bonds which are shorter in this work [1.47(2) Å and 1.44(2) Å compared to 1.504(6) Å].[7] The structure of the four membered ring $C_3H_4O_2S$ has been determined by the microwave technique.[8] The bond lengths and angles of the four mem-

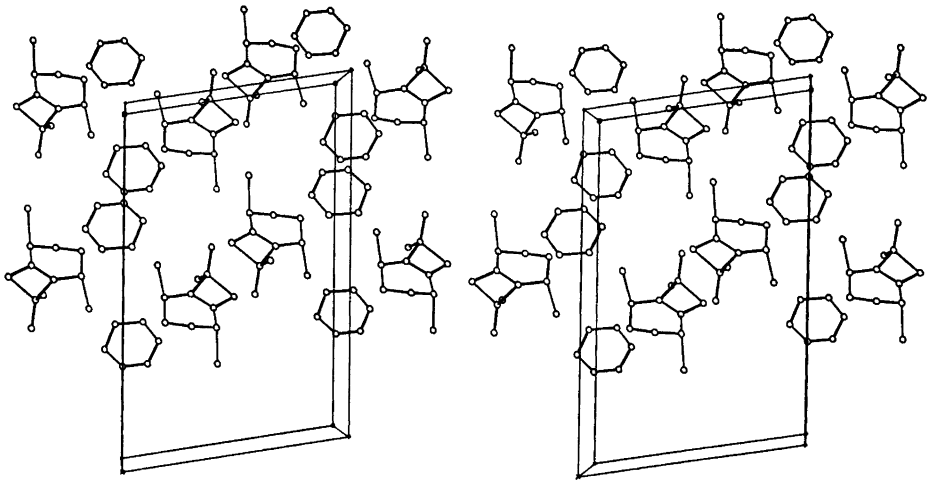


Fig. 2. Stereoscopic drawing of the packing in the crystal. The *b*-axis is directed upwards perpendicular to the plane of the paper and the *c*-axis is vertical.

bered ring part of the present molecule compare well with those found in Ref. 8 except for the double bond which is significantly longer in Ref. 8 (1.39 Å).

The high temperature factors and the comparatively large standard deviations in the coordinates of the benzene carbon atoms are to be expected since the benzene molecules are obviously weakly bonded.

The molecular packing of the crystal is shown in Fig. 2 and can be described as consisting of layers of molecules parallel to the $y-z$ plane. The layers are packed two by two, the benzene molecules lying cross-wise between these double layers, thus separating them but allowing the Br(2) atoms of neighbouring double layers to be in van der Waals contact (4.0 Å). The distance between the Br(1) atom of one molecule and the O(1) atom of its equivalent related by the glide plane is 3.15(1) Å which is somewhat shorter than expected from pure van der Waals interaction (3.35 Å).

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Intermolecular Hydride Transfer Reactions. V. Acid Induced Redox Reaction of 4-Phenylflav-3-ene

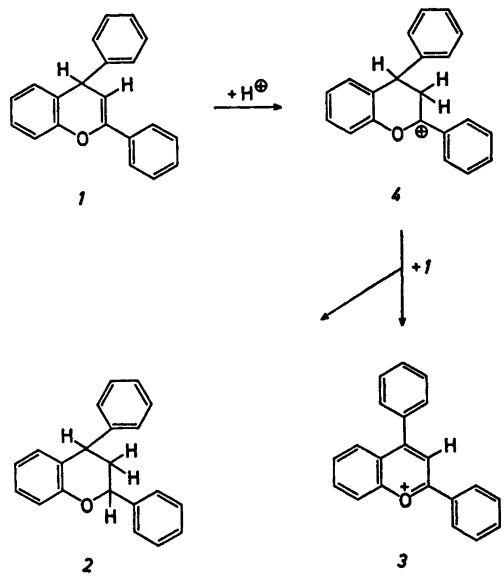
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4-Phenylflav-3-ene was found to undergo intermolecular hydride transfer reaction in perchloric or trifluoroacetic acid. The main product besides the 4-phenylflavylium cation was *trans*-1-(*o*-hydroxyphenyl)-1,3-diphenylprop-1-ene. Traces of 4-phenylflavanes were also formed. A reaction mechanism accounting for these products was proposed.

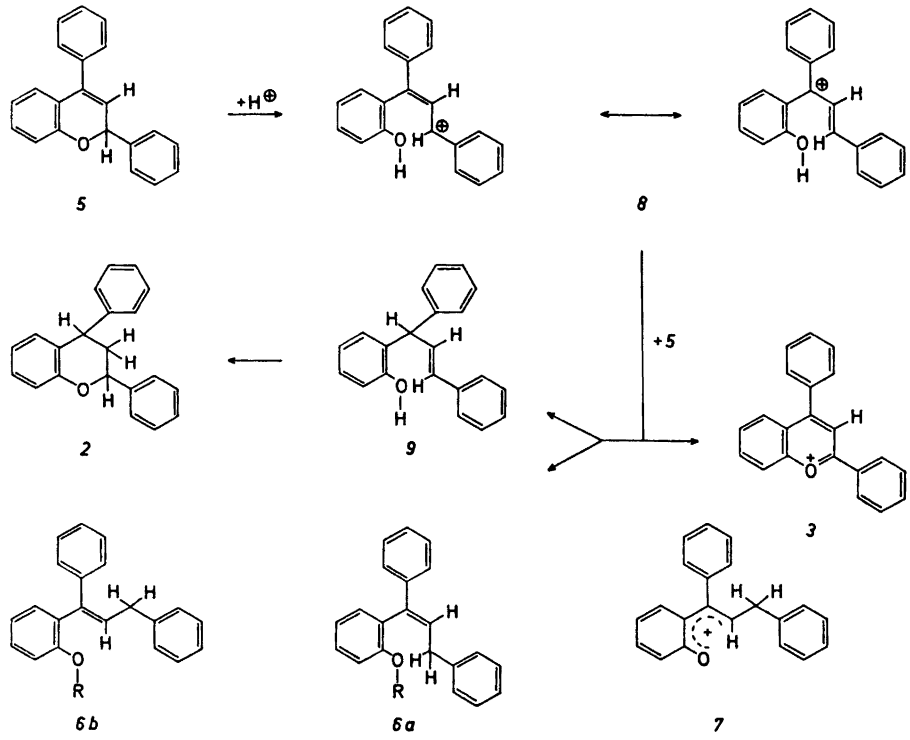
2-Flavenes undergo intermolecular hydride transfer in acid solution to yield equimolar mixtures of the flavylium salt and the flavane. Thus the reaction of 4-phenylflav-2-ene (*1*) with trifluoroacetic acid (TFA) was found to be nearly quantitative and gave both *cis*- and *trans*-4-phenylflavanes (*2*) in 10:1 ratio besides the 4-phenylflavylium salt (*3*).[1] The course of the reaction (Scheme 1) was assumed to proceed through the intermediate formation of the carbonium ion *4*, formed by protonation of *1* at C-3. Subsequent hydride transfer from unprotonated *1* to the carbonium ion would account for the formation of the products. 4-Alkyl- and 3,4-dialkyl substituted 3-chromenes [2] as well as their thio-analogues [3] undergo similar intermolecular hydride transfer reactions in acidic solutions.

The present work deals with the behaviour of 4-phenylflav-3-ene (*5*) in acidic solutions. The reaction of this compound with perchloric-acetic acid mixture led to the formation of 4-phenylflavylium perchlorate (*3*), the yield of which approximates 50 % which is consistent with an acid-induced disproportionation of *5*. Examination of the reaction mixture by TLC and GLC showed the presence of 4-phenylflavanes (*2*) using authentic samples previously prepared.[1] The other main reaction product (about 35 %) which was isolated by preparative TLC afforded an acetyl derivative on treatment with acetic anhydride in pyridine. Its ^{1}H NMR spectrum in deuteriochloroform exhibited a doublet at δ 3.38 (2 H, J 8 Hz), a triplet at δ 6.55 (1 H, J 8 Hz) and a multiplet at δ 7.0 – 7.3 (14 H). The ^{1}H NMR spectrum of the acetate was similar to that of the parent compound except for a slight upfield shift of the olefinic proton triplet and a



Scheme 1.

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Scheme 2.

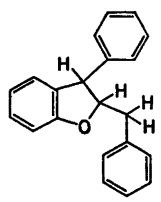
down-field shift of the methylene doublet, besides a singlet at δ 1.92 (3 H) for the acetate group. On the basis of these data, the structure of the parent compound was suggested to be either the *cis*- or the *trans*-1-(*o*-hydroxyphenyl)-1,3-diphenylprop-1-ene (*6a* or *b*, R = H) given in Scheme 2. The structure was further confirmed by mass spectrometry. The spectrum of *6a* or *b* (R = H) showed a base peak at *m/e* 286 which corresponds to its molecular ion, and a fragmentation pattern explainable on the basis of the proposed structure. The molecular ion in the mass spectrum of the acetate *6a* or *b* ($R = CH_3CO$) appeared at *m/e* 328 (relative abundance 41 %). The base peak which appeared at *m/e* 285 corresponds to the resonance-stabilised cation *7* shown in Scheme 2. Its formation from the molecular ion is supported by the presence of a metastable peak at *m** 247.5.

4-Phenylflav-3-ene (*5*) was also found to undergo disproportionation in trifluoroacetic acid (TFA). The oxidation product was isolated in 94 % yield as the perchlorate salt *3* by addition of perchloric acid to the reaction mixture after removal of TFA. The phenolic compound *6* was isolated in 64 % yield by preparative TLC. The mentioned yields were calculated according to the stoichiometry of a disproportionation reaction of *5*. The ^1H NMR spectrum of the reaction mixture showed signals due to the flavylium cation *3*, and the phenolic compound *6a* or *b* (R = H). The ^1H NMR spectrum carried out about 10 min after addition of drops of TFA to a solution of *5* in deuteriochloroform, apart from the signals of the flavylium cation showed two doublets centered at δ 3.63 (J 8 Hz) and 3.38 (J 8 Hz). The spectrum of the same solution after 24 h revealed the disappearence of the low-field doublet with increasing intensity of the other one. These two doublets may be assigned to *6a* (R = H) and *6b* (R = H), respectively, since the methylene protons of these two isomers are expected to give doublets of nearly the same coupling constant but with different chemical

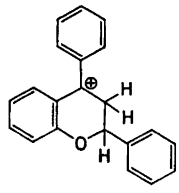
shifts. It seems likely that the observed process is isomerisation of *6a* (R=H) to the more stable *6b* (R=H) with the benzyl and *o*-hydroxyphenyl groups *trans* oriented. Hence the isolated phenolic compound should be the *trans* isomer *6b* (R=H).

4-Phenylflavanes (*2*) were also formed in the above reaction as minor products, the detection of which was evident by GLC. The failure of their identification by ^{1}H NMR is probably due to their relatively low concentration in the reaction mixture.

A probable reaction mechanism which accounts for the observed products *3*, *6* (R=H) and *2* (Scheme 2) involves initial protonation of the heteroatom of *5* and subsequent ring opening to the carbonium ion *8*. A hydride transfer from unprotonated *5* to this carbonium ion would lead to the flavylium cation *3*. The carbonium ion *8* offers two sites for the transferred hydride ion. Attack at the secondary carbon would result in the formation of the phenolic compound *6* (R=H). Meanwhile hydride transfer to the tertiary carbon should give *9*, ring closure of which may explain the observed formation of 4-phenylflavanes (*2*). Moreover *9*, the precursor of *2*, can be considered to arise by a shift of the double bond of the phenolic compound *6* (R=H). This is supported by the formation of *trans*-4-phenylflavane (*2*) on boiling *6b* with TFA. The isomerisation is similar to that reported for the conversion of 1-(*o*-hydroxyphenyl)-3-phenylprop-1-ene into flavane in boiling acid.[4] Such an acid catalysed isomerisation probably involves the dihydrobenzofuran intermediate *10* (Scheme 3 A).



10



11

A $6b \rightleftharpoons 10 \rightleftharpoons 9 \rightarrow 2$

B $5 \xrightarrow{H^+} 11 \xrightarrow{+5} 2+3$

Scheme 3.

With respect to the formation of the carbonium ion *8*, it should be mentioned that the acid catalysed reaction of 2,2-diphenylchromene with 1,1-diphenylethylene to yield 2,2-diphenyl-4-(2,2-diphenylvinyl)chromane was shown to proceed through an intermediate carbonium ion formed by fission of the chromene ring. Attack of this carbonium ion on the double bond of the olefin leads to a new cation which subsequently cyclises to the chromane derivative.[5] Moreover the conversion of certain isoflavenes into indenes under acid conditions, was assumed to take place by fission of the heterocyclic ring.[6]

Alternatively, the 4-phenylflavanes (*2*) may also be formed *via* the cyclic carbonium ion *11* produced by protonation of the double bond in *5* (Scheme 3 B). This reaction path is similar to the mechanism proposed for the acid-induced disproportionation of 4-alkyl- and 3,4-dialkyl substituted 3-chromenes.[3] It should be pointed out that disproportionation reactions proceeding *via* acyclic carbonium ion intermediate like *8* which gave rise to the unsaturated phenolic derivative *6* further susceptible to cyclisation, were not discussed before. It seems likely that fission of the heterocyclic ring should be more favoured in the 2-substituted 3-chromenes. As indicated from our results, a facile ring opening may be attributed to the benzylic group attached to ring oxygen of *5*.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Varian A-60A instrument with TMS as internal standard. A Perkin Elmer F11 instrument equipped with an OV-17 column was used for GLC analysis. Mass spectra were recorded on a AEI MS902 instrument. TLC was performed on silica plates using hexane, ethyl acetate baths.

Reaction of 4-phenylflav-3-ene (5)[7] *with perchloric acid.* To a mixture of perchloric acid (70 %) and acetic acid (1:10; 15 ml), 4-phenylflav-3-ene (*5*, 0.5 g) was added and the reaction mixture was stirred at 20 °C for 72 h. On addition of ether (100 ml), 4-phenylflavylium perchlorate (*3*), m.p. 222 °C[1] precipitated and was collected by filtration (0.3 g; 90 % yield). ^{1}H NMR spectrum of *3* in TFA: δ 8.58 (s, 1 H), 7.7–8.4, (m, 14 H). The ethereal solution was washed with water, 10 % $NaHCO_3$ and dried ($MgSO_4$). GLC and TLC analysis of this solution showed the presence of 4-phenylflavanes (*2*) besides traces of unreacted *5*. The ether was evaporated and the residue gave after pre-

parative TLC (hexane: ethyl acetate 10:1), *trans*-1-(*o*-hydroxyphenyl)-1,3-diphenylprop-1-ene (*6b*, R=H) (0.18 g; 72 % yield) as an oil. Molecular weight by MS: obs. 286.1356. Calc. for $C_{21}H_{18}O$: 286.1358. ^{1}H NMR ($CDCl_3$): δ 3.38 (d, 2 H, J 8.0 Hz), 6.55 (t, 1 H, J 8.0 Hz), 7.0–7.3 (m, 14 H).

trans-*1-(o-Acetoxyphenyl)-1,3-diphenylprop-1-ene* (6b, *R=CH$_3$CO*): A solution of *trans-1-*(*o*-hydroxyphenyl)-1,3-diphenylprop-1-ene (*6b*, R=H) (0.20 g) in pyridine (12 ml) was treated with acetic anhydride (1 ml) and kept overnight at room temperature. The reaction mixture was poured into water, extracted with ether and the ethereal solution washed successively with 10 % HCl, 10 % $NaHCO_3$, dried ($MgSO_4$) and evaporated. From the residue the pure acetate was obtained as an oil by preparative TLC (hexane: ethyl acetate 7:1). Molecular weight by MS: obs. 328.1468, calc. for $C_{23}H_{20}O_2$ 328.1463. ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3 H), 3.43 (d, 2 H, J 8.0 Hz), 6.36 (t, 1 H, J 8.0 Hz), 7.0–7.5 (m, 14 H).

Reaction of 4-phenylflav-3-ene (5) with TFA. A solution of 4-phenylflav-3-ene (*5*) (0.5 g) in TFA (10 ml) was stirred at 20 °C for 48 h, evaporated and the residue dissolved in chloroform. GLC analysis showed the presence of 4-phenylflavanes (*2*) besides traces of unreacted *5*. On addition of a mixture of perchloric acid (70 %) and acetic acid (1:10, 15 ml), followed by ether (100 ml) and then keeping overnight, 4-phenylflavylium perchlorate (*3*), m.p. 222 °C, was obtained in 94 % yield (0.32 g). The residue from the ethereal solution obtained after washing with 10 % $NaHCO_3$, drying ($MgSO_4$) and evaporation gave by preparative TLC (hexane:ethyl acetate 10:1), the *trans*-1-(*o*-hydroxyphenyl)-1,3-diphenylprop-1-ene (*6b*, R=H) (0.15 g, 64 %).

Reaction of trans-*1-(o-hydroxyphenyl)-1,3-diphenylprop-1-ene* (6b, *R=H) with TFA.* A solution of *trans*-1-(*o*-hydroxyphenyl)-1,3-diphenylprop-1-ene (0.1 g) in TFA (5 ml) was refluxed for 2 h, poured into water and extracted with ether. The ethereal solution after washing with 10 % $NaHCO_3$, drying ($MgSO_4$) was analysed by GLC. The *trans*-4-phenylflavane (*2*) was identified by comparison with authentic sample previously prepared.[1] Traces of *cis*-4-phenylflavane [1] (*2*) could not be detected.

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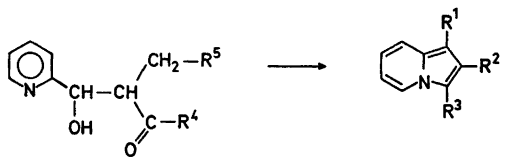
Indolizine Derivatives. III. Cyclization of the Aldol Product of 2-Pyridinecarbaldehyde and β-Dimethylamino- (or β-Hydroxy-)ketones

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A novel synthesis of 2-acylindolizines *via* cyclization of the aldol product of 2-pyridinecarbaldehyde and β-dimethylamino- (or β-hydroxy-) ketones is described. Cyclizations through reaction of the amino (or hydroxy) group or the carbonyl group are observed. Thus, 3-dimethylaminomethyl-4-hydroxy-4-(2-pyridyl)-2-butanone (Ia) gives, on treatment with acetic anhydride, several other indolizines in addition to 1-(2-indolizinyl)ethanone (IIa), including those formed through reaction of the carbonyl carbon. 2-Dimethylaminomethyl-3-hydroxy-1-phenyl-3-(2-pyridyl)-1-propanone (Ib) behaves analogously. With 3-hydroxymethyl-4-hydroxy-4-(2-pyridyl)-2-butanone (Ic), IIa is the sole indolizine product. The divergent reactions of Ia and Ic are discussed on the basis of the finding that 3-dimethylaminomethyl-4-(2-pyridyl)-3-buten-2-one gives with acetic anhydride the same products as Ia. In boiling acetic acid, both Ia and Ic give 2-methyl-1-(2-pyridyl)-1,3-butanedione, which with acetic anhydride cyclizes to 1-acetoxy-3-methyl-2-indolizinylmethyl acetate.

The cyclization of 3-*tert*-amino-1-(2-pyridyl)-1-alkanols or 3-*tert*-amino-1-(2-pyridyl)-1-alkenes on treatment with boiling acetic anhydride to afford indolizines was discussed by Barrett in detail.[1] Especially significant were his findings that pyridylalkenyl acetates cannot be intermediates and that the reaction of aminopyridylalkanols does not proceed through aminopyridylalkenes. Michalski *et al.* reported on the formation of 3-(2-pyridyl)indolizine from 1,3-di-(2-pyridyl)-1,3-propanediol when refluxed with acetic anhydride/sodium acetate.[2] On the other hand, 3-(2-pyridyl)-1-propanones are known to give indolizine derivatives through cyclocondensation.[3–5]



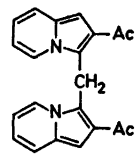
I II

I/	R^4	R^5
a	Me	NMe_2
b	Ph	NMe_2
c	Me	OH

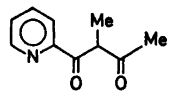
II/	R^1	R^2	R^3
a	H	Ac	H
b	Ac	Ac	H
c	H	Ac	Ac
d	H	CHO	Me
e	OAc	Me	Me
f	H	PhCO	H
g	Ac	PhCO	H
h	H	PhCO	Ac
i	H	CHO	Ph
j	OAc	Me	Ph
k	OAc	CH_2OAc	Me
l	OAc	Me	Ac
m	OAc	Me	CH_2OAc
n	H	Ac	CH_2CH_2Ac

The multifunctional aldols I, possessing simultaneously 3-(2-pyridyl)-1-propanone and 3-*tert*-amino- (or 3-hydroxy-) 1-(2-pyridyl)-1-propanol properties, may be expected to cyclize to indolizines in two ways: the 3-carbon of the resultant indolizines could originate from the carbonyl carbon or from the carbon carrying the amino (or hydroxy) group. In this paper it is shown that in heating with acetic anhydride the β-aminoketones Ia and Ib are converted in both ways, whereas the β-hydroxyketone Ic is changed only through reaction of the hydroxymethyl group. Herewith a practical route to several new indolizines is demonstrated. Thus, the aldol Ia when refluxed with acetic anhydride gives rise to several indolizines, namely: IIa, IIb, IIc, and III through reaction of the carbon carrying the dimethylamino group, and IId and IIe through reaction of the carbonyl carbon. The analogous products IIf, IIg, IIh and IIi, IIj are obtained from Ib, whereas Ic yields only IIa and III. All three aldols Ia, Ib, and Ic produce some 2-pyridylmethyl acetate. In boiling acetic acid, neither Ia nor Ic give indolizines, but the pyridine IVa, probably through IVb. With boiling acetic acid, IVa in turn cyclizes to the indolizine IIk.

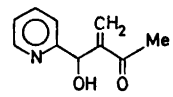
At room temperature, with acetic anhydride, Ia is rapidly acetylated to Va, from which acetic acid is then eliminated to afford the unsaturated pyridineketone Vb. At early stages of the reaction of Ic with acetic anhydride the NMR monitoring suggests acetylation to VIa, but on further reaction the presence of any subsequent intermediates could not be demonstrated. In an acetic acid solution at 60 °C, Vb is soon partly isomerized. Comparison between the NMR



III



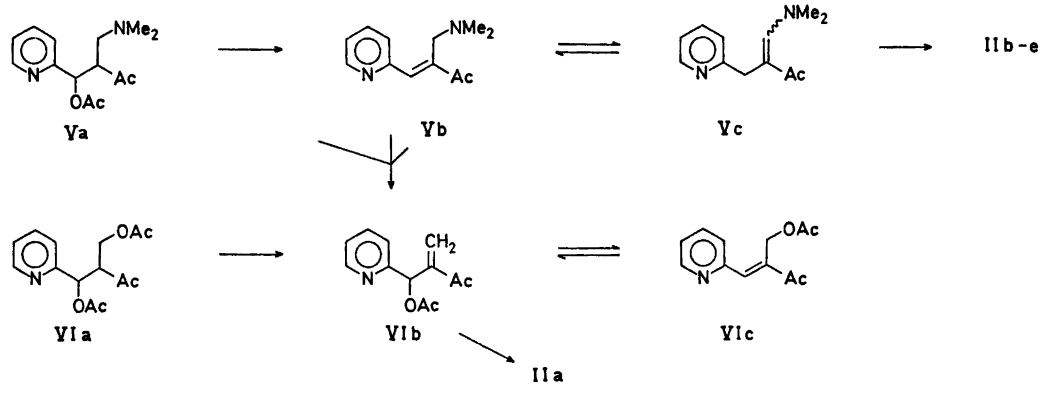
IVa



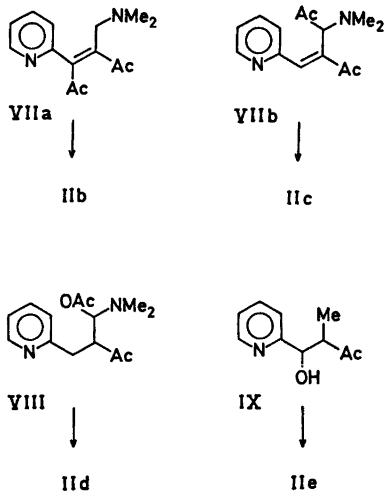
IVb

spectrum of the starting solution and that obtained after 0.5 h showed that Vb had been partly changed into another pyridine, probably Vc. The methylene peak of Vc appears at lower field (0.25 ppm) and the C-6 ring proton of Vc at higher field (0.35 ppm) than those of Vb, indicating that Vc cannot be a conjugated pyridine. On further heating with acetic acid, Vb yields only IIa and III. With boiling acetic anhydride, Vb gives the same products, IIa–e and III, in practically identical ratio as Ia. This strongly suggests that Vb is an intermediate in the formation of the indolizines IIb–e from Ia. No decomposition through the retroaldolization of the aldols I to 2-pyridinecarbaldehyde and β-amino- (or β-hydroxy) ketones occurs. This is proved (TLC) by the absence of the indolizinediketone IIn in the product mixtures, as 2-pyridinecarbaldehyde and 4-dimethylamino- (or 4-hydroxy-) 2-butanone should give IIn.[6]

The cyclization of *tert*-aminopyridylalkanols and *tert*-aminopyridylalkenes without an acyl group may be explained in terms of a simple



substitution on the carbon carrying the amino group. In the case of the aldols Ia, Ib, and Ic the possibility of such a substitution is not entirely ruled out, of course, since IIa is also formed when Ia is heated in water containing some oxalic acid. However, it is more plausible that the five-membered ring of IIa, for example, is formed through addition to the carbon-carbon double bond of the intermediate unsaturated ketone VIb, which in turn can be generated from Ia, Ic, Vb, and Vc by several means, as indirectly stated above. The exclusive cyclization of VIb to IIa hence accounts for the absence of the ketones VIb and VIc in the product mixtures.



It is currently assumed that the diacetylindolizines IIb and IIc are formed analogously to IIa through the cyclization of acetyl derivatives of Va or Vb, such as VIIa and VIIb, rather than through the acetylation of IIa; for the indolizines having electron-withdrawing substituents are very reluctantly acetylated.[7,8]

The indolizinecarbaldehyde IId is undoubtedly formed from Vb or Vc, but before the addition step to the carbonyl carbon giving rise to the five-membered ring can take place, the unsaturated ketone clearly has to change into a saturated one, probably VIII. The reduced indolizine product IIe can also be prepared by the cyclocondensation of the pyridylbutanone IX, and its origin is obviously analogous to that of the reduced indolizine derivatives from the Perkin reaction of 2-pyridinecarbaldehyde.[9] The second reduced reaction product, 2-pyridylmethyl acetate, may have a similar origin. The formation of methylenebisindolizines, such as III, was discussed by Barrett.[1]

The structure of IIk is consistent with the finding that IVa in the presence of acetic anhydride/potassium acetate yields as the main product IIk, which is stable on further reaction, accompanied by a small amount of the acetylindolizine III. It is assumed that acetic anhydride effects the cyclization of IVa to the pyridinium compound X, which rearranges into the indolizines IIk or IIm. The latter indolizine, which was not isolated, gives III through the cleavage of formaldehyde and subsequent acetylation catalyzed by acetic anhydride/potassium acetate.

The structures of the new indolizines were unambiguously deduced from their analyses and spectral data. Especially informative are the NMR spectra, for the position and nature of the substituents of indolizines are in most cases easily determined by inspection of the chemical shifts of the indolizine ring protons.[8,10] To facilitate comparison, the NMR data are collected in Table 1. The appearance of the C-1 proton of 2,3-diacylindolizines (IIc, IIh) and C-3 proton of 1,2-diacylindolizines (IIb, IIg) at higher field than those of 2-acylindolizines (IIa, IIf) is due to the distortion of the 2-acyl group of diacylindolizines out of the ring plane.

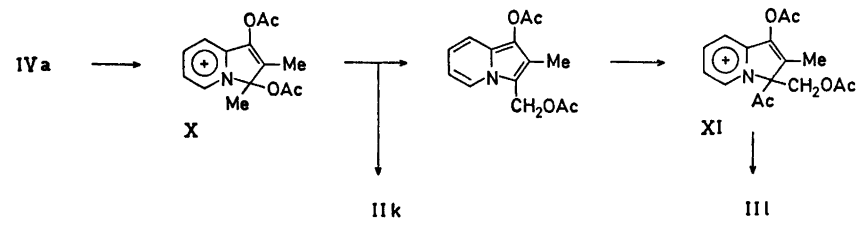


Table 1. NMR-Data of the new indolizines II and III. (δ-values, $J_{5,6}=J_{6,7}=ca.$ 7 Hz, $J_{7,8}=ca.$ 9 Hz).

	R^1	R^2	R^3	H^5	H^6	H^7	H^8
IIa	6.76(s)	2.53(3 H s)	7.75(br s)	7.84	6.35	——6.8	7.33
IIb	2.64 or 2.60 (3 H s)	2.64 or 2.60 (3 H s)	7.64(s)	7.96	6.65	——7.25	8.17
IIc	6.55(s)	2.51 or 2.40 (3 H s)	2.51 or 2.40 (3 H s)	9.59	6.5	——7.1	7.32
IId	6.65(s)	10.04(1 H s)	2.73 (3 H s)	7.60	6.3	——6.75	7.27
IIe	2.10(3 H s)	2.31 or 2.26 (3 H s)	2.31 or 2.26 (3 H s)	7.40	6.15	——6.65	7.09
IIf	6.69(br s)	7.8(2 H) and 7.35(3 H)	7.60(d 1.5)	7.73	6.2	——6.7	7.20
IIg	2.18(3 H s)	7.9(2 H) and 7.5(3 H)	7.35(s)	7.97	6.7	——7.25	8.29
IIh	6.45(s)	7.9(2 H) and 7.5(3 H)	2.24(3 H s)	9.92	6.7	——7.4	7.45
IIi	6.77(s)	9.79(1 H s)	7.37(5 H s)	7.81	6.15	——6.75	7.26
IIj	2.09(3 H s)	2.28(3 H s)	7.36(5 H s)	7.83	6.05	——6.65	7.06
IIk	2.34 or 2.28 (3 H s)	5.04(2 H s) and 1.94(3 H s)	2.34 or 2.28 (3 H s)	7.40	6.15	——6.6	7.06
III	2.33(3 H s)	2.38(3 H s)	2.47(3 H s)	10.05	6.6	——7.4	7.15
III	6.78(s), 2.74(s), 5.50(s), 8.05(br d 7), 6.2——6.8(m), 7.22(br d 9), (1/3/1/1/2/1)						

EXPERIMENTAL

Acetic anhydride contained less than 3 % acetic acid. Silica chromatography columns were made up in benzene and eluted with benzene containing increasing amounts of methylene chloride. The fractionations were followed by TLC (silica, benzene containing 2 – 5 % of methanol). Melting points are uncorrected. Elemental analyses were performed by Mrs. M. Horko. The solid products were recrystallized from ethanol or light petroleum (b.p. 40 – 65 °C). UV spectra were obtained for solutions in ethanol. IR spectra were obtained for KBr-tablets or liquid films. NMR spectra were measured for solutions in $CDCl_3$ or CCl_4 at 60 MHz. Mass spectra were recorded at 70 eV through the cooperation of Mr. L. Hiltunen, Dipl. Eng.

Preparation of the aldols I

3-Dimethylaminomethyl-4-hydroxy-4-(2-pyridyl)-2-butanone, (Ia). Method A. 2-Pyridinecarbaldehyde (10.7 g, 0.10 mol), 4-dimethylamino-2-butanone (11.5 g, 0.10 mol), 2 ml of water and 0.5 g finely powdered $Ca(OH)_2$ were kept at – 15 °C for 10 h with occasional shaking. The product was extracted with ether and dried over Na_2SO_4. The remaining oil (19.0 g, 81 %) consisted of almost pure Ia, according to its NMR spectrum: δ 8.40 (1 H, br d), 7.9 – 6.95 (3 H, m), 5.5 (1 H, br s), 5.10 and 5.08 (1 H, d 6.5 and d 5), 3.7 – 2.3 (3 H, m), 2.3 – 2.15 (9 H). *Method B.* 2-Pyridinecarbaldehyde and 4-dimethylamino-2-butanone were allowed to stand at room temperature without any added catalyst for 3 – 4 days, giving a dark oil which contained, besides Ia, about 10 % unchanged starting materials.

2-Dimethylaminomethyl-3-hydroxy-1-phenyl-3-(2-pyridyl)-1-propanone, (Ib). From 2-pyridinecarbaldehyde (10.7 g, 0.10 mol) and 3-dimethylamino-1-phenyl-1-propanone (17.7 g, 0.10 mol) by the method A 24.1 g (85 %) almost pure Ib was obtained; NMR spectrum: δ 8.39 and 8.28 (1 H, d 5 and d 5), 6.20 (1 H, br s), 5.4 – 5.15 (1 H, d and d), 4.6 – 4.1 (1 H, m), 3.1 – 2.4 (2 H, m), 2.28 and 2.18 (6 H, s and s).

4-Hydroxy-3-hydroxymethyl-4-(2-pyridyl)-2-butanone, (Ic). 2-Pyridinecarbaldehyde (10.7 g, 0.10 mol), and 4-hydroxy-2-butanone (8.8 g, 0.10 mol) gave Ic (15.4 g, 79 %) by the method A; NMR spectrum: δ 8.43 (1 H, d), 7.9 – 7.0 (3 H, m), 5.20 and 5.07 (1 H, d 5.5 and 6.5), 4.7 (1 H, br s), 2.22 and 2.14 (3 H, s and s).

The aldols I were used without further purification.

Reactions of the aldols I

Ia with Ac_2O. The aldol Ia (22.2 g, 0.10 mol) and Ac_2O (150 ml) were boiled under reflux for 2 h. Most volatile materials (AcOH, the excess of Ac_2O, *N,N*-dimethylacetamide) were removed *in vacuo*, the temperature not exceeding 40 °C. The black tar was chromatographed on silica. The following components could be identified, listed in order of elution: *2,3-Dimethyl-1-indolizinyl acetate, (IIe)*, (0.8 g, 4 %), m.p. 111 °C. (Found C 70.85; H 6.60; N 6.95. Calc. for $C_{12}H_{13}NO_2$: C 70.90; H 6.45; 6.90). UV, λ_{max} (nm) and log ε: 361 (2.96), 301 (3.07), 289 (3.10), 279 (3.05), 239 (4.49). IR, ν_{max}(cm^{-1}): 1760, 1745. MS, *m/e* (%): 203 (40), 161 (100), 160 (98), 78 (74), 53 (17), 52 (11), 51 (17), 43 (13). *3,3'-Methylenebis-1-(2-indolizinyl)ethanone, (III)*, (0.3 g, 1 %), m.p. 218 °C. (Found C 76.35; H 5.30; N 8.55. Calc. for $C_{21}H_{18}N_2O_2$: C 76.35; H 5.50; N 8.50). UV, λ_{max}(nm) and log ε: 394 sh (3.31), 377 (3.48), 363 (3.43), 350 sh (3.31), 311 (2.41), 302 (3.36), 267 sh (4.13), 247 (4.67), 243 (4.68). IR, ν_{max}(cm^{-1}): 1645. MS, *m/e* (%): 330 (27), 288 (24), 287 (100), 245 (16), 243 (25), 43 (15). *3-Methyl-2-indolizinecarbaldehyde, (IId)*, (1.4 g, 9 %), m.p. 75 °C. (Found C 75.35; H 5.55; N 8.75. Calc. for $C_{10}H_9NO$: C 75.45; H 5.70; N 8.80). UV, λ_{max} and log ε: 399 sh (3.09), 381 (3.17), 365 (3.11), 310 (2.40), 267 sh (3.75), 251 (4.31), 246 (4.27). IR, ν_{max}(cm^{-1}): 2780, 2720, 2700, 1670, 1655, 1645, 1625. MS, *m/e* (%): 159 (77), 158 (60), 130 (100), 78 (18), 77 (15), 29 (40). *1-(2-Indolizinyl)ethanone, (IIa)*, (3.2 g, 20 %), m.p. 127 °C. (Found C 75.45; H 5.70; N 8.80. Calc. for $C_{10}H_9NO$: C 75.45; H 5.70; N 8.80). UV, λ_{max}(nm) and log ε: 397 sh (2.64), 382 sh (3.08), 365 (3.30), 351 sh (3.30), 340 sh (3.20), 311 (3.15), 299 (3.08), 275 (3.82), 266 (3.97), 245 (4.55). IR, ν_{max}(cm^{-1}): 1645, 1630. MS, *m/e* (%): 159 (65), 144 (93), 116 (100), 89 (37), 63 (31), 62 (15), 51 (21), 50 (15), 43 (50), 39 (29), 38 (15). *1,1'-(2,3-Indolizinediyl)bisethanone, (IIc)*, (0.6 g, 3 %), m.p. 65 °C. (Found C 71.45; H 5.70; N 7.20. Calc. for $C_{12}H_{11}NO_2$: C 71.60; H 5.50; N 6.95). UV, λ_{max}(nm) and log ε: 375 (3.89), 261 (4.11), 237 (4.36). IR, ν_{max}(cm^{-1}): 1670, 1610. MS, *m/e* (%): 202 (14), 201 (93), 187 (20), 186 (100), 144 (14), 130 (42), 116 (16), 43 (14). *2-Pyridylmethyl acetate*, (0.8 g, 5 %). *1,1'-(1,2-Indolizinediyl)bisethanone, (IIb)*, (4.8 g, 24 %), m.p. 123 °C. (Found C 71.60; H 5.75; N 6.80. Calc. for $C_{12}H_{11}NO_2$: C 71.60; H 5.50; N 6.95). UV, λ_{max}(nm) and log ε: 381 sh (3.58), 366 (3.64), 354 sh (3.63), 313 (3.43), 304 (3.38), 260 (3.65). IR, ν_{max}(cm^{-1}): 1660, 1635, 1615. MS, *m/e* (%): 201 (26), 186 (100), 130 (16), 89 (16), 43 (68).

Ib with Ac_2O. The aldol Ib (28.4 g, 0.10 mol) and Ac_2O (200 ml) gave analogously: *2-Methyl-3-phenyl-1-indolizinyl acetate, (IIj)*, (1.3 g, 5 %), m.p. 42 °C. (Found C 76.80; H 6.00; N 5.45. Calc. for $C_{17}H_{15}NO_2$: C 76.95; H 5.70; N 5.30). IR, ν_{max}(cm^{-1}): 1765, 1740. *3-Phenyl-2-indolizinecarbaldehyde, (IIi)*, (1.8 g, 8 %), m.p. 77 °C. (Found C 81.60; H 5.25; N 6.05. Calc. for $C_{15}H_{11}NO$: C 81.45; H 5.00; N 6.35). UV, λ_{max}(nm) and log ε: 382 (2.56), 324 (2.64), 269 sh (3.24), 251 (3.11). IR, ν_{max}(cm^{-1}): 2820, 2790, 2730, 1660. MS, *m/e* (%): 221 (100), 220 (19), 192 (23), 191 (33). *2-Indolzinylphenylmethanone, (IIf)*,[10] (5.7 g, 26 %), m.p. 95 °C. (Found C 81.35; H 4.95; N 6.50. Calc. for $C_{15}H_{11}NO$: C 81.45; H 5.00; N 6.35), UV, λ_{max}(nm) and log ε: 376 (3.01), 278 sh (4.38), 263 (4.55), 237 sh (4.05). IR, ν_{max}(cm^{-1}): 1625, 1595. MS, *m/e* (%): 221 (100), 194 (44), 193 (17), 144 (49), 116 (30), 77 (17). *1-(2-Benzoyl-3-indolizinyl)ethanone, (IIh)*. (1.3 g, 5 %), m.p. 81 °C. (Found C 77.45; H 5.15; N 5.45. Calc. for $C_{17}H_{13}NO_2$: C 77.55; H 5.00; N 5.30). UV, λ_{max}(nm) and log ε: 361 sh (3.78), 351 (3.80), 319 (3.71), 273 sh (4.04), 255 (4.22), 233 (4.13). IR, ν_{max}(cm^{-1}): 1655, 1650, 1630, 1615. MS, *m/e* (%): 264 (15), 263 (100), 248 (89), 234 (19), 191 (21), 77 (45), 51 (20). 2-Pyridylmethyl acetate, (0.8 g, 5 %). *1-(2-Benzoyl-1-indolizinyl)ethanone, (IIg)*, (4.2 g, 16 %), m.p. 114 °C. (Found C 77.50; H 4.95; N 5.60. Calc. for $C_{17}H_{13}NO_2$: C 77.55; H 5.00; N 5.30). UV, λ_{max}(nm) and log ε: 361sh (3.71), 352 (3.73), 320 (3.64), 274 sh (4.00), 255 (4.18), 232 (4.19). IR, ν_{max}(cm^{-1}): 1650, 1630, 1610, 1590. MS, *m/e* (%): 263 (49), 249 (15), 248 (100), 191 (15), 77 (29).

Ic with Ac_2O. Similarly the aldol Ic (19.5 g, 0.10 mol) and Ac_2O gave: III (0.7 g, 2 %) and IIa (6.7 g, 42 %).

Ia or Ic with AcOH. The aldols Ia (22.2 g, 0.10 mol) or Ic (19.5 g, 0.10 mol) were refluxed with AcOH (100 ml) for 2 h. After evaporation the residue was put through a silica column and *2-methyl-1-(2-pyridyl)-1,3-butanedione, (IVa)*, (8.4 g, 47 %), collected. Recrystallization from petroleum gave a white solid IVa, m.p. 35 °C. (Found C 67.85; H 6.00; N 8.05. Calc. for $C_{10}H_{11}NO_2$: C 67.80; H 6.25; N 7.90). IR, ν_{max}(cm^{-1}): 1720, 1695. NMR: δ 8.60 (1 H, br d 5), 8.1–7.6 (2 H, m), 7.6–7.3 (1 H, m), 4.82 (1 H, q 7), 2.28 (3 H, s), 1.35 (3 H, d 7). MS, *m/e* (%): 177 (2), 162 (3), 134 (54), 133 (24), 106 (40), 79 (73), 78 (100), 51 (25), 43 (55).

Vb with Ac_2O. The aldol Ia (22.2 g, 0.10 mol) was treated with Ac_2O (51 g, 0.5 mol) at 20 °C. After 1 h the mixture was neutralized with $NaHCO_3$, the precipitated oil extracted with ether and dried over Na_2SO_4. The residue (14.1 g, 69 %) consisted of rather pure *3-dimethylaminomethyl-4-(2-pyridyl)-3-buten-2-one, (Vb)*. UV, λ_{max}(nm) and log ε: 286 (4.11), 253 (4.09). IR, ν_{max}(cm^{-1}): 1690–1650, 1615. NMR: δ 8.55 (1 H, br d 5), 7.65 (2 H, m), 7.34 (1 H, s), 7.3–6.9 (1 H, m), 3.69 (2 H, s), 2.32 (3 H, s), 2.12 (6 H, s). The unsaturated pyridylaminoketone, Vb (20.4 g, 0.10 mol) without further purification was boiled with Ac_2O (150 ml) for 2 h giving the same products as Ia with Ac_2O: IIe (0.6 g, 3 %), III (0.15 g, 0.5 %), IId (1.75 g, 11 %), IIa (2.9 g, 18 %), IIc (1.0 g, 5 %), 2-pyridylmethyl acetate (0.8 g, 5 %), IIb (5.4 g, 27 %).

Vb with AcOH. The unsaturated pyridylaminoketone Vb (20.4 g, 0.10 mol) and AcOH (100 ml) were kept overnight at 60 °C yielding only III and IIa.

IVa with Ac_2O. The pyridinediketone IVa (17.7 g, 0.10 mol) and Ac_2O (150 ml) were refluxed for 1 h. After evaporation *in vacuo* the residue was purified by column chromatography giving *1-acetoxy-3-methyl-2-indolizinylmethyl acetate, (IIk)*, (12.3 g, 47 %), m.p. 96 °C. (Found C 64.05; H 5.80; N 5.45. Calc. for $C_{14}H_{15}NO_4$: C 64.35; H 5.80; N 5.35). UV, λ_{max}(nm) and log ε: 360 (3.06), 300 (3.21), 288 (3.17), 279 (3.06), 237 (4.39). IR, ν_{max}(cm^{-1}): 1775, 1755, 1720. MS, *m/e* (%): 261 (10), 160 (21), 159 (100), 158 (23), 130 (15), 78 (23), 43 (15). Similar amounts of IVa and Ac_2O together with AcOK (50 g, 0.5 mol) yielded IIk (11.7 g, 45 %) and *1-(1-acetoxy-2-methyl-3-indolizinyl)-ethanone, (III)*, (1.8 g, 7), m.p. 75 °C. (Found C 67.35; H 5.85; N 6.00. Calc. for $C_{13}H_{13}NO_3$: C 67.50; H 5.65; N 6.05). UV, λ_{max}(nm) and log ε: 365 (4.10), 297 (3.22), 269 (3.32), 263 sh (4.28), 226 (4.33). IR, ν_{max}(cm^{-1}): 1750, 1735, 1600. MS, *m/e* (%): 231 (24), 189 (100), 188 (36), 174 (36), 159 (23), 146 (15), 105 (15), 78 (48), 43 (15).

IX with Ac_2O. 2-Pyridinecarbaldehyde (10.7 g, 0.10 mol), 2-butanone (14.4 g, 0.20 mol), K_2CO_3 (1.4 g, 0.1 mol) and 100 ml of water were stirred at 10 °C for 12 h. The precipitated oil was extracted with ether and dried over Na_2SO_4. Evaporation delivered a mixture (12.1 g) consisting mainly of *4-hydroxy-3-methyl-4-(2-pyridyl)-2-butanone, (IX)* and *1-(2-pyridyl)-1-penten-3-one (XII)* (NMR). The treatment of this mixture with Ac_2O gave after evaporation and chromatography IIe (12.3 g, 11 %, based on 2-pyridinecarbaldehyde) and XII, m.p. 13 °C. NMR: δ 8.56 (1 h, br d 5), 7.8–7.0 (3 H, m), 7.34 (1 H, d 15.7), 7.14 (1 H, d 15.7), 2.62 (2 H, q 7.5), 1.08 (3 H, t 7.5).

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Synthesis of Methyl 2-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside. A New Route to 2-Acetamido-2-deoxy-α-D-glucopyranosides

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A new route to 2-acetamido-2-deoxy-α-D-glucopyranosides has been worked out and exemplified by the synthesis of methyl 2-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside. Condensation of 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-β-D-mannose with methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside, followed by deacetylation, yielded a disaccharide derivative with a free hydroxyl at C-2 in the α-D-mannopyranosyl residue. Oxidation to the corresponding carbonyl derivative, oximation, stereoselective reduction with simultaneous removal of protecting groups, acetylation and *O*-deacetylation yielded the title compound.

We have recently developed a route for the synthesis of 1,2-*cis*-glycosides [1,2] which involves the preparation of the corresponding epimer with the reversed configuration at C-2, with all hydroxyls except that at C-2 suitably protected. The condensation was performed either by a Koenigs-Knorr reaction [1] or an orthoester synthesis, using a fully benzylated 1,2-*O*-methylorthoacetyl derivative.[2] Oxidation to the carbonyl compound followed by stereoselective reduction yielded the 1,2-*cis*-glycoside. The method has been used for the synthesis of β-D-mannopyranosides [1–3] and α-D-glucopyranosides.[2] Oximation of the carbonyl derivative followed by stereoselective reduction should yield a 1,2-*cis*-2-amino-2-deoxyglycoside.

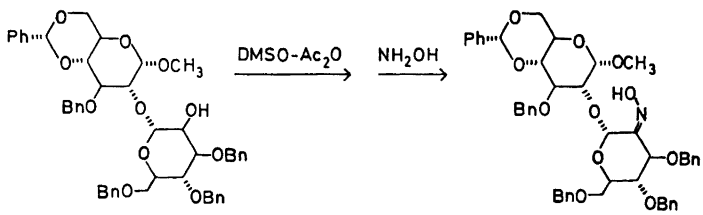
The disaccharide methyl 2-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside (VII, title compound), contains the same sugars and linkages as a structural element [α-D-GlcNAc*p*-(1→2)-α-D-Glc*p*-(1→] of the common core oligosaccharide of *Salmonella* lipopolysaccharides.[4,5] The substance was needed in studies of the specificity of the bacteriophage ΦX 174 receptor [6] against this core. We therefore chose to demonstrate the new route to α-D-glucosaminides by the synthesis of this substance.

RESULTS and DISCUSSION

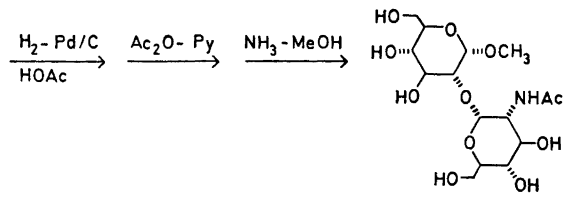
The aglycone, methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucoside (II) was prepared from methyl 4,6-*O*-benzylidene-2-*O*-*p*-toluenesulfonyl-α-D-glucoside [7] by benzylation, using benzyl bromide and silver oxide in dimethylformamide,[8,9] followed by treatment with lithium aluminium hydride.

The orthoester, 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-β-D-mannose was prepared as previously described,[2] and condensed with the aglycone (II) as devised by Kochetkov *et. al.*[10] Deacetylation of the product (III) to IV, and oxidation of IV to V, using dimethyl sulfoxide-acetic anhydride, was also performed as previously described.[1,2] The carbonyl derivative was not purified but transformed directly into the oxime (VI). The yield of crystalline oxime, starting from IV, was 82 %. A mixture of two stereoisomeric oximes should be formed, and the results indicate that one of these forms predominates.

The stereoselectivity on reduction of oximes analogous to VI has been investigated by Lemieux *et al.*[11] The low solubility of the oxime VI in methanol or ethanol precluded the use



IV VI



VII

of catalytic hydrogenation in these solvents and in the presence of hydrazine, a method which has shown good stereoselectivity. When tetrahydrofuran was added, in order to increase the solubility of VI, no reduction was obtained. However, hydrogenation of VI in acetic acid, using palladium as catalyst, was rapid and produced the α-D-glucosaminide in good yield. Acetylation of the reaction mixture and chromatography on silicic acid afforded the major component as a chromatographically pure syrup, identified by NMR as fully acetylated VII. *O*-Deacetylation of this product yielded VII, which was purified by gel filtration. The yield of crystalline VII, m.p. 240 – 245 °C (dec.), $[\alpha]_{578}^{23}$ + 169°, starting from the oxime (VI) was 38 %. Attempts to find a solvent from which it could be recrystallized were unsuccessful.

Acid hydrolysis of VII yielded equimolar amounts of D-glucose and D-glucosamine, analysed by GLC of their corresponding alditol acetates. No D-mannosamine was observed. That VII is an α-D-glucosaminide is evident from its high specific optical rotation and from chemical shifts, δ 5.04 and 4.94, and coupling constants, $J_{1,2}$ 3,0 Hz and 2.5 Hz, respectively, of its two anomeric protons.

Although the route described above for the synthesis of α-D-glucosaminides involves several steps, it provides a feasible alternative to the method developed by Lemieux and coworkers, starting from the dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride.[11] Our method could also be applicable for the synthesis of β-D-mannosaminides.

EXPERIMENTAL

General methods were the same as previously reported,[3] except that NMR spectra were also recorded with a Varian XL 100 instrument. For spectrum determination in D_2O, 3-trimethylsilyl-1,1,2,2,3,3-hexadeuteriopropane-sulfonic acid sodium salt was used as internal standard.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-p-toluenesulfonyl-α-D-glucoside (I). Methyl 4,6-*O*-benzylidene-2-*O*-*p*-toluenesulfonyl-α-D-glucoside [7] (65 g) was dissolved in dimethylformamide (130 ml) and benzylated with benzyl bromide (36 ml) and silver oxide (36 g) with stirring in the dark at room temperature for 20 h. Methanol (30 ml) was added and the mixture stirred for another 2 h. The mixture was filtered through kieselguhr. The filtrate was concentrated and the resulting syrup was crystallized from ethanol to give I (62.5 g; 80 %). Recrystallization from ethanol yielded the pure substance, m.p. 121.5 – 123.5 °C, $[\alpha]_{578}^{23}$ + 2° (*c* 1.1, chloroform). (Found: C 63.8; H 5.80; S 6.21. $C_{28}H_{30}O_6S$ requires: C 63.9; H 5.74; S 6.09). NMR ($CDCl_3$, 100 MHz): δ 7.70 (2 H, d, $J_{H,H}$ 9.0 Hz, *p*-$MeC_6H_4SO_2$-O, other 2 H in other aromatics), 7.05 – 7.55 (12 H, m, other aromatics), 5.45 (1 H, s, benzylidene proton), 4.91 (1 H, d, $J_{1,2}$ 4 Hz, H-1), 3.35 (3 H, s, OCH_3), 2.31 (3 H, s, aryl methyl).

Methyl 3-O-benzyl-4,6-O-benzylidene-α-D-glucoside (II). A solution of I (30 g) and lithium aluminium hydride in diethyl ether (750 ml) was refluxed overnight. Excess hydride was decomposed by addition of ethyl acetate, ethanol and water. The organic layer was separated and the aqueous layer extracted with chloroform (500 ml). The combined organic phases were washed with saturated aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$) and evaporated to yield crystalline II (16.3 g; 82 %). Crystallization from ethanol afforded the pure substance, m.p. 183–185 °C. $[\alpha]_{578}^{23}$ +87° (*c* 0.9, chloroform). (Found: C 67.9; H 6.58 $C_{21}H_{24}O_6$ requires: C 67.7; H 6.50). NMR ($CDCl_3$, 100 MHz): δ 7.10–7.55 (10 H, m, aromatics), 5.51 (1 H, s, benzylidene proton), 4.74 (1 H, d, $J_{1,2}$ 3 Hz, H-1), 3.39 (3 H, s, OCH_3).

Methyl 2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannosyl)-3-O-benzyl-4,6-O-benzylidene-α-D-glucoside (III). *II* (6.8 g) and 3,4,6-tri-*O*-benzyl-1,2-*O*-methylorthoacetyl-β-D-mannose [2] (7.4 g) were dissolved in nitromethane (180 ml), mercuric bromide (0.62 g) was added and the mixture was distilled. The volume was kept constant by addition of nitromethane. After 3 h, when TLC indicated a complete reaction, the solvent was evaporated. Preparative TLC (silica gel, ethyl acetate-toluene, 1:4) yielded III (2.5 g; 20 %) as a homogeneous syrup (R_F = 0.44), having $[\alpha]_{578}^{22}$ +16° (*c* 2.2, chloroform). NMR ($CDCl_3$, 100 MHz): δ 7.00–7.60 (25 H, m, aromatics), 5.50 (1 H, s, benzylidene proton), 3.43 (3 H, s, OCH_3), 2.13 (3 H, s, $OCOCH_3$).

Methyl 3-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannosyl)-4,6-O-benzylidene-α-D-glucoside (IV). III (1.37 g) was dissolved in 7 % methanolic ammonia (30 ml) and was left at room temperature for 72 h. After concentration the product was purified by preparative TLC (silica gel, ethyl acetate-light petroleum, 2:3) to yield IV (0.98 g, 75 %) as a homogeneous syrup (R_F = 0.23), $[\alpha]_{578}^{23}$ +31° (*c* 1.4, chloroform). NMR ($CDCl_3$, 60 MHz): δ 7.00–7.50 (25 H, m, aromatics), 5.51 (1 H, s, benzylidene proton); 5.05 (1 H, s, $J_{1,2}$ < 1 Hz, D-mannosyl residue), 4.93 (1 H, d, $J_{1,2}$ 2 Hz, H-1 D-glucosyl residue), 3.42 (3 H, s, OCH_3).

Methyl 3-O-benzyl-(3,4,6-tri-O-benzyl-α-D-arabino-hexoside-2-ulose)-4,6-O-benzylidene-α-D-glucoside (V). IV (0.91 g) was dissolved in a mixture of dimethyl sulfoxide (90 ml) and acetic anhydride (4.5 ml). The solution was kept under nitrogen at room temperature for 2 days and the reagents were removed by lyophilization. The crude product was used without purification in the following step. IR (KBr) showed, *inter alia*, a strong absorption at 1740 cm^{-1} (C=O).

Methyl 3-O-benzyl-(3,4,6-tri-O-benzyl-2-oximino-α-D-arabino-hexosyl)-4,6-O-benzylidene-α-D-glucoside (VI). V (from 0.91 g of IV) was dissolved in tetrahydrofuran (11.5 ml) and methanol (3 ml). Hydroxylamine hydrochloride (0.36 g) in water (2.25 ml) and pyridine (2.25 ml) were added. After 7 h at room temperature, the solvents were evaporated and the residue suspended in toluene (125 ml). The organic phase was washed with water (100 ml), 10 % sulfuric acid (2 × 50 ml), saturated aqueous sodium hydrogen carbonate (2 × 50 ml) and water (50 ml), dried over magnesium sulfate and evaporated. The crude product was purified by preparative TLC (silica gel, ethyl acetate-light petroleum, 2:3) to yield VI (0.75 g; 82 %). Crystallization from methanol yielded the pure substance, m.p. 164.5–166 °C, $[\alpha]_{578}^{23}$ +44° (*c* 0.6, chloroform). (Found: C 70.3; H 6.22; N 1.80. Calc. for $C_{48}H_{51}O_{11}N$: C 70.5; H 6.29; N 1.71). NMR ($CDCl_3$, 100 MHz): δ 8.54 (1 H, s, oxime hydroxyl), 7.00–7.60 (25 H, m, aromatics), 6.10 (1 H, s, H-1 oximated residue), 5.51 (1 H, s, benzylidene proton), 3.35 (3 H, s, OCH_3).

Methyl 2-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside (VII). The oxime VI (0.65 g) was dissolved in glacial acetic acid (80 ml) and hydrogenated at atmospheric pressure over 10 % palladium on charcoal (1.30 g). After processing, a ninhydrine-positive product was obtained. Acetylation with acetic anhydride (5 ml) and pyridine (8 ml) yielded a crude product (0.52 g) which on TLC (silica gel, methanol-chloroform, 5:95) showed one major (R_F = 0.45) and several minor, faster moving components. The major component (0.31 g) was isolated by preparative TLC, and showed an NMR-spectrum compatible with peracetylated VII. This product was *O*-deacetylated by treatment with 5 % methanolic ammonia (8 ml) for 17 h, to yield crude VII (0.16 g). After gel filtration (Sephadex G-15, water), pure VII (0.12 g, 38 %) was obtained as a slowly crystallizing syrup, which could not be recrystallized. M.p. 240–245 °C (dec.), $[\alpha]_{578}^{23}$ +169° (*c* 0.7, water). NMR (D_2O, 100 MHz): δ 5.04 (1 H, d, $J_{1,2}$ 3 Hz, anomeric proton), 4.94 (1 H, d, $J_{1,2}$ 2.5 Hz, anomeric proton), 3.41 (3 H, s, OCH_3), 2.06 (3 H, s, $N\text{-}COCH_3$).

Sugar analysis of VII. The disaccharide (5 mg) was hydrolysed first with 90 % formic acid (100 °C, 2 h) and then with 0.25 M sulfuric acid (100 °C, 15 h), neutralized ($BaCO_3$) and the sugars were transformed into their alditol acetates by reduction (sodium borohydride) and acetylation (acetic anhydride-pyridine, 1:1). The mixture was analysed by GLC.[12]

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Short Communications

New Syntheses of Indazole and 2-Substituted Indazoles

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The chemistry of indazole and its derivatives has been reviewed by Behr.[1] Popular synthetic methods include the ring closure of *ortho* disubstituted benzene derivatives.

We have lately been interested in the reactions of *ortho* substituted nitroarenes.[2] One such compound, *o*-nitrobenzonitrile seemed to be a possible starting material for the synthesis of indazole by a reductive cyclisation.

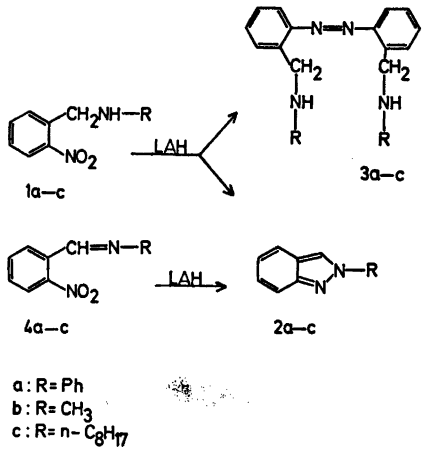
Both nitro groups and cyano groups may be reduced by a variety of reagents, *e.g.* metals or complex metal hydrides. A stepwise reduction of both the nitro group and cyano group might give intermediates able to cyclise to indazole or one of its precursors. Reduction by complex metal hydrides was judged to be the more convenient method.

By reduction of *o*-nitrobenzonitrile with lithium aluminium hydride (LAH), indazole was indeed formed in *ca.* 40 % yield. This yield was obtained when the reduction was performed in refluxing diethyl ether or tetrahydrofuran. Reductions at lower temperatures, down to − 50 °C, gave less indazole and increased yields of by-products. Sodium borohydride reduction of *o*-nitrobenzonitrile did not give indazole.

Catalytic hydrogenation of cyano groups usually demands elevated pressure,[3] and hydrogenation (1 atm., Pd/C) of *o*-nitrobenzonitrile gave only *o*-aminobenzonitrile as product.

o-Aminobenzonitrile did not give indazole on reaction with butyl lithium or LAH. This indicated that the formation of indazole from *o*-nitrobenzonitrile did not proceed *via* this compound.

In an attempt to synthesise 2-substituted indazoles and also to obtain information on the reaction path of the reduction of *o*-nitrobenzonitrile, LAH reduction of *N*-substituted *o*-nitrobenzylamines (*i.e.* *1a*, *1b* and *1c*) and of *N*-substituted *o*-nitrobenzylidenamines (*i.e.* *4a*, *4b* and *4c*) was studied. Moderate (50 %) or poor yields have been reported on tin reductions of *N*-aryl-*o*-nitrobenzylamines, the yields depending on the substituents in the *N*-aryl ring.[1]



LAH reductions of three *N*-substituted *o*-nitrobenzylamines (*1a*, *1b* and *1c*, made from *o*-nitrobenzyl chloride and the corresponding amines) gave varying results. The aniline derivatives (*1a*) gave a low yield (31 %) of 2-phenylindazole (*2a*) together with *o,o'*-di-(*N*-phenylaminomethyl)azobenzene (*3a*) (37 %).

N-Methyl-*o*-nitrobenzylamine (*1b*) and *N*-octyl-*o*-nitrobenzylamine (*1c*) did not give any 2-alkylindazoles. The only isolated substance was *o,o'*-di-(*N*-methylaminomethyl)azobenzene (*3b*) (63 %) from the reduction of *1b*. These attempts to synthesis 2-substituted indazoles from the corresponding *N*-substituted *o*-nitrobenzylamines thus met with only limited success.

The LAH reduction of the three *N*-substituted *o*-nitrobenzylidenamines (*4a*, *4b* and *4c*, made from *o*-nitrobenzaldehyde and the corresponding amines) [4] gave rather better results. The products from all three imines were the corresponding 2-substituted indazoles: 2-phenylindazole (*2a*) (74 %), 2-methylindazole (*2b*) (71 %) and 2-octylindazole (*2c*) (74 %).

The indazoles *2a* [5–7] and *2b* [8,9], were identified by comparison with authentic samples. Compound *2c* was identified from its spectral properties which were closely related to those of *2b*. No 1-substituted indazoles were found from these reactions, however, low yields of these cannot be excluded.

An attempt to obtain indazole itself by LAH reduction of *o*-nitrobenzylidenamine (in an equilibrium mixture with *o*-nitrobenzaldehyde and ammonia) was unsuccessful. Neither did

catalytic hydrogenation of *o*-nitrobenzylideneaniline give any 2-phenylindazole.

The poor results from the reduction of the *o*-nitrobenzylamines *1* and the ready formation of the indazoles *2* from the *o*-nitrobenzylideneamines *4* may indicate an imine to be an important intermediate in the reductive cyclisation of *o*-nitrobenzonitrile to indazole.

The described reactions offer new syntheses of indazole and 2-substituted indazoles from readily available starting materials.[4] It may also be possible to use such reactions for the formation of further substituted indazoles.

Experimental. The general instrumentation has been described.[2] NMR signals are given in δ values. The products described were identified by comparison with authentic samples or by their spectroscopic properties.

Syntheses of 2-substituted indazoles (2). The imine *4* was dissolved in diethyl ether (200 ml) and dripped into a refluxing suspension of LAH (2 g) in diethyl ether (200 ml) during 40 min. After 1 h continued reflux, the product was obtained by hydrolysis of the reduction mixture followed by chromatography (dry column, silica gel, chloroform). 2-Phenylindazole (*2a*) (74 % yield) had m.p. 82 – 82.5 °C (lit.[5] 83 – 84 °C) and gave a picrate with m.p. 91 °C (lit.[6,7] 93 – 94 °C). 2-Methylindazole (*2b*) (71 % yield) had m.p. 53 °C (lit.[8] 56 °C). UV (EtOH):[9] λ_{max} 268 nm, ε_{max} 590 m^2 mol^{-1}; λ_{max} 288, ε_{max} 550. IR: 3100, 3050, 2950, 1630, 1520, 1390, 1300, 1160;, 1010, 910, 820, 790, 760 cm^{-1}. NMR ($CDCl_3$): δ 3.84 (3 H, s), 6.4 – 7.8 (5 H, m). 2-Octylindazole (*2c*) (70 % yield). UV (EtOH): λ_{max} 268 nm, ε_{max} 700 m^2 mol^{-1}; λ_{max} 286, ε_{max} 660. IR: 3050, 2950, 1660, 1620, 1500, 1460, 1150, 750 cm^{-1}. NMR ($CDCl_3$): δ 0.85 (3 H, t), 1.0 – 1.3 (10 H), 1.9 (2 H m), 4.2 (2 H, t), 6.8 – 7.8 (5 H, m), MS: *m/e* 230 (58 %, $C_{15}H_{22}N$).

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Adenylyl Cyclase in Isolated Plasma Membranes of Granulation Tissue

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In an earlier paper [1] we have reported on the activities of 5′-nucleotidase (EC 3.1.3.5), Na^+,-K^+-activated Mg^{2+}-dependent adenosine triphosphatase (EC 3.6.1.3) and leucine-β-naphthylamidase (EC 3.4.11.1) in the plasma membranes isolated from experimental granulation tissue and then incubated in various conditions. The purpose of this note is to extend the observations to adenylyl cyclase (EC 4.6.1.1).

The purification of the adenylyl cyclase activity in the plasma membranes was of the same magnitude (18 ×) as calculated from the activities of 5′-nucleotidase, Na^+,K^+-activated Mg^{2+}-dependent adenosine triphosphatase and leucine-β-naphthylamidase [2] and slightly higher in the preparations from mature (3 week) granuloma than from proliferating (1 week) tissue (Table 1). Adenylyl cyclase is relatively insoluble;[3] only about 10 % of the total activity could be solubilized from the membranes with Lubrol WX.[4] This is in agreement with the earlier observations.

Cyclic AMP (10^{-3} – 10^{-6} M) stimulated by 15 – 30 % the activity of 5′-nucleotidase [5] of the plasma membrane both in incubated slices and isolated membrane preparations. Neuraminidase (EC 3.2.1.18) (12.5 U/sample) inhibited the activity of adenylyl cyclase in plasma membranes by 20 – 30 %, whereas hyaluronidase (EC 4.2.99.1) and collagenase (EC 3.4.4.19) were without any effect. Serotonin stimulated the

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[a] "Light" plasma membranes. [b] "Heavy" plasma membranes (the bulk).

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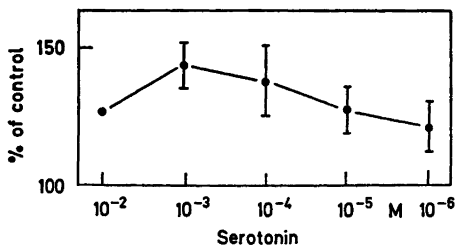


Fig. 1. Effect of added serotonin on the activity of adenylyl cyclase in incubated plasma membranes. For the details see the text. The means ± S.E.M. ($n = 5-8$, except $n = 2$ at 10^{-2} M) are given.

activity of adenylyl cyclase in incubated isolated plasma membranes (Fig. 1). The combined action of PGE_1 (15 μg/sample) and serotonin (10^{-3} M) was about 2-fold the straight summation. All the other additions mentioned in Table 1 of the earlier paper [1] were found to have no effect on the activity of adenylyl cyclase.

It is relevant that cyclic AMP is known to increase the synthetic functions in connective tissue [6-8] and that serotonin has been claimed to stimulate connective tissue both to proliferate [9] and to synthetize collagen.[10]

Experimental. The production of experimental granulation tissue by the subcutaneous implantation of viscose-cellulose sponges has been described previously [11] as have the details of the preparation of plasma membranes.[1,2]

For the assay of adenylyl cyclase, 150 μl of plasma membrane suspension were added to a substrate solution containing 1 mM unlabelled ATP, 45 pM ATP-^{14}C (25 μCi or about 3×10^4 cpm), 10 mM NaF, 10 mM theophylline, 3.3 mM $MgSO_4.7H_2O$ dissolved in 40 mM tris-HCl buffer, pH 7.3, to make a final volume of 600 μl. The temperature was 30 °C and the reaction time 10 min. When the effects of the additions were being studied, the added substances were preincubated with the plasma membrane preparation for 60 min. The enzyme action was stopped by immersing the tube into a boiling-water bath for 3 min and then cooling it in ice-cold water. The precipitated protein was removed by centrifugation (10 000 rpm, 10 min) and the supernatant analyzed for cAMP according to Krishna *et al.*[12] The sample was fractionated on Dowex 1-x8 (200/400 mesh, Cl^--form)-columns (0.6 × 3.5 cm). The ATP and other impurities in the sample were first eluted with 20 ml of water and then cAMP with 0.2 N formic acid. For the assay of radioactivity 200 μl of the sample were mixed with 10 ml of hydrophilic scintillation fluid [13] and counted with a Packard Model 3320 Liquid Scintillation Spectrometer. Protein was determined according to the method given by Lowry *et al.*[14] and its concentration in the sample was usually adjusted to 333 μg/ml.

Special reagents were obtained from various sources: cAMP, Fluka (Buchs, Switzerland); ATP, Fluka, or E. Merck (Darmstadt, Germany); AMP E. Merck; theophylline, Sigma (St. Louis, Mo. USA); serotonin Fluka; prostaglandin PGE_1, Upjohn; neuraminidase from *Vibrio cholerae*, 500 U/ml, Behringwerke (Marburg/Lahn, Germany), Code ORKD; adenosine-^{14}C (U)-5′-triphosphate CFB. 91 and adenosine/base-^{14}C(U)-3′,5′-cyclic phosphate, CFA. 442 from The Radiochemical Centre, Amersham, Bucks., U.K.

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Heterocylic Compounds from Malonyl Chlorides. V.* Further Investigation on Cyclization of 2-Alkylthiopyrimidones

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Malonyl chlorides with nitriles or thiocyanates give pyridines and pyrimidines.[1-3] The present work deals with further reactions of some of these products. 2-(2-Bromoethylthio)- and 2-(3-bromopropylthio)-4,5-dichloropyrimidin-6-one (*1* and *2*) have been shown to cyclize in dimethylformamide to the corresponding compounds *4* with the *ortho*-quinoid structures ($\nu_{C=O}$ 1675–1686 cm^{-1}).[4] In polar nonbasic solvents, however, they gave quaternized products which could be dehydrobrominated to compounds *5* with the *para*-quinoid structures ($\nu_{C=O}$ 1638 cm^{-1}).[4]

The utilization of a stronger base in the cyclization process would probably lead to removal of the proton before the ring closure and by-pass the quaternized intermediates. Ring closure ought to be possible also with other functional groups than bromide in the alkyl chain. When the pyrimidine *1*, *2*, or *3* was treated with diethylamine at different temperatures products were isolated according to Table 1.

The pyrimidine *3* at room temperature gave the ammonium salt *6* which must be an intermediate in the cyclization process since heating the mixture at 56 °C resulted in the pyrimidothiazine *4* ($n=3$). The reactivity was significantly increased by ionization. In most cases N(1) represented the attacking center; at 22 °C, however, the thiazolopyrimidine product appeared as a mixture of the isomeric compounds *4* and *5* in a ratio of 8:1.

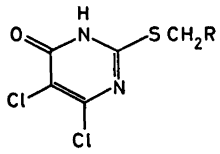
Table 1. Products from reaction of the pyrimidines *1*, *2*, and *3* in diethylamine at different temperatures.

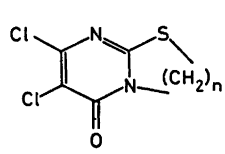
Pyrimidine	Temp. [a] °C	Products	Yields %
3	22 [b]	*6*	95
3	56 [c]	*4* ($n=3$)	60
2	0 [d]	*7*	88
2	22 and 56 [b]	*4* ($n=3$)	81
1	0 [b]	*4* ($n=2$)	90
1	22 [b]	*4* ($n=2$)	80
		5 ($n=2$)	10

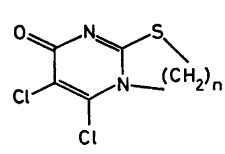
[a] Also the temperature of the reactants before mixing. [b] Instantaneous reaction. [c] Reaction time 6 h. [d] Reaction time 2 days.

The condensation to the 5-membered ring proceeded more easily than to the 6-membered ring in agreement with earlier findings.[4] At 0 °C the thiazolopyrimidine *4* ($n=2$) was formed from the pyrimidine *1* whereas no pyrimidothiazine *4* ($n=3$) was produced from compound *2* at this temperature. In the latter case reaction with the solvent gave the zwitterion *7* while at 22 and 56 °C only the pyrimidothiazine *4* ($n=3$) was formed.

Several attempts were made to cyclize the esters *8* ($n=0$, 1, and 2)[3] without success. The



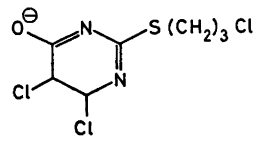


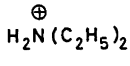


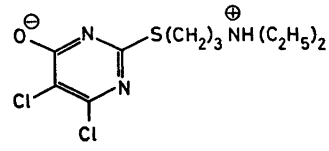
1 R = CH_2Br *8* R = $(CH_2)_nCOOC_2H_5$ *4*

2 R = $(CH_2)_2Br$ *9* R = $(CH_2)_nCOOH$

3 R = $(CH_2)_2Cl$



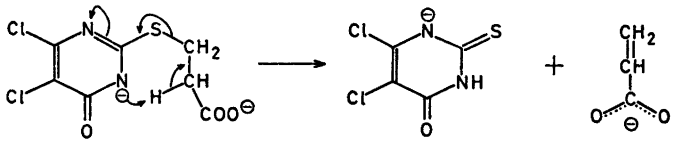




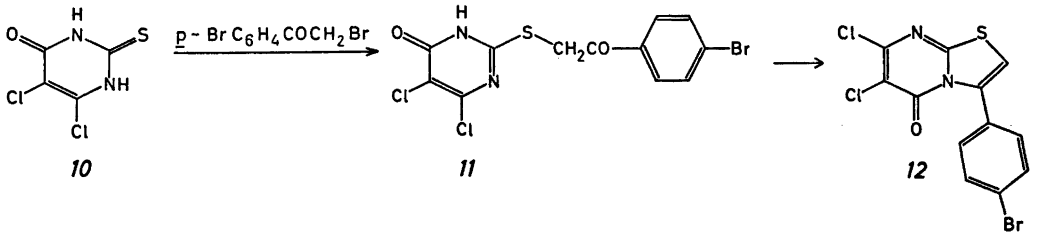
6 *7*

Scheme 1.

* Part IV: Hegrand, R., Stensrud, T. and Bernatek, E. *Medd. Norsk Farm. Selskap 32* (1971) 37.



Scheme 2.



Scheme 3.

three esters were therefore hydrolyzed under basic conditions. Hydrolysis of the ester *8* ($n=1$) with 1.5 – 2 equiv. of sodium hydroxide gave the acid *9* ($n=1$) as the sole product; with 2.25 – 3 equiv. the product was a mixture of the acid and 5,6-dichloro-2-thiouracil (*10*); larger quantities of sodium hydroxide gave the thiouracil *10* exclusively. The acid *9* ($n=1$) treated as the ester *8* ($n=1$) gave the same amount of the thiouracil *10*. The base abstracts both the carboxylic and the ring proton which leaves the nitrogens with higher electron density. The thione is formed with elimination of acrylic acid which is found in the filtrate.

The hydrolysis of the esters *8* ($n=0$ and 2) stopped at the acid stage even with a large excess of the hydroxide. In these cases no conjugative stabilized species such as acrylic acid could be eliminated. No cyclization of the acids *9* ($n=0$, 1, and 2) was observed with dicyclohexylcarbodiimide, acetic anhydride, or thionyl chloride.

p-Bromophenacyl bromide reacted with the thiouracil *10* to the pyrimidine *11*. This was cyclized in concentrated sulfuric acid to 3-*p*-bromophenyl-6,7-dichloro-5*H*-thiazolo[3,2-*a*]-pyrimidin-5-one (*12*).

Experimental. Melting points were determined on a micro hot-stage. IR spectra were recorded on a Perkin-Elmer 457 Grating Infrared Spectrophotometer (in KBr), NMR spectra on Varian A-60 A and HA 100 Spectrometers in DMSO-d_6 with TMS as an internal standard and the mass spectra on an AEI/EC MS 902 instrument. Elemental analyses were performed by I. Beetz, West Germany and were correct within 0.5 %.

Reaction of the pyrimidines 1, 2, *and* 3 *in diethylamine. (Table 1). General procedure.* The pyrimidine (0.5 g) was dissolved in diethylamine (10 ml) at the reaction temperature. Compound *5* ($n=2$) was insoluble in diethylamine and was separated from the isomeric compound *4* ($n=2$) by filtration. The compounds *4* ($n=2$ and 3) and *5* ($n=2$) were identified by comparison with authentic samples,[4] mixed m.p. and IR spectra.

4,5-Dichloro-2-(3-diethylaminopropylthio)-pyrimidin-6-one (7). M.p. 207 – 208 °C (from dioxane), NMR (TFA): δ 1.46 (t, J 7 Hz, CH_3), 2.20 – 2.55 and 3.06 – 3.78 (m, CH_2). The IR spectrum had no absorption due to carbonyl and the X-ray diffraction pattern revealed the zwitterion structure.[5]

5,6-Dichloro-2-thiouracil (10). The ester *8* ($n=1$) (3.0 g, 10 mmol) was refluxed in sodium hydroxide solution (40 ml, 10 %) for 1 h, acidified to pH = 1 and cooled. The product *10* (1.8 g, 90 %) was filtered off, m.p. 236 – 238 °C. (The filtrate was kept). IR: 3500, 3390, 1670 cm^{-1}. MS: *m/e* 196 (M^+) with two chlorine atoms.

Acrylic acid was isolated from the filtrate from above and identified by comparison with an authentic sample, mixed m.p. and IR spectrum.

2-(2-Carboxylethylthio)-4,5-dichloropyrimidin-6-one (9, n = *1).* The ester *8* ($n=1$) (3.0 g, 10 mmol) was refluxed in sodium hydroxide solution (15 ml, 4 %) for 1 h, acidified to pH = 1, cooled and filtered to give compound *9* ($n=1$) (1.9 g, 70 %), m.p. 205 – 207 °C (from acetic acid/water). NMR: δ 2.70 (t), 3.28 (t); J 6.5 Hz. IR: 1705 and 1650 cm^{-1}. MS: *m/e* 268 (M^+) with two chlorine atoms.

2-(p-Bromophenacylthio)-4,5-dichloropyrimidin-6-one (11). A solution of the thiouracil *10* (1.0 g, 5 mmol) and *p*-bromophenacyl bromide (1.4 g, 5 mmol) in acetone (50 ml) was refluxed for 3 h. The acetone was removed to give compound *11* (1.2 g, 60 %) m.p. 232 – 235 °C (from acetic acid/water), sublimation started at 210 °C. NMR: δ 4.80 (s, CH_2), 7.44 (d) and 7.96

(d, J 8.5 Hz, aromatic H). IR: 1690 and 1655 cm^{-1}. MS: m/e 392 (M^+) with one bromine and two chlorine atoms.

3-p-Bromophenyl-6,7-dichloro-5H-thiazolo-[3,2-a]pyrimidin-5-one (12). A solution of the pyrimidine *11* (0.5 g) in concentrated sulfuric acid (7.5 ml) was kept at room temperature for 20 min and then cautiously poured into ether (10 ml). Solid sodium bicarbonate was added together with some water until pH = 6 – 7. The separated compound *12* was filtered off (0.35 g, 72 %) m.p. 204 – 206 °C (from acetic acid/water). NMR: δ 7.51 (s, ethylenic H), 7.30 (d) and 7.55 (d, J 8.5 Hz, aromatic H). IR: 1691 cm^{-1}. MS: m/e 374 (M^+) with one bromine and two chlorine atoms.

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