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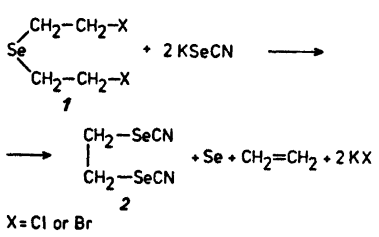
Synthesis of Aryl 2-Haloethyl Selenides and their Reactions with Potassium Selenocyanate

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A number of selenide dihalides, such as 2-bromoethyl phenyl selenide dibromide (*3*), 2-chloroethyl phenyl selenide dichloride (*4*) and 2-bromoethyl 4′-tolyl selenide dibromide (*5*), have been synthesized. Two of these dihalides (*3* and *5*) have been dehalogenated to give the corresponding selenides, 2-bromoethyl phenyl selenide (*6*) and 2-bromoethyl 4′-tolyl selenide (*7*), respectively. 2-Bromoethyl 2′,4′-dinitrophenyl selenide (*10*) has also been prepared, but by another synthetic route. These 2-halogen substituted selenides have been allowed to react with potassium selenocyanate giving mixtures of normal and abnormal substitution products. A mechanism is presented for the reaction between aryl 2-haloethyl selenides and potassium selenocyanate.

The reaction of bis(2-bromoethyl) selenide (*1*; X = Br) and its chlorine analogue with potassium selenocyanate has been studied earlier (Scheme 1).[1]



Scheme 1.

The question arose as to whether this reaction is specific for the bis(2-haloethyl) selenides (*1*), or if it could be generalized to systems of analogous structure. In order to investigate this a number of aryl 2-haloethyl selenides were synthesized and their reactions with potassium selenocyanate studied.

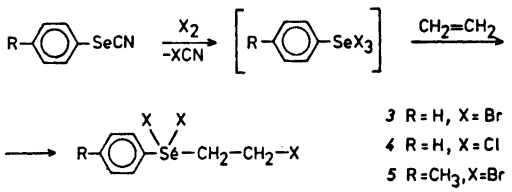
Alkyl or aryl 2-halogen substituted selenides are most conveniently synthesized by adding olefins to alkyl- or arylselenenyl halides.[2–4] In all the cases mentioned above the selenenyl halides were synthesized in advance and then allowed to react with the olefin. The *in situ* generation of selenenyl bromides and addition of olefins to the reaction mixture, however, seemed to be a more rational method.[5]

RESULTS AND DISCUSSION

In accordance with the method mentioned above, efforts to generate selenenyl bromides *in situ* from a selenocyanate and bromine were made.[6] The product isolated when ethylene was passed through the solution, however, consisted of a selenide dibromide and unreacted selenocyanate.

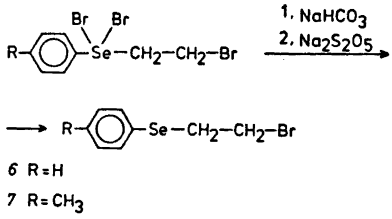
The direct preparation of aryl 2-haloethyl selenides according to the above method was thus not possible. On the other hand it was feasible to synthesize aryl 2-haloethyl selenide dihalides by the *in situ* generation of arylselenium trihalides and passing ethylene through the reaction mixture until completion of the reaction. 2-Bromoethyl phenyl selenide dibromide (*3*), 2-chloroethyl phenyl selenide dichloride (*4*) and 2-bromoethyl 4′-tolyl selenide dibromide (*5*) were prepared in this way (Scheme 2).

No reaction occurred when negatively substituted aryl selenocyanates, such as 2-nitrophenyl selenocyanate and 2,4-dinitrophenyl selenocyanate, were used as substrates.[6] 2,4-Dinitrophenylselenium trichloride has, however, been found to add to other olefinic substrates.[7,8]



Scheme 2.

The dihalides were dehalogenated by dissolving them in an aqueous sodium hydrogen carbonate solution and reducing the generated selenoxides with sodium pyrosulfite.[9,10] 2-Bromoethyl phenyl selenide (*6*) and 2-bromoethyl 4′-tolyl selenide (*7*) were prepared in this way (Scheme 3).



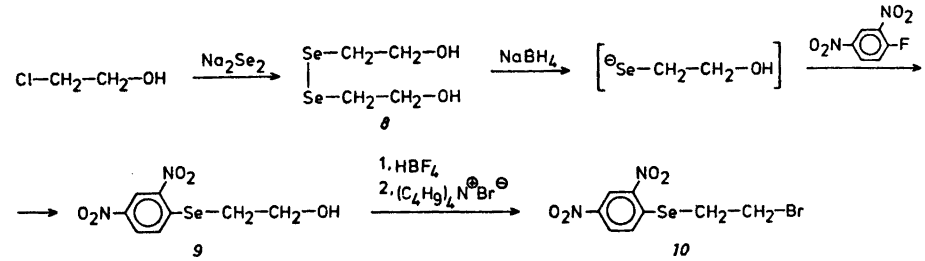
Scheme 3.

The negatively substituted selenide 2-bromoethyl 2′,4′-dinitrophenyl selenide (*10*) was synthesized by a reaction sequence starting from 2-chloroethanol and 1-fluoro-2,4-dinitrobenzene. Bis(2-hydroxyethyl) diselenide (*8*) was prepared from 2-chloroethanol and sodium diselenide. The diselenide was reduced with sodium borohydride in ethanol and to this reaction mixture, 1-fluoro-2,4-dinitrobenzene was added. 2-Hydroxyethyl 2′,4′-dinitrophenyl selenide (*9*) was isolated. The alcohol was treated with fluoboric acid and then allowed to react with tetrabutylammonium bromide in dry chloroform to give 2-bromoethyl 2′,4′-dinitrophenyl selenide (*10*) (Scheme 4).

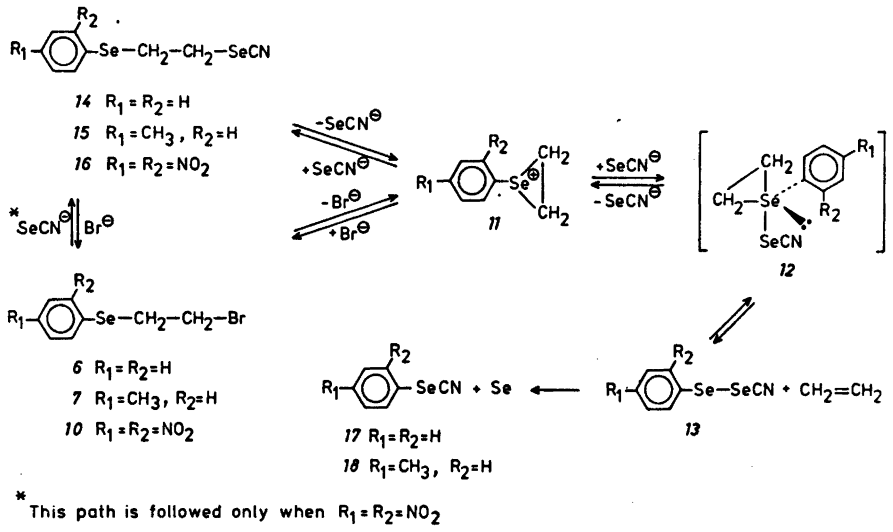
When 2-bromoethyl phenyl selenide (*6*) and 2-bromoethyl 4′-tolyl selenide (*7*) were allowed to react with potassium selenocyanate, using acetone as solvent, phenyl selenocyanate (54 %) and 4-tolyl selenocyanate (69 %), respectively, were isolated. The reaction between *6* and potassium selenocyanate was followed by ^{1}H NMR spectroscopy using acetone-d_6 as solvent. The original A_2B_2 spectrum, centred around a δ value of 3.48 and corresponding to the two methylene groups of the starting material, rapidly disappeared when potassium selenocyanate was added to the NMR tube. Two singlets at δ 5.33 and 3.41 were formed during the reaction. The singlet at δ 5.33 corresponded to ethylene.

The crude product from the reaction between *6* and potassium selenocyanate was also studied by mass spectroscopy. A peak was obtained at *m/e* 183 corresponding to phenyl selenocyanate as well as a peak at *m/e* 291 originating from the molecular ion of the normal substitution product phenyl 2-selenocyanatoethyl selenide (*14*).

The ^{1}H NMR spectrum of *14* should give rise to an A_2B_2 spectrum corresponding to the two non-equivalent methylene groups. From investigations on A_2B_2 spectra, however, it is possible that these can appear as singlets in certain solvents.[11] The singlet at δ 3.41 might thus originate from the two methylene groups of the normal substitution product. Alternatively, the singlet at δ 3.41 could correspond to the two methylene groups of the episelenurane (*12*) (Scheme 5), which may be proposed as an intermediate when a selenocyanate ion attacks the positive selenium atom of the episelenonium ion (*11*).[4,12] During the reaction the intensity of the singlet increased but on completion its intensity remained unchanged. From this and the fact that phenyl seleno-



Scheme 4.



Scheme 5.

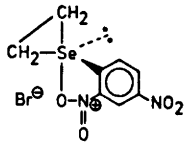
cyanate was isolated as the end product, the peak could not correspond to an intermediately formed episelenurane (*12*). From the ^{1}H NMR spectrum of the raw product it was obvious that the formation of the normal substitution product could not constitute more than 30 % of the complete reaction.

The mechanistic interpretations of the above results are all in accordance with the proposed mechanism for the reaction between bis(2-bromoethyl) selenide (*1*; X = Br) and potassium selenocyanate (Scheme 5).[1]

The reaction between 2-bromoethyl 4′-tolyl selenide (*7*) and potassium selenocyanate was also studied by ^{1}H NMR spectroscopy using acetone-d_6 as solvent. It was found that *7* behaved similarly to *6*. The aliphatic region of the ^{1}H NMR spectrum of *7* consisted of an A_2B_2 spectrum centred around a value of δ 3.43 and a singlet at δ 2.29 corresponding to the methyl group. When potassium selenocyanate was added to the NMR tube containing the starting material, immediate formation of ethylene was observed. A singlet at δ 3.38 was obtained, corresponding to the A_2B_2 spectrum of the methylene groups of the normal substitution product 2-selenocyanatoethyl 4′-tolyl selenide (*15*). The appearance of the A_2B_2 spectrum as a singlet could be explained as above for compound *14*. Two singlets corresponding to methyl groups were also obtained. The singlet at δ 2.36 corresponds to the methyl group of 4-tolyl selenocyanate (*18*) and the singlet at δ 2.31 originates from the methyl group of the normal substitution product *15*.

2-Bromoethyl 2′,4′-dinitrophenyl selenide (*10*) was also used as starting material for the reaction with potassium selenocyanate. The reason for choosing *10* for this reaction was that intermediate *13*, corresponding to 2,4-dinitrobenzeneselenenyl selenocyanate, would be stable if formed.[13] The motive for this experiment, was therefore that any attack on the positive selenium atom of the cyclic selenonium ion *11* would give *13* as the product and not the corresponding selenocyanate as in earlier reactions. The reaction between *10* and potassium selenocyanate was studied by ^{1}H NMR spectroscopy using acetone-d_6 as solvent. No ethylene was formed during the reaction. It was, however, obvious that a reaction took place from changes of the ^{1}H NMR spectrum. The product had an elemental composition corresponding to the normal substitution product *16*. An ^{1}H NMR spectrum of the product in acetone-d_6 showed a singlet at δ 3.71. By similar reasoning as before, this singlet could be attributed to an A_2B_2 spectrum. Furthermore, an episelenurane structure (*12*) for the product in the reaction between *10* and potassium selenocyanate could not be excluded. From a mechanistic point of view the forma-

tion of an episelenurane is, however, not very likely since the destabilizing effect of the two nitro groups on the episelenonium ion *11* makes preliminary formation of such a cyclic selenonium ion improbable. The most likely explanation is that starting material *10* is attacked on the β-carbon giving the normal substitution product 2',4'-dinitrophenyl 2-selenocyanatoethyl selenide (*16*) without preliminary formation of *11*. Alternatively, intermediate *11*, by interaction with the nitro group in the *ortho* position, may be represented as *19*, as has been proposed for analogous sulfur compounds.[14]



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The possibility of a nucleophilic attack on the selenium atom of *19* and the formation of an episelenurane is thereby reduced. The reactions of the bis(2-haloethyl) or aryl 2-haloethyl selenides mentioned here and potassium selenocyanate have thus been shown to give analogous products and the reactions most probably proceed *via* the same mechanism except for 2-bromoethyl 2',4'-dinitrophenyl selenide (*10*). When *10* is used as starting material, it is obvious that the introduction of nitro groups in the aromatic part of the molecule may exclude the formation of an episelenonium ion or change its appearance, which is accompanied by exclusive formation of the normal substitution product *16*.

EXPERIMENTAL

The selenium analyses were performed by a microanalytical method developed by Bengtsson[15] and very similar to that of Gould.[16] The melting points are uncorrected.

2-Bromoethyl phenyl selenide dibromide (3). To a solution of 36.4 g (0.2 mol) of phenyl selenocyanate in 80 ml of carbon tetrachloride 64 g (0.4 mol) of bromine in 80 ml of carbon tetrachloride was added dropwise. The reaction mixture was kept at room temperature for 30 min with stirring. Ethylene was passed into the reaction mixture until the solution had turned pale yellow and the evolution of heat had ceased. Evaporation of the reaction mixture left a yellow residue, which was washed with ether and recrystallized from carbon tetrachloride. Yield 74.0 g (87 %), m.p. 110–111.5 °C. (Found: C 22.66; H 2.07; Br 56.58; Se 18.65. Calc. for $C_8H_8Br_2Se$: C 22.67; H 2.14; Br 56.56; Se 18.63).

2-Bromoethyl 4'-tolyl selenide dibromide (5). The synthetic procedure was similar to that used for *3* described above, starting from a solution of 19.6 g (0.1 mol) of 4-tolyl selenocyanate in 40 ml of carbon tetrachloride. The crude product was recrystallized from carbon tetrachloride. Yield 21.2 g (48 %), m.p. 112–114.5 °C. (Found: C 24.67; H 2.54; Br 54.87; Se 18.08. Calc. for $C_9H_{11}Br_2Se$: C 24.68; H 2.54; Br 54.75; Se 18.03).

2-Chloroethyl phenyl selenide dichloride (4). A solution of 4.55 g (0.025 mol) of phenyl selenocyanate in 10 ml of chloroform was prepared. Chlorine was passed into the solution with stirring at room temperature. Evolution of heat occurred and a yellowish precipitate was obtained. After some minutes the reaction was complete. Ethylene was passed through the reaction mixture, heat was developed and the solid product slowly dissolved. When the evolution of heat had ceased, the reaction was considered complete and the solvent was evaporated giving a solid residue, which was washed with ether and recrystallized from carbon tetrachloride. Yield 4.3 g (59 %), m.p. 89–90.5 °C. (Found: C 32.87; H 3.18; Cl 36.50; Se 26.93. Calc. for $C_8H_9Cl_2Se$: C 33.08; H 3.13; Cl 36.61; Se 27.18).

2-Bromoethyl phenyl selenide (6). To a solution, heated to 40–50 °C, of 9.25 g (0.11 mol) of sodium hydrogen carbonate in 250 ml of water, 21.2 g (0.05 mol) of 2-bromoethyl phenyl selenide dibromide (*3*) was added. The substance dissolved with evolution of carbon dioxide. The reaction mixture was cooled to 0 °C and poured into a separatory funnel containing 75–100 g of ice. To this solution was added 250 ml of ice cold ether and a cold solution of 5.75 g (0.03 mol) of sodium pyrosulfite in 125 ml of water. When the pyrosulfite solution was added an instantaneous reaction took place. The ether layer was separated and the extraction was repeated. The combined extracts were washed with water and dried. The dried solvent was evaporated at reduced pressure and at a low temperature giving 12.1 g (92 %) of raw product. The product was recrystallized at −50 °C from light petroleum, m.p. 18–19 °C. (Found: C 36.34; H 3.41; Br 30.46; Se 29.97. Calc. for C_8H_9BrSe: C 36.39; H 3.44; Br 30.26; Se 29.90).

2-Bromoethyl 4'-tolyl selenide (7). The synthetic procedure was similar to that of *6* described above, starting from 21.9 g (0.05 mol) of 2-bromoethyl 4'-tolyl selenide dibromide (*5*). Yield 9.0 g (65 %) of raw product, which was recrystallized from light petroleum, m.p. 41–42 °C. (Found: C 38.89; H 4.01; Br 28.89; Se 28.43. Calc. for $C_9H_{11}BrSe$: C 38.87; H 4.00; Br 28.74; Se 28.40).

The reaction between 2-bromoethyl phenyl selenide (6) and potassium selenocyanate. To a solution of 5.2 g (0.02 mol) of *6* in 100 ml of acetone 3.2 g (0.022 mol) of potassium selenocyanate dissolved in 30 ml of acetone was added. The reaction mixture was refluxed for 2 h with stirring.

After filtering the mixture the solvent was evaporated leaving a residue. Distillation of the residue gave 1.95 g (54 %) of phenyl selenocyanate, b.p. 96–98 °C/3.0 mmHg, $n_D^{17.5}$ 1.6043 (verified by IR spectroscopy). Elemental selenium (0.8 g) was regained from the reaction mixture.

The reaction between 2-bromoethyl 4'-tolyl selenide (7) and potassium selenocyanate. To a solution of 2.8 g (0.01 mol) of *7* in 50 ml of acetone 1.6 g (0.011 mol) of potassium selenocyanate dissolved in 15 ml of acetone was added. After refluxing the mixture for 2 h, filtration and evaporation of the solvent, the residual product was recrystallized from light petroleum giving 1.35 g (69 %) of 4-tolyl selenocyanate, verified by ^{1}H NMR and IR spectroscopy. Elemental selenium (0.5 g) was regained from the reaction mixture.

Bis(2-hydroxyethyl) diselenide (8).[17] In a 1 l three-necked flask, equipped with a dropping funnel, reflux condenser and a gas inlet, 16 g (0.4 mol) of sodium hydroxide and 25 g (0.162 mol) of sodium formaldehydesulfoxylate were dissolved in 250 ml of water. To the solution 32 g (0.4 mol) of elemental selenium was added. The reaction was performed in a nitrogen atmosphere. When the selenium was completely reduced, 32.2 g (0.4 mol) of 2-chloroethanol was added. The reaction mixture was left at room temperature for 5 h with stirring. A heavy oil containing some elemental selenium was obtained. The oil was separated and dissolved in acetone. The water phase was extracted with chloroform and the extracts were combined with the acetone solution. After drying the obtained solution, the solvents were evaporated giving an orange-coloured oil. Distillation gave 19.5 g (39 %) of bis(2-hydroxyethyl) diselenide (*8*), b.p. 169–179 °C/1.6–4.0 mmHg, n_D^{25} 1.6412. Some decomposition of the product was obtained during the distillation, but the purity of the product was acceptable for further synthetic use.

2-Hydroxyethyl 2',4'-dinitrophenyl selenide (9). To an alcoholic solution of 12.4 g (0.05 mol) of bis(2-hydroxyethyl) diselenide (*8*) was added 1.0 g (0.025 mol) of sodium borohydride or sufficient so that the colour of the solution disappeared. Large excess of borohydride should be avoided. The reaction was performed in a nitrogen atmosphere. When the reaction had terminated, 20.0 g (0.105 mol) of 1-fluoro-2,4-dinitrobenzene in the least possible amount of ethanol was added to the mixture containing the reduced diselenide. The reaction mixture was allowed to stand at room temperature under stirring and in a nitrogen atmosphere overnight. After filtration the solvent was evaporated, and 16.2 g of a solid residue was obtained. The raw product was recrystallized from ethyl acetate/light petroleum. Yield 12.5 g (43 %), m.p. 104–105.5 °C. (Found: C 33.02; H 2.74; Se 27.35. Calc. for $C_8H_8N_2O_5Se$: C 33.00; H 2.78; Se 27.12).

2-Bromoethyl 2',4'-dinitrophenyl selenide (10). A suspension of 4.0 g (0.014 mol) of dry 2-hydroxyethyl 2',4'-dinitrophenyl selenide (*9*) in dry chloroform was prepared. The suspension was cooled to −40 °C and 15.0 g (0.09 mol) of a solution of fluoboric acid in ether (54 %) was added. A cooled solution of 5.3 g (0.016 mol) of tetrabutylammonium bromide in dry chloroform was added and the temperature of the reaction mixture was allowed to rise to room temperature. The reaction mixture was refluxed for 1 1/2 h and washed with an aqueous sodium hydrogen carbonate solution. The organic solution was dried over magnesium sulfate. The dry solvent was evaporated to give an oily residue which was extracted with ether. The ether extract was dried over magnesium sulfate and the solvent evaporated. A solid product was obtained which was recrystallized from ethyl acetate/light petroleum. Yield 1.8 g (36 %), m.p. 110.5–111.5 °C. (Found: C 27.34; H 1.99; Br 22.54; Se 22.34. Calc. for $C_8H_7BrN_2O_4Se$: C 27.14; H 2.00; Br 22.57; Se 22.30).

The reaction between 2-bromoethyl 2',4'-dinitrophenyl selenide (10) and potassium selenocyanate. To a solution of 1.05 g (0.003 mol) of *10* in 30 ml of acetone 0.48 g (0.0033 mol) of potassium selenocyanate dissolved in 15 ml of acetone was added. The reaction mixture was refluxed for 4 h and then filtered. The solvent was evaporated and a residue was obtained. The raw product was recrystallized from benzene/light petroleum. Yield 0.95 g (83 %), m.p. 123–125 °C. (Found: C 28.59; H 1.86; Se 41.65. Calc. for $C_9H_7N_3O_4Se_2$: C 28.51; H 1.86; Se 41.66). ^{1}H NMR (60 MHz, acetone-d_6): δ 3.71 (4 H, s), 8.17 (1 H, d, J 9.0), 8.49 (1 H, dd, J 9.0 and 2.5), 9.07 (1 H, d, J 2.5).

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Synthesis of D,L-Chalcose and D,L-Desosamine

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Cyclization of methyl 2-*cis*-5-hexadienoate (*1*) with polyphosphoric acid (PPA) gives parasorbic acid (*2*) which on epoxidation, acid catalyzed opening of the epoxide ring in refluxing methanol and reduction with diisobutylaluminium hydride (DIBAH), gives D,L-chalcose (*6*). Treatment of the epoxide *3* with aqueous dimethyl amine results in a mixture of the amino acid *8* and the amide *7*. The lactone *11* is reduced to D,L-desosamine (*9*) by DIBAH.

It seemed to us that methyl 2-*cis*-5-hexadienoate [1a] (*1*), a product of the Ni-catalyzed reaction of carbon monoxide, allyl chloride, and acetylene, could serve as a suitable starting material for a general synthesis of several hexoses. The *cis*-structure should enable a facile acid catalyzed ring closure to the δ-lactone parasorbic acid (*2*) and further hydroxylations, rearrangements, and reductions would then lead to sugar derivatives.

It turned out that cold conc. sulfuric acid [1b] or polyphosphoric acid (PPA) at 70 – 80 °C was a suitable reagent for accomplishing the cyclization. The epoxidation of *2* had been carried out earlier [2] but in our hands the method gave variable yields. Reliable yields of the epoxide *3* were obtained by epoxidation at *ca.* 20 °C with 1.9 equiv. of H_2O_2 in methanol using solid sodium bicarbonate as catalyst. By controlling reaction time and temperature the formation of the methyl ester *4*, which occurs as a side product, could be brought to a minimum. Only the *trans* isomer *3* was formed and the crude product was used without purification for the further reactions. Refluxing *3* in methanol with a small amount of *p*-toluenesulfonic acid opened the epoxide ring in the expected direction and afforded the lactone *5* in a good yield. Reduction with diisobutylaluminium hydride (DIBAH) gave D,L-chalcose *6*, Scheme 1. Lithium aluminium hydride or sodium bis[2-methoxyethoxy]aluminium hydride gave lower yields. The ^{1}H NMR spectrum of *6* in D_2O was identical to that published earlier.[3] Chalcose has been prepared earlier by several routes.[4–10]

Opening of the epoxide ring with dimethylamine was accomplished by heating *3* in an autoclave with anhydrous dimethylamine.[11] We found that this reaction could be carried out simply by letting *3* stand for 10 days in aqueous dimethylamine. A mixture of the amide *7* and the carboxylate *8* was obtained with the latter as major product which by treatment with hydrochloric acid gave the lactone *11*. This was then reduced by DIBAH to D,L-desosamine (*12*), the ^{1}H NMR spectrum of which was identical to that published by Woo *et al.*[12] (the scale of the published spectrum is shifted by *ca.* 0.45 ppm).

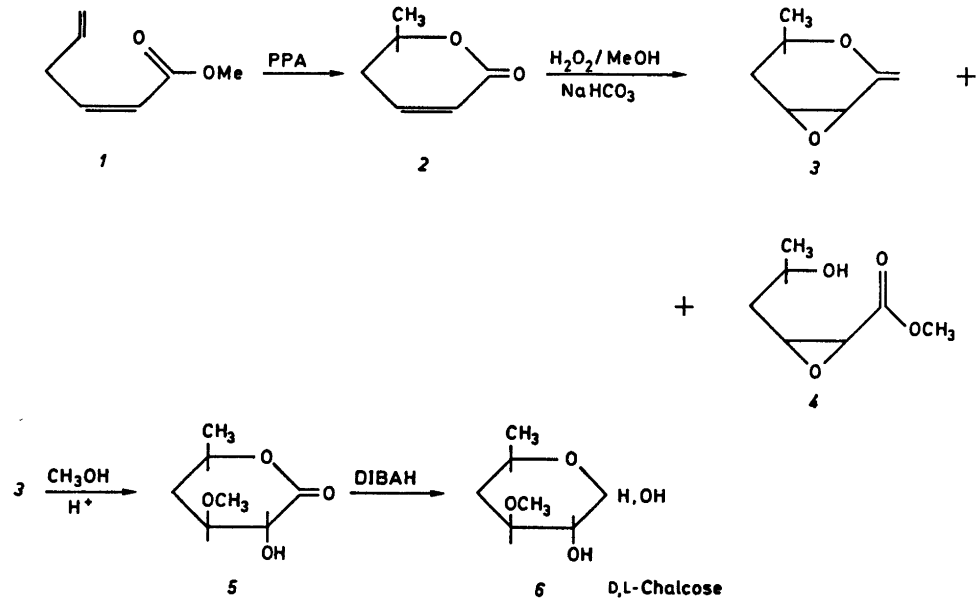
Treatment of *3* with concentrated ammonia gave the analogous amino compounds *9* and *10*.

Synthesis of desosamine has been accomplished earlier by several groups.[9,11,13–17]

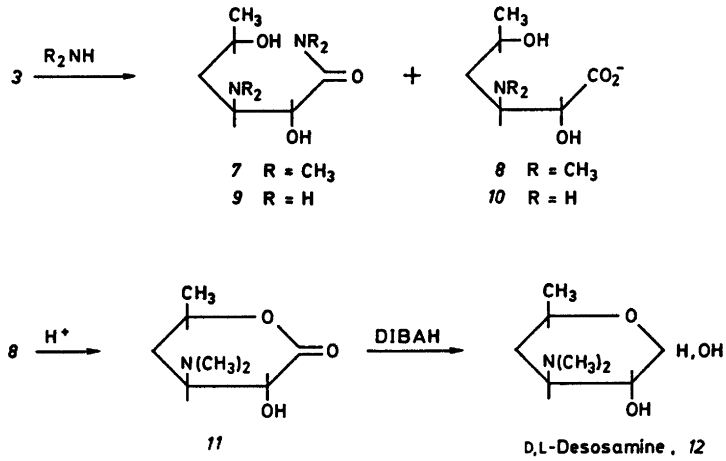
EXPERIMENTAL

Boiling and melting points are uncorrected. *J*-values given are first order splittings.

Parasorbic acid (*2*). A mixture of PPA (400 g) and methyl 2,5-hexadienoate *1* (50 g) was heated in an oil bath (70 – 80 °C) for 4 h with vigorous stirring. The reaction mixture was allowed to cool down to 30 – 35 °C and the PPA hydrolyzed by adding excess of crushed ice – water to the reaction flask. The organic material was extracted several times with CH_2Cl_2. The combined extract was washed



Scheme 1.



Scheme 2.

with water, dried over Na_2SO_4, and the solvent evaporated under reduced pressure. On distillation the residue gave pure parasorbic acid, *2*, b.p. 102–104 °C/12 mmHg (lit.[2] 110–113 °C/13 mmHg); yield 35 g (70 %). ^{1}H NMR (CCl_4): δ 1.39 (CH_3, d, J_{56} 6.3 Hz), 2.3 (2 H_4, m), 4.5 (H_5, m), 5.87 (H_2, d, t, J_{23} 9.7 Hz, $J_{24a} = J_{24e} = 1.5$ Hz), 6.90 (H_3, ddd, J_{34ae} 5.0 and 3.4 Hz).

Epoxidation of parasorbic acid. (A) *Sodium hydroxide method.*[2] To parasorbic acid (5.6 g, 0.05 mol) was added a solution of H_2O_2 (8 g, 40 % solution, 0.094 mol) dissolved in methanol (25 ml). The mixture was cooled to 10 °C and stirred. Sodium hydroxide solution (10 M, 1.75 ml) was added to the stirred mixture during 90 min so that the pH did not exceed 8, and the temperature was kept below 15 °C.

After stirring the reaction mixture for an additional period of 2 h, it was diluted with water (20 ml), saturated with $(NH_4)_2SO_4$ and extracted with ether (6 × 25 ml). The ether extract was cooled in an ice-water bath and the peroxide destroyed by gradually adding $NaHSO_3$ in excess while stirring. The decanted ether was dried with Na_2SO_4, solvent evaporated under vacuum, and the residue distilled to give pure epoxide *3*, b.p. 90 – 94 °C/2 mmHg (lit.[2] 59 °C/0.1 mmHg); yield 2 g (31 %). IR (film): 1745, 1270, 850 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.32 (CH_3, d, J_{56} 6.4 Hz), 1.95 (H_{4a}, dd, J_{4ae} 15.2 Hz, J_{4a5} 11.2 Hz, $J_{4a3} \sim 0$ Hz), 2.39 (H_{4e}, d, t, J_{4e3} 2.9 Hz, J_{4e5} 3.4 Hz), 3.55 (H_3, d, J_{23} 3.8 Hz), 3.70 (H_5, dd), 4.66 (H_6, ddq).

(B) *Sodium bicarbonate method.* To a cooled mixture of parasorbic acid (5.6 g, 0.05 mol), methanol (15 ml), sodium bicarbonate (4.2 g, 0.05 mol) was added in one portion a solution of H_2O_2 (9 g, 35 %) in methanol (25 ml). The reaction mixture was stirred for 48 h at 14 – 16 °C, diluted with water (40 ml), saturated with solid $(NH_4)_2SO_4$, and extracted with ether (6 × 25 ml). The extract, on working up as described in method (A), gave 3.8 g (60 %) of pure epoxide *3* which required no distillation.

trans-*3-Hydroxy-4-methoxy-tetrahydro-pyran-2-one*, *5*. A mixture of epoxide *3* (2.56 g), methanol (50 ml), and *p*-toluenesulfonic acid (100 mg) was refluxed for 48 h and methanol removed under reduced pressure. The residue was taken up in CH_2Cl_2, washed twice with sodium bicarbonate solution (10 %) and then with water, dried over Na_2SO_4, and solvent evaporated under vacuum. The residue distilled to provide *trans*-3-hydroxy-4-methoxy-6-methyl-tetrahydropyran-2-one, *5*, b.p. 104 – 105 °C/0.44 mmHg; yield 1.8 g (56 %). Calc. for $C_7H_{12}O_4$: C 52.50; H 7.5. Found: C 52.82; H 7.66. IR (film) 3450, 1740, 965, 850 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.41 (CH_3, d, J_{56} 6.5 Hz), 1.67 (H_{4a}, J_{4ae} 14.0 Hz, J_{4a3} 10.7 Hz, J_{4a5} 11.3 Hz), 2.33 (H_{4e}, J_{4e3} 4.5 Hz, J_{4e5} 3.0 Hz), 3.65 (H_3, J_{32} 9.0 Hz), 4.08 (H_2, d), 4.46 (H_6, ddq).

Chalcose (2,3-dihydroxy-4-methoxy-6-methyl-tetrahydropyran). A solution of *trans*-3-hydroxy-4-methoxy-6-methyl-tetrahydropyran-2-one (1.6 g, 0.01 mol) in dry THF (15 ml) was cooled to – 25 to – 30 °C under N_2 and a solution of DIBAH (18 ml, 20 % toluene solution, 3.06 g, 0.021 mol) was added whilst stirring for 40 min. After additional stirring for 2 h at – 25 to – 30 °C the temperature was allowed to rise to 0 °C and the complex decomposed by adding $Na_2SO_4.10H_2O$:celite mixture so that the temperature did not exceed 20 °C. The reaction mixture was filtered and the residue extracted a few times with boiling chloroform. The chloroform solution, dried with Na_2SO_4, was evaporated under vacuum, and the residue (800 mg) on chromatographic separation (TLC silica/$CHCl_3$) gave (500 mg) of pure chalcose as the first fraction (31 %). The IR in $CHCl_3$ and ^1H NMR in D_2O were identical with the spectra of a reference sample of natural D-chalcose.

Reaction of parasorbic acid epoxide 3 *with dimethylamine.* A mixture of epoxide *3* (5.0 g) and dimethylamine solution (12 g, 40 %) was kept at room temperature for 10 days. The excess of dimethylamine and water were removed under vacuum at 50 °C. The syrupy residue was allowed to stand at room temperature for a few hours and then chloroform was added. A solid separated (2.8 g) which was removed by filtration. The filtrate on keeping in the cold room for 2 days deposited further 0.8 g of the same solid, D,L-2,5-dihydroxy-3-*N*-dimethylaminohexonic acid *8*. It was recrystallized from ethanol, m.p. 145 – 147 °C; yield 3.5 g (50 %). IR (Nujol): 1630 cm^{-1} (COO^-); (KBr), 1630 cm^{-1} (lit.[11] 1600 cm^{-1}, L-configuration). ^1H NMR (D_2O): δ 1.24 (CH_3, d, J_{56} 6.2 Hz), 1.96 (2 H_4, t, $J_{34} = J_{45} = 6.1$ Hz), 2.90 [$N(CH_3)_2$, s], 3.55 (H_2, q, $J_{23} \sim J_{43} \sim 6$ Hz), 4.02 (H_5, m), 4.16 (H_3, d, J_{23} 5.7 Hz), 4.7 (OH, s).

The chloroform solution on removal of the solvent furnished 2,5-dihydroxy-3-dimethylaminohexonic acid dimethylamide *7* as a thick syrup (2.5 g, 25 %). ^1H NMR ($CDCl_3$): 1.17 (CH_3, d, *J* 6.1 Hz), 2.41 [$N(CH_3)_2$, s], 2.99 and 3.13 [$CON(CH_3)_2$, d]. *7* was not hydrolyzed by boiling water.

Hydrochloride of the 3,4,6-trideoxy-3-dimethylaminohexonic acid lactone 11. A solution of 2,5-dihydroxy-3-dimethylaminohexonic acid (2 g) and hydrochloric acid (21 ml, 0.5 M) was evaporated to dryness to give 3,4,6-trideoxy-3-dimethylaminohexonic acid lactone hydrochloride (1.8 g, 90 %). It crystallized from methanol, m.p. 213 – 216 °C. IR (Nujol): 1740 cm^{-1} (δ-lactone). The ^1H NMR D_2O agreed with literature values.[11]

Desosamine. A DIBAH solution (4.4 ml, 0.0044 mol, 20 % in toluene) was gradually added to *11* (420 mg, 0.002 mol) in dry THF (20 ml) at – 20 °C with stirring. After stirring the reaction mixture for another hour at – 20 to – 25 °C the complex was decomposed by adding celite and sodium sulfate decahydrate mixture so that the temperature did not rise above 20 °C. The mixture was filtered and the residue washed a few times with hot chloroform. The combined filtrate was dried over sodium sulfate, the solvent evaporated under vacuum to give desosamine (130 mg, 30 %), the ^1H NMR spectrum of which was identical with the published one.[12]

Reaction of parasorbic acid epoxide 3 *with ammonia.* A mixture of epoxy parasorbic acid *3* (4 g) and ammonia solution (50 ml, 25 %) was kept at room temperature for 10 days. Excess base and water was removed under vacuum at 50 °C. The thick syrupy residue was treated with methanol and the insoluble portion separated by filtration. On crystalliza-

tion from ethanol, water mixture (3:1) it gave pure 2,5-dihydroxy-3-amino-hexoic acid, (*10*), m.p. 215–217 °C; yield 2.4 g, 48 %. Found: C 43.70; H 8.09. Calc. for $C_6H_{13}NO_4$: C 44.11; H 7.97. IR (KBr): 1640, 1575 cm^{-1} (COO^-, lit.[11] 1640 cm^{-1}). ^1H NMR (D_2O): δ 1.23 (CH_3, d, J_{56} 6.3 Hz), 1.83 (H_4, t, $J_{34} \sim J_{45} \sim 6.3$ Hz), 3.67 (H_5, m), 4.10 (H_3, m), 4.72 (NH_3^+, OH, s) The methanol solution gave on evaporation of the solvent under vacuum a solid which was not investigated.

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The Crystal and Molecular Structure of 2,5-Dihydroxy-1,4-benzoquinone at —162 °C

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The crystal structure of 2,5-dihydroxy-1,4-benzoquinone has been determined at −162 °C from three-dimensional counter data. The space-group is $C2/c$ with $a=7.801(1)$, $b=6.951(1)$, $c=9.939(2)$ Å, and $\beta=90.04°$. Full matrix least-squares refinements have yielded a conventional R value of 0.035 for 916 observations having $\sin\theta/\lambda>0.55$. The molecules are situated at the centers of inversion, and there are two different C−O bonds [1.235(1) and 1.327(1) Å] and three different C−C bonds [1.506(1), 1.442(1) and 1.355(1) Å]. The planar structure shows some quinoid character although the double bonds are only partially localized. The hydrogen atom attached to the hydroxyl group appears to participate in two weak hydrogen bonds, one of which ties the molecules into infinite planar sheets, the other is a weak intramolecular interaction.

The crystal structure of the dipotassium salt of 2,5-dihydroxy-1,4-benzoquinone has recently been reported.[1] The anion $C_6H_2O_4^{2-}$ was found to have D_{2h} symmetry in the crystal within experimental error, thus there is virtually complete delocalization of the π electrons along the 1-oxopropen-3-olat fragment. Intermediate character although not complete conjugation in corresponding bonds has also been found in the crystal structures of fluoro- and chloroanilic acids.[2,3] To further investigate the effects of conjugation in this type of molecule, the crystal structure of 2,5-dihydroxybenzoquinone was determined. The determination was also carried out as part of a series of structure investigations of compounds containing the 1-oxo-propen-3-ol chain.[4,5]

EXPERIMENTAL

The compound was synthesized by the method given by Jones and Shonle (Dec. 217 °C),[6] and suitable deep-red to orange crystals were grown by slow cooling of a solution of the compound in absolute alcohol. The crystals were frequently found to be twinned with (101) as the twin plane. Oscillation and Weissenberg photographs indicated monoclinic symmetry. ($a=7.89$, $b=6.95$, $c=10.10$ Å, $\beta=91.4°$, $t=25$ °C). Systematic absence of reflections hkl for $h+k=2n+1$ and $h0l$ with l odd determined the space group to be either Cc or $C2/c$. The latter was assumed and proved to be correct by the structure refinements. Cell dimensions at −162 °C were determined from the setting angles of 30 reflections with 2θ-values larger than 35°. Three-dimensional intensity data were collected from an almost rectangular crystal of dimensions $0.35\times0.45\times0.30$ mm on a SYNTEX P$\bar{1}$ diffractometer with graphite monochromated Mo$K\alpha$ radiation ($K\alpha_1$, $\lambda=0.70926$ Å; $K\alpha_2$, $\lambda=0.71354$ Å). The diffractometer was operated in the ω-2θ scanning mode and equipped with an Enraf-Nonius liquid nitrogen cooling device (modified by H. Hope). The temperature at the position of the crystal was -162 ± 2 °C. Data were collected in one quadrant of reciprocal space to $2\theta_{max}=80°$ with a variable scan speed dependent on intensity. Below $2\theta=50°$ the scan speed was varied between 2 and 8° min^{-1}, above $2\theta=50°$ between 2 and 4° min^{-1}. In the latter shell a threshold value was applied to avoid measurement of very weak reflections ($<2\sigma(I)$). A symmetric scan range of 2° corrected for angular dependence of spectral dispersion were performed, the ratio of background time to scan time was 0.6. A total of 1406 intensities excluding space group absences were measured, of which 1294 were greater than $2\sigma(I)$ and regarded as observed, the remaining 112 were rejected from the final structure analysis. The intensities and their standard deviations were corrected for Lorentz

Table 1. Fractional coordinates and thermal parameters with estimated standard deviations: A, for the refinement with an inner cut-off at sin $\theta/\lambda = 0.55$ Å^{-1}, B, for the refinement of all data. Expression for anisotropic vibration is exp $[-2\pi^2(h^2a^{*2}U11 + \cdots + 2klb^*c^*U23)]$.

A

ATOM	X	Y	Z	U11	U22	U33	U12	U13	U23
O1	.29174(7)	.52877(7)	.71852(6)	.0156(2)	.0131(2)	.0122(2)	.0008(1)	.0077(1)	.0005(1)
O2	.39934(8)	.85262(7)	.59547(6)	.0216(2)	.0069(2)	.0168(2)	.0008(1)	.0106(2)	-.0013(1)
C1	.38792(7)	.50784(8)	.61384(5)	.0113(2)	.0092(2)	.0099(2)	.0002(2)	.0035(1)	.0007(1)
C2	.45292(7)	.66643(8)	.54478(5)	.0129(2)	.0088(2)	.0112(2)	-.0002(1)	.0047(2)	-.0001(1)
C3	.44118(8)	.32136(9)	.56307(6)	.0151(2)	.0083(2)	.0129(2)	-.0003(2)	.0066(2)	.0002(1)

B

ATOM	X	Y	Z	U11	U22	U33	U12	U13	U23
O1	.29177(8)	.52886(9)	.71841(6)	.0161(3)	.0138(2)	.0130(2)	.0010(2)	.0075(2)	.0003(2)
O2	.39971(9)	.85335(9)	.59537(6)	.0224(3)	.0092(2)	.0174(3)	.0008(2)	.0110(2)	-.0012(2)
C1	.38803(10)	.50691(11)	.61288(7)	.0112(3)	.0101(3)	.0104(3)	.0002(3)	.0038(2)	.0005(2)
C2	.45295(10)	.66613(11)	.54469(7)	.0132(3)	.0089(3)	.0119(3)	-.0000(2)	.0041(2)	-.0003(2)
C3	.44123(10)	.32158(11)	.56331(8)	.0154(3)	.0099(3)	.0134(3)	-.0005(3)	.0060(3)	.0006(2)

ATOM	X	Y	Z	B	ATOM	X	Y	Z	B
HO	.3397(21)	.8172(22)	.6682(17)	3.4(3)	HC	.4035(19)	.2187(21)	.6288(14)	2.9(3)

and polarization effects and a 2 % uncertainty to account for experimental uncertainties was included in the latter. Atomic form factors used were those of Doyle and Turner[7] for oxygen and carbon atoms and those of Stewart *et al.*[8] for hydrogen atoms.

Computer programs employed during the present study are those of Refs. 9 and 10.

CRYSTAL DATA (−162 °C)

2,5-Dihydroxy-1,4-benzoquinone, $C_6H_4O_4$, monoclinic. Space group $C2/c$, $a = 7.8008(14)$, $b = 6.9405(13)$, $c = 9.987(19)$ Å, $\beta = 90.044(10)$, $V = 538.1$ Å^3, $M = 140.10$ g mol^{-1}, $F_{(000)} = 288$, $Z = 4$, $D_{calc} = 1.729$ g cm^{-3}, D_{obs}(25 °C) = 1.65 g cm^{-3}.

SOLUTION AND REFINEMENTS

The structure was solved by direct methods[7] and further refined by full matrix least squares methods. The function minimized was $\sum w(F_o - F_c)^2$, $w = \sigma(F_o)^{-2}$. The hydrogen atoms were located in a difference Fourier map at the end of the isotropic refinement. However, the hydrogen atom (H_O) attached to the hydroxylic oxygen was initially placed on the line joining the donor and acceptor of the intermolecular hydrogen bond. During the subsequent refinements the isotropic temperature factor of H_O increased from 3.0 to 5.5 Å^2, while the atom position shifted near to that determined from the difference map, whereupon the value of the thermal parameter again declined to 3.4 Å^2. Refinement with all data terminated with the residuals $R = 0.036$, $R_w = 0.044$ and $S = 2.16$.* In the final refinements only data for which sin $\theta/\lambda > 0.55$ were included, leaving 916 structure factors for a refinement of heavy atoms only. During these refinements the positional parameters shifted by up to 7 standard deviations, while all U_{ii} were reduced by amounts up to 4 standard deviations. The residuals obtained were $R = 0.030$, $R_w = 0.030$ and $S = 1.2$. Positional and thermal parameters from the refinement of "high" angle data are given in Table 1 together with the values from the refinement of all data.

In the following discussion heavy atom parameters from the high angle refinement will be considered, though only small changes in bond lengths (maximum 3×10^{-3} Å) were observed.

An analysis of the molecular motion in terms of rigid body vibrations indicated that the molecule may be regarded as rigid since $\Delta U_{rms} = 0.0003$ Å^2.[11,12] Corrections for librational motions range from 1×10^{-3} (C1 − O1) to 4×10^{-3} Å (C1 − C2) and have not been applied. The r.m.s. amplitudes range from 0.173 to 0.078 Å with the larger component perpendicular to the molecular plane (see Fig. 1). The structure factors can be obtained from the author upon request.

* Standard deviations of unit weight $S = [\sum W(F_o - F_c)^2/(m-n)]^{\frac{1}{2}}$.

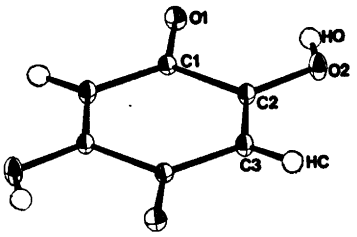


Fig. 1. Perspective drawing of the molecule with atom numbering as indicated. The shapes of the heavy atoms represent 50 % probability contours of thermal motion.

DESCRIPTION AND DISCUSSION

The molecular structure of 2,5-dihydroxy-1,4-benzoquinone in the crystalline state is shown in Fig. 1,[13] which also shows the atom numbering scheme. The molecules are situated at crystallographic centers of inversion. Bond lengths and angles from both refinements are given in Table 2 together with hydrogen bond parameters.

The molecular structure of 2,5-dihydroxy-1,4-benzoquinone corresponds to that found previously in the chloro- and fluoroanilic acids.[2,3] The structure is basically of quinoid character, however, some degree of conjugation exists along the sequence of atoms O1*–C1*–C3–C2–O2.† Thus C1*–C3 [1.442(1) Å] is definitely shorter than C1–C2 [1.506(1) Å], indicating only minor conjugation across the latter bond. There is therefore markedly less conjugation in the 1-oxo-propen-3-ol chain than in the 1-oxo-propen-3-olat chain in the free acid and in the dipotassium salt.[1] The C1–C2 bond in the present study is also probably shorter than the corresponding value in the salt [1.543(7) Å]. The bond lengths in the conjugated chain are similar to those found in the *trans*-enol arrangements of dimedone [4] and phenylmalondialdehyde.[5]

A slight tendency towards higher conjugation in the O1–C1–C3–C2–O2 skeleton upon substitution of hydrogen for the more electron withdrawing halogens may be noted. The C–O double bond increases from 1.215(3) Å in fluoroanilic acid [2] to 1.235(1) Å in the present work, while the C–O single bond decreases from 1.334(3) to 1.327(1) Å. Further the C–C double bond increases from 1.332(4) to 1.355(1) Å, while the C1–C3 single bond contracts from 1.456(3) to 1.442(1) Å. On the other hand the slight expansion of the C1–C2 bond from 1.496(4) to 1.506(1) Å may indicate a decrease in conjugation across this bond. The distances found in chloroanilic acid [3] lie between those found for fluoroanilic acid [2] and the title compound except that the C–O single bond length does not differ significantly from that found in the present work.

The molecule is planar within the estimated standard deviations. The average absolute discrepancy from the root-mean-square plane through the heavy atoms in the asymmetric unit is only 0.001 Å, and the largest deviation (0.002 Å for C1) is not significant. The hydrogen atoms also lie in this plane with deviations for H_C and H_O of 0.035 and 0.026 Å, respectively.

A packing diagram of the structure is shown in Fig. 2.[13] The molecules are linked together to form infinite sheets of hydrogen bonded layers [O---O, 2.728(1) Å] extending in the (101) plane. The stacking of layers perpendicular to (101) is such that C–O bonds of adjacent molecules overlap in an approximately antiparallel manner with an average layer separation of 3.16 Å. This suggests that the attractive

Table 2. Interatomic distances, bond angles and hydrogen bond parameters, with estimated standard deviations: A, for the refinement with an inner cut off at sin $\theta/\lambda = 0.55$ Å^{-1}, B, for the refinement of all data.

A

DISTANCE		(Å)	DISTANCE		(Å)
O1 - C1		1.2353(6)	O2 - C2		1.3267(7)
C1 - C2		1.5064(7)	C1 - C3		1.4423(8)
C2 - C3		1.3547(7)	HO - O2		.7987
HC - C3		.9392	O1 - O2		2.6583(7)
O1 - HO		2.2273	O1 - O2		2.7275(6)
O1 - HO'		2.1199			
ANGLE		**(°)**	**ANGLE**		**(°)**
C3 - C1 - C2		119.1(1)	C1 - C2 - C3		122.8(1)
C1 - C3 - C2		118.9(1)	O1 - C1 - C2		117.2(1)
O1 - C1 - C3		123.7(1)	O2 - C2 - C1		116.2(1)
O2 - C2 - C3		121.9(1)	C2 - O2 - HO		111.6
C1 - O1 - HO		88.6	O2 - HO - O1		114.4
C1 - O1 - HO'		113.4	O2 - HO - O1'		114.3

B

DISTANCE		(Å)	DISTANCE		(Å)
O1 - C1		1.2360(8)	O2 - C2		1.3308(9)
C1 - C2		1.5045(10)	C1 - C3		1.4398(10)
C2 - C3		1.3533(10)	HO - O2		.804(10)
HC - C3		.942(15)	O1 - O2		2.6618(8)
O1 - HO		2.229(16)	O1 - O2'		2.7296(8)
O1 - HO'		2.116(17)			
ANGLE		**(°)**	**ANGLE**		**(°)**
C3 - C1 - C2		119.1(1)	C1 - C2 - C3		122.8(1)
C1 - C3 - C2		118.9(1)	O1 - C1 - C2		117.1(1)
O1 - C1 - C3		123.7(1)	O2 - C2 - C1		116.4(1)
O2 - C2 - C3		121.7(1)	C2 - O2 - HO		111.5(11)
C1 - O1 - HO		88.8(5)	O2 - HO - O1		114.1(14)
C1 - O1 - HO'		113.4(2)	O2 - HO - O1'		114.2(12)

† Atoms marked with an asterisk are those centrosymmetrically related within the molecule.

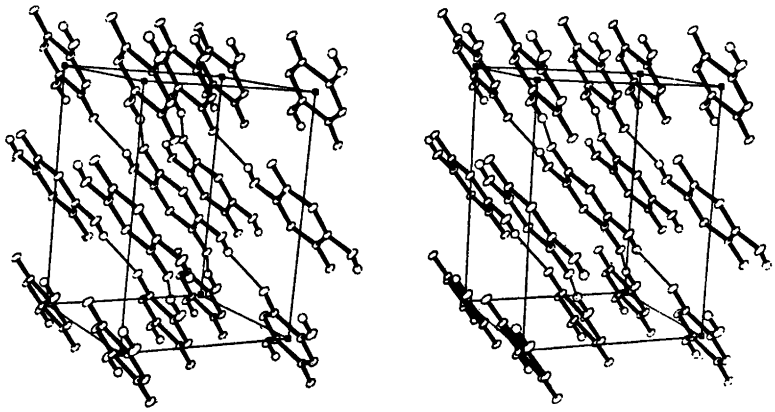


Fig. 2. Stereoscopic illustration of the crystal structure of 2,5-dihydroxy-1,4-benzoquinone. Thermal ellipsoids are scaled to include 50 % probability.

forces between layers may be partly due to dipole-dipole interactions.[14] The intermolecular contacts between these atoms do, however, correspond to normal van der Waals distances (C1---O1, 3.06 Å; C2---O2; 3.09 Å).[15] In addition to the O---O hydrogen bonds there are two other intermolecular interactions within the sheets. These are from H_C to O1 and O2 of lengths 2.67 and 2.49 Å, respectively, and are similar to CH---O contacts commonly occurring in quinoid compounds.[14]

The intermolecular hydrogen bond [O---O, 2.728(1) Å], is weak and of a similar length to those found in fluoro- and chloroanilic acids (2.720 and 2.769 Å, respectively).[2,3] The hydrogen atom is displaced significantly off the line joining the two oxygen atoms ($\angle$O–H···O = 114°) with an H_O---O separation of 2.12(2) Å. An examination of intramolecular contacts reveals that the H_O---O1 distance within the molecule is only 2.23(2) Å, and although longer than the intermolecular contact, it is shorter than the sum of van der Waals radii for hydrogen (1.0 Å)[16] and oxygen (1.4 Å),[15] suggesting the presence of a bifuracted hydrogen bond arrangement. The corresponding intramolecular O---O distance is 2.658(1) Å and the O–H···O angles i 114°. The intramolecular interaction constituting a five-membered ring system is certainly weak, however somewhat similar arrangements have previously been found in the crystal structure of tropolone [17] and there is also evidence of such interaction in (–)-noradrenaline and some related compounds.[18]

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Organic Electrosyntheses. IX.* Condensation of 2-Aminophenylhydrazine with Ortho Esters

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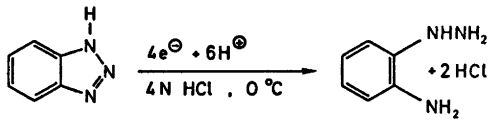
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A procedure for the preparation of 2-aminophenylhydrazine (*2*) in high yield by large-scale cathodic reduction of benzotriazole (*1*) in acidic medium has been developed. With *2* as an easily available starting material a simple and efficient synthesis of 3-substituted benzo-1,2,4-triazines (*4*) by condensation with the corresponding ortho esters is presented. The optimum conditions for this reaction were found from kinetic measurements and variation of the reaction parameters, determining the product concentration by means of cathodic DC-polarography.

Benzo-1,2,4-triazines have been prepared by several methods,[1–12] but few of these are both general and give satisfactory yields. Kwee and Lund [11] have briefly reported on the formation of 3-substituted benzo-1,2,4-triazines by condensation of electrochemically prepared 2-aminophenylhydrazine dihydrochloride (*2*) with carboxylic acid derivatives in rather low yields. In the present work we have scaled up the electrolytic reduction of benzotriazole (*1*) from a 1 g scale to about 50 – 100 g to make *2* an available starting material for synthetic purposes, and optimized the condensation of *2* with ortho esters to produce the corresponding 3-substituted benzo-1,2,4-triazines (*4*) in high yields (90 – 95 %).

RESULTS AND DISCUSSION

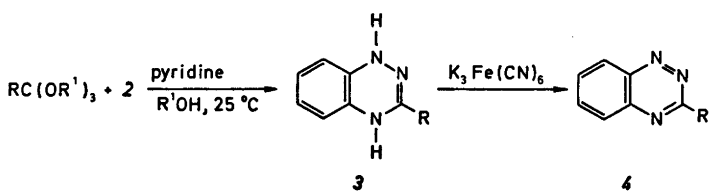
The procedure for the cathodic reduction of benzotriazole to *2* in acidic medium is similar to that of Lund and Kwee,[11] and details are to be found in the experimental section (eqn. 1).



1 *2* (80 - 85%) (1)

The methyl and ethyl ortho esters of formic, acetic, propionic, and benzoic acid were found to react with *2* in the presence of a tertiary amine (eqn. 2). The intermediate dihydro derivative *3* has not been isolated, but could be detected by anodic polarography,[11] and was oxidized by excess potassium hexacyanoferrate(III) to *4* which was isolated by continuous extraction with benzene.

The formation of *4* could conveniently be followed by cathodic polarography in alkaline



3 *4* (90 - 95% overall yield) (2)

* For Part VIII, see Ref. 14.

Table 1. Yields, solvents, and second order rate constants (k_2) for the formation of 3-substituted benzo-1,2,4-triazines (*4*) from the condensation of ortho esters with 2-aminophenylhydrazine (*2*) in the presence of pyridine and subsequent oxidation with potassium hexacyanoferrate(III).

Ortho ester $RC(OR^1)_3$			Sol-	Yields (%) of *4* (based on *2*)			k_2 (25 °C)
Case	R	R^1	vent	Anal.	Crude	Isol.	(dm^3 mol^{-1} min^{-1})
aa	H	Me	MeOH	87	82	77	4.7 ± 0.2
ab	H	Et	EtOH	99	93	89	23.2 ± 1.9
ba	Me	Me	MeOH	98	94	88	4.0 ± 0.4
bb	Me	Et	EtOH	99	96	92	20.2 ± 1.7
ca	Et	Me	MeOH	97	93	93	2.6 ± 0.3
cb	Et	Et	EtOH	99	93	93	7.4 ± 0.8
da	Ph	Me	MeOH	95	90	84	1.0 ± 0.2
db	Ph	Et	EtOH	96	91	86	2.0 ± 0.2

medium where the hexacyanoferrate(III) ion is not reducible. The wave-height was found to be independent of the concentrations of $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, KOH, and added amine up to 10 times that of the concentrations used in any of the samples investigated. This facilitated kinetic measurements (Fig. 1) which showed that the reaction is first order in both 2-aminophenylhydrazine and ortho ester. Results are given in Table 1 and some of the properties of *4* in Table 2. The procedure described seems to be the method of choice for the preparation of *4*, provided the automatic equipment for the electrochemical reduction of *1* into *2* is available. The most reliable other procedures are probably those of Kwee and Lund [11] or Adgar *et al.*[12]

Compound *2* has been prepared by reduction of 2-nitrophenylhydrazine with tin and hydrochloric acid,[13] but is difficult to obtain pure in a reasonable yield. The yield of *2* from cathodic reduction of *1* is probably nearly quantitative, but certain losses are inevitable during longtime electrolysis and work-up due to the very easy oxidation of *2* even in acidic media as described in the experimental section.

The optimum conditions for the formation of *4* were investigated by changing variables such as amount of oxidant, temperature, added base, reaction time, and solvent, the yields being determined by cathodic DC-polarography.

It was unnecessary to use excess of $K_3Fe(CN)_6$ in the oxidation step (*cf.* Fig. 1), but reaction times could of course be shortened. Some experiments were made using air as the oxidating agent, by passing a stream of air through the reaction mixture. Though it was possible to get relatively high yields of *4* by this method, it extended the duration of the oxidation step by a factor of at least 10 – 15.

The temperature had a significant influence on the pathway of the reaction, most pronounced in the case of the formation of *4a* where the yield was nearly halved by increasing the temperature from 25 to 65 °C (case aa) or to 78 °C (case ab, see Table 1). At these temperatures about 15 % of benzimidazole was also isolated. In the case of formation of *4b* or *4c*, the yields decreased by about 25 % under identical conditions, and about 5 % of 2-methylbenzimidazole was isolated together with *4b*. The formation of *4d* seemed to be insensitive to variations of temperature. In none of these cases were the yields increased by lowering the temperature to 0 °C.

Using Et_3N or Na_2CO_3 as base in equivalent amounts had no significant effect on the yields of *4*. These yields seemed to be independent of the type of base used, as long as this was not used in too large an excess. In the limiting case where pyridine was used both as a solvent and a base, the yields decreased by 40 – 60 %.

From a preparative point of view it is preferable to use the triethyl ortho esters, both because the reaction time is shorter which can be seen from the rate constants given in Table 1, and because the yields of *4* are generally higher, especially for the cases aa and ab where an increase in the yield of 12 % was found by using triethyl orthoformate instead of trimethyl orthoformate (Table 1).

Fig. 1 shows the results from typical kinetic runs for the oxidation step on a 0.0256 mol scale of *2* with equivalent amounts of pyridine,

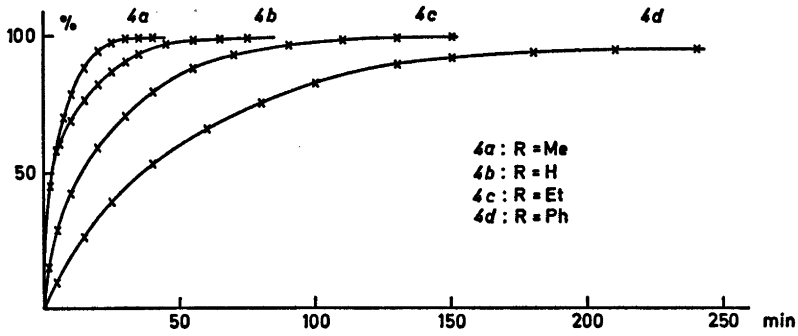


Fig. 1. Results from typical kinetic runs of the oxidation step of the reaction (2) with equivalent amounts of 2-aminophenylhydrazine, ortho ester, pyridine, and potassium hexacyanoferrate(III) (for details see the experimental section).

ortho ester, and $K_3Fe(CN)_6$. It is seen that the reaction requires an oxidation time between 40 min and 4 h which was much less than with air oxidation where at least 24 h were required for the full conversion of *3*. As expected, the duration of the oxidation step was independent of whether trimethyl or triethyl ortho esters were used.

As expected the yields decreased rapidly when the water content of the reaction medium was raised. Using water as solvent gave about 3 – 10 % of *4*, and using 96 % EtOH decreased the yields by 20 – 60 %.

Condensation of *1* with formic acid, acetic acid, propionic acid, benzoic acid, acetic anhydride, propionic anhydride, methyl acetate, ethyl acetate, and methyl benzoate gave yields of *4* only in the range 5 – 20 % using the optimum conditions given in the experimental section. Raising the temperature also decreased these yields.

Instead of using a large scale procedure for the preparation of *2* (which included a purification step, details are to be found in the experimental section), a one-step procedure involving the reduction of *1* on a 5 g scale was worked out in which the resulting crude *2* was used without further purification before the condensation reaction with triethyl ortho acetate under the optimum conditions found. The overall analytical, crude, and recrystallized yields of *4b* (based on *1*) were 96, 93, and 90 %, respectively. This result further indicated that the electrochemical reduction of benzotriazole is an almost quantitative reaction.

Attempts to condense *2* with carbonyl compounds using *in situ* technique [14] with electrolytic reduction of *1* in an aqueous acetate buffer of pH 5 in the presence of excess acetaldehyde or acetone were not very promising, as no cyclized product could be found after consumption of 4F/mol. However, a rather fast condensation reaction must have taken place during the electrolysis, as azomethine bonds were detected spectroscopically in the isolated product mixture which was not further separated and characterized.

Preliminary experiments to condense *2* with biacetyl to form 3,4-dimethylbenzo-1,2,5-triazepine using conditions similar to the ortho ester reaction gave complicated reaction mixtures which were difficult to work up, and this possibility was not further explored. However, yields of up to 60 % 2,3-dimethylquinoxaline were isolated.

EXPERIMENTAL

Apparatus. An H-type 3-electrode cell of conventional design [15] was used in combination with a JUUL Electronic 40V/25A potentiostat. The cell was cooled by means of a HETOFRIG CB122 cryostat. Spectra were recorded on a Varian A60 NMR spectrometer, a Perkin-Elmer Infracord or a Beckmann IR 18-A IR spectrophotometer, a Perkin-Elmer 402 UV spectrophotometer, and a CEC 21-104 mass spectrometer. DC-polarograms were recorded on a Radiometer PO4 polarograph. Melting and boiling points are uncorrected. GLC purity checks were performed on a Hewlett-Packard 5711A gas chromatograph in combina-

tion with a Hewlett-Packard 3370B integrator using 15 % SE 30 on Chromosorb WAW (80–100 mesh) on a 2 m SS-column. Coulometric measurements were done by means of an LK 5A electromechanical integrator.

Materials. Benzotriazole (*1*) was a commercial product. Ortho esters were either commercial products (cases aa, ab, ba, bb, ca, db, see Table 1) or prepared according to published procedures.[16] The commercial products were used as received (checked by GLC). Our yields of triethyl orthopropionate b.p. 43–44 °C/12 mmHg, n_D^{25} 1.3941 and trimethyl orthobenzoate b.p. 69–71 °C/12 mmHg, n_D^{25} 1.4900 were 49 and 16 %, respectively (purity checked by GLC).

Electrolytic reduction of benzotriazole (1). Electrolyses were performed in an H-type cell of about 400 ml catholyte volume and 65 cm^2 of mercury surface immersed in a methanol bath thermostated around 0 °C by means of a magnetic valve and a contact thermometer in the cathode compartment. *1* (50–75 g) was reduced in 4 N aqueous hydrochloric acid at −1.2 V (*vs.* Ag/AgCl in 4 N HCl) under nitrogen which was purified by passing over a BASF R3-11 catalyst at 120–150 °C. The hydrochloride of *1* precipitated by cooling, thus making magnetic stirring difficult and limiting the amount of *1* which could be used in the beginning. More *1* could be added after 5–6 h of electrolysis at a relatively low current (0.8–1.2 A), the total electrolysis time being 2–3 days with consumption of 4 F/mol. Efficient cooling and temperature control was indispenpensable, as a much more impure product was obtained from electrolysis at 10 °C, and below 0 °C magnetic stirring was virtually impossible due to the high viscosity of the semisolid catholyte mixture. In practice it is very difficult to avoid the formation of deep reddish coloured by-products, probably caused by the presence of trace amounts of oxygen in the catholyte, as seen by the pink colour developed in the neighbourhood of the mercury surface shortly after the current had been switched on. It was *not* due to oxidation by compounds formed at the anode, as the red colour diffused through the glass frit into the middle compartment from the cathode side. The anolyte was a 1:1 4 N hydrochloric acid/ethanol mixture as earlier reported.[17]

After electrolysis the clear, reddish catholyte was thoroughly evaporated *in vacuo* (1 mmHg) at a bath temperature not exceeding 40 °C. The pink residue was purified by heating (5–10 min) in deaerated methanol to dissolve unreduced *1* (hydrochloride) and strongly coloured impurities, cooled to −20 °C, rapidly filtered, washed with cold deaerated methanol, and dried to constant weight *in vacuo* over silica gel. *2* was isolated in 80–85 % yield (based on *1*) as a pinkish material, identified by IR,[18] showing an anodic polarographic wave and assayed by potentiometric titration with standard silver nitrate solution, giving mol.wt. 196.0 ± 1.5 (normally 195.5). The compound was unstable against air and must be stored *in vacuo* or under nitrogen. A direct DC-polarographic determination[19] of the concentration of *2* in an aliquot of the catholyte was not attempted due to the difficulties in obtaining a standard sample of known purity. Probably the formation of *2* by electrolytic reduction of *1* is nearly quantitative, but some losses must be envisaged due to air oxidation during work-up and a finite solubility of *2* in methanol.

General procedure for the preparation of benzo-1,2,4-triazines (4). *2* (0.025 mol) was added to a deaerated mixture of the ortho ester (0.025 mol) and pyridine (0.060 mol) in 50 ml of the corresponding alcohol (methanol or abs. ethanol) at room temperature under nitrogen. After an appropriate time (usually 2–18 h, *cf.* Table 1) the reaction mixture was poured into 100 ml of an aqueous solution of potassium hexacyanoferrate(III) (0.090 mol) and left for 1–4 h at room temperature. The benzo-1,2,4-triazine (4) was extracted continuously by benzene (175 ml) for 16 h. After drying over anhydrous potassium carbonate the benzene solution was evaporated *in vacuo* (12 mmHg) at a bath temperature not exceeding 40 °C in order to avoid sublimation (distillation). The residue was either recrystallized from light petroleum (b.p. < 50 °C), or chromatographed on alumina with a 1:1 mixture of chloroform and redistilled light petroleum, or fractionated *in vacuo*. Usually the crude products *4* were very pure (as checked by TLC) so that chromatographic purification was unnecessary before recrystallization (or sublimation), except if a trace impurity of some strongly yellowish material should be removed. The yields of *4* (Table 1) are based on purified *2*, assuming the material to be the pure dihydrochloride, mol.wt. 196. Identification of *4* was done by comparison with spectroscopic data for authentic samples except for the 3-ethylbenzo-1,2,4-triazine (*4c*), which has not been reported in the literature. Some properties of *4* are indicated in Table 2. It should be noticed that compounds *4a*, *4b*, and *4d* easily sublime at reduced pressures which could also be used for their purification.

One-step procedure for the preparation of benzo-1,2,4-triazines (4). *1* (4.65 g, 0.0378 mol) was reduced during 8 h according to the above procedure at −1.1 V (*vs.* Ag/AgCl) with the H-cell (catholyte volume about 175 ml) immersed in an ice-water bath. After evaporation to dryness *in vacuo* (1 mmHg) the pink residue was dissolved in 60 ml of deaerated ethanol, poured into a solution of triethyl orthoacetate (0.0378 mol) and pyridine (0.0756 mol) in 40 ml of ethanol at room temperature under nitrogen. After 2 h of magnetic stirring the reaction mixture was poured into 100 ml of an aqueous solution of potassium hexacyanoferrate(III) (0.0756 mol) and left for 1 h. The

Table 2. Properties of 3-substituted benzo-1,2,4-triazines (*4*). (No ^{1}H NMR, IR, or MS data have been stated in the literature).

Compound	R	M.p. (°C) or b.p./mmHg	60 MHz ^{a}H NMR δ ($CDCl_3$)	UV (96 % ethanol) λ_{max} (nm)	log ε	IR ($CHCl_3$) cm^{-1} [a]	MS (*m/e*)[a]
4a	H	71–72	9.98 (s)	241	3.80	940 (v), 965 (v), 1015 (s), 1095 (m), 1105 (v)	131 (M^+), 103 (M^+-N_2)
				306	3.55	1125 (m), 1170 (v), 1210 (b), 1280 (m), 1375 (m), 1435 (v), 1480 (m), 1570 (m), 1620 (v), 3000 (m), 3400 (b)	
4b	Me	90–91	3.18 (s)	243	3.93	880 (m), 955 (m), 1000 (v), 1065 (s), 1130 (v)	145 (M^+), 117 (M^+-N_2)
				305	3.56	1175 (m), 1210 (b), 1300 (s), 1370 (m), 1395 (m)	
				330	3.42	1455 (m), 1520 (s), 1570 (m), 1620 (v), 3000 (m), 3400 (b)	
4c	Et	101/1 (n_D^{25}= 1.5920)	1.69 (t, J=8) 3.45 (q, J=8)	304	3.87	960 (m), 1000 (v), 1040 (m), 1080 (m), 1125 (v)	159 (M^+), 131 (M^+-N_2)
				328	3.36	1210 (b), 1280 (v), 1325 (v), 1390 (v), 1460 (m)	
				341	3.50	1520 (s), 1570 (v), 1620 (v), 3000 (m), 3400 (b)	
4d	Ph	122–123	7.30–8.35 (m)	219	3.70	705 (s), 925 (v), 960 (m), 1015 (s), 1070 (v)	179 (M^+-N_2)
				269	4.12	1100 (m), 1125 (v), 1175 (m), 1210 (b), 1270 (v)	
				254	3.38	1325 (s), 1395 (v), 1445 (m), 1510 (s), 1570 (v), 1615 (v), 3000 (m), 3400 (b)	

[a] Only main peaks are given.

work-up procedure was as above, and the analytical yield of *4b* (based on *1*) was 96 %, the crude yield 93 %, and the yield after recrystallization 90 %.

Kinetic measurements. Determination of the oxidation time. Reactions were performed with equivalent amounts of *2*, ortho ester and pyridine (0.0256 mol scale) in 60 ml of the appropriate alcohol according to the above procedure (reaction time 24 h). The reaction mixture was poured into 150 ml of an aqueous solution of potassium hexacyanoferrate(III) (0.0256 mol), diluted by water to a total volume of 250.0 ml and shaken. Periodically 1.0 ml samples of the solution were withdrawn and added to 24.0 ml of an alkaline sulfite solution [19] (9 g of KOH and 20 g of Na_2SO_3 per l of water), and the analytical yield was determined by DC-polarography by means of a standard curve. The analytical results are depicted in Fig. 1 from which the time of oxidation was determined.

Determination of the reaction time. 2-Aminophenylhydrazine dihydrochloride (*2*) was added to a deaerated mixture of the ortho ester and pyridine in equivalent amounts (0.0513 mol scale) in the appropriate alcohol (total volume of the reaction mixture: 250.0 ml). Periodically 1.0 ml samples were withdrawn, added to 24.0 ml of an aqueous solution of potassium hexacyanoferrate(III) (0.00041 mol) and the mixture polarographed as above.

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Studies on Ligand Binding of Kidney Bean Leghemoglobin

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Absorption spectra of different ligand derivatives of kidney bean leghemoglobin *a* have been recorded.

The effect of pH on the absorption spectra of kidney bean leghemoglobin *a* has been studied. The *pK* of the acid-alkaline transition of the heme-linked water molecule is 8.25 and the pK for the acid dissociation of the heme group is 4.03.

Affinities of kidney bean leghemoglobin for two different types of ligands have been studied in comparison with soybean leghemoglobins *a* and *c* and sperm whale myoglobin. All these leghemoglobins have similar affinities for the small anionic ligand fluoride ion, and they are only slightly more accessible to this ligand than is sperm whale myoglobin. Differences in the primary structure or in conformation of these proteins are reflected in the affinity for the bulky ligand imidazole. The accessibility to imidazole increases in the order sperm whale myoglobin ≪ soybean Lb*a* < soybean Lb*c* < kidney bean Lb*a*, and also low spin Lb*a* < high spin Lb*a*.

The results are discussed with respect to the amino acid sequences of the leghemoglobins.

Leghemoglobin, the monomeric oxygen-carrying hemoprotein of leguminous root nodules, belongs to the globin family of proteins. The amino acid sequences of four different leghemoglobins are known: soybean (*Glycine max*) Lb*a*,[1,2] kidney bean (*Phaseolus vulgaris*) Lb*a*[3,4] broad bean (*Vicia faba*) Lb*a*,[5] and lupin (*Lupinus luteus*) Lb*a*.[6] Kidney bean Lb*a* and soybean Lb*a* differ at 35 of the 142 residues, and the primary structures of leghemoglobins differ as much as 80 – 90 % from the animal globin sequences, although the tertiary structures are closely related.[7] A high oxygen affinity (see Refs. 8, 9) and a low isoelectric point (see Ref. 10) are considered to be characteristic of leghemoglobins but not of animal globins. The spectral properties of soybean leghemoglobins *a* and *c*[11–19] resemble those of animal globins, but spectral data of other leghemoglobins have been lacking. The aim of this study was to elucidate the correlation between the primary structure and molecular properties of different leghemoglobins.

MATERIALS AND METHODS

Leghemoglobins. Kidney beans (*Phaseolus vulgaris* var. Kaiser Wilhelm) were grown in a greenhouse at 26 – 32 °C in sea sand as described previously,[10,22] using a 12 h day produced by artificial light. Leghemoglobin was extracted and purified as described previously [10] but on DE-52 cellulose (Whatman, Maidstone, Kent.) instead of DEAE-Sephadex A-25. Soybeans (*Glycine max*) were grown in the conditions described above. The soybean leghemoglobin components *a* (high spin and low spin) and *c* were purified by the method used for the kidney bean leghemoglobin, but the chromatography was performed on a DE-52 cellulose column equilibrated with pH 5.6 sodium acetate buffer, and increasing acetate molarity from 0.01 to 0.1 at the same pH was used for elution.

Myoglobin. Lyophilized sperm whale myoglobin type II was from Sigma Chemical Co., St. Louis, Mo.

Heme protein concentrations were determined by the pyridine hemochrome method.[23]

Absorption spectra were recorded in 1 cm quartz microcuvettes equipped with beam masks on a Cary 15 recording spectrophotometer thermostated at 20 °C. The sample volume was 200 – 300 µl. A 1 mM stock solution of kidney bean leghemoglobin in water was diluted with appropriate buffer to about 50 µM (700 – 450 nm) or to about 5 µM (450 – 350 nm). The spectra of liganded leghemoglobin were recorded in sodium phosphate buffer, pH 6.0, µ 0.1, with the exceptions of the acetate complex in acetate buffer of the same pH and ionic strength and the alkaline form in sodium borate pH 10.1. Concentrated solutions of potas-

sium fluoride, potassium cyanide, sodium azide, nicotinic acid, and imidazole were brought to pH 6.0 with phosphoric acid. The ligand concentration in the cuvette was 0.87 M for fluoride, 8.5 mM for nicotinate and 0.20 M for the other ligands.

Acid-alkaline transition of kidney bean leghemoglobin was studied by recording the absorption spectra (750 – 350 nm) in the following buffers at an ionic strength of 0.1: sodium phosphate pH 6.00 and 7.00, Tris-HCl pH 7.98 and 8.55, and sodium borate from pH 9.18 to pH 10.59.

Heme group dissociation was determined from the spectral changes in the 450 – 350 nm region in sodium citrate buffers from pH 3.00 to pH 6.22. The spectrum of dissociated heme plus apoprotein was recorded in approximately 0.1 N HCl, pH 1.10.

Equilibrium constants for fluoride and imidazole dissociation were determined spectrophotometrically with a Beckman DU-2 in sodium phosphate buffer pH 6.0, μ 0.1 at room temperature (23 °C) using sperm whale myoglobin to check the method. Potassium fluoride binding was studied from spectral changes at 605 nm (leghemoglobins) or 607 nm (myoglobin) and imidazole binding at 533 nm (leghemoglobins) or 565 nm (myoglobin). Imidazole equilibria of the high and low spin forms of soybean Lb*a* were studied similarly in phosphate buffers, μ 0.1, pH 5.9, 7.1 and 7.5. Titrations were carried out by successive additions of concentrated ligand solution. The absorbances were corrected for dilution, and the end point of each titration was checked by extrapolation of [L] $\rightarrow\infty$ in the graph of $\Delta A/[L]$ *versus* ΔA, where ΔA is the absorbance change produced by the added amount of the ligand and [L] is the corresponding ligand concentration. All the pK values obtained had standard errors of ± 0.02 or less.

pH values were measured with a Radiometer pH meter 27 calibrated with Beckman pH 7.0 standard buffer, 0.05 M potassium biphthalate and 0.05 M sodium tetraborate.

Abbreviations. Hb, hemoglobin; Mb, myoglobin; Lb, leghemoglobin; CT, charge transfer bands. Lb^{2+} refers to leghemoglobin in the ferrous state and Lb^{3+} in the ferric state.

The term low spin is used for ferric hemoproteins with one unpaired iron electron and high spin for those with five unpaired electrons.

RESULTS

Absorption spectra. The spectral properties of kidney bean Lb*a* closely resemble those of hemoglobins and myoglobins in general (Table 1). Detailed comparison with the absorption spectra of soybean [11–19] or lupin [20–21] leghemoglobins is difficult because previously published data are not expressed in numerical values. Spectral differences between kidney bean Lb*a* and soybean leghemoglobins can be detected. For example, the oxy forms differ slightly in the wavelength of their Soret maxima and the ratio of the α and β band intensities. The $Lb^{3+}H_2O$ spectra also differ from each other. The Soret absorption maximum obtained here for soybean high spin Lb*a* is at 403.5 nm ($\varepsilon_{mM} = 155$) and at 404.5 nm ($\varepsilon_{mM} = 142$) for both low spin Lb*a* and kidney bean Lb*a*, the soybean Lb-components having been purified by ion exchange chromatography, which does not give the pure low spin form. The ε_{mM} (400 nm) for soybean $Lba^{3+}F^-$ was found to be 160 (in contrast to Ref. 18), so both soybean and kidney bean leghemoglobins have more intense Soret absorption for the fluoride complexes than for the $Lb^{3+}H_2O$ forms. This feature is not present in animal globins.

The major difference between the absorption spectra of the different leghemoglobins is the spin state of the ferric form below pH 7, where kidney bean leghemoglobin is much more in the low spin state than soybean or lupin leghemoglobins. Unlike kidney bean Lb*a*,[10] soybean Lb*a* and Lbc can be resolved to pure high and low spin forms by isoelectric focusing in sucrose gradient, where the isoelectric points of the high and low spin forms differ by about 0.1 pH units.[24] Nicotinic acid has been shown to be the natural hemochrome ligand of soybean leghemoglobin,[16] and it seems that kidney bean Lb*a* is isolated mainly as the nicotinate complex.

Compared with the spectra of animal globins, some of the absorption maxima of leghemoglobins are shifted by a few nanometers towards lower wavelengths.

Acid-alkaline transition. The pK value of the acid-alkaline transition of kidney bean ferric leghemoglobin *a* was determined spectrophotometrically using eqn. (1).

$$pK = n\text{pH} + \log([Lb^{3+}OH^-]/[Lb^{3+}H_2O]) \quad (1)$$

where $Lb^{3+}OH^-$ and $Lb^{3+}H_2O$ are the alkaline and acid forms, respectively, and n is the Hill constant, which gives the number of protons involved in the transition. The spectral changes at both 572 nm and 404 nm give a pK of 8.25, and the data are consistent with a single pro-

Table 1. Spectral values for kidney bean leghemoglobin *a*. The wavelengths (nm) of the absorption maxima or the shoulders (s) are given, and in parentheses are the corresponding millimolar absorption coefficients (cm^{-1} M^{-1}) based on heme concentration. The values for myoglobin and hemoglobin are from Ref. 25.

Ligand	Source [a]	Absorption bands					
		CT	α		β	CT	γ
Ferrous	PLb*a*			556(13.4)			427(98.4)
unliganded	GLb*a*[16]			555(13.0)			427(103)
	s.w.Mb			556(11.8)			434(115)
O_2	PLb*a*		574(14.7)		539(14.4)		409(134)
	GLbc[12]		574(15.1)		541(15.0)		411(139)
	s.w.Mb		581(13.3)		543(13.6)		418(128)
CO	PLb*a*		563(13.8)		538(14.0)		417(210)
	s.w.Mb		579(12.2)		542(14.0)		423(187)
Ferric	PLb*a* (pH 6.0)	625(3.12)	565s(7.40)		532(10.1)	490s(9.10)	404.5(142)
H_2O	GLb (pH 5.2)[16]	625(3.5)	560(5.0)		530(7.6)	496(8.5)	403.5(151)
	s.w.Mb (pH 6.0)	635(3.55)				505(9.47)	409.5(157)
OH^-	PLb*a* (pH 10.1)		572(11.2)		539(12.2)		413(110)
	s.w.Mb (pH 11.5)		582(9.10)		542(9.48)		414(97.2)
N_3^-	PLb*a*		573(9.97)		539(12.6)		414.5(123)
	Horse Mb		570(8.7)		540(11.2)		420(123)
CN^-	PLb*a*			538(11.6)			417(130)
	s.w.Mb			540(10.7)			423(109.7)
Imidazole	PLb*a*		562s(10.0)		533(12.8)		411(138)
	Horse Hb		560(12.5)		534(14.7)		411(105)
Nicotinate	PLb*a* (pH 6.0)		557(11.0)		528(12.9)		407.5(121)
	GLb (pH 5.3)[16]		557(10.1)		528(11.9)		407.5(119)
Acetate	PLb*a*	622(5.95)	570(5.04)		532s(6.51)	494(9.08)	403.5(169)
	Horse Hb	620(5.5)				497(10.5)	404(178)
F^-	PLb*a*	605(9.02)	576(7.00)		526(6.72)	485(9.56)	400(170)
	s.w.Mb	609(7.82)				490(8.31)	406(133)

[a] Abbreviations: PLb*a*, kidney bean (*Phaseolus*) leghemoglobin *a*; GLb*a* and GLbc; soybean (*Glycine*) leghemoglobin *a*, and *c*; s.w.Mb, sperm whale myoglobin.

ton equilibrium, $n = 1$ (Fig. 1). The pK values for the acid-alkaline transition of different hemoglobins and myoglobins all lie in a relatively narrow range,[25] and leghemoglobins are no exception to the general pattern. The pK values for soybean Lb*a* and Lbc are 8.34 and 8.16, respectively,[11] whereas lupin leghemoglobin has a pK value of 8.7.[21]

No other heme-linked ionization could be demonstrated from the optical absorption spectra in the pH range 6.5 – 10, although the isosbestic points (at about 602, 525 and 479 nm) were not sharp enough to rule out this possibility. The slight instability of the absorption spectra above pH 10 indicated alkaline denaturation.

Dissociation of the heme group at low pH and ionizations below pH 7. The observed pK value for the cleavage of the prosthetic group from the protein is 4.03 and the stoichiometry of the reaction is 1.0 (Fig. 1). The slightly higher acid stability of kidney bean Lb*a* compared to soybean Lb*a* [14] may reflect minor differences in the environment of the heme group. Fig. 1 shows that subjecting kidney bean leghemoglobin to pH values below 5.5 causes dissociation of the heme group, and the pH range where the protein is spectrally stable is pH 6 – 10.

At pH 5 – 6.5 some ionizations occur in myoglobins and hemoglobins that may give rise to small changes in the spectrum of the acid form.[25] Soybean Lb*a* of the low spin type [24] and lupin Lb [21] have a spectrally distinguishable ionization in this pH region, whereas the absorption spectra of kidney bean leghemoglobin remain essentially unaffected by this pH change.

Equilibrium with fluoride. Fluoride is a small anionic ligand (p$K_{HF} = 3.13$) that binds to the

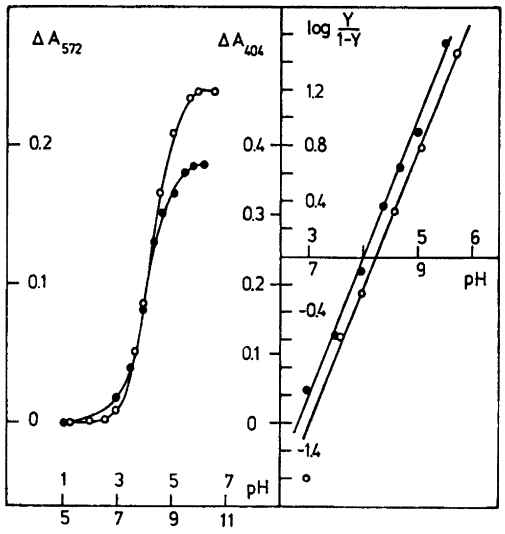


Fig. 1. pH stability curves and the corresponding Hill plots for kidney bean leghemoglobin *a*. Dissociation of the heme group at acid pH is measured at 404 nm (●) and the acid-alkaline transition at 572 nm (○). Y is the fraction of undissociated and alkaline Lb, respectively.

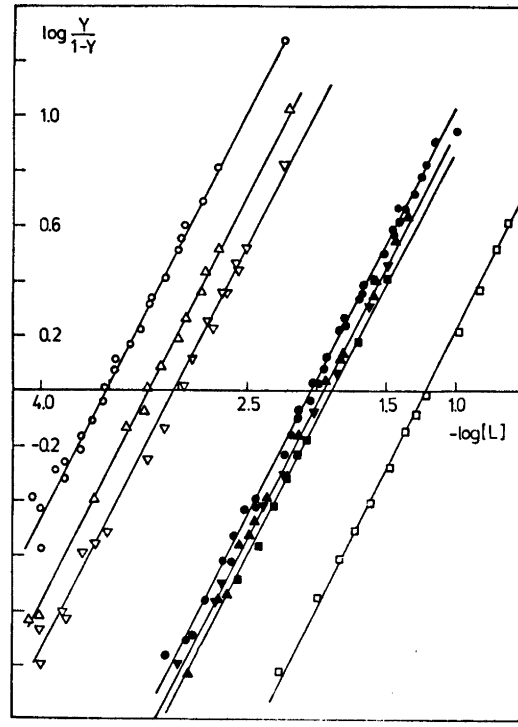


Fig. 2. Hill plots of the binding of fluoride (filled symbols) and imidazole (open symbols) to different leghemoglobins: kidney bean Lb*a* (○), soybean leghemoglobin components *a* (△) and *c* (▽) and to sperm whale myoglobin (□). Y is the fractional saturation of the protein with the ligand.

sixth coordination position of the leghemoglobin iron, to form a purely high spin ligand complex.[26] Titrations of kidney bean leghemoglobin *a* as well as soybean leghemoglobins *a* and *c* with fluoride showed only minor differences in the affinities for fluoride of these proteins. The Hill plots of the formation of the fluoride complexes are shown in Fig. 2. Sperm whale myoglobin has the pK value 1.89, which agrees with the literature value.[27] Leghemoglobins have a slightly greater affinity for fluoride: kidney bean Lb*a* has a pK value of 2.04, and soybean Lb*a* and Lb*c* 1.95. An equilibrium constant obtained elsewhere for the soybean Lb*a* fluoride complex [28] differs markedly from the value obtained here. The present results show that no differences between the primary structures of leghemoglobins or even between leghemoglobins and myoglobin are reflected in the affinity for fluoride.

Equilibrium with imidazole. The reaction of ferric leghemoglobin with imidazole can be described by the following simplified equations:

$$Lb^{3+}H_2O + Im \rightleftharpoons Lb^{3+}Im + H_2O \qquad (2)$$

$$K_{app} = [Lb^{3+}H_2O][Im]/[Lb^{3+}Im] \qquad (3)$$

where K_{app} represents the observed dissociation constant for the reaction and the imidazole concentrations in eqn. (3) include all possible ionized species. At pH 6.0 the concentration of ferric leghemoglobin hydroxide is negligible compared to the concentration of acidic ferric leghemoglobin (1:200), but since the pK for imidazole dissociation is 6.98, the mixture contains 90.5 % of the positively charged imidazolium ion and 9.5 % of the uncharged imidazole. It has generally been assumed that the neutral form of imidazole reacts with the acidic form of the hemoprotein (*e.g.* Ref. 29), although it also has been postulated that imidazole reacts as if it were a charged ligand.[30]

Imidazole has different affinities for the proteins tested. Sperm whale myoglobin has a

pK value of 1.22 for imidazole dissociation, which agrees well with the literature value.[29] Leghemoglobins from soybean have much greater affinity for imidazole than myoglobin, the corresponding pK values being 3.04 for Lba and 3.22 for Lbc. Kidney bean leghemoglobin is even more accessible to the ligand with a pK of 3.52. The corresponding association constants of sperm whale myoglobin, soybean Lba, soybean Lbc, and kidney bean Lba in the reaction with imidazole are in the ratio 1:66:100:200.

The slight difference between the imidazole equilibria of the high and low spin forms of soybean Lba is a result of the conformation differences, for the amino acid sequences are expected to be identical. The pK values for imidazole dissociation are 2.99 and 2.88 at pH 5.9, 3.85 and 3.73 at pH 7.1 and 3.98 and 3.98 at pH 7.5 for the high and low spin forms, respectively. At pH 7.5 the high and low spin forms of Lba have identical affinities for imidazole probably because of the dissociation of nicotinate from the low spin form.

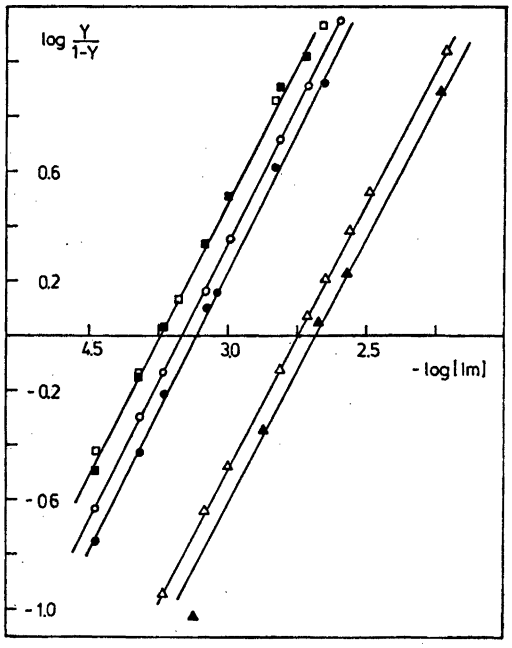


Fig. 3. Hill plots of binding of imidazole to high spin (open symbols) and low spin (filled symbols) soybean leghemoglobin a at pH 5.9 (△), pH 7.1 (○) and pH 7.5 (□).

DISCUSSION

The absorption spectra of porphyrins are shifted towards lower wavelengths when the electron-attracting power of the substituents on the porphyrin nucleus decreases.[31] This kind of shift in the absorption spectra of ferric leghemoglobins, compared with myoglobins, may reflect weaker hydrophobic interactions between the heme and the globin. This would be consistent with the observation that at neutral pH the heme in ferric leghemoglobin is less tightly bound to the globin than in ferric myoglobin.[32]

The tertiary structure of lupin leghemoglobin [7] shows that the heme group is embedded in a hydrophobic cavity, like the heme in all other globins. The most serious hindrances to approaching bulky ligands such as imidazole are the practically invariable globin side chain groups of His E7, Val E11 and Phe CD1 (see Ref. 25). It seems that the residue E11 can be still bulkier without impairing ligand binding. Exceptionally E11 is leucine in kidney bean and soybean leghemoglobins and isoleucine in the invertebrate midge larva (with E7 Glu) and lamprey globins,[33] and all these globins have a high affinity for imidazole.[34,35] Flexing of the helices and bending of the ligand permit binding even in these cases.

Residue B10 as well seems to be of interest in leghemoglobins. An invariable leucine is found in animal globins at this position,[33] which is occupied by an aromatic residue in all the leghemoglobins studied.[1-6] This residue is in heme contact at the ligand binding side [25] and it may well have an effect on the spectral and ligand binding properties of leghemoglobins.

Soybean Lbc is slightly more accessible to imidazole than soybean Lba, the ratio of the association constants being 100:66. The difference between nicotinate binding of these two proteins [17] resembles the results obtained here. The amino acid sequence of soybean Lbc (Ellfolk and Sievers, in preparation) seems to differ in at least 9 amino acid residues from the soybean Lba sequence. In four of these replacements soybean Lbc is identical with kidney bean Lba, and in a further three the resemblance between soybean Lbc and kidney bean Lba is very close. Thus, soybean Lbc is more closely related to kidney bean Lba than

is soybean Lb*a*, which is reflected in the affinities of these proteins for imidazole. It seems possible that some of the accessibility of the heme to bulky ligands may be attributed to the specific amino acid residues that are identical or closely related in kidney bean Lb*a* and soybean Lbc, but dissimilar in soybean Lb*a* and Lbc. The spin state of leghemoglobin also has an effect on the binding of a bulky ligand, as demonstrated by the slightly greater affinity for imidazole of the high spin form. This probably reflects greater flexibility of the high spin form.

Kidney bean leghemoglobin is mainly in the low spin state, but despite this it is more accessible to imidazole than the high spin form of soybean Lb*a*. This difference must therefore be due to variations in the amino acid sequences, which are different at 35 positions of the total 142 residues.[4] Most of these substitutions are conservative. Assuming that the leghemoglobins in question have the same three-dimensional structure as lupin leghemoglobin and myoglobin, an estimate can be made of the positions of the differences.

Interesting differences between soybean and kidney bean leghemoglobin structures seem to be at the following positions, respectively: A12 tryptophan or phenylalanine, E10 serine or lysine, H9 leucine or tryptophan, and the penultimate tyrosine instead of the termination of the protein chain. The residues in the E10 position in the vicinity of the ligand differ in size and charge, the first of which is probably not important if the residue projects into the solvent. Kidney bean leghemoglobin may have a greater affinity for positively charged ligands partly because the residue at E10 is neutral, whereas it has a positive charge in soybean leghemoglobin. The other amino acid residues mentioned above result in a slightly different distribution of aromatic residues in soybean and kidney bean leghemoglobins. Kidney bean leghemoglobin has more aromatic character than soybean Lb*a* at the AB corner because of Trp A12, and also at the opposite side of the molecule because of the penultimate tyrosine. Soybean Lb*a*, however, has more aromatic character in the middle of the H helix (Trp H9) close to the plane of the heme. These differences in the orientation of some aromatic side chains could possibly affect the ligand binding of these two closely related proteins by changing the electronic structure of the heme.

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On Formaldehyde *O*-Methyloxime and its Trimer 1,3,5-Trimethoxyhexahydro-1,3,5-triazine

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Formaldehyde *O*-methyloxime has been obtained from the reaction between methoxyamine and formaldehyde and also by methylation of formaldehyde oxime.

The compound has been found to be monomeric on the basis of its mass spectrum and ^{1}H NMR spectra in D_2O. However, in acid solution, the trimerization product, 1,3,5-trimethoxyhexahydro-1,3,5-triazine, is formed. In contrast to trimeric formaldehyde oxime, this compound could be extracted from the neutralized solution. Its mass spectrum showed a molecular ion with *m/e* 177, in agreement with its formulation as a trimer.

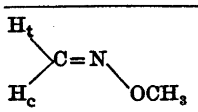
In another paper,[1] we have shown by NMR spectroscopic measurements that an equilibrium exists in aqueous solution between monomeric formaldehyde oxime, $H_2C{=}NOH$, and its trimer, 1,3,5-trihydroxyhexahydro-1,3,5-triazine. An investigation of formaldehyde *O*-methyloxime has shown that this compound is, similarly, able to trimerize and moreover, it has been possible to isolate the trimer by extraction of the solution and to determine its molecular weight by mass spectrometry.

The NMR spectrum of formaldehyde *O*-methyloxime has been studied earlier by Shapiro *et al.*[2,3] but the compound was not isolated. It was extracted with various solvents and the solutions used directly for the NMR spectroscopic measurements. Furthermore, the possibility of polymerization was disregarded.

We have isolated formaldehyde *O*-methyloxime as a lowboiling liquid by distillation of an aqueous solution of methoxyamine and formaldehyde. Its identity was proved by mass spectrometry and by infrared spectroscopy, the IR spectrum being similar to that of gaseous monomeric formaldehyde oxime.[4] It was also formed in low yield by methylation of formaldehyde oxime with dimethyl sulfate or methyl iodide in strongly basic solution; in ether solution formaldehyde oxime reacts with methyl iodide to form *N*-methyl-1,3,5-trihydroxyhexahydro-1,3,5-triazinium iodide.[1]

The ^{1}H NMR spectrum of formaldehyde *O*-methyloxime in CCl_4 shows two doublets at δ 6.31 and 6.92, due to the CH_2 protons, with a coupling constant $J = 9.0$ Hz; these values agree well with those reported by Shapiro *et al.*[2,3] The CH_3 protons give rise to a singlet at δ 3.84. In D_2O, the CH_2 signals appear at δ 6.62 and 7.09 (10 °C); further, the spectrum was unchanged after an interval of several days (Table 1). However, when the solution was acidified, two new signals appeared at δ 4.44 and 3.61 in a ratio of 2:3. These signals are assigned to the CH_2 and CH_3 groups, respectively, of 1,3,5-trimethoxyhexahydro-1,3,5-triazine, the trimer of formaldehyde *O*-methyloxime. Thus, it may be concluded that hydrogen ions catalyze the trimerization. This process is reversible in acid media with the equilibrium being shifted towards the monomer at higher pH and higher temperature. A solution of equimolecular amounts of formaldehyde and methoxyammonium chloride in D_2O at pH = *ca.* 1 immediately showed only the signals of the trimer at δ 3.86 and 3.61 (39 °C) but on neutralization the signals of the monomer appeared. However, no change in the ratio of the concentrations of the two compounds was observed when an acid solu-

Table 1. ^{1}H NMR spectra (δ values) of formaldehyde *O*-methyloxime (*1*) and its trimer, 1,3,5-trimethoxyhexahydro-1,3,5-triazine (*2*).

Solvent	°C	pH	Chemical shifts					% trimer[e]
						$(CH_2NOCH_3)_3$		
			1			*2*		
			H_t	H_c	CH_3	CH_2	CH_3	
$CDCl_3$	39					3.94	3.54	
CCl_4	39		6.31	6.92[a]	3.84	3.77	3.48	0
CF_3COOD	39		6.48	7.04	3.88	3.73	3.56	25
D_2O	39	7.00				4.12	3.52[c]	
D_2O	10	6.25	6.62	7.09[b]	3.87			0
D_2O	10	1.20	6.62	7.09[b]	3.87	4.44	3.61	26[d]
D_2O	39	1.20	6.62	7.09	3.88	—	3.63	2.4

[a] $J(H_cH_t)=9.0$ Hz. [b] $J(H_cH_t)=7.1$ Hz. [c] No transformation to the monomer occurred in a solution of the trimer in pure water. [d] Unchanged on addition of NaOD to pH = 12 and heating to 39 °C, but the signals of the trimer shifted to 4.12 and 3.53 (*cf.* Ref. 1). [e] % trimer in solution of monomer.

tion of the two compounds in equilibrium was made alkaline (Table 1). The trimer was isolated by neutralizing an acid solution of the trimer, and extracting with ether. On evaporation of the ether, the trimer was obtained as a white crystalline substance which could be recrystallized from pentane. Its ^{1}H NMR spectrum in D_2O at 39 °C showed two singlets at δ 4.12 and 3.52 (ratio of integrals 2:3) in agreement with the values found for the trimer in the solution containing both monomer and trimer at 39 °C and pH = 12. Its mass spectrum showed a molecular ion with *m/e* 177, in agreement with its formulation as a trimer.

The polymerization of formaldehyde oxime seems to be unique among the aldehyde oximes. We have investigated, by NMR spectroscopy, the possibility that acid would catalyze trimerization of acetaldehyde oxime but there was no indication of this. Its ^{1}H NMR spectrum was studied in D_2O and CD_3OD solution at various temperatures and pH values, but the spectra showed only the signals of the monomer, studied earlier by Karabatsos and Taller.[5]

This behaviour may be explained in terms of steric hindrance in the case of the higher homologues, and an increased carbonium ion character in formaldehyde oxime.

EXPERIMENTAL

The NMR spectral data (Table 1) were obtained at 60 MHz with a Varian A-60A spectrometer. $(CH_3)_3SiCH_2CH_2CH_2SO_3Na$ (DSS) was used as internal standard in D_2O; TMS was used for the measurements in CCl_4 and $CDCl_3$. The IR spectra were recorded with a Perkin-Elmer Model 337 grating spectrophotometer. The mass spectra were obtained with an A.E.I. MS 902 mass spectrometer. For the pH measurements, a pH meter 22 from Radiometer, Copenhagen, was used.

Formaldehyde O-methyloxime. Methoxyamine[6] (2.4 g), placed in a distillation flask, was cooled in acetone-dry ice, and 40 % aqueous formaldehyde (3.5 ml) was added. The mixture was heated slowly, the receiver being cooled in acetone-dry ice. A colourless liquid passed over and was collected until the thermometer of the column had reached 25 °C. Yield 2.55 g (87 %). The b.p. of the pure compound was 12 – 12.5 °C/760 mmHg.

The use of free methoxyamine is not essential in this preparation Formaldehyde *O*-methyloxime was obtained in 78 % yield by the addition of sodium hydroxide and formaldehyde to a solution of methoxyammonium chloride (4.2 g) in water (20 ml).

The same compound was also obtained, but in low yields (4 – 22 %) and in an impure state, by methylation of an aqueous solution of triformoxime hydrochloride with dimethyl sulfate and sodium hydroxide, or by methylation of formaldehyde oxime in 1-butanol with methyl iodide and sodium 1-butanolate. IR

(CCl_4 and CS_2): 3005 m ($\nu_s CH_2$), 2978 m ($\nu_{as} CH_2$), 2946 s ($\nu_{as} CH_3$), 2908 m ($\nu_s CH_3$), 2832 m, 1900 w ($2 \times \omega CH_2$), 1873 w ($\nu NO + \nu OCH_3$), 1641 w ($\nu C=N$), 1604 m ($\nu OCH_3 + \delta CNO$), 1460 m (δCH_2), 1432 w ($\delta_{as} CH_3$), 1373 w ($\delta_s CH_3$), 1208 w, 1151 w (ϱCH_2), 1055 vs (νOCH_3), 950 s (ωCH_2), 823 s (νNO), 562 m (δCNO).

The assignments have been made on the basis of those made for formaldehyde oxime by Califano and Lüttke.[4]

MS: The mass spectrum was obtained at 70 eV with an ion source temperature of 150 °C and using the glass inlet system; (*m/e* (% rel. int.): 60 (2.4), 59 (100,[M]), 58 (5.3), 44 (34.4, [CH_2NO^+]), 43 (2.8), 31 (24.8, [CH_3O^+]), 30 (5.1), 29 (11.4), 28 (29.1), 27 (8.1).

1,3,5-Trimethoxyhexahydro-1,3,5-triazine. Methoxyammonium chloride[6] (2.1 g) was dissolved in 3 ml of water, the solution was cooled at *ca.* 10 °C and 1.75 ml of aqueous formaldehyde (40 %) was added. An exothermic reaction occurred (the temperature rose to *ca.* 30 °C). The solution was cooled and adjusted to pH = 7 with 2 M NaOH (to complete the reaction with formaldehyde) and was left for 10 min in ice. Then 4 ml of 4 M hydrochloric acid was added and the solution was cooled in ice for 2 h. The solution was neutralized, saturated with NaCl and extracted with 3 × 25 ml of ether. The dried ($MgSO_4$) ether solution on evaporation gave 340 mg of white crystals (43 %), which were recrystallized from pentane, m.p. 137 – 138 °C. Anal. $C_6H_{15}N_3O_3$: C, H, N.

The trimer was also obtained when hydrochloric acid was added to an aqueous solution of monomeric formaldehyde *O*-methyloxime and the neutralized solution was extracted with ether but yields were lower because a solution of the monomer in equilibrium with the trimer contains only *ca.* 25 % of the trimer.

The molecular weight of this compound follows from its mass spectrum: The mass spectrum was obtained at 70 eV with an ion source temperature below 90 °C and using the direct inlet system; *m/e* (% rel. int.): 177 (2.2, [M]), 119 (5.4), 118 (100), 86 (2.4), 66 (2.8), 60 (47.3, [$CH_2NHOCH_3^+$]), 59 (90, [$CH_2NOCH_3^+$]), 58 (7.9), 44 (12.5), 42 (20.7, [$CH_2NCH_2^+$]), 41 (2.0). The base peak *m/e* 118 is assigned to the ionized dimer.

IR (KBr): 2985 s, 2940 s, 2895 s, 2865 s, 2813 s, 1460 s, 1450 m, 1417 w, 1362 s, 1327 vw, 1300 w, 1179 m, 1151 m, 1047 vs, 1013 w, 990 w sh, 978 m, 950 s sh, 943 s, 925 s, 905 m, 862 w, 841 w, 771 s, 740 s, 644 w, 609 w, 562 s, 548 w sh, 516 w, 471 m.

Acetaldehyde oxime was prepared according to Wieland.[7] After two distillations it still contained some paraldehyde, according to NMR spectroscopy and gas chromatography. It was rendered analytically pure by cooling until it had crystallized, followed by recrystallization from hexane. M.p.46 – 47 °C. (lit.[7] 47).

^1H NMR spectra. Weighed amounts of the compounds were dissolved in D_2O and the solutions adjusted to the desired pH value by the addition of 1 M DCl in D_2O or 1 M NaOD in D_2O.

The results obtained with formaldehyde *O*-methyloxime are shown in Table 1. The NMR spectrum of acetaldehyde oxime was identical to that reported by Karabatsos and Taller,[5] there being no indication of the formation of a cyclic trimer, either on addition of acid or on cooling a CD_3OD solution to – 55 °C.

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Electron Impact Promoted Fragmentation of Some 4-Quinolone Alkaloids and Related Compounds*

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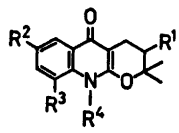
The complete high resolution mass spectra and low resolution mass spectra of sixteen alkaloids and related compounds containing the 4-quinolone ring system fused to either a dihydropyran or a dihydrofuran ring system have been recorded, and rationalizations of their decomposition modes subsequent to electron impact have been suggested. Mass spectrometry provides a powerful tool for the recognition of these 4-quinolone bases, but differentiation between isomeric compounds having fused dihydropyran or dihydrofuran ring systems is more difficult; however, quantitative and in some cases also qualitative differences distinguish their respective mass spectra.

Mass spectrometry has been widely applied to structure elucidation problems in natural products chemistry,[1] being especially powerful in the field of alkaloid structure determination.[2] No systematic study of the mass spectral fragmentation of bases containing the 4-quinolone ring system, fused either to a dihydropyran or dihydrofuran ring, has appeared. However, some fragmentation processes have been described for a few alkaloids or derivatives bearing these skeletons.[3–6] To study the mass spectral reactions of these two types of quinolone bases the complete high resolution spectra of the sixteen compounds studied, *1–16*, were recorded and the data analyzed with the aid of the recently developed computer program, INTSUM.[7] Automated treatment of the large amount of information present in a series of high resolution spectra of complicated molecules has been shown [7,8] to be a valuable tool in reducing and summarizing the data in a form useful in the subsequent analysis. For the present series of compounds the INTSUM treatment proved less useful than expected. A preliminary examination of the spectra did, however, reveal the occurrence of a number of reactions that we would not have predicted *a priori*, and it was therefore decided to study the spectra more closely, using the traditional approach of organic mass spectrometrists, to elucidate the reactions upon electron impact of these compounds.

Nearly all reactions that give rise to abundant ions take place in the dihydropyran or dihydrofuran rings, which in itself reduces the number of possible fragmentations to consider; furthermore, many of the reactions that give rise to abundant ions are in fact rearrangement processes. A thorough examination of the course of these rearrangements would conceivably require extensive isotope- and substituent-labeling, which was, however, beyond the scope of this investigation. The present report describes the basic fragmentation processes occurring upon electron impact in the quinolone ethers examined, to show how mass spectrometry can differentiate within these two classes of alkaloid; it also provides further examples of how isomerization processes subsequent to electron impact may give rise to

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Khaplofoline (*1*): $R^1 = R^2 = R^3 = R^4 = H$.
N-Methylkhaplofoline (*2*): $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$.
Ribalinine (*3*): $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = CH_3$.
Ribalinidine (*4*): $R^1 = R^2 = OH$, $R^3 = H$, $R^4 = CH_3$.
Isobalfourodine (*5*): $R^1 = OH$, $R^2 = H$, $R^3 = OCH_3$, $R^4 = CH_3$.
7-*O*-Methylribalinidine (*6*): $R^1 = OH$, $R^2 = OCH_3$, $R^3 = H$, $R^4 = CH_3$.
3′-*O*-Acetyl-7-*O*-methylribalinidine (*7*): $R^1 = OCOCH_3$, $R^2 = OCH_3$, $R^3 = H$, $R^4 = CH_3$.

fragmentation modes that are ambiguous or even misleading when using mass spectrometry alone for structure elucidation. Ten of the compounds studied, *1*, *3*–*5*, *8*, *9*, *11*, *13*, *15*, and *16*, are natural bases whilst the others have not been found in nature.

4-Quinolone bases containing a fused dihydropyran ring system. All the alkaloids of this group exhibit abundant molecular ion peaks in their mass spectra. Loss of a hydroxyl radical from the molecular ion is common to all bases studied, except the C(3′) acetoxy derivative, *7*, and could well encompass the 4-quinolone oxygen atom, since the compounds lacking an oxygen function at C(3′), *1* and *2*, also show this fragmentation, and since a similar reaction was absent in the mass spectra of several natural products containing the 2,2-dimethylchroman ring system.[9]

Elimination of a methyl radical from their respective molecular ions gives rise to abundant ions for compounds *1* and *2*. The CH_3 group is presumably lost from the *gem*-dimethyl group by cleavage α to the ether oxygen atom. Methyl loss is considerably less pronounced for the C(3′) oxygenated compounds, *3*–*7*, possibly because scission of the C(2′)–C(3′) bond, which is in these compounds α to both oxygen atoms, becomes more favorable. The oxygenated compounds do, however, give rise to characteristic ions corresponding to the combined loss of H_2O and CH_3 (for *7*, of CH_3COOH and CH_3). Presumably, these ions arise by elimination of a methyl radical followed by dehydration of that species, but no metastable ion evidence was found to clarify this point. The 3′-acetoxy derivative, *7*, eliminates CH_3COOH prior to loss of CH_3·, to yield a species formally corresponding to the 2,2-dimethylchromene ring system; loss of CH_3· is characteristic of 2,2-dimethylchromenes.[9,10]

Expulsion of 29 a.m.u. from the molecular ion occurs for bases *1*–*6*. The high resolution data show that for *1* and *2*, the fragment eliminated is an ethyl radical, possibly C(3′), C(4′) plus an additional hydrogen atom, whereas for the oxygenated bases *3*–*6* the 29 a.m.u. fragment eliminated is CHO. This difference

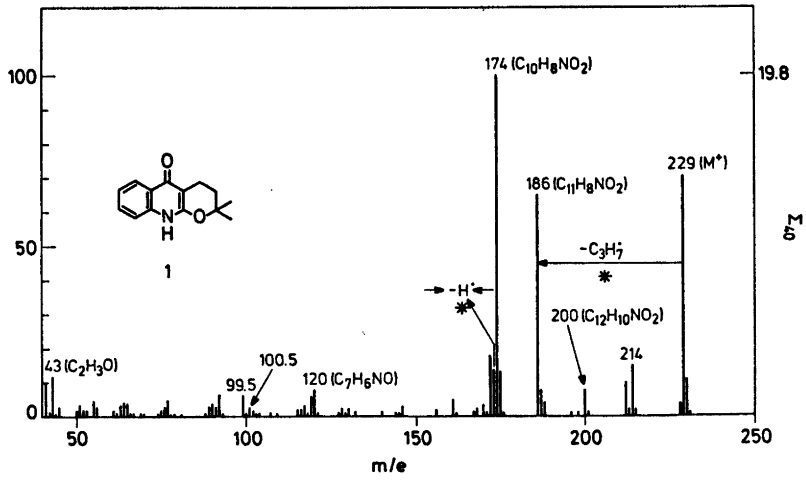
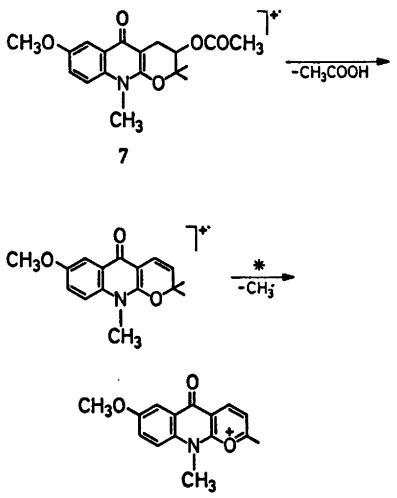


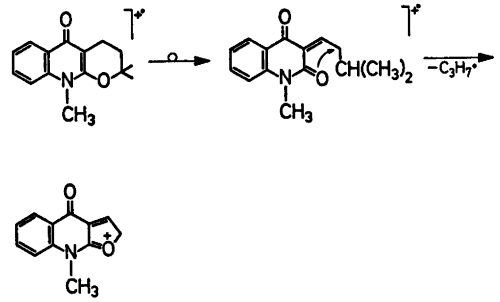
Fig. 1. Mass spectrum (70 eV) of khaplofoline (*1*).



Scheme 1.

in fragmentation is surprising since *N*-methyl-4-quinolone itself eliminates CHO,[11] and it was indeed overlooked in a previous study [6] of these compounds. The CHO fragment may incorporate the carbonyl group of the quinolone system, or it may be C(3′) with its attached oxygen function, less the hydroxyl hydrogen atom, since the mass spectrum of *6*-OD shows greater than 80 % retention of the isotopic label in the $[M-CHO]^+$ ion.

Loss of a C_3H_7 radical from the molecular ions of bases *1* and *2* gives rise to very abundant ions. This can be rationalized in terms of extrusion of the *gem*-dimethyl entity, as shown in Scheme 2; isomerization by ring contraction prior to $C_3H_7^{\cdot}$ elimination to an isopropyl-dihydrofuran structure (see below) is a viable mechanistic alternative. Convincing evidence that the analogous loss of a CH_3 fragment from chroman occurs by ring con-



Scheme 2.

traction has been presented by Djerassi and coworkers [12] and by Budzikiewicz and Lenz.[13] Also tetrahydropyrans have been shown to undergo ring contraction upon electron impact.[14] The spectra of compounds *3*–*6* show peaks corresponding to the loss of C_3H_7O rather than C_3H_7; 3-hydroxychroman analogously eliminates a CH_3O fragment.[13] This reaction may occur *via* transfer of the C-3′

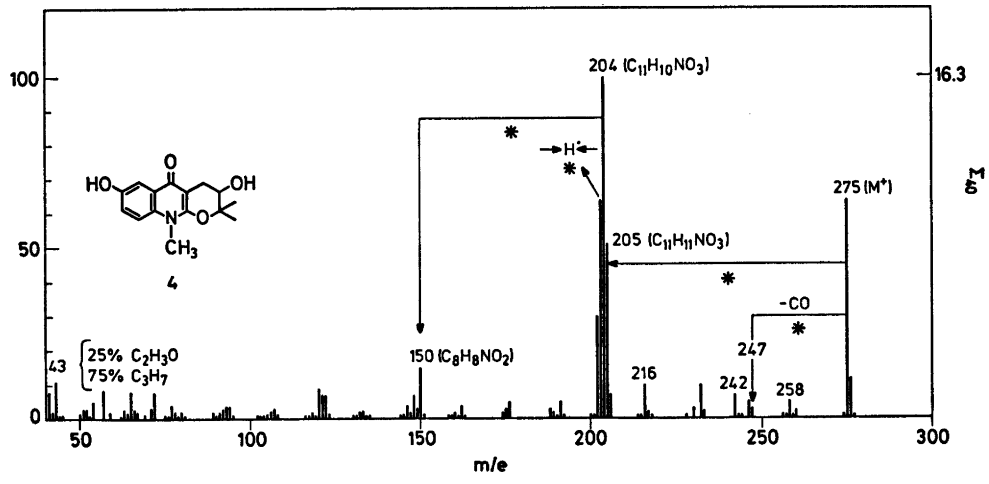
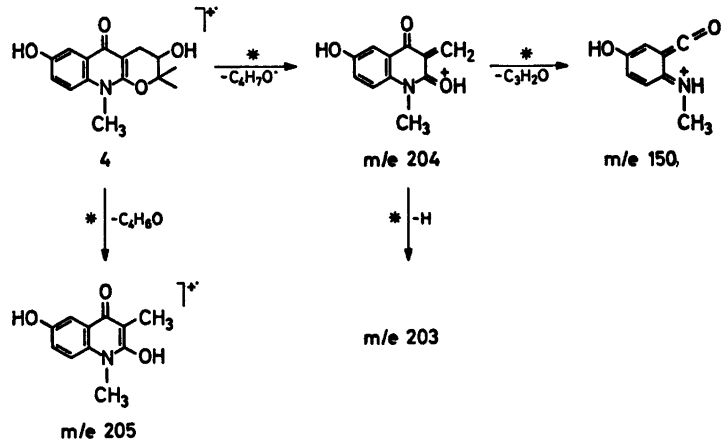


Fig. 2. Mass spectrum (70 eV) of ribalinidine (*4*).



Scheme 3.

hydroxyl to C-2′ as suggested for the latter compound,[13] or *via* ring contraction with formation of species similar to the dihydrofuran bases described below. Once again it should be noted that these reactions would not have been predicted from previous studies[9] of the related 2,2-dimethylchroman ring system.

Very intense peaks in the spectra of compounds *1* – *6* correspond to ions formed through elimination, after hydrogen migration, of fragments consisting of C(2′), C(3′), and their substituents (reactions of this type are sometimes classified as 'RDA + H' processes), and to ions formed herefrom by further loss of a hydrogen atom, as shown for compound *4* in Scheme 3 (see ions *m/e* 204 and 203). Analogous reactions have been observed for 2,2-dimethylchromanes[9] and other similar oxygen heterocycles.[15] The bases carrying a C(3′) hydroxyl group (*3* – *6*) in addition suffer extrusion of the C(2′) – C(3′) fragment after double hydrogen transfer (formation of *m/e* 205 in Scheme 3).

A characteristic ion present in all spectra corresponds in elemental composition to the molecular ion less the dihydropyran ring, plus one hydrogen atom (for *4*, *m/e* 150; see Scheme 3). Metastable ion evidence suggests that this ion is generated stepwise as shown, by loss of initially two, then four of the dihydropyran ring atoms, in what may be considered two successive RDA fragmentations.

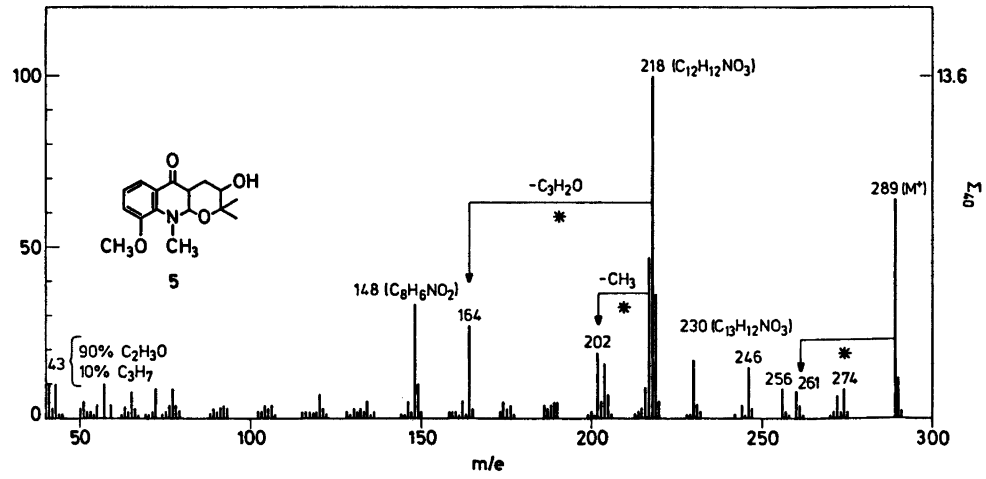
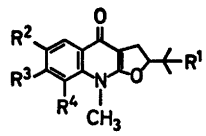
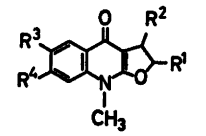


Fig. 3. Mass spectrum (70 eV) of isobalfourodine (*5*).



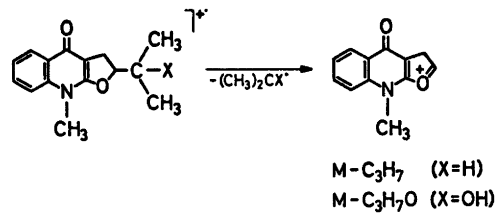
Lunacrine (*8*): $R^1=R^2=R^3=H$; $R^4=OCH_3$.
Lunine (*9*): $R^1=R^2=H$; R^3, $R^4=-OCH_2O-$.
6-Deoxyribaline (*10*): $R^1=OH$; $R^2=R^3=R^4=H$.
Ribaline (*11*): $R^1=R^3=OH$; $R^2=R^4=H$.
6-*O*-Methylribaline (*12*): $R^1=OH$; $R^2=OCH_3$; $R^3=R^4=H$.
Balfourodine (*13*): $R^1=OH$; $R^2=R^3=H$; $R^4=OCH_3$.

4-Quinolone alkaloids containing a fused dihydrofuran ring. The two alkaloids of this group which contain a 2′-isopropyl group, *8* and *9*, yield mass spectra that closely resemble those of the 2,2-dimethyldihydropyran analogs, *1* and *2* (*cf.* Figs. 1 and 4). Thus, bases *8* and *9* show reasonably intense peaks in their mass spectra corresponding to loss of OH, CH_3 and C_2H_5 radicals from the molecular ion, and very intense peaks for $[M-C_3H_7]^+$ and $[M-C_4H_4]^+$ ions, as do *1* and *2*. The occurrence of these reactions for both pairs of compounds, giving rise to spectra that are also quantitatively very similar, suggests isomerization to a common structure or mixture of structures. The facile methyl loss from the molecular ions of both pairs suggests 2,2-dimethyldihydropyran as the preferred structure; on the other hand, the presence of abundant $[M-C_3H_7]^+$ ions may conveniently be rationalized as α-cleavage from a 2-isopropyldihydrofuran structure.



Isodihydrokokusaginine (*14*): $R^1=R^2=H$; $R^3=R^4=OCH_3$.
Lemobiline (*15*): $R^1=(CH_3)_2$; $R^2=CH_3$; $R^3=R^4=H$.
Ifflaiamine (*16*): $R^1=CH_3$; $R^2=(CH_3)_2$; $R^3=R^4=H$.

Four of the dihydrofuran bases examined, *10*–*13*, contain a hydroxy substituted isopropyl group in the 2′-position. The fragmentation of these compounds is reminiscent of that of the two isopropyl compounds, *8* and *9*, except that the fragments lost are nearly all oxygen containing. Analogous to the loss



Scheme 4.

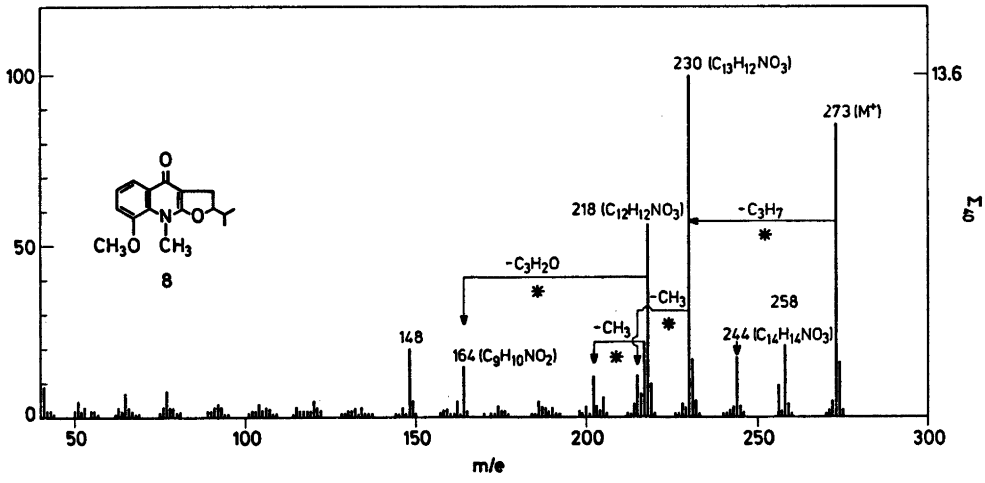


Fig. 4. Mass spectrum (70 eV) of lunacrine (*8*).

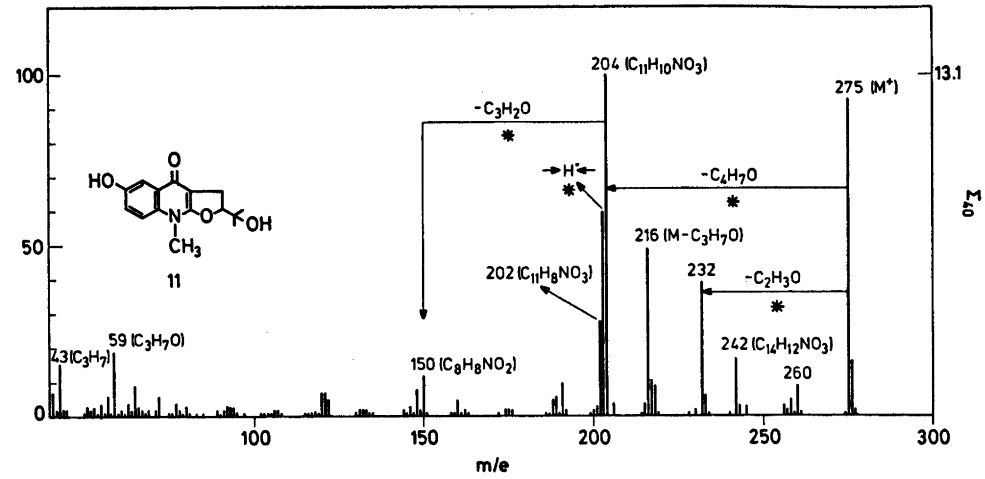


Fig. 5. Mass spectrum (70 eV) of ribaline (*11*).

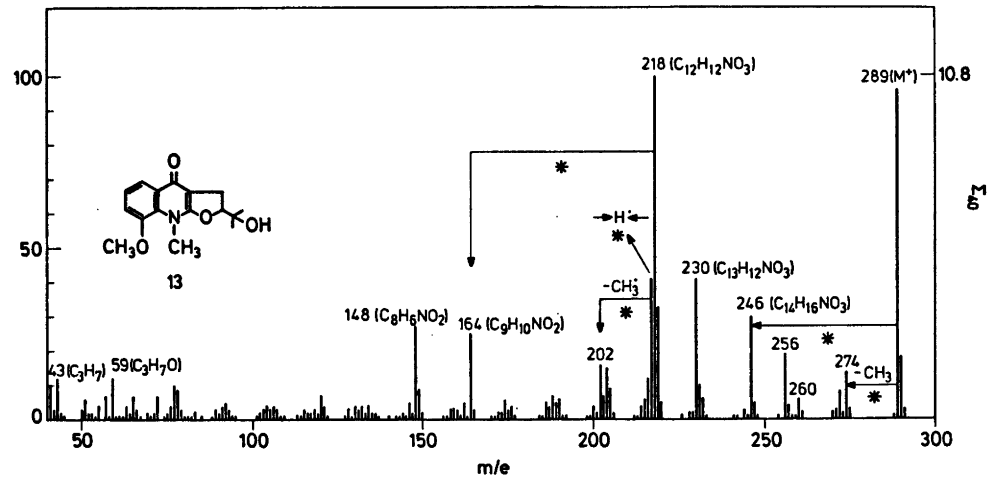


Fig. 6. Mass spectrum (70 eV) of balfourodine (*13*).

of C_3H_7 and C_4H_7 radicals from *8* and *9*, the spectra of *10*–*13* show intense peaks for $[M-C_3H_7O]^+$ and $[M-C_4H_7O]^+$ ions. The loss of $C_3H_7O^\cdot$ is visualized as cleavage α to both oxygen atoms in *10*–*13* (see Scheme 4). Similar elimination of $C_3H_7O^\cdot$ from 2-hydroxyisopropyl substituted di- and tetrahydrofurans has been observed previously.[16,17] The presence of $[M-C_3H_7O]^+$ peaks in the spectra of the 3′-hydroxydihydropyran bases *3*–*6* (see above) could be taken as evidence for the occurrence of a ring contraction process prior to fragmentation also in these compounds. It is, however, not very likely that complete isomerization to a common structure occurs prior to loss of $C_3H_7O^\cdot$ for the bases that have an oxygen function attached to the fused dihydrofuran (*10*–*13*) or dihydropyran ring (*3*–*6*), since the abundances of the corresponding ions differ considerably. The intensities of the $[M-C_3H_7O]^+$ peaks are on an average four times higher in the dihydrofuran spectra than in the dihydropyran spectra. Furthermore, only the dihydrofuran bases show appreciable

Table 1. Principal ions observed in the mass spectra of the 4-quinolone bases studied.[a]

N-Methylkhaplofoline (*2*)

M+ 243 (82), 242 (3), 228 (14), 226 (7), 214 (11), 200 (93), 188 (100), 187 (20), 186 (17), 175 (5), 134 (11), 132 (6), 130 (5), 107 (8), 105 (5), 104 (7), 77 (14), 51 (6), 41 (12).

Ribalinine (*3*)

M+ 259 (40), 242 (12), 230 (3), 226 (4), 216 (4), 200 (5), 189 (39), 188 (100), 187 (10), 186 (8), 160 (3), 134 (12), 104 (4), 77 (8), 72 (8), 57 (7), 43 (6), 41 (4).

7-*O*-Methylribalinidine (*6*)

M+ 289 (75), 274 (3), 272 (6), 261 (4), 260 (6), 256 (10), 246 (12), 244 (3), 230 (15), 219 (54), 218 (100), 217 (95), 216 (10), 205 (7), 204 (11), 203 (6), 202 (30), 164 (9), 148 (15), 136 (7), 120 (5), 77 (5), 72 (7), 59 (4), 57 (7), 43 (9), 41 (7).

3′-*O*-Acetyl-7-*O*-methylribalinidine (*7*)

M+ 331 (31), 272 (20), 271 (47), 256 (100), 254 (6), 242 (2), 230 (11), 219 (15), 218 (47), 217 (45), 216 (7), 202 (23), 164 (7), 148 (10), 136 (5), 128 (5), 120 (5), 92 (5), 77 (5), 72 (7), 65 (5), 57 (5), 43 (32), 41 (8).

Lunine (*9*)

M+ 287 (87), 272 (19), 270 (7), 258 (15), 244 (94), 232 (100), 231 (25), 230 (11), 219 (8), 178 (6), 176 (4), 148 (4), 147 (5), 91 (5), 90 (4), 65 (7), 41 (10).

6-Deoxyribaline (*10*)

M+ 259 (73), 244 (9), 242 (3), 230 (3), 226 (24), 216 (26), 201 (17), 200 (61), 189 (38), 188 (100), 187 (11), 186 (10), 175 (5), 173 (6), 144 (6), 134 (15), 132 (5), 106 (6), 77 (13), 72 (8), 59 (19), 57 (6), 51 (5), 43 (16), 41 (6).

6-*O*-Methylribaline (*12*)

M+ 289 (100), 274 (8), 272 (6), 260 (5), 256 (21), 246 (29), 230 (48), 219 (53), 218 (99), 217 (89), 216 (13), 204 (12), 202 (33), 188 (6), 164 (10), 148 (17), 136 (8), 120 (6), 92 (6), 77 (6), 72 (8), 65 (7), 59 (14), 57 (7), 43 (12), 41 (10).

Isodihydrokokusaginine (*14*)

M+ 261 (100), 246 (62), 232 (4), 230 (3), 218 (21), 216 (4), 200 (4), 190 (7), 188 (5), 178 (4), 150 (14), 122 (4), 82 (6), 69 (8), 53 (5).

Lemobiline (*15*)

M+ 243 (52), 228 (100), 226 (8), 214 (5), 200 (27), 187 (12), 183 (7), 134 (5), 77 (10), 51 (4), 41 (6).

Table 1. Continued.

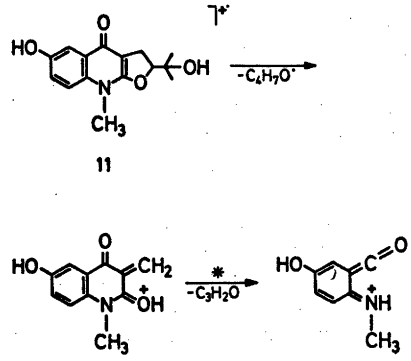
Ifflaiamine (*16*)

M+ 243 (31), 228 (100), 226 (5), 214 (12), 200 (5), 188 (5), 77 (7), 51 (3), 43 (3), 41 (3).

[a] This table is constructed of ion (intensity) pairs using relative intensity values (100 = base peak). Mass values below 3 % relative abundance have not been recorded. See also Figs. 1–6.

peaks corresponding to $[M-C_3H_7O]^+$ and $[M-C_3H_6O]^+$ ions.

A similar difference in abundance is not found for the $[M-C_4H_7O]^+$ ions, which are in both series the most abundant fragment ions, carrying between 15 and 30 % of the total ion current. This ion is formed by loss of C(2′) with its attached substituent, less one hydrogen atom for those bases carrying a fused dihydrofuran ring. This process presents an intriguing mechanistic problem, inasmuch as two skeletal bonds to the same carbon atom appear to be broken in the course of the reaction. It is not possible, in the absence of isotopic labeling data, to establish the origin of the migrating hydrogen atom, nor can the mechanism be established with certainty. One possibility would again be isomerization to a dihydropyran structure. It should be noted that the isomerization reaction taking the dihydrofuran bases *10–13* into their dihydropyran analogs, *3–6*, and *vice versa*, may be regarded as an intramolecular transetherification of a glycol mono-ether. The structure suggested (Scheme 5) for the $[M-C_4H_7O]^+$ ion, as well as the subsequent decomposition reactions of



Scheme 5.

this species, are the same as for the dihydropyran analog (Scheme 3).

In the high mass range the spectra of *10–13* show peaks corresponding to elimination of OH and CH_3 radicals from the molecular ion, as well as peaks corresponding in elemental composition to $[M-CH_3O]^+$ and $[M-C_2H_3O]^+$. The methyl fragment expelled probably originates in the C(2′) side chain, where it may be eliminated in an α-cleavage reaction. The $[M-CH_3O]^+$ is presumably formed by sequential loss of CH_3 and H_2O fragments, but no metastable ion evidence was found in support of this assumption. Loss of CH_3O has been observed in other systems incorporating a hydroxyisopropyl group attached to C-2 in a fused dihydrofuran ring.[16] Similarly, the $[M-C_2H_3O]^+$ ion is believed to arise through successive losses of CH_3 and CO neutrals, even though a rearrangement-decomposition reaction resulting in expulsion of an acetyl radical cannot be ruled out. This ion is diagnostically significant when attempting to distinguish between isomers in the present series, as it is nearly ten times more abundant for the dihydrofuran bases *10–13* than for their dihydropyran analogs *3–6*.

Three 4-quinolone bases lacking substitution or with only methyl substituents in the dihydrofuran ring, *14–16*, have been investigated in this study. All three compounds eliminate a methyl radical from their respective molecular ions, and the M − 15 species attain 62, 100 and 100 % relative intensity, respectively. In the case of *14* the eliminated methyl group could originate from the 6-methoxy group, in line with previous results [18] obtained from 6,7-dimethoxycoumarin. Subsequent loss of CO from the M − 15 species is observed and this is also in agreement with the behaviour of 6,7-dimethoxycoumarin on electron impact.[18] Compounds *15* and *16* both eliminate ethyl and propyl radicals from the molecular ion. The relative intensities of the M − 29 and M − 43 peaks in the two spectra show that extrusion of C(2′) plus a hydrogen atom is a favorable reaction for these compounds; for the remaining dihydrofuran bases extrusion of C(2′) with its attached substituents *less* a hydrogen atom is the observed reaction (see above).

CONCLUSION

The mass spectra of dihydropyran- and dihydrofuran-4-quinolone alkaloids can be of considerable value in the elucidation of the structure of these compounds. The presence of substituents in either the aromatic ring or in the dihydropyran or dihydrofuran rings is easily recognized, and the cleavage reactions of the cyclic ether portion of the molecule permit positional assignment of substituents to be made with confidence. Distinction between isomeric bases containing either a 2-isopropyl dihydrofuran ring or a 2,2-dimethyl dihydropyran ring system has, however, only been possible with certainty in the present series when the molecules possess an additional oxygen function (at C(3′) in the dihydropyran ring, or in the isopropyl group appended to C(2′) in the dihydrofuran ring. In this case the compounds containing a dihydrofuran ring are recognized by the presence in their mass spectra of abundant $[M-C_2H_3O]^+$ ions, which are nearly absent for the dihydropyran analogs, and by prominent $[M-C_3H_7O]^+$ ions, which are also of considerably reduced intensity in the dihydropyran spectra.

EXPERIMENTAL

Low resolution mass spectra were recorded by Mr R. G. Ross on an A.E.I. MS-9 mass spectrometer operating at 70 eV.

Samples were introduced into the ion source (temperature 180 °C) using the direct probe. Complete high resolution mass spectra were recorded by Ms A. Wegmann using a Varian MAT 711 mass spectrometer (ion source 200 °C). All high resolution measurements were accurate to ±4 ppm. Samples used in this investigation were of analytical purity, and except for the following compounds they are described in a previous publication.[19] The remaining bases were obtained from the following investigators: balfourodine (*13*) and isobalfourodine (*5*), Prof. H. Rapoport, Univ. of Calif., Berkeley, U.S.A.; lunacrine (*8*) and lunine (*9*), Dr. J. A. Lamberton, CSIRO, Melbourne, Australia; khaplofoline (*1*) and *N*-methylkhaplofoline (*2*), Prof. M. F. Grundon, The New University of Ulster, Coleraine, N. Ireland; ifflaiamine (*16*), Dr. W. C. Taylor, Univ. of Sydney, Australia; lemobiline (*15*), Dr. S. K. Talapatra, Univ. College of Science and Technology, Calcutta, India.

Preparation of isodihydrokokusaginine (*14*). 2,3-Dihydrokokusaginine (75 mg) and methyl iodide (1 ml) were heated in a sealed tube at

100 °C for 24 h. After evaporation to dryness anhydrous pyridine (1 ml) was added and the heating repeated at 80 °C for 12 h. The pyridine was removed *in vacuo* and the residue partitioned between chloroform (3 ml) and 5 % aqueous sodium sulfite solution (0.5 ml). The organic phase was dried ($MgSO_4$) and evaporated. The residue was crystallized from alcohol to constant m.p. 222–225 °C; yield 25 mg. M^+ found 261.1008; calc. for $C_{14}H_{15}NO_4$: 261.1001.

Preparation of 3'-O-deuterio-7-O-methylribalinidine. The parent base (3 mg) was dissolved in CH_3OD (1 ml) and the solvent removed *in vacuo*. This procedure was repeated twice and the mass spectrum immediately recorded. The product consisted of 65 % d_1 and 35 % d_0 species.

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β-Thioxoketones. I. Preparation and Structure of Thioacetylacetone

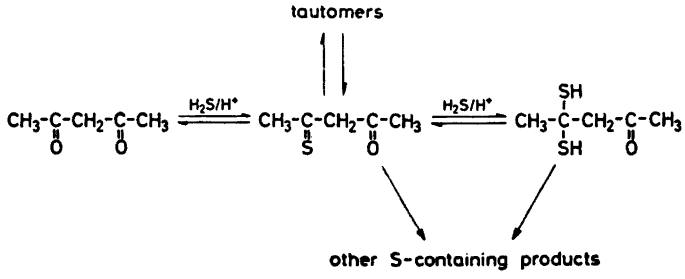
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The acid catalysed reaction of acetylacetone with H_2S has been investigated, and conditions leading to complete conversion of the diketone into thioacetylacetone have been found. ^1H NMR, IR, Raman, and UV spectral investigations of the title compound and its *S*-methyl derivative have established that the former exists as an equilibrium mixture of two rapidly interconverting tautomers, (*Z*)-4-mercaptopent-3-en-2-one and (*Z*)-4-hydroxypent-3-ene-2-thione (predominant tautomer). Similar tautomeric features have been found for 1-phenylbutan-1-one-3-thione and 1,4-diphenyl-butan-1-one-3-thione, which were also obtained in good yields by acid catalysed reaction of the corresponding diketones with H_2S.

β-Thioxoketones have attracted considerable attention in the past as chelating agents with promising applications especially within analytical chemistry.[1–3] However, these compounds are also interesting for structural reasons, and because they may be of utility in the synthesis of a variety of heterocyclic compounds (exemplified recently [4]) just as are their oxygen analogues. Against this background thioacetylacetone, being related to the simplest β-diketone, is of particular interest.

Synthesis. Thioacetylacetone has been synthesized by basepromoted condensation of acetone with ethyl thionoacetate in 8 % yield,[5] by base catalysed reaction of acetylacetone with H_2S (yield: 24 %),[6–8] and by acid catalysed reaction of acetylacetone with H_2S (yield: 20 %).[9,10] Evidently, none of the reported methods can be satisfactory, if thioacetylacetone is required with a high degree of purity (*gem*-dithiol formation is a prevailing process under the reaction conditions stated in the two last mentioned cases) and in a quantity appropriate for further synthetic use. Referring to our experiences with the synthesis of β-thioxo esters [11,12] we decided to study the reaction of acetylacetone with H_2S under acidic conditions in order to find easily reproducible conditions leading to a complete conversion of the diketone into merely its monothio derivative (Scheme 1). The ambiguity of this type of reaction is well documented.[7,11,13] It should also be noticed that, in the hands of Fromm and Ziersch,[14] the reaction of acetylacetone with H_2S in acidic ethanol afforded a dimer of 2,4-pentanedithione with tetrathiaadamantane structure as the only isolated product. Our investigations led to the conclusion that the critical factors controlling the reaction course are the reaction temperature and the nature



Scheme 1.

of the solvent used, whereas a decisive dependence on the concentration of the catalyst (HCl), as stated by other authors,[9] could not be confirmed. Treatment of acetylacetone in acetonitrile solution (0.6 – 0.7 M) with excess of both H_2S gas and HCl gas at −40 °C leads to the complete and exclusive formation of thioacetylacetone within 6 h. The crude product (yield: 80 – 89 %) is sufficiently pure for further, immediate synthetic use. However, thioacetylacetone is thermally unstable, and distillation of the crude product (at reduced pressure) inevitably leads to partial decomposition, the degree of decomposition depending on the distillation conditions (Experimental). Decomposition also takes place slowly at room temperature, but freshly distilled thioacetylacetone can be kept for months in a closed ampoule at −20 °C.

The above-mentioned reaction conditions were found applicable also to the synthesis of 1-phenylbutan-1-one-3-thione (*2*), but the conversion of 1,4-diphenylbutane-1,3-dione into 1,4-diphenylbutan-1-one-3-thione (*3*) required a somewhat higher reaction temperature (−20 °C) as well as a prolonged reaction time.

The methylation of thioacetylacetone (*1*) with sodium hydride and iodomethane afforded as the only product the *S*-methyl derivative (*Z*)-4-(methylthio)pent-3-en-2-one (*4*).

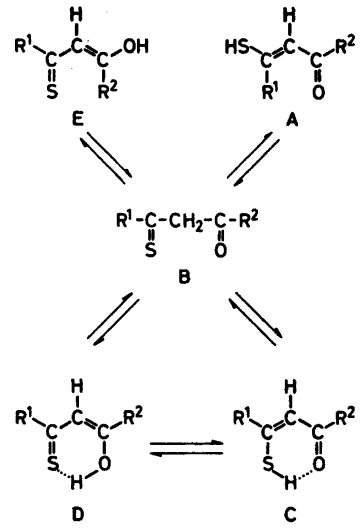
Structure. A survey over the potential tautomeric forms of thioacetylacetone and the likely pathways of their interconversion is given in Scheme 2 ($R^1 = R^2 = Me$). In general, tautomeric interconversion is sufficiently slow to allow for NMR spectroscopic distinction between the tautomers.[15] The ^1H NMR spectrum of thioacetylacetone shows only four sharp signals, at δ 2.09 (3 H), 2.37 (3 H), 6.20 (1 H), and 13.53 (1 H) (Table 1).* Immediately, this points to the existence of either of the tautomers *C* and *D*. The low-field signal at δ 13.53 occurs in the region characteristic for enolic protons engaged in intramolecular hydrogen bonding (δ 12 – 17).[17–20] Chelated enethiolic protons give rise to signals in the region δ 4.8 – 8.5.[11,12,21] On this background the enolic structure *D* seems preferable, as also suggested by Klose *et al.*[22] On the other hand, the existence of a weak coupling ($J = 0.4$ Hz) between the vinylic proton and the methyl group protons (R^1) lends support to the preference of the enethiol structure *C*, as chelated enethiols,[11,12,21]

* Possible minor peaks at δ 1.83, 2.13 (2.17[16]), 2.97 (3.03[16]), and 3.27 (3.23[16]) are indicative of the presence of the *gem*-dithiol 4,4-dimercaptopentan-2-one (MeC<, Ac, CH_2, and SH, resp.) and not, as stated previously,[7,16] of the presence of the thioxo ketone tautomer (*B*). For comparison, the ^1H NMR spectrum of ethyl 3,3-dimercaptobutyrate exhibits signals at δ 1.89 (MeC<), 2.94 (CH_2), and 3.25 (SH).[7]

Table 1. ^1H NMR chemical shifts and coupling constants (in brackets) of β-thioxoketones and derivatives. The solvent is CCl_4.

	R^1	R^2	$\delta(R^1)$	$\delta(R^2)$	$\delta(=CH-)$	$\delta(H^{chel})$	δ(SMe)
1	Me	Me	2.37 d (0.4)	2.09 s	6.20 q (0.4)	13.53[a] s[b]	
2	Me	Ph	2.54 m[c,d]	7.2 – 7.9 m	6.91 m[c]	15.09[a] m[c]	
3	$PhCH_2$	Ph	3.97 s[b](2H) 7.13 s(5H)	7.1 – 7.8 m	6.78 s[b]	14.86[a] s[b]	
4	Me	Me	2.30 d (1.1)	2.08 s	5.73 q (1.1)		2.26 s

[a] Value at infinite dilution.[11] [b] Any higher multiplicity could not be detected by signal expansion. [c] Multiplicity uncertain due to limitations in the resolution capacity of the apparatus. [d] Apparently dd (0.2/0.2).



Scheme 2.

but not chelated enols,[17–20] exhibit such a coupling. Structure *C* is plausible also because of the fact that the degree of enethiolization of thioketones in general is considerably higher than the degree of enolization of the corresponding ketones.[23] However, the coupling constant in question is remarkably smaller than that usually found for structurally related enethiols (as an example, $J = 1.2$ Hz in the (*Z*)-enethiol tautomer of ethyl thioacetoacetate)[11] as well as that found for the *S*-methyl derivative (*4*) (Table 1). The occurrence of a very rapid intramolecular proton exchange in (*Z*)-enolized β-diketones has been established,[24–26] and it is attractive to assume the occurrence of a similar proton exchange also among β-thioxo ketones, *i.e.* the occurrence of a very rapid interconversion between the equilibrated tautomers *C* and *D* by simple chelate proton transfer. If the interconversion $C \rightleftharpoons D$ takes place at a rate much higher than the difference in resonance frequencies between the NMR signals from the two tautomers, a single spectrum will be observed, where the positions of the resonance signals are weighted averages of the chemical shifts of the separate tautomers.[27] Weighted averages of couplings may also be expected. In fact, any apparent discrepancy is removed, if the NMR spectrum of thioacetylacetone is interpreted in terms of an equilibrium mixture of the two rapidly interconverting tautomers *1C* and *1D*. The small coupling ($J = 0.4$ Hz) between the vinylic proton and the methyl protons (R^1) suggests the contribution of about 33 % of the enethiol tautomer (*1C*) (*J* estimated to ~1.2 Hz[11]) and about 67 % of the enol tautomer (*1D*) (*J* estimated to ~0 Hz) according to the principle of weighted averages. Similarly, the chelate proton chemical shift is between those normally observed for chelated enethiols[11] and that found for enolic acetylacetone (δ 15.57)[17,20] Assuming the δ-values 15.57 and 7.30 (the chemical shift of the mercapto-proton of the (*Z*)-enethiol tautomer of ethyl thioacetoacetate[11]) as the representative chelate proton chemical shifts of *1D* and *1C*, respectively, the observed average shift at δ 13.53 would reflect the existence of about 25 % of *1C*. However, according to predictions based on a theoretical study of hydrogen bondings by means of the Schroeder-Lippincott potential function model,[28] intramolecular O–H···S hydrogen bondings are stronger than the corresponding O–H···O hydrogen bondings, *i.e.* presumably the chemical shift of the enolic proton of *1D* is even higher than δ 15.57. Consequently, the equilibrium percentages of *1C* should be estimated to be somewhat higher than 25 %. The ^{1}H NMR spectra of *2* and *3* (Table 1) are also consistent with the existence of solely the $C \rightleftharpoons D$ equilibrium system. In both cases increased chelate proton chemical shifts and reduced allylic couplings are observed, thus indicating relatively smaller equilibrium percentages of the enethiol tautomer (*C*).

The conclusion that thioacetylacetone must exist as an equilibrium mixture of the tautomers *1C* and *1D* has been drawn very recently also by Fabian[29] on the basis of UV spectroscopic investigations combined with LCAO-MO calculations. Our UV spectral data (Table 2) are in good agreement with those of Fabian,[29] although smaller deviations with respect to band intensities are notable. The UV spectra of *1–3* are all characterized by two significant bands of which the band at shorter wavelength (with absorbance A_s) is attributed to a $\pi \rightarrow \pi^*$ transition in the S–C=C–C=O chromophore (*C*), and that at longer wavelength (with absorbance A_l) to a $\pi \rightarrow \pi^*$ transition in the O–C=C–C=S chromophore (*D*). The solvent

Table 2. UV spectral data of β-thioxoketones and derivatives. Calculated percentages of enethiol tautomer (*C*) in different solvents.

	Solvent	Concentration 10^{-5} mol/l	λ_{max}(nm)[a] A_1	A_2	A_3	A_4	%*C*[b]
1	C_6H_{12}	6.63	455	354	296	237	27
			0.0026	0.600	0.226	0.110	(30)
	CCl_4	6.65	455	356	296		32
			0.0028	0.524	0.243		(34)
	EtOH	7.61	448	354	293	236	39)
			0.0024	0.579	0.374	0.120	(50)
	MeCN	8.92	445	357	291		44
			0.0026	0.562	0.440		(48)
4	C_6H_{12}	3.23			286		
					0.610		
2	C_6H_{12}	8.31		389	322		19
				1.205	0.280		
3	C_6H_{12}	4.44		392	307		20
				0.766	0.190		
	EtOH	5.30		394	314		30
				0.792	0.344		
	MeCN	3.89		394	311		40
				0.452	0.308		

[a] A_n = Absorbance of band No. *n*. [b] The percentages have been calculated under the assumption of approximately similar molar absorption coefficients of the bands 2 and 3 (% $C = 100A_3/(A_2+A_3)$ %).[29] The values in brackets are those determined by Fabian.[29]

interdependence of the two bands is interpreted in terms of a solvent promoted displacement in the $C \rightleftharpoons D$ equilibrium system. The assumption of approximately similar molar absorption coefficients for the two bands may lead to calculations of equilibrium percentages of the tautomers (Table 2), which are in good agreement with those estimated on the basis of the NMR data. However, for a more comprehensive discussion of the UV spectral properties of thioacetylacetone, the paper of Fabian [29] is recommended.

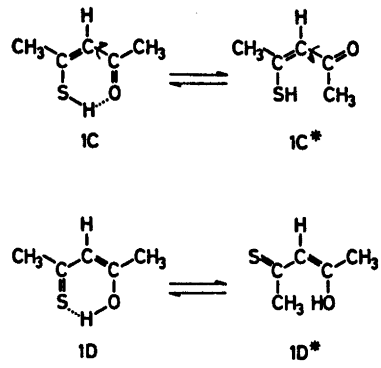
In contrast to the findings above, Siiman and his coworkers,[30] on the basis of IR and Raman

Table 3. Characteristic IR and Raman absorption bands (cm^{-1}) of thioacetylacetone (*1*) and its *S*-methyl derivative (*4*).

1			*4*		
IR	Raman	ΔI[a]	IR	Raman	ΔI[a]
2500(w,br.)	[b]				
1672(w,sh)	1671(w)	(−)	1683(s)	1682(s)	(−)
1652(m)	[b]				
1571(vs)	1573(vs)	(+)	1570(s)	1572(vs)	(+)
1422(m)	1422(w,sh)	(−)	1431(m)	1433(w)	(−)
1376(w,sh)	1378(w,sh)	(=)	1382(s)	1382(w)	(−)
1363(m)	1365(m)	(=)	1362(s)	1365(w)	(−)
1264(m)	1266(m)	(=)			
1218(w,sh)	1220(m)	(+)			
1202(s)	1202(w,sh)	(−)	1200(s)	1200(w)	(−)
1125(m)	1120(s)	(+)	1124(m)	1125(w)	(−)
1027(m)	1028(m)	(+)	1010(m)	1010(s)	(+)

[a] Intensity of Raman band compared with the corresponding IR band, increased: (+); decreased: (−); approximately unchanged: (=). [b] Absorption not detectable.

spectroscopic investigations combined with normal-coordinate analyses, reached the conclusion that thioacetylacetone should exist predominantly in the enethiol form (*1C*). The existence of a few percentages of the thioxo ketone form (*1B*) was also recognized, but the enol structure (*1D*) was not taken into consideration.[30] We have recorded the IR and Raman spectra of thioacetylacetone (*1*) and, for comparison, also of its *S*-methyl derivative (*4*), and we wish to proclaim that the vibrational spectra of thioacetylacetone clearly indicates the co-existence of the two tautomeric forms *1C* and *1D*. Some characteristic IR and Raman spectral data of *1* and *4* are found in Table 3.



Scheme 3.

The presence of the enethiol tautomer *1C* should be reflected by at least 3 characteristic absorptions bands, arising from the S–H stretching vibration, the C=O stretching vibration, and the C=C stretching vibration. A weak, broad band at ~2500 cm^{-1} indicates the intramolecularly chelated mercapto-group,[7,11,12,21] and the very intense band at 1571 cm^{-1} (1573 cm^{-1} in the Raman spectrum) reflects unambiguously a carbonyl-conjugated C=C double bond (compare with the vibrational spectral data of the *S*-methyl derivative *4* in Table 3). The two less intensive bands at 1652 cm^{-1} and 1672 cm^{-1} are ascribed to conjugated C=O stretchings in the intramolecularly H-bonded structure *1C*, and its rotamer *1C**, respectively (Scheme 3). It has been demonstrated[11,12] that α,β-unsaturated β-mercapto-esters ((*Z*)-enethiol form of β-thioxo esters) do exhibit such a rotameric effect, thus giving rise to two distinct ester carbonyl stretching absorption bands. With respect to the more abundant tautomer *1D*, bands arising from the O–H, the C=C, and the C=S stretchings should be immediately expected. However, the O–H stretching vibration bands of hydroxy-groups engaged in strong intramolecular H-bondings (as in enolized β-diketones) are known to be extremely broad and weak, and may very often escape detection.[31] The absence of a well-defined O–H stretching vibration band may therefore very probably simply reflect a strong intramolecular chelation. The C=C stretching absorption band of *1D* may be expected to coalesce with that of *1C*. It has been shown very recently that the C=S stretching vibration band of simple aliphatic thioketones occurs in the region 1244–1270 cm^{-1} with an intensity considerably lower than that of the C=O stretching band of the corresponding ketone.[32] In the actual case, the C=S band(s) should appear at a somewhat lower frequency due to conjugation and chelation. Using the values 1670 cm^{-1} (frequency of the conjugated, non-chelated carbonyl group, as in *1C**) and 1600 cm^{-1} (frequency of the conjugated, chelated carbonyl group in enolized β-diketones[33]) as references, the equation[32] $\nu(C=O)/\nu(C=S) = 1.38$ predicts C=S stretching vibration bands at 1212 cm^{-1} (structure *1D**) and 1160 cm^{-1} (structure *1D*), respectively. Furthermore, these bands should be of a strongly enhanced intensity in the Raman spectrum.[32–34] On this background, we ascribe the weak IR band at 1218 cm^{-1} (1220 cm^{-1} in the Raman spectrum) to ν(C=S) of *1D**, and the medium intensity band at 1125 cm^{-1} (1120 cm^{-1} in the Raman spectrum) to ν(C=S) of *1D*.

EXPERIMENTAL

^{1}H NMR spectra were recorded on 10–20 % solutions on a Jeol C-60 HL spectrometer. TMS was used as internal reference. The chemical shifts are correct within ±0.02 ppm. The coupling constants were measured on expanded signals and are expressed numerically with an accuracy within ±0.1 Hz. UV spectra were measured on a Beckman Acta III spectrophotometer. IR spectra were recorded on 5–20 % CCl_4 solutions on a Perkin-Elmer 457 grating spectrophotometer. Raman spectra were recorded on 20 % CCl_4 solutions on a

spectrometer equipped with a Jarrell-Ash model 25–101 double grating monochromator, and the exciting line was 514.5 nm from Spectra-physics model 165–00 argon ion laser.

Boiling and melting points are uncorrected. Unless stated otherwise the yields refer to the isolated quantities of the pure products. The purity was checked by NMR and elemental analysis. Elemental analyses were carried out in the micro-analytical laboratory of the Department of General and Organic Chemistry, the H. C. Ørsted Institute, University of Copenhagen.

Thioacetylacetone (1). A solution of 20.0 g (0.2 mol) of acetylacetone in 300 ml of MeCN was cooled to −50 °C, and a stream of H_2S gas was passed through it for 1.5–2 h, during which the temperature was allowed to rise to −40 °C. Then a stream of HCl gas was passed through the solution for 1.5 h, whilst the reaction temperature was kept carefully at −40 °C. After a further supply of H_2S gas for 2.5–3 h at −40 °C, the reaction solution was poured into a mixture of 500 ml of ice-water, 300 ml of pentane, and 100 ml of ether under manual stirring. The layers were separated, the aqueous layer was extracted with 200 ml of a 3:1 mixture of pentane and ether, and the combined organic extracts were washed twice with water (until neutral), and dried ($CaSO_4$). The solvents were removed by evaporation at reduced pressure at room temperature, leaving 18.5–20.5 g of a yellow oil, which by NMR analysis was found to consist of the desired product together with minor quantities of non-evaporated solvents (5–10 %). Subsequent distillation *in vacuo* afforded pure thioacetylacetone, but the yield was drastically reduced by this purification due to partial thermal decomposition. In four experiments (carried out under identical reaction conditions) the yields of pure, distilled *1* were found ranging from 11.9 g (51 %) (b.p. 58–59 °C/11 mmHg) to 7.8 g (33 %) (b.p. 73–75 °C/22 mmHg). Yellow oil, b.p. (see above), 59–60 °C/13 mmHg, 66–67 °C/18 mmHg (lit. 50–55 °C/10 mmHg,[9] 68 °C/14 mmHg,[35] 62 °C/15 mmHg [5]), n_D^{25} 1.5681 (lit. n_D^{19} 1.556,[9] n_D^{25} 1.559 [35]). Anal. C_5H_8OS: C, H, S.

The treatment of acetylacetone with H_2S and HCl at −50 °C, but otherwise under the same conditions as described above, resulted in incomplete conversion of the diketone into thioacetylacetone. At −30 °C the same reaction led to a mixture of thioacetylacetone and 4,4-dimercaptopentan-2-one (~10 %, identified by NMR and IR). A series of experiments, in which ethanolic solutions of acetylacetone were treated with H_2S and HCl at temperatures from −50 to 0 °C, resulted in less well-defined product mixtures of thioacetylacetone, unreacted starting material, 4,4-dimercaptopentan-2-one, and at temperatures above −20 °C, also other sulfur-containing products (not identified).

1-Phenylbutan-1-one-3-thione (2). A solution of 8.1 g (50 mmol) of benzoylacetone in 200 ml of MeCN was treated with H_2S and HCl according to the procedure given above. Following also the same working-up procedure, the crude product (8.4–8.9 g, containing ~10 % of non-evaporated solvents) was obtained as a yellow oil that solidified rapidly on cooling (refrigerator). Recrystallization (light petroleum) afforded 4.1–4.3 g (46–48 %) of the pure product. M.p. 26 °C (lit. m.p. 25–26 °C,[5] 28 °C[9]). Anal. $C_{10}H_{10}OS$: C, H, S. IR (CCl_4): 1580 (w, sh), 1548 (s), 1230 (s) cm^{-1}.

1,4-Diphenylbutan-1-one-3-thione (3): A solution of 5.95 g (25 mmol) of 1,4-diphenylbutane-1,3-dione in 250 ml of MeCN was cooled to −50 °C. H_2S gas was supplied for 1.5 h, during which the temperature was allowed to rise to −40 °C. Then dry HCl gas was supplied for 1.5 h (at −40 °C), followed by H_2S gas for further 3 h, during which the temperature was raised gradually to −20 °C. The reaction solution was kept at −20 °C for 16 h before working-up according to the usual procedure. The crude product (6.3 g) appeared as a reddish oil that rapidly solidified on cooling (refrigerator). Recrystallization (hexane) gave 3.44 g (54 %) of the pure product. Yellow crystals, m.p. 50–50.5 °C. Anal. $C_{16}H_{14}OS$: C, H, S. IR (CCl_4): 1608 (w, sh), 1593(m), 1558 (s), 1250 (s) cm^{-1}.

(Z)-4-(Methylthio)pent-3-en-2-one (4). A solution of 4.64 g (40 mmol) of thioacetylacetone in 20 ml of dry benzene was added dropwise at room temperature during 0.5 h to a stirred suspension of 1.00 g of NaH in 50 ml of dry benzene. Stirring was continued until the evolution of H_2 had ceased (1 h). Then a solution of 8.52 g (>40 mmol) of MeI in 20 ml of dry benzene was added dropwise under stirring during 0.5 h. The stirring was continued at room temperature overnight (16 h). 50 ml of ether and, successively, 200 ml of water (caution!) were added, and the reaction mixture was stirred until the two layers were completely clear. The layers were separated, and the organic layer was washed twice with 200 ml of water, and then dried ($CaSO_4$). The solvents were evaporated, and the residual oil (3.5 g) subjected to PLC (2 plates, silica gel $PF_{254+366}$ (Merck) support (450 × 250 × 3 mm), one elution with a 1:1 mixture of ether and light petroleum). The material absorbed in the R_F-region 0.64–0.84 was isolated to give 2.02 g (39 %) of the pure product. Colourless oil, b.p. 84–85 °C/12 mmHg (lit.[39] 60 °C/0.8 mmHg). Anal. $C_6H_{10}OS$: C, H, S.

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Cyclopentadienylcopper Reactions with Some Benzylic Halides, α-Haloketones and Pyrylium Ion

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Cyclopentadienylcopper(I) tributylphosphine ($CpCuPBu_3$) reacts with chlorotriphenylmethane and dibromodiphenylmethane under mild conditions to give cyclopentadienyltriphenylmethane and dicyclopentadienyldiphenylmethane, respectively. With 2-bromo-2-phenylacetophenone and trimethylpyrylium perchlorate it gives 2-cyclopentadienyl-2-phenylacetophenone and trimethylazulene, respectively. The mechanism of these reactions are briefly discussed.

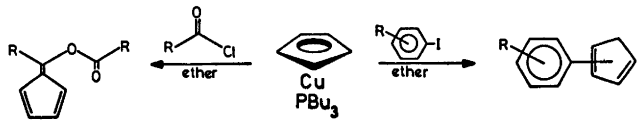
During the last decade organocopper reactions have been developed into valuable synthetic tools by several groups. Various lithium cuprates have been successfully applied as conjugate-addition reagents for the synthesis of natural products. Other organocopper compounds often generated "*in situ*" in the presence of suitable ligands also show promising utility as new and selective reagents. The field has been reviewed recently.[1–4] In contrast to most other organocopper compounds cyclopentadienylcopper(I) tributylphosphine is monomeric and is reasonably stable.[5–7] It is a weak base but rather reactive towards organic halides. Previously we have reported on the reactions of $CpCuPBu_3$ with iodoarenes [8,9] and with acid chlorides [10] to give arylcyclopentadienes and acyloxyfulvenes, respectively (Scheme 1).

The reactivity of $CpCuPBu_3$ towards these organic halides has prompted us to investigate its reaction with other organic halides.

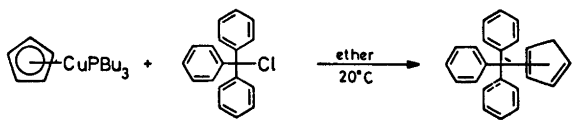
RESULTS AND DISCUSSION

Cyclopentadienylcopper(I) tributylphosphine ($CpCuPBu_3$) reacts at room temperature with chlorotriphenylmethane in ether to give cyclopentadienyltriphenylmethane in a good yield (Scheme 2). The product is a mixture of isomers with the cyclopentadienyl ring bonded in the 1- and 2-positions. A report on an analogous reaction with nickelocene has been published.[11]

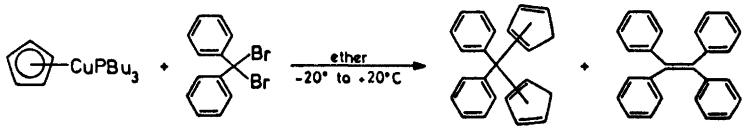
More interesting is the reaction of $CpCuPBu_3$ with dibromodiphenylmethane which gives dicyclopentadienyldiphenylmethane and tetraphenylethene (Scheme 3).



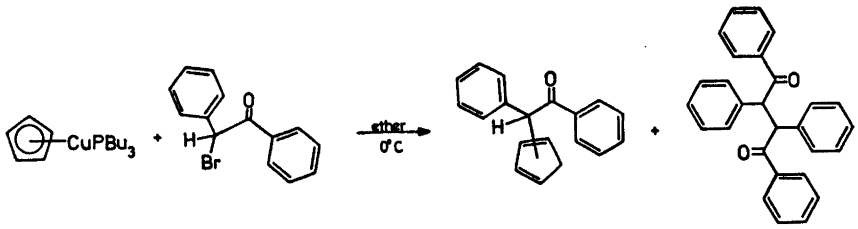
Scheme 1.



Scheme 2.



Scheme 3.



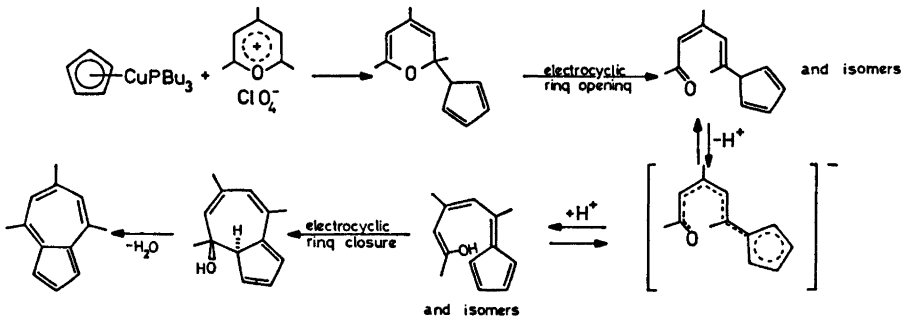
Scheme 4.

In the reaction between $CpCuPBu_3$ and dibromodiphenylmethane two reaction paths with different mechanisms seem to compete. One leads to tetraphenylethene *via* an initial one-electron transfer reaction followed by a radical dimerisation or possibly by a carbene dimerisation. The other reaction path leads to dicyclopentadienyldiphenylmethane by a route that is more likely to involve an oxidative addition of a carbon-bromine bond to copper or a nucleophilic substitution of bromine by a cyclopentadienyl group originally bonded to copper.

We have also reacted $CpCuPBu_3$ with various α-halocarbonyl compounds. Reduction and reductive dimerisation of the α-halocarbonyl compounds occur frequently. However 2-bromo-2-phenylacetophenone reacts with $CpCuPBu_3$ to give 2-cyclopentadienyl-2-phenylacetophenone as the major product, together with some 1,2,3,4-tetraphenylbutan-1,4-dione (Scheme 4).

So far the reactions described have been specific for cyclopentadienylcopper or in a few cases nickelocene. However, other less specific reactions can still be of some interest when discussing reaction mechanisms. Cyclopentadienyl sodium is known to react with trimethylpyrylium perchlorate to give trimethylazulene in 60 % yield.[14] No detailed mechanism has been proposed for this reaction, although the initial attack of a cyclopentadienide ion on the pyrylium ring and a subsequent ring opening has several analogies. With trimethylpyrylium perchlorate, $CpCuPBu_3$ gives trimethylazulene in 60 % yield. The following reaction scheme is suggested since $CpCuPBu_3$ is a weak base and is not expected to undergo metal-hydrogen exchange reactions (Scheme 5).

The initial nucleophilic attack is followed by an electrocyclic ring opening (6 π-system) to give a mixture of ketones. These are isomerized to the enols one of which can undergo an un-



Scheme 5.

usual electrocyclic ring closure (10 π-system) to form the azulene skeleton. The last reaction is the elimination of water "releasing" the resonance energy of azulene.

We have found that $CpCuPBu_3$ is a rather selective reagent for the introduction of cyclopentadienyl groups by reaction with organic halides, such as iodoarenes, acid chlorides, benzyl halides and some α-halocarbonyl compounds. The reactions are run under neutral conditions in ether from -25 °C to reflux.

Several mechanisms have been proposed for organocopper reactions, the more common ones being the oxidative addition of a substrate to an organocopper compound followed by reductive elimination,[12,13] one-electron transfer reaction with oxidation and reduction of copper(I) and copper(II) species [18] and "copper-assisted nucleophilic substitution" where the organic part of the organocopper compound acts as a nucleophile.[19]

No single mechanism seems to fit our experimental results. Tetraphenylethylene and tetraphenylbutandione are most likely formed *via* one-electron transfer processes whereas nucleophilic substitutions or alternatively oxidative additions occur during the formation of the cyclopentadienyl derivatives.

EXPERIMENTAL

Melting points were determined on a Reichert Termophan hot stage melting point apparatus. NMR spectra were recorded on a Varian A60 or a Bruker WH 270, UV spectra on a Beckman DK-2A, IR spectra on a Beckman IR-10 and MS on an AEI MS 902 instrument.

Cyclopentadienylcopper(I) tributylphosphine ($CpCuPBu_3$) was prepared by the method suggested by Cotton and Marks.[15] All reactions with $CpCuPBu_3$ were run in a dried apparatus under dry, oxygen-free nitrogen.

$CpCuPBu_3$ and chlorotriphenylmethane. A solution of chlorotriphenylmethane (0.01 mol) in ether (50 ml) was added dropwise to a freshly prepared solution of $CpCuPBu_3$ (0.01 mol) in dry diethyl ether (200 ml). The mixture was stirred at room temperature for 5 h. The white precipitate of cyclopentadienyltriphenylmethane (1.0 g) was formed slowly and collected. The filtrate was concentrated and chromatographed on silica gel (Merck. Kieselgel 60, 70–230 mesh ASTM) with tetrachloromethane as eluent to give more cyclopentadienyltriphenylmethane (0.8 g, m.p. 190–194 °C lit.[11] 199–200 °C, total yield 58 %). The product, which was identified by IR and NMR spectra, was an isomeric mixture of 1- and 2-triphenylmethylcyclopentadienes. NMR (270 MHz, $CDCl_3$): δ 7.29–7.15 (15, m, aromatic protons), 6.41, 6.34, 6.27, 6.21, and 6.06 (3, m, olefinic protons) and 3.03, 2.90 (2, m, methylene protons).

$CpCuPBu_3$ and dibromodiphenylmethane. A solution of dibromodiphenylmethane (0.01 mol) in ether (50 ml) was added dropwise to a freshly prepared solution of $CpCuPBu_3$ (0.02 mol) in ether (150 ml). The mixture was stirred for 15 h at -25 °C and placed in a refrigerator (+5 °C) for another 24 h. The precipitate was removed and the filtrate was concentrated to 10 % of the starting volume. A second filtration yielded tetraphenylethene (0.61 g). The resulting filtrate was evaporated and chromatographed on silica gel with tetrachloromethane giving an isomeric mixture of di(1,3-cyclopentadien-1-yl)- or di(1,4-cyclopentadien-1-yl),- and (1,3-cyclopentadien-1-yl)-(1,4-cyclopentadien-1-yl)diphenylmethane in a 3:2 ratio, as faintly yellow crystals (0.63 g, 21 %, m.p. 115–127 °C). NMR ($CDCl_3$): δ 7.25–7.15 (25, m, aromatic protons), 6.45–6.29 (10, m, cyclopentadienyl olefinic protons), 6.19 (1, sextet, cyclopentadienyl olefinic protons), 5.98 (3, quintet, cyclopentadienyl olefinic protons), 5.87 (1, quintet, cyclopentadienyl olefinic protons), 3.00 (6, quintet, methylene protons), 2.97 (2, q, methylene protons), 2.93 (2, q, methylene protons). IR (KBr): 3350 (s), 1598 (m), 1490 (s), 1448 (s), 1376 (m), 1350 (s), 1036 (m), 905 (s), 755 (m), 734 (s), 703 (s), 662 (s) and 652 cm^{-1} (s). MS (70 eV): *m/e* 296 (M^+, 100 %), 205 (42), 167 (53) and 155 (42).

Later fractions yielded more tetraphenylethene (0.72 g, total yield 43 %, m.p. 224–225 °C, lit.[17] 225 °C). The structure was checked by IR and NMR spectra. The same reaction, run at +25 °C, gave essentially the same results.

$CpCuPBu_3$ and 2-bromo-2-phenylacetophenone. A solution of 2-bromo-2-phenylacetophenone (0.036 mol) in ether (50 ml) was added to a freshly prepared solution of $CpCuPBu_3$ (0.036 mol) in ether (150 ml) at 0 °C. After 1.5 h the stirred mixture was placed in a refrigerator (+5 °C) for another 15 h. The reaction mixture was filtered, the filtrate evaporated, and the residue chromatographed on silica gel with tetrachloromethane/toluene as eluent to give an isomeric mixture of 2-(1,3-cyclopentadien-1-yl)- and 2-(1,4-cyclopentadien-1-yl)-2-phenylacetophenone as faint yellow crystals (4.39 g, 47 %, m.p. 73–76 °C). NMR ($CDCl_3$): δ 8.0 (4, m, aromatic protons), 7.3 (16, m, aromatic protons), 6.4 (4, m, cyclopentadienyl olefinic protons), 6.3 (1, m, cyclopentadienyl olefinic protons), 5.8 (2, m, methine proton) and 3.1 ppm (4, m, cyclopentadienyl methylene protons). IR (KBr): 3250 (m), 1715 (m), 1675 (s), 1598 (s), 1580 (s), 1496 (m), 1447 (m), 1215 (s), 765 (m), 725 (s), 702 (s) and 646 cm^{-1} (s). UV (EtOH): $\lambda = 207$ nm (log $\varepsilon = 4.34$) and 246 (4.15). MS (70 eV): *m/e* 260 (M^+, 8 %), 155 (19), 105 (base peak) and 72 (71). Abs.

mass; found 260.120, calc.for $C_{19}H_{16}O$ 260.120.

Later fractions yielded 1,2,3,4-tetraphenylbutan-1,4-dione (0.9 g, 13 %, m.p. 158 °C, lit.[15] 159–160 °C).

CpCuPBu$_3$ and trimethylpyrylium perchlorate. In a series of experiments trimethylpyrylium perchlorate (0.01 mol) was suspended in ether (100 ml) together with cyclopentadienylthallium and copper(I) iodide tributylphosphine (0.02, 0.01, 0.005, and 0.0025 mol) and stirred at room temperature for 48 h. The solvent was evaporated and the residue chromatographed on silica gel with dichloromethane as eluent to give violet 4,6,8-trimethylazulene (spectroscopic yields, based on CpCuPBu$_3$, were 14, 26, 56, and 60 %, respectively). The product was identified by its NMR and mass spectra.

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The Structure of (11ξ,12ξ)-11,12-Di(7-drimen-11-oxy)-11,12-epoxy-7-drimene

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A new terpenoid – isolated from *Drimys winteri* Forst – was assigned the structure (11ξ,12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene on the basis of spectral and chemical data including hydrolysis to the known sesquiterpenoids drimenol and polygodial. The absolute configurations were established for all but two asymmetric centres, C(11) and C(12), which were tentatively deduced as *R*, *R* from ^{13}C and ^{1}H NMR considerations.

The stem bark of the South American tree *Drimys winteri* Forst (family Winteraceae) has been shown to produce sesquiterpenoids of the drimane type, *i.e.* drimenol (*1*) and three isomeric lactones, drimenin, isodrimenin and confertifolin, which are oxygenated at C(11) and C(12).[1–3] The present communication deals with the structure of a new constituent isolated from *D. winteri* Forst, (11ξ,12ξ)-11,12-di(7-drimen-11-oxy-)-11,12-epoxy-7-drimene (*2*).

The new compound was obtained as the major component of the methanol-insoluble fraction of the light petroleum extract. It proved to be unstable under acidic conditions and required precautions like pretreatment of adsorbents with pyridine to avoid decomposition during isolation. Elemental analysis and a very weak ion at *m/e* 660.5, observable only in the low voltage mass spectrum (15 eV, low resolution), indicated the composition $C_{45}H_{72}O_3$ (= *m/e* 660.547); in the 70 eV high resolution

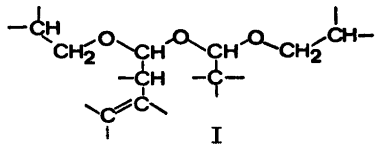
Table 1. Carbon-13 chemical shift data.[a]

Compound			2[b]				1[c]	3[c]	4[c]
Carbon	δ_C	Carbon	δ_C	Carbon	δ_C	Carbon	δ_C	δ_C	δ_C
1	40.14	1′	40.14	1″	40.14	1	39.37	39.62	39.35
2	18.82	2′	19.33	2″	19.33	2	18.78	18.06	18.82
3	42.38	3′	42.67[d]	3″	42.54[d]	3	42.17	41.78	42.03
4	33.50	4′	33.16[d]	4″	32.89[d]	4	32.92	32.93	32.98
5	49.52	5′	50.28	5″	50.28	5	49.92	49.05	49.43
6	23.74	6′	24.05	6″	24.05	6	23.62	25.26	23.59
7	138.69	7′	134.56	7″	134.56	7	123.94	154.22	127.54
8	120.22	8′	122.85[d]	8″	122.65[d]	8	133.03	138.37	136.97
9	58.52	9′	54.89[d]	9″	54.74[d]	9	57.27	60.38	54.51
10	36.32	10′	36.32	10″	36.32	10	36.05	36.91	35.66
11	106.15	11′	65.89	11″	68.07	11	60.85	201.94	61.50
12	104.13	12′	23.05[d]	12″	22.90[d]	12	21.95	193.24	67.51
13	33.73	13′	33.73	13″	33.73	13	33.37	33.14	33.19
14	21.60	14′	22.25[d]	14″	22.13[d]	14	22.04	21.95	21.91
15	14.41	15′	14.80[d]	15″	14.65[d]	15	14.84	15.28	14.55

[a] δ-Values relative to TMS. [b] Run in C_6D_6. [c] Run in $CDCl_3$. [d] These assignments of primed and corresponding doublyprimed carbons may be interchanged.

spectrum, the ion of highest mass occurred at *m/e* 438 and had the composition $C_{30}H_{46}O_2$.*

The presence of 45 carbon atoms was confirmed by the ^{13}C and ^1H NMR spectra (*cf.* Table 1) which also disclosed that twelve of these were methyl groups on quaternary carbons and three olefinic methyl groups. Two singlets, one of which corresponded to two carbon atoms, and three doublets in the olefinic region (120 – 140 ppm) of the ^{13}C NMR spectrum and three olefinic proton signals in the ^1H NMR spectrum (*ca.* δ 5.56) revealed the presence of three trisubstituted double bonds. A one-proton doublet at δ 5.10 and a one-proton singlet at δ 5.39 in the latter spectrum and two doublets at δ 104.1 and 106.2 in the ^{13}C NMR spectrum implied the occurrence of two carbon atoms carrying two oxygens and one proton each. The doublet at δ 5.10 was coupled (J 4.5 Hz), as confirmed by spin decoupling experiments, to a one-proton multiplet at δ 2.70 attributable to an allylic proton. Two triplets in the ^{13}C NMR spectrum at δ 65.9 and 68.1 and two AB-parts of two ABX-systems positioned at δ 3.99 and 3.73 (J_{AB} 10 Hz, J_{AX} 6.5 Hz, J_{BX} 3 Hz) and at δ 4.29 and 3.37 ($J_{A'B'}$ 9.5 Hz, $J_{A'X'}$ 7.5 Hz, $J_{B'X'}$ 2 Hz) in the ^1H NMR spectrum suggested the presence of two oxygenated methylene groups attached to tertiary carbon atoms. Since the compound comprised totally three oxygens and its IR spectrum showed no hydroxyl absorption, these data could be summarized as shown in the partial structure I.

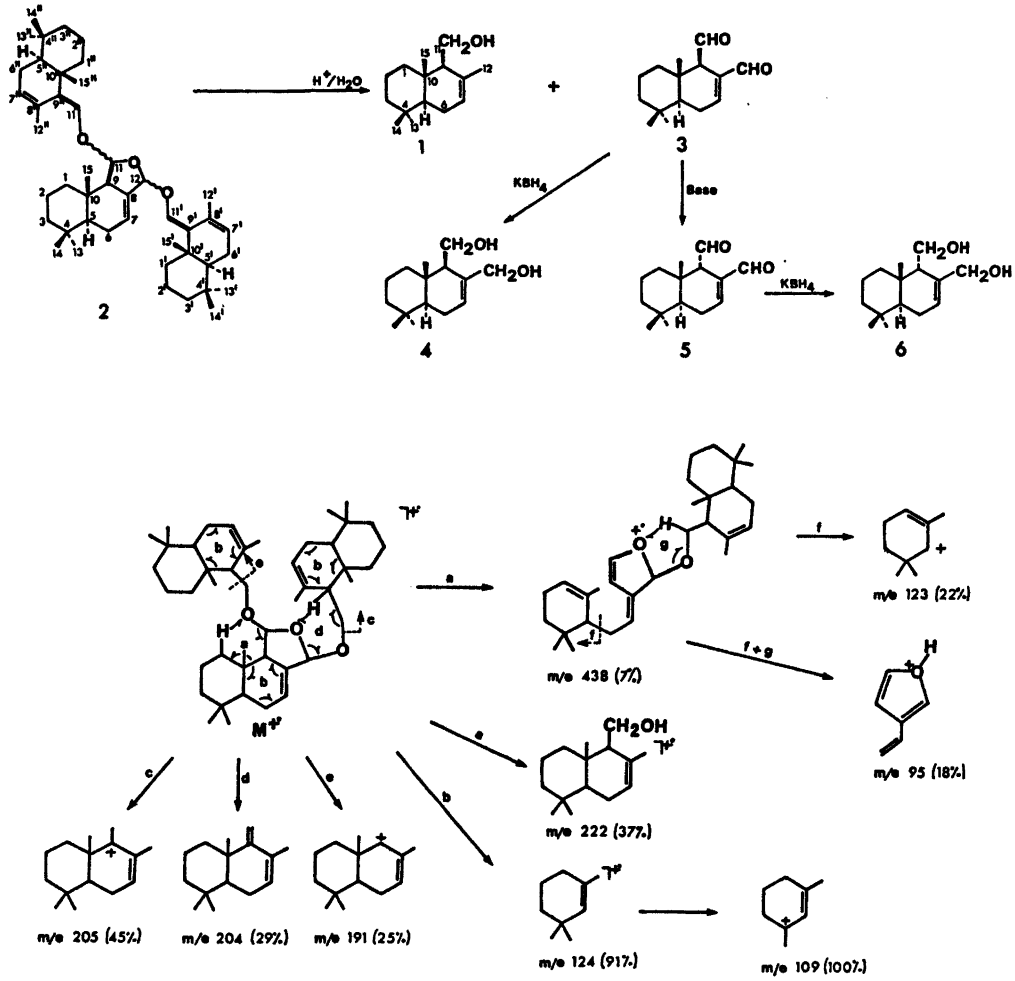


I

Acid hydrolysis of the new compound yielded two products in a 1:2 ratio. The major product was readily recognized as drimenol (*1*) by direct comparison with an authentic sample, while the minor product was identified as polygodial (*3*) on the basis of comparison of its spectral data with those for natural [5,6] (*e. Polygonum hydropiper* L.) and synthetic [7] polygodial, kindly provided by Drs. Loder and Kato. The identity of polygodial was further manifested by hydride reduction to the known drim-7-en-11,12-diol (*4*), and by base-catalysed epimerization to the more stable 9α-drim-7-en-11,12-dial (*5*), which on reduction furnished 9α-drim-7-en-11,12-diol (*6*). The ^{13}C NMR assignments of drimenol (*1*), polygodial (*3*), and drim-7-en-11,12-diol (*4*) are given in Table 1 and were made on the basis of chemical shift considerations and comparison of data for structurally related diterpenoids.[8,9] The data thus obtained for drimenol (1) were used to assist the assignments given for the new compound (Table 1), which on the basis of the above chemical and spectral data could be formulated as (11ξ, 12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene (*2*).

Its fragmentation on electron impact provided confirmatory evidence since the formation of the eight most abundant ions could on the basis of results for structurally related diterpenoids [10] readily be accounted for in terms of this structure, as indicated in Scheme 1. Although several routes of similar probability exists in many cases, only one – preferentially that involving the molecular ion as the parent species – is detailed here. Thus decomposition of the molecular ion by a cyclic reaction encompassing rupture of the C(11) – O and C(9) – C(10) bonds with transfer of a hydrogen at C(1) to the oxygen (path a) yields the *m/e* 438 ion and the drimenol ion (*m/e* 222). Retro-Diels-Alder fragmentation of the molecular ion, furnishes the *m/e* 124 species (path b), which by loss of a methyl generates the *m/e* 109 fragment. Simple cleavage of the C(11′) – O [or the C(11″) – O] bond in the molecular ion, accompanied by a 1,2-hydride shift in the smaller charged fragment, produces the *m/e* 205 species (path c), while rupture of the same bond with hydrogen transfer to the larger neutral fragment accounts for the formation of the *m/e* 204 ion (path d). Simple cleavage of the 9′ – 11′ (or 9″ – 11″) bond in the molecular ion gives the *m/e* 191 ion (path e), while simple cleavage of the 5,6-bond in the *m/e* 438 species accounts for the formation of the *m/e* 123 ion (path f). When the last reaction is accompanied by elimination of drimenal

* A preliminary report on the present compound, then designated Acetal-B and erroneously assumed to have a molecular weight of 438, was presented at the XI Congreso Latino-americano de Quimica, Santiago, 1972; *cf.* Ref. 4.



Scheme 1.

through rupture of the C(12)−O bond and transfer of 11′−H to the smaller fragment the *m/e* 95 species (path f + g) is generated.

The absolute configuration of all but two asymmetric centres, C(11) and C(12), follows from the known absolute stereochemistry of drimenol and polygodial. It might be expected, if (11ξ,12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene (*2*) is formed *in vivo* from polygodial (*3*) and drimenol (*1*), that the sterically less congested 11α-isomer would result. While no conclusive evidence could be obtained from the coupling constant (4.5 Hz) for the vicinal 9α- and 11ξ-hydrogens, it can be inferred that the interaction from an 11β ether oxygen on the C(15) methyl group, if present, would probably have caused one of the three angular methyl signals to appear further downfield (about 2 ppm) of the other two, C(15′) and C(15″), in the ^{13}C NMR spectrum than actually encountered (observed difference 0.4 ppm).[9] A corresponding difference in chemical shift, about 0.2 ppm, between the methyl signals in the ^{1}H NMR spectrum would also be expected if the exocyclic 11-oxygen were β-oriented, but is not observed since all occur in the δ 0.91−1.02 region ($CDCl_3$).[11]

The difference between the 12α and 12β isomers with respect to steric congestion is small as judged by Dreiding models. However,

examination of these models suggests that the upfield-shift of one (δ 3.37) and the downfield shift of the other (δ 4.28) of the two protons on one of the oxygenated methylene groups relative to those on the other oxygenated methylene group (δ 3.99 and 3.73) in the ^{1}H NMR spectrum (in C_6D_6) is best explained if the oxygen linked to this methylene group, C(11′), is β-oriented. Thus these chemical shifts are most readily accounted for as a result of a long-range anisotropic shielding by the 7,8-double bond.* Moreover, such an orientation of the C(11′) methylene group, which makes it essentially anti-periplanar to the tetrahydrofuran oxygen, is also consistent with the occurrence of the C(11′) signal in the ^{13}C NMR spectrum 2.2 ppm upfield of that due to C(11″).[12] It seems likely therefore that the compound is (11*R*, 12*R*)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene.

Polygodial has not previously been detected in *D. winteri*, but it has been isolated by Loder [6] from a related shrub, *Drimys lanceolata*, which is confined to the mountains of south-east Australia and Tasmania. The recent isolation of scalaradial,[13] and several related hemiacetals such as scalarin,[14] deoxoscalarin,[15] and heteronemin [16] from sponges of the order Dictyoceratida is also of interest in the present context because of the close structural relationship with the compounds considered here and the less frequent occurrence of dialdehydes in Nature.

EXPERIMENTAL

IR spectra, rotations and mass spectra were recorded on Digilab FTS-14, Perkin-Elmer 141 and LKB 2091 instruments, respectively. ^{13}C and ^{1}H NMR spectra were obtained in $CDCl_3$ or C_6D_6 solutions in FT-mode on a Varian XL-100-12 spectrometer equipped with S-124 FT accessory and controlled by a Varian 620 L computer. Accurate mass determinations were carried out at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm. GLC–MS was performed on a Varian 1700 chromatograph attached to the LKB 2091 instrument using glass capillary columns (50 × 0.25 mm) coated with Emulphor.

Extraction. The bark of *Drimys winteri* was cut into small pieces and exhaustively extracted with light petroleum (b.p. 70–80 °C) in a Soxhlet apparatus. Removal of the solvent yielded a brown, oily residue. The yield varies with the sample of the bark, but is usually between 20 and 30 %.

Purification. The crude extract (50 g) was extracted at room temperature with methanol (3 × 100 ml) which removed about 50 % of the material (including drimenol). The methanol-insoluble residue was dissolved in a mixture of pyridine/butanol (4:96) and methanol added to turbidity. The mixture was kept at −15 °C for 24 h and the amorphous slightly yellow precipitate (*ca.* 18 g) collected. TLC revealed the presence of three components (ratio about 1:2:3) of which (11ξ,12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene was the major constituent. An aliquot (4 g) of the precipitate was chromatographed on neutral alumina (May and Baker) which had been pretreated for 24 h with pyridine-light petroleum (4:96) to avoid decomposition which readily takes place on acidic adsorbents. Pure (11ξ,12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene (0.9 g) was recrystallised from a mixture of dioxane and methanol by evaporation of part of the solvent mixture at room temperature. M.p. 128–131 °C. $[\alpha]_D$ −24.0° (*c* 1; benzene). Found C 81.87; H 11.13. Calc. for $C_{45}H_{72}O_3$: C 81.75, H 11.00. MS at 15 eV: M^+ at *m/e* 660.5 (*ca.* 0.2 %, calc. for $C_{45}H_{72}O_3$ 660.547) $(M+1)^+$ at *m/e* 661.5 (48.8 % of M^+; calc. 49.5 %); MS at 70 eV: M^+ not visible, 109 (100), 124 (91), 205 (45), 222 (37), 204 (29), 191 (25), 123 (22), 95 (18), 98 (16), 135 (12), 234 (11), ions at *m/e* > 300: 438 (7), 439 (5); accurate mass determination: found 438.3506, calc. for $C_{30}H_{46}O_2$ 438.3498; UV: no absorption above 210 nm; IR: ν_{max} (KBr): 2949 (s), 2918 (s), 2845 (s), 1459 (m), 1440 (m), 1389 (m), 1365 (m), 1257 (w), 1218 (w), 1145 (w), 1105 (m), 1069 (m), 1021 (m), 992 (m), 957 (s), 920 (m), 880 (w), 825 (m) cm^{-1}. δ_C (C_6D_6): Table 1. δ_H($CDCl_3$): 0.91 (3 H, s), 0.93 (3 H, s), 0.96 (3 H, s), 1.01 (12 H, s), 1.02 (6 H, s), 1.87 (9 H, broad s), 3.38 (1 H, dd, *J* 7 and 10 Hz), 3.71 (2 H, m), 4.16 (1 H, dd, *J* 1 and 10 Hz), 5.07 (1 H, d, *J* 4 Hz), 5.31 (1 H, s), *ca.* 5.56 (2 H, br), 5.86(1 H, m). δ_H(C_6D_6): 0.69 (3 H, s). 0.79 (6 H, s), 0.83 (3 H, s), 0.87 (6 H, s), 0.89 (6 H, s), 1.02 (3 H, s) 1.98 (3 H, broad s), 2.07 (6 H, broad s), *ca.* 2.70 (1 H, m), 3.37 (1 H, dd, *J* 7.5 and 9.5 Hz), 3.73 (1 H, dd, *J* 3 and 10 Hz), 3.99 (1 H, dd, *J* 6.5 and 10 Hz), 4.29 (1 H, dd, *J* 2 and 9.5 Hz), 5.10 (1 H, d, *J* 4.5 Hz), 5.39 (1 H, s), *ca.* 5.55 (3 H, m). Irradiation at δ 2.70 simplified the signal at δ 5.10 to a singlet. On irradiation at δ 4.29 the doublet of doublets centered at δ 3.38 collapsed to a doublet. Irradiations at δ 1.98 and 2.07, respectively, sharpened the multiplet at δ 5.55. Irradiation

* The same magnitude of the shift differences for these protons [4.16 (dd, *J* 10.1); 3.38 (dd, *J* 10.7); 3.71 (2 H, m)] were observed when $CDCl_3$ was used as solvent, which implies that the shift difference mentioned above was not due to an aromatic solvent induced shift but of intramolecular origin.

at δ 2.18 simplified the two doublets of doublets at δ 3.73 and 3.99 to an AB-system with J 10 Hz.

Hydrolysis of (11ξ,12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene (2). A mixture of (11ξ, 12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene (92 mg), ether (15 ml) and water (1 ml) containing 2 drops of acetic acid was stirred for 3 h at room temperature. Water was added and the products extracted with ether. The extract was dried over Na_2SO_4 and the solvent removed *in vacuo* leaving an oil which was chromatographed on a silica gel column yielding drimenol (*1*, 25 mg) and polygodial (*3*, 13 mg). The identity of drimenol was confirmed by direct comparison, optical activity included, with an authentic sample of the naturally occurring compound. The more polar polygodial (*3*) had the following properties: $[\alpha]_D^{20}$ −121° (589 nm), −126° (578), −146° (546), −271° (436) (*c* 0.44 in $CHCl_3$); lit.[5] $[\alpha]_D^{19}$ −131° (*c* 0.96 in EtOH); lit.[6] $[\alpha]_D^{19}$ −126°(*c* 2.07 in $CHCl_3$); MS: *m/e* 234 (M^+, 6 %), 206 (100), 43 (68), 121 (59), 41 (59), 109 (58), 110 (50), 191 (49); MS kindly provided by Dr. Loder: *m/e* 234 (M^+ 5 %), 206 (100), 109 (100), 121 (80), 191 (49); $\delta_H(CDCl_3)$: 0.82 (3 H, s), 0.93 (3 H, s), 0.95 (3 H, s), *ca.* 7.12 (1 H, m), 9.47 (1 H, s), 9.56 (1 H, d, J 5 Hz); chemical shifts of olefinic and aldehydic protons in the spectrum kindly provided by Dr. Loder: *ca.* δ 7.15 (m), 9.45 (s), and 9.52 (d, J 5 Hz), respectively (these values differ from those originally published by Barnes and Loder[5] for polygodial); δ_C ($CDCl_3$): Table 1. IR: ν_{max}(film): 2929 (s), 2721 (w), 1724 (s), 1680 (s), 1645 (w), 1391 (w), 1369 (w), 1269 (w), 1180 (w), 1136 (w), 1117 (w), 1072 (w), 837 (w), 824 (w), 697 (w) cm^{-1}; the spectrum was nearly identical with those of natural (CS_2) and synthetic (CCl_4) polygodial. An aliquot of the dialdehyde isolated from the hydrolysis of (11ξ,12ξ)-11,12-di(7-drimen-11-oxy)-11,12-epoxy-7-drimene was reduced with KBH_4 to a diol which was identified as drim-7-en-11,12-diol by comparison with an authentic sample: m.p. 73−75 °C; $[\alpha]_D$ −2° (*c* 2.47, $CHCl_3$); IR: ν_{max}(KBr): 3330, 1655, 1385, 1360, 680 cm^{-1}; MS: *m/e* 238 (M^+, 7 %), 109 (100), 190 (47), 124 (35), 44 (32?), 41 (23), 69 (20), 55 (18), 81 (17), 105 (17), 119 (13), 175 (10); δ_C ($CDCl_3$): Table 1; $\delta_H(CDCl_3)$: 0.77 (3 H, s), 0.88 (6 H, s), 3.68 (1 H, dd, J 7.5 and 11 Hz), 3.91 (1 H, dd, J 2.5 and 11 Hz), 3.98 (1 H, d, J 12.5 Hz), 4.34 (1 H, d, J 12.5 Hz), 5.81 (1 H, m); on treatment with basic Al_2O_3 the dialdehyde epimerized to 9α-drim-7-en-11,12-dial which on reduction with KBH_4 furnished 9α-drim-7-en-11,12-diol: m.p. 128−129.5 °C. $[\alpha]_D$ −136° (*c* 2.05, $CHCl_3$); IR: ν_{max}(KBr): 3330, 1660, 1380, 1360 cm^{-1}.

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The Geometry of an *N*-Aryl-alkylnitrilium Ion. Low Temperature X-Ray Study of *N*-(2,6-Dimethylphenyl)-acetonitrilium Tetrafluoroborate

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The crystal structure of the title compound has been determined by X-ray methods using 1628 observed reflections collected by counter methods from a crystal kept at −169 °C. The crystals are orthorhombic, space group *Pnma*, with unit cell dimensions $a = 19.858(14)$ Å; $b = 6.780(2)$ Å; $c = 8.042(2)$ Å. The structure was solved by direct methods and refined to a conventional *R*-factor of 0.073; standard deviations in bond lengths are 0.003 – 0.004 Å and in angles 0.2° where non-hydrogen atoms are involved. The tetrafluoroborate ion is disordered both at room temperature and at liquid nitrogen temperature. The nitrilium group has a nearly linear C–N–C–C arrangement with the central C–N bond length corresponding to a triple bond. The charge distribution is discussed on the basis of results from CNDO calculations.

The term "nitrilium salt" was adopted by Hantzsch in 1931 for compounds which he assumed would be formed by the reaction of nitriles with acids.[1] *N*-Substituted nitrilium salts were actually isolated 25 years later;[2,3] the existence of protonated nitrilium salts of the type $[R-C\equiv NH]^+X^-$ (where X^- is an anion of the type $SbCl_6^-$) has also been reported.[4]

Nitrilium ions have been assumed to be intermediates in certain reactions, *e.g.* in the Ritter reaction[5] and others involving nitriles and organic halides. Their electronic structure is usually described by formulae *1a* or *1b*

$$R^1-\overset{+}{C}=N-R^2 \longleftrightarrow R^1-C\equiv\overset{+}{N}-R^2$$

1a *1b*

The ion reacts normally as a carbonium ion (*1a*) with nucleophilic reagents. From NMR data (including ^{13}C and ^{15}N resonance data) Olah and Kiovsky concluded that both protonated nitriles and *N*-alkylnitrilium ions appear to have linear configuration (*1b*) in solution (SO_2), however, and that the iminocarbonium form (*1a*) is only a minor contributor to the structure.[6]

To investigate the geometry of the nitrilium group in the solid state an *N*-phenylacetonitrilium ion was chosen for X-ray structure determination. In order to avoid ring closure through condensation to a quinazolinium salt the *ortho* positions of the phenyl ring were substituted by methyl groups.[7] Although the tetrafluoroborate is extremely hygroscopic, this salt was used in order to avoid the heavier atoms of the more stable salts (*e.g.* $SbCl_6^-$) which would impair the accuracy of determination of the structural parameters of the cation.

EXPERIMENTAL

Crystals of the title compound were prepared from 2,6-dimethylphenyldiazonium tetrafluoroborate and acetonitrile.[6] It was found necessary to work with substances of high purity and to control the temperature carefully in the last stages of the synthesis. The optimal temperature for the reaction of the diazonium salt with acetonitrile was found to be 35 ± 2 °C in order to avoid excessive formation of by-products. To form crystals of good quality the reaction was stopped as soon as the product started to precipitate. Colourless crystals were

isolated as long hexagonal needles or hexagonal plates by slow diffusion of ethyl ether into the filtered solution at 4 °C. The crystals are very reactive; in contact with air a layer of white powder is immediately formed on the surface, and decomposition of the crystals is complete after a couple of seconds.

X-Ray data were collected at 18 ± 1 and −169 ± 2 °C. At the higher temperature the crystal (approximately of cubic shape, edge 0.4 mm) was kept in a sealed capillary; at the low temperature the crystal was fairly stable in the cold nitrogen stream without protection.

Oscillation and Weissenberg photographs showed the crystal to be orthorhombic; systematically absent reflections indicated the space group $Pna2_1$ or $Pnma$. For the measurement of cell dimensions and collection of intensity data a SYNTEX $P\bar{1}$ diffractometer was used with graphite crystal monochromated Mo$K\alpha$ radiation (λ = 0.71069 Å).

The room temperature data were collected using the $\theta/2\theta$ scanning mode with scan speeds (2θ) of 3 − 8° min^{-1} depending on the peak 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 ± 1.0° appropriately expanded to cover the effects of α_1-α_2 splitting; background counts were taken for 0.35 times the scan time at each end of the scan. Three standard reflections were measured after every 50 reflections; the data set was adjusted to compensate for a slow decrease in the intensity of the standard reflections during the data collection. The estimate of the standard deviations of the measurements was based on counting statistics with an additional term of 2 % of the net intensity.

Reflections having an intensity larger than $2.5\sigma(I)$ were regarded as observed and used for the structure determination. No correction for absorption or secondary extinction was carried out.

Owing to severe thermal effects only 508 unique reflections were observed; this is less than one third of the theoretical number for $\sin\theta/\lambda > 0.7$ Å^{-1}. In order to obtain a better data set the experiment was repeated with a crystal held at −169 ± 2 °C. The procedure was the same as for the room temperature data collection except for the scan range (± 1.2°) and the scan speed (2 − 8°). The resulting low temperature data set comprises 1628 observed reflections with $\sin\theta/\lambda < 0.85$ Å^{-1}.

A description of the computer programs used is given in Ref. 8. The quantity minimized in the full-matrix least-squares program was $\sum w\Delta F^2$ where w is the inverse of the variance of the observed structure factor. Atomic form factors of Doyle and Turner [9] for F, N, B, and C and of Stewart, Davidson and Simpson [10] for H were used.

CRYSTAL DATA

N-(2,6-Dimethylphenyl)-acetonitrilium tetrafluoroborate, $C_{10}H_{12}NBF_4$, orthorhombic.

18 ± 1 °C : a = 20.242(2) Å; b = 7.035(1) Å; c = 8.116(1) Å;
V = 1155.7 Å^3; D_{obs} = 1.3 g cm^{-3}; D_{calc} = 1.339 g cm^{-3}

−169 ± 2 °C: a = 19.858(14) Å; b = 6.780(2) Å; c = 8.042(2) Å;
V = 1082.8 Å^3; D_{calc} = 1.429 gcm^{-3}

M = 233.03; $F(000)$ = 480; Z = 4. Absent reflections: ($0kl$) for $k+l$ odd and ($hk0$) for h odd.

Space group $Pnma$ (No. 62).

STRUCTURE DETERMINATION

The crystal structure was determined from the room temperature data. Intensity statistics gave strong evidence for a centric structure and thus for space group $Pnma$. The structure was solved by a combination of Patterson and direct methods and refined by Fourier and least-squares techniques.

The initial model was one of a nitrilium ion with all the atoms of the carbon-nitrogen skeleton situated on a crystallographic mirror plane and a tetrahedral tetrafluoroborate ion with the boron and two fluorine atoms in the mirror plane. As the refinement procedure progressed, it emerged that although the parameters of the cation were satisfactory, abnormally high thermal parameters for the BF_4^- ion suggested a disordered anion. A new model for this ion was therefore tried, *viz.* splitting the

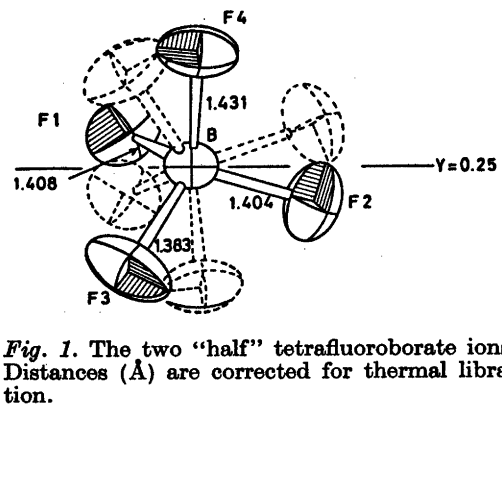


Fig. 1. The two "half" tetrafluoroborate ions. Distances (Å) are corrected for thermal libration.

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations obtained from the low temperature data. The temperature factor is exp $-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)$ for the nonhydrogen atoms.

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
F1	.2898(1)	.2987(4)	.2482(3)	.0333(8)	.242(11)	.096(3)	.044(4)	−.043(2)	−.060(8)
F2	.3882(1)	.1917(4)	.3527(3)	.0194(6)	.330(12)	.169(4)	.046(3)	.025(3)	.061(9)
F3	.2920(2)	.1067(5)	.4785(5)	.0362(9)	.195(8)	.129(5)	−.078(6)	.038(5)	−.037(9)
F4	.3278(2)	.4197(5)	.4933(4)	.0351(9)	.116(5)	.087(3)	−.014(5)	−.010(4)	−.011(7)
B	.3249(1)	.25	.3923(3)	.0161(7)	.115(5)	.074(3)		−.004(3)	
N	.1424(1)	.25	.7159(2)	.0167(6)	.098(4)	.068(3)		−.053(2)	
C1	.0762(1)	.25	.6553(3)	.0143(6)	.088(4)	.066(3)		−.039(2)	
C2	.0245(1)	.25	.7725(3)	.0161(6)	.100(4)	.062(3)		−.006(2)	
C3	−.0403(1)	.25	.7110(3)	.0153(7)	.115(5)	.077(3)		.086(2)	
C4	−.0518(1)	.25	.5402(3)	.0147(7)	.106(4)	.084(3)		−.024(2)	
C5	.0003(1)	.25	.4286(3)	.0160(6)	.110(4)	.065(3)		−.085(2)	
C6	.0669(1)	.25	.4830(3)	.0154(6)	.090(4)	.063(3)		−.043(2)	
C7	.1948(1)	.25	.7708(3)	.0175(7)	.100(4)	.065(3)		.049(2)	
C8	.2617(1)	.25	.8381(3)	.0166(7)	.145(5)	.086(4)		−.127(3)	
C9	.0390(2)	.25	.9556(3)	.0224(9)	.171(6)	.059(3)		−.044(3)	
C10	.1239(2)	.25	.3623(3)	.0174(7)	.159(6)	.084(3)		.112(3)	

Atom	x	y	z	B	Atom	x	y	z	B
H3	−.078(2)	.25	.781(4)	2.7(4)	H91	−.007(2)	.25	1.013(5)	4.4
H4	−.096(2)	.25	.507(4)	2.7	H92	.066(1)	.132(4)	.983(3)	4.4
H5	−.013(2)	.25	.306(5)	2.7	H101	.160(3)	.25	.397(6)	4.4
H81	.259(2)	.25	.952(6)	4.4(3)	H102	.124(1)	.140(4)	.284(3)	4.4
H82	.285(1)	.134(4)	.812(3)	4.4					

fluorine atoms into two "half" atoms to account for the thermal motion following the argument of Kartha and Ahmed.[11] A drawing of this model, in which the tetrahedral arrangement is maintained for each of the two halves, and seen along the mirror plane, is shown in Fig. 1. Even after the introduction of anisotropic thermal parameters for all non-hydrogen atoms, refinement did not reduce the R-factor to below 0.13. The goodness of fit $S = [\sum w\Delta^2/m-n]^{\frac{1}{2}}$ was 8.5, indicating systematic errors in ΔF, probably originating in a poor structure model. The resulting bond lengths and angles were not acceptable.

The low-temperature data set collected at this stage was introduced. It turned out, however, that the refinement of an ordered model converged to a conventional R-factor as high as 0.15, but introduction of the disordered BF_4^--model lowered the R-value to 0.10. Obviously the cooling of the crystals had not resulted in an ordering of the structure. The hydrogen atoms were located and refined using isotropic thermal parameters; the least-squares calculations terminated with a conventional R-factor of 0.073, $R_w = 0.086$ and $S = 3.6$.

The final atomic parameters are listed in Table 1. The observed and calculated structure factor data are available from the authors.

A difference Fourier map showed electron densities between −0.35 and 0.20 e Å^{-3}. The structure refinement was repeated with necessary modifications in space group $Pna2_1$ but the results gave no justification for a change of space group. Interchange of atomic scattering factors for the C and N atoms of the nitrilium group resulted in abnormal thermal parameters for both atoms. We conclude therefore that the original assignment was correct.

Analysis of the thermal parameters in terms of rigid-body motion yielded r.m.s. ΔU values of 7×10^{-4} Å^2 and 18×10^{-4} Å^2 for the tetrafluoroborate ion and the nitrilium ion, respectively.[12] For the BF_4^- ion the largest r.m.s. amplitudes of libration are those about directions nearly parallel to the a and b axes, 10.5 and 8.1°, respectively, whereas that about the c axis is 3.8°. The fairly large amplitudes tend

Table 2. Bond lengths (Å) and angles (°).

Bonds		Angles	
B–F1	1.391	F1–B–F2	109.7
B–F2	1.356	F1–B–F3	110.7
B–F3	1.360	F1–B–F4	107.9
B–F4	1.410	F2–B–F3	110.9
C1–C2	1.394	F2–B–F4	109.6
C2–C3	1.380	F3–B–F4	108.0
C3–C4	1.393	C1–C2–C3	116.5
C4–C5	1.371	C2–C3–C4	120.5
C5–C6	1.393	C3–C4–C5	121.4
C6–C1	1.398	C4–C5–C6	120.8
C1–N	1.400	C5–C6–C1	115.9
N–C7	1.131	C6–C1–C2	124.9
C7–C8	1.434	C1–C6–C10	123.0
C2–C9	1.501	C1–C2–C9	121.5
C6–C10	1.490	C2–C1–N	117.1
		C6–C1–N	118.0
		C1–N–C7	177.4
		N–C7–C8	179.2

to bring this ion towards the other half ion through the mirror plane (*cf.* Fig. 1) indicating that the disorder may be dynamic even at temperatures below 100 K. The libration motion of the nitrilium ion is far less extreme, the largest r.m.s. amplitude being 3.1° about the C4–C8 direction in the molecule; corrections to the bond lengths in this ion are thus quite small.

DISCUSSION

Bond lengths and angles are given in Table 2; Figs. 1 and 2 show the structure of the ions, thermal ellipsoids and bond lengths corrected for thermal motion. Carbon-hydrogen bond lengths were found in the range 0.77(5) to 1.03(4) Å with a mean value of 0.95 Å. Estimated standard deviations in distances and angles were calculated from the correlation matrix to be 0.003–0.004 Å and 0.2°, respectively, when not involving hydrogen atoms.

Tetrafluoroborate ion. Owing to the disorder, the structure of this ion is not well determined. The mean value of the B–F bond length is 1.38 Å (1.41 Å corrected for libration) with standard deviation from the mean of 0.02 Å. The F–B–F angles are found to be approximately tetrahedral.

N-(2,6-Dimethylphenyl)acetonitrilium ion. All atoms of the nitrilium ion except for two hydrogen atoms of each methyl group are coplanar by crystal symmetry requirements. Thermal parameters indicate that the ion is little affected by the disorder of the anion.

The carbon–carbon bond lengths in the dimethylphenyl part are normal. The bond angles in the ring deviate from 120° as expected in the presence of a very strongly electron-withdrawing substituent such as a nitrilium group, and the effect is of the same magnitude as that found in aryldiazonium ions. The internal angle at the carbon atom carrying the electropositive substituent is 124.9° as compared to the corresponding angle in benzenediazonium chloride of 124.8° [13] and in *p*-benzenediazonium sulfonate of 125.4°.[14] The internal angles at the neighbouring carbon

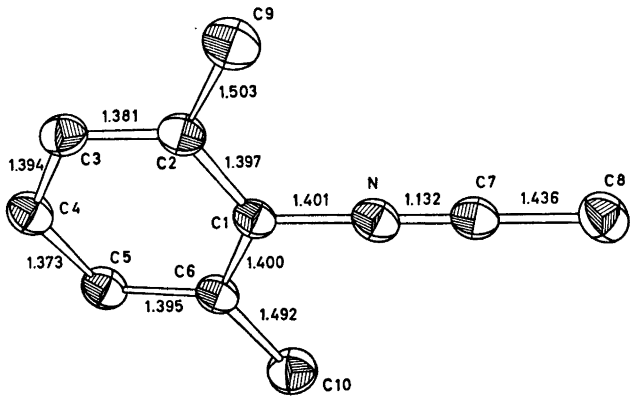


Fig. 2. Perspective view of the nitrilium ion not including hydrogen atoms. Distances (Å) are corrected for thermal libration.

atoms are correspondingly less than 120, 116.2° in the nitrilium salt and 117.6 and 116.3°, respectively, in the diazonium salts. The internal angles at C3, C4 and C5 are only slightly larger than 120°. The methyl groups at C2 and C6 are not symmetrically arranged about the long axis of the molecule; the hydrogen atom bonded to C9 situated in the mirror plane is pointing away from the nitrilium group, whereas that bonded to C10 is pointing parallel to the C1–N bond. The latter hydrogen atom is thus situated fairly close to the nitrogen atom; repulsion between these atoms is probably responsible for a larger external angle C1–C6–C10 (123.0°) than C1–C2–C9 (121.5°) and for the asymmetry in external angles at C1.

The acetonitrilium chain, C1–N–C7–C8, is almost linear and form *1b* is thus the major contributor to the structure in the solid state as well as in solution.[6] The N≡C7 bond length is found to be 1.13 Å, the C1–N bond 1.40 Å and the C7–C8 bond 1.44 Å. However, in structure determinations by X-ray methods applying mainly low-angle data valence electrons may influence the results. One would expect the high electron density in the C–N triple bond to result in a measured bond distance shorter than the real internuclear separation. The high-angle data are more dependent on the inner-shell electrons and less on the valence electrons and may therefore be used to determine the nuclear positions more correctly.[15] Least-squares refinement of the parameters of the heavy atoms using data with $\sin\theta/\lambda >$ 0.45 Å^{-1} (1200 reflections) gave the following bond lengths in the nitrilium chain: N≡C7 1.15 Å; C1–N 1.39 Å, and C7–C8 1.43 Å. Although the standard deviations in the bond lengths are slightly larger (0.005 Å), we believe that the latter bond lengths are the more correct.

CNDO-calculations. In order to obtain an indication of the charge distribution along the nitrilium ionic chain, the CNDO method was employed to calculate the net charges on the atoms. A description of the standard version CNDO/2 is given in Ref. 16, which also gives the parameters needed in the calculations; the molecular geometry used was that found in the present low-temperature structure determination. Somewhat surprisingly the largest positive charge, +0.25, was found to be on C7. On the nitrogen atom, which classically, might be expected to carry the positive charge the calculated net charge was +0.10; the remaining positive charge was found to be distributed mainly among the methyl hydrogen atoms. The calculations were repeated with a crystal field simulated by placing four negative charges (F^-) in positions relative to the nitrilium ion corresponding to those of the BF_4^- ions in the crystal. This resulted in a net charge on C7 of +0.36, on the nitrogen atom of only +0.05 and a small increase in the positive charge on the hydrogen atoms nearest to the negative ions. The nitrilium ionic chain is clearly easily polarized, as expected for a triple bond.

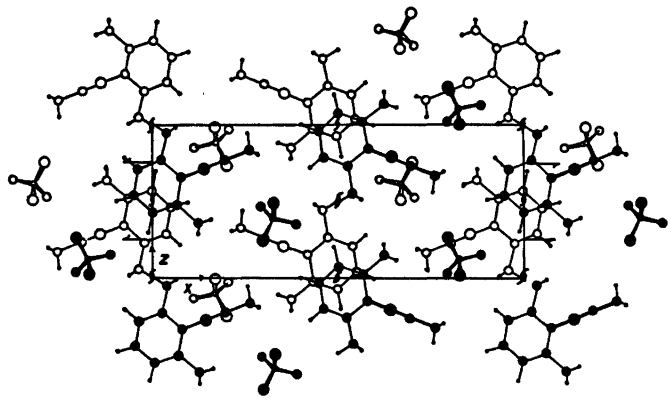


Fig. 3. The packing as seen along the *b*-axis. Filled circles are atoms of layer in $y = \frac{1}{4}$, open circles of layer in $y = \frac{3}{4}$.

Crystal packing. The crystals consist of nitrilium and tetrafluoroborate ions in layers parallel to the (010) plane in $y = \frac{1}{4}$ and $y = \frac{3}{4}$. The layers coincide with the crystallographic mirror planes; the layers are visualized in Fig. 3 as seen along the *b* axis.

Within each layer the nitrilium ions form chains along the *a* axis with van der Waals' contacts between phenyl hydrogen atoms in one ion and the hydrogen atoms of the C8 methyl group in another. Between the chains there are van der Waals' contacts between phenyl methyl groups. The tetrafluoroborate ions are situated in cavities formed between four nitrilium ions. This cavity seems to be too small to accomodate a tetrafluoroborate ion in the ordered situation in the mirror plane. The separation of the layers is $\frac{1}{2}b$, *i.e.* 3.39 Å corresponding to the normal packing distance between aromatic rings.

Each dimethylphenylacetonitrilium ion is surrounded by six tetrafluoroborate ions with their boron atoms at the corners of a distorted octahedron. Four of these are situated in the same layer as the nitrilium ion, one in the layer above and one in the layer below. Similarly, each tetrafluoroborate ion is surrounded by eight nitrilium ions.

The acetonitrilium part of the organic ion, N – C7 – C8, which apparently carries most of the positive charge, is coordinated to four BF_4^- ions in a distorted tetrahedral arrangement; two of these are situated in the same mirror plane and the others above and below the nitrilium group in neighbouring mirror planes. The latter BF_4^- ions appear to have stronger interactions with the nitrilium moiety; C7 – F and C8 – F distances are in the range 2.87 to 3.08 Å and there are several short distances between fluorine and methyl hydrogen atoms. The separations between the nitrilium group and the tetrafluoroborate ions in the same layer are larger, probably for steric reasons. The interionic forces are mainly those between fluorine atoms and the C8 methyl group.

Comparison of the high and low temperature structures

Owing to the scarcity of data obtained at room temperature the bond distances and angles are not accurate enough for a discussion to be of interest, and no particular differences in ionic geometry would be expected. Some differences in the packing may be noted, however. As the cell volume decreases by 6.3 % when going from +18 to −169 °C, the packing is obviously denser at low temperatures. The decrease in cell parameters is 1.9, 3.6, and 0.9 % in the *a*, *b*, and *c*-axes, respectively. The large change in the *b*-axis reduces the inter-layer distance from 3.52 to 3.39 Å; this is reasonable since this separation is mainly determined by the comparatively weak forces between phenyl groups.

Although the separation of nitrilium ions also decreases within the layers when the compound is cooled, the C – F and N – F interionic distances show little variation due to changes in orientation of the BF_4^- ion. The equilibrium positions of the two half ions with a common position of the central boron atom seem to shift in the direction of an ordered situation. At room temperature the two fluorine atoms closest to the mirror plane are found 0.45 and 0.65 Å out of the plane, respectively. At low temperature the deviations from the plane have decreased to 0.25 and 0.40 Å, and one might expect that they coincide with the plane at even lower temperatures.

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Oxidation of Carbohydrate Derivatives with Silver Carbonate on Celite. XI. Oxidation of 4,6-*O*-Alkylidene Derivatives of D-Galactose and D-Glucose

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Oxidation of 4,6-*O*-alkylidene derivatives of D-galactose and D-glucose in methanol with silver carbonate on Celite leads to 2,4-*O*-alkylidenetetroses as major products. In benzene–*N*,*N*-dimethylformamide, oxidation of the galactose derivatives gives mainly 3,5-*O*-alkylidene derivatives of D-lyxose; a galactonolactone derivative is also formed. Oxidation of 4,6-*O*-alkylidene-D-glucoses in benzene or benzene–*N*,*N*-dimethylformamide results in the formation of 4,6-*O*-alkylidene-D-gluconolactones, degradation to arabinose derivatives is negligible.

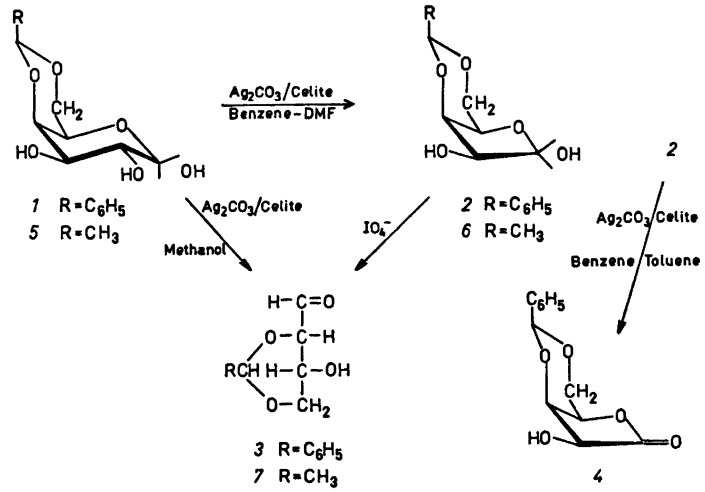
The relative reactivities of the anomers of the 4,6-*O*-ethylidenehexoses in benzene–*N*,*N*-dimethylformamide are investigated, and in the light of the results obtained, the difference in the product compositions obtained from the galactose and the glucose derivatives in this solvent mixture is discussed.

The formation of 3,5-*O*-isopropylidene-D-lyxose on oxidation of 4,6-*O*-isopropylidene-D-galactopyranose with silver carbonate on Celite in benzene, was reported in a previous paper in this series.[1] In methanol, the oxidation product was mainly 2,4-*O*-isopropylidene-D-threose. The initially formed *O*-formyl group at C-4 of the lyxose derivative, resulting from cleavage of the galactopyranose derivative between C-1 and C-2, is unstable in methanol, and the liberated hydroxyl group at C-4 may form a hemiacetal with the aldehyde group by cyclisation. The next degradation step of the sugar derivative may possibly occur in this furanose form.[2] Another possible way to the threose derivative is a degradation of the acyclic α-hydroxy hemiacetal formed between the lyxose derivative and methanol. The use of benzene as a solvent in the oxidation reaction prevents both these reaction paths, and hence makes possible a one-step degradation of the galactose derivative. Five different 4,6-*O*-alkylidene derivatives of D-galactose and D-glucose have been oxidized with silver carbonate on Celite in order to obtain information about the effect of fused rings on the reactivity of aldopyranoses, and also with the purpose to investigate the possible general applicability of the method in preparation of 3,5-*O*-alkylidenepentoses. The results are reported in this paper.

RESULTS AND DISCUSSION

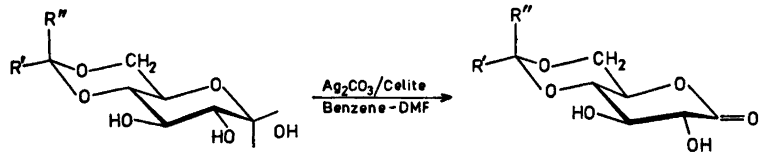
4,6-*O*-Benzylidene-D-galactose (*1*) on oxidation with silver carbonate on Celite in benzene containing a little *N*,*N*-dimethylformamide (DMF), followed by treatment of the product mixture with an anion exchange resin, gave 3,5-*O*-benzylidene-D-lyxose (*2*) in about 55 % yield. The identity was established by hydrolysis to lyxose, and by periodate oxidation which gave 2,4-*O*-benzylidene-D-threose (*3*). Further oxidation of *2* in benzene-toluene yielded the known[3] 3,5-*O*-benzylidene-D-lyxono-1,4-lactone (*4*); in benzene alone this oxidation occurred very slowly. Oxidation of 4,6-*O*-benzylidene-D-galactose (*1*) in methanol gave mainly 2,4-*O*-benzylidene-D-threose (*3*).

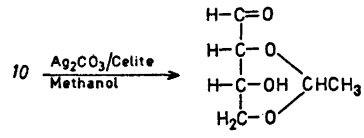
4,6-*O*-Ethylidene-D-galactose (*5*) in benzene-DMF reacted analogously to give 3,5-*O*-ethylidene-D-lyxose (*6*) as the main product, periodate oxidation of which gave 2,4-*O*-ethylidene-



D-threose (*7*). Small amounts of galactonolactone were detected in this case by treatment of the anion exchange resin used after oxidation of *5* with trifluoroacetic acid. 2,4-*O*-Ethylidene-D-threose (*7*) was also obtained from *5* by silver carbonate oxidation in methanol.

Oxidation of 4,6-*O*-alkylidene derivatives of D-glucose in benzene-DMF did not proceed in the same way as oxidation of the galactose derivatives; the main reaction was oxidation to gluconolactone derivatives. Thus, 4,6-*O*-benzylidene-D-glucose (*8*) gave crystalline 4,6-*O*-benzylidene-D-glucono-1,5-lactone (*9*) in about 50 % yield; trace amounts only of arabinose were detected after hydrolysis of the mother liquor. 4,6-*O*-Ethylidene- (*10*) and 4,6-*O*-isopropylidene- (*11*)-D-glucopyranose were also oxidized to the corresponding aldonolactones (*12* and *13*, respectively). Isolation of these compounds was difficult when the oxidation had been carried out in benzene – DMF, due to instability of the lactone ring. The use of benzene as solvent facilitated, however, the isolation of the lactones. Also in these cases, small amounts only of degradation products were detected. Since the oxidation in benzene – DMF gave 3,5-*O*-alkylidenearabinoses in negligible amounts, oxidation of 4,6-*O*-ethylidene-D-glucopyranose (*10*) in methanol



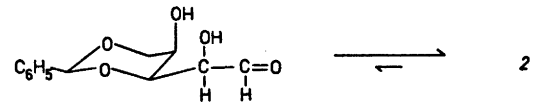


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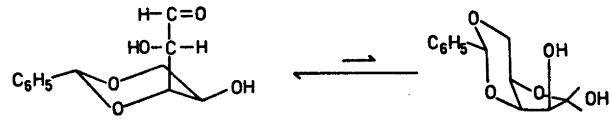
was tried. Degradation beyond the pentose stage occurred, however, too rapidly to make this a suitable method for preparation of 3,5-*O*-ethylidene-D-arabinose. 2,4-*O*-Ethylidene-D-erythrose (*14*) was the only product after the reaction mixture had been treated with an anion exchange resin, in analogy with the results obtained on oxidation of 4,6-*O*-alkylidene derivatives of D-galactose in methanol.

A synthesis of a 3,5-*O*-benzylidene-D-lyxose has been described,[4] in which the primary hydroxyl group of 1,3-*O*-benzylidene-D-arabinitol was selectively oxidized to give the lyxose derivative. The product was described as a syrup, and based on IR spectroscopy it was supposed to exist in *aldehydo* form. To this compound was assigned the configuration and preferred conformation *2b*. In the 3,5-*O*-benzylidene-D-lyxose prepared here, the configuration at the benzylic carbon atom should be expected to be the same as in the starting compound 4,6-*O*-benzylidene-D-galactopyranose (*1*), in which the phenyl group most likely is equatorially attached to the 1,3-dioxan ring in a chair conformation.[5] The expected configuration at the benzylic carbon atom in the produced 3,5-*O*-benzylidene-D-lyxose (*aldehydo* form *2a*) is the opposite of that of the 3,5-*O*-benzylidene-D-lyxose *2b*. The facts that the IR spectrum shows no carbonyl absorption, and that the compound is oxidized to a 1,4-lactone (*4*) in benzene–toluene, suggest that this 3,5-*O*-benzylidene-D-lyxose exists in a furanose form (*2*). The fact that cyclization of the benzylidenelyxose *2b* to a furanose form (*2c*) is not observed, may be a result of the unfavourable interactions of the hydroxyl group at C-2 in the furanose ring with the benzylic carbon atom and with C-5 in the lyxose part. These interactions are of the *syn* 1–3 diaxial type if the 1,3-dioxan ring exists in the chair conformation (*2c*) with the phenyl group equatorially attached.

The observed differences between 4,6-*O*-alkylidene derivatives of glucose on one hand and those of galactose on the other, in the oxidation with silver carbonate on Celite in benzene–DMF could arise from steric differences in the pyranose rings caused by the *trans*-fused 1,3-dioxan ring in the glucose derivatives, resulting in a very rigid, *trans*-decalin type conformational situation,[6] whereas the dioxan ring is *cis*-fused in the galactose derivatives, and hence leads to a much more flexible, *cis*-decalin type situation. The explanation could, however, also be found simply in differences in the anomeric ratio of the reacting hexose derivatives, and a product composition dependent on this ratio. It was therefore of interest to determine the relative reactivity of the anomers of 4,6-*O*-alkylidenehexoses. Samples withdrawn from the reaction mixtures during the oxidation of the ethylidene derivatives (*5* and *10*), were therefore trimethylsilylated and analysed by GLC (Table 1). A rapid decrease in the amounts of the β-anomeric forms was observed, and the α-anomer of 4,6-*O*-ethylidene-D-galactopyranose was also relatively rapidly oxidized, whereas the α-anomer of the glucose derivative disappeared



2a ⇌ *2*



2b ⇌ *2c*

Table 1. GLC of the anomers of 4,6-*O*-ethylidenehexoses and their oxidation products as *O*-trimethylsilyl derivatives. For conditions see experimental part.

Compound	Relative retention time
4,6-*O*-Ethylidene-α-D-glucopyranose	1.00
4,6-*O*-Ethylidene-β-D-glucopyranose	1.20
4,6-*O*-Ethylidene-D-glucono-1,5-lactone	1.79
4,6-*O*-Ethylidene-α-D-galactopyranose	1.24
4,6-*O*-Ethylidene-β-D-galactopyranose	1.53
Major product [a]	1.44
Minor product [b]	3.09

[a] Presumably 4-*O*-formyl-3,5-*O*-ethylidene-*aldehydo*-D-lyxose. [b] Presumably 4,6-*O*-ethyliden-D-galactono-1,5-lactone.

slowly. The minor oxidation product from the galactose derivative, presumably 4,6-*O*-ethylidene-D-galactono-1,5-lactone, was found to be formed rapidly in the initial phase of the reaction, the rate being comparable to that with which the β-anomeric form of the 4,6-*O*-ethylidenegalactose disappeared. From the glucose derivative (*10*), almost exclusively one product, indistinguishable from 4,6-*O*-ethylidene-D-glucono-1,5-lactone (*12*), was observed.

The results obtained indicate that the β-anomeric forms of the 4,6-*O*-ethylidenehexoses are oxidized to the corresponding lactones. The α-anomer of the galactose derivative is cleaved between C-1 and C-2 more rapidly than it is anomerized, the reactivity of the α-anomer of the glucose derivative, on the other hand, seems to be very low, and anomerization to the β-form, followed by oxidation of this anomer to the lactone, occurs more rapidly than degradation.

The low reactivity of 4,6-*O*-alkylidene-α-D-glucopyranoses in degradation to pentose derivatives in benzene – DMF could be due to a pronounced difference in energy between the preferred chair conformation and a conformation with the C-1 – O-1 and C-2 – O-2 bonds in the pyranose ring co-planar, caused by the *trans*-fused 1,3-dioxan ring. A related phenomenon is the relatively great deviation from planarity of the same bonds in 1,2:4,6-di-*O*-alkylidene-α-D-glucopyranoses.[7] On the other hand, in the 4,6-*O*-alkylidene-α-D-galactopyranoses the *cis*-fused 1,3-dioxan ring results in a high conformational flexibility in the pyranose ring and presumably in a much lower energy difference between the preferred conformation of the pyranose ring and the transition state conformation. Previous results support the proposal that co-planarity of the groups involved in degradation by silver carbonate on Celite is favourable, since a preference for oxidative degradation of sugars in furanose forms has been observed,[2,8] in which co-planarity of these groups is readily attained by the anomer with *cis*-configuration at C-1, C-2 or for ketoses C-2, C-3.

The resistance against flattening of the pyranose ring in the 4,6-*O*-alkylideneglucoses might, however, also be expected to lead to a decreased reactivity in oxidation to lactones, since introduction of a lactone group in a six-membered ring causes a change towards planarity of the carbon and oxygen atoms involved in order to allow resonance stabilisation.[9] As no difference in reactivity was observed between the β-anomers of the 4,6-*O*-ethylidenehexoses, the effect of such a resistance must be considerably less important than it is in the degradation of the α-anomers. The observed instability of the lactone rings of 4,6-*O*-alkylidene-D-glucono-1,5-lactones is, on the other hand, presumably a result of the tendency to release the strain in the lactone ring in the compounds.

That the oxidation of 4,6-*O*-ethylidene-D-glucopyranose (*10*) in methanol, contrary to what is observed in benzene – DMF, leads to degradation may possibly be explained by the possibility of hemiacetal formation between the sugar and the solvent and degradation of the resulting α-hydroxy hemiacetal, which is present in equilibrium with the pyranose forms.

The proposed need for co-planarity of the reacting C – O bonds in degradation with silver carbonate on Celite, may also explain why 3,5-*O*-benzylidene-D-lyxose (*2*) is oxidized to the corresponding lactone (*4*) in benzene – toluene, and not degraded to 2,4-*O*-benzylidene-

D-threose (*3*). The β-anomer, which has the needed *cis*-configuration at C-1, C-2 for degradation, is unlikely to be present in substantial amounts since four substituents on the same side of a furanose ring are energetically unfavourable. An additional explanation could be that for the β-anomer, the approach to the oxidant surface of the reacting hydroxyl groups is hindered by the 3,5-*O*-benzylidene group.

The results obtained in the present work show the importance of conformational effects on the product composition obtained from sugar derivatives with silver carbonate on Celite in benzene or benzene–DMF.

From the 4,6-*O*-alkylidenegalactoses, where the *cis*-fused 1,3-dioxan ring leads to a conformationally flexible pyranose ring, the product ratio seems to be determined mainly by the ratio of the anomers present. The 4,6-*O*-alkylideneglucoses, on the other hand, seem to be oxidized almost exclusively in the β-form, presumably due to resistance against degradation of the α-form caused by the puckering effect of the *trans*-fused 1,3-dioxan ring.

The importance of the choice of solvent is also clearly demonstrated. The earlier finding [1,10] that oxidation in methanol gives the most extensive degradation, presumably due to the possibility of this solvent to form hemiacetals with the α-hydroxyaldehydes and hence allowing degradation in acyclic form to occur, is supported by the present results.

EXPERIMENTAL

Paper chromatography was performed on Whatman No. 1 paper with butanol–pyridine–water 5:3:1 (v/v), and electrophoresis in borate buffer at pH 10. TLC was carried out on Silica gel G plates in A, benzene–ethanol 3:1; B, benzene–ethanol 5:1; and C, chloroform–methanol 10:1. The compounds were detected with aniline oxalate (PC), diphenylamine–aniline–phosphoric acid [11] (electrophoreses and TLC), and hydroxylamine–iron(III) chloride [12] (TLC). GLC was performed on a Perkin-Elmer F 11 gas chromatograph, equipped with a flame-ionization detector, using a glass column (180 × 0.15 cm) with 3 % XE 60 on Chromosorb G AW-DMCS (80–100 mesh). The operating temperatures were 120 °C for 5 min, and then programmed at 2 °C/min for *O*-isopropylidene derivatives, prepared for identification of degradation products, and 140–215 °C programmed at 3 °C/min for *O*-trimethylsilyl derivatives. The nitrogen flow-rate was 20 ml/min. Mass spectrometry was carried out with an AEI MS-902 and a Micromass 12 F mass spectrometer.

3,5-O-Benzylidene-D-lyxose (2). 4,6-*O*-Benzylidene-D-galactopyranose [5] (*1*, 100 mg) in DMF (40 ml) and benzene (200 ml) was refluxed and stirred with silver carbonate on Celite (2 g) for 90 min. The solution was filtered and the solvents removed under reduced pressure. The residue was treated with Dowex 1 (HCO_3^-) ion exchange resin in water (10 ml) and methanol (10 ml) for 30 min. Filtration and evaporation of the solvents gave a chromatographically homogeneous (TLC, solvents B and C) residue which crystallized on standing. Yield 49 mg (55 %), after recrystallization from acetone the product had m.p. 148–150 °C, $[\alpha]_D^{20}$ +18° (*c* 1, acetone). Found: C 59.70; H 5.88. Calc. for $C_{12}H_{14}O_5$: C 60.50; H 5.88. IR (KBr): No carbonyl absorption.

Hydrolysis of a few mg of the product in 0.5 M sulfuric acid at 90 °C for 1 h, neutralization with Dowex 1 (HCO_3^-) ion exchange resin and subsequent paper chromatographic and electrophoretic investigation of the solution showed a single component, indistinguishable from lyxose.

Periodate oxidation of 3,5-O-benzylidene-D-lyxose (2). 3,5-*O*-Benzylidene-D-lyxose (*2*, 40 mg) in water (5 ml) was treated with sodium periodate (50 mg) for 1 h, the solution being maintained at pH 7 by addition of small amounts of sodium hydrogen carbonate. Barium acetate (0.5 M) was added until precipitation was complete, and after centrifugation of the solution and evaporation of the solvent, the residue was extracted with chloroform. Removal of the chloroform gave solid 2,4-*O*-benzylidene-D-threose (*3*), m.p. 110–115 °C (reported [13] for the L-enantioner 119–120 °C), $[\alpha]_D^{20}$ +66° (*c* 0.3, pyridine) (lit.[14] +77.6°). The product was indistinguishable (TLC) from a sample of 2,4-*O*-benzylidene-D-threose (m.p. 110–113 °C) prepared from 4,6-*O*-benzylidene-D-galactose (*1*) by periodate oxidation. From both preparations two components were seen, each giving a yellow colour when visualized with diphenylamine–aniline–phosphoric acid (R_F 0.63 and 0.69 in solvent B, R_F 0.77 and 0.85 in solvent C), presumably corresponding to a monomeric and a dimeric form of 2,4-*O*-benzylidene-D-threose.

Treatment of the product with acetone containing 1 % sulfuric acid for 2 h, neutralization with solid sodium hydrogen carbonate, filtration and evaporation of the acetone, gave 1,2-*O*-isopropylidene-β-D-*threo*-furanose, m.p. 81–84 °C (lit.[15] 84 °C), $[\alpha]_D^{20}$ −11° (*c* 1, acetone) (lit.[15] −15.3°). The product was indistinguishable from an authentic sample [16] (TLC, solvent C).

Oxidation of 3,5-O-benzylidene-D-lyxose (2) with silver carbonate on Celite in benzene–toluene. 3,5-*O*-Benzylidene-D-lyxose (*2*, 10 mg) in benzene (40 ml) and toluene (10 ml) was

stirred with silver carbonate on Celite at reflux temperature until TLC (solvent B) showed complete conversion of the starting material. The solution was filtered and the solvents were evaporated to give crystalline 3,5-*O*-benzylidene-D-lyxono-1,4-lactone (*4*), m.p. 205 – 207 °C, (lit.[3] 203.5 – 206 °C). The product was detectable on the TLC-plate with the ester detecting reagent only. IR (chloroform): 1780 cm^{-1} (s) (C = O, 1,4-lactone).

Oxidation of 4,6-O-benzylidene-D-galactopyranose (1) in methanol. 4,6-*O*-Benzylidene-D-galactopyranose (*1*, 300 mg) was stirred with silver carbonate on Celite (6 g) in methanol (60 ml) at 55 °C. After 1 h, TLC (solvent B) showed almost complete oxidation of the starting material, and after 3 h also the initially formed *O*-benzylidenelyxose was oxidized. The solution was filtered, and after addition of water (30 ml), it was stirred with Dowex 1 (HCO_3^-) ion exchange resin for 30 min. Filtration of the solution and evaporation of the solvents gave 2,4-*O*-benzylidene-D-threose (*3*), yield 138 mg (59 %). TLC (solvents B and C) showed also in this case the presence of two components, indistinguishable from those obtained on periodate oxidation of 4,6-*O*-benzylidene-D-galactopyranose. The product was extracted with ethanol, and evaporation of the solvent gave a solid residue, m.p. 110 – 113 °C, $[\alpha]_D^{20}$ +70° (*c* 0.3, pyridine).

Treatment of a part of the product with acetone containing 1 % sulfuric acid as described above, gave a compound indistinguishable from 1,2-*O*-isopropylidene-β-D-threofuranose (m.p. and TLC, solvent C).

3,5-O-Ethylidene-D-lyxose (6). 4,6-*O*-Ethylidene-D-galactopyranose (*5*, 500 mg) in DMF (5 ml) was poured into a stirred, boiling suspension of silver carbonate on Celite (5 g) in benzene (150 ml). After 4 h of continued boiling, the solution was filtered and the solvents were evaporated under reduced pressure. The residue was stirred with Dowex 1 (HCO_3^-) ion exchange resin in methanol (10 ml) and water (10 ml) for 30 min, the solution was filtered and the solvents were evaporated to give syrupy 3,5-*O*-ethylidene-D-lyxose (*6*). Yield 330 mg (77 %), $[\alpha]_D^{20}$ +12° (*c* 3, methanol). MS [IP 70 eV; *m/e* (% rel inst.)]: 175 (1.5), 161 (1.5), 159 (2), 149 (5), 117 (24), 102 (24), 73 (56), 45 (100), 43 (67). Mol. wt. M – H obs. 175.0617, calc. for $C_7H_{11}O_5$ 175.0606.

Hydrolysis as described for 3,5-*O*-benzylidene-D-lyxose (*2*) gave a product indistinguishable (paper chromatography and electrophoresis) from lyxose.

Identification of D-galactonic acid. The ion exchange resin from the previous experiment was treated with 1 M trifluoroacetic acid, the water and acid were evaporated under reduced pressure and the residue was treated with acetic acid (30 %) at 90 °C for 18 h. Evaporation of the solvents gave a syrup (80 mg), indistinguishable from D-galactono-1,4-lactone by TLC (solvent A), $[\alpha]_D^{20}$ −42° (*c* 2, water, final) (lit.[18] −48.2°), m.p. of the derived phenylhydrazide 198 – 200 °C (lit.[19] 200 – 205 °C).

Periodate oxidation of 3,5-O-ethylidene-D-lyxose (6). 3,5-*O*-Ethylidene-D-lyxose (*6*, 40 mg) was oxidized as described for 3,5-*O*-benzylidene-D-lyxose (*2*) to give a crystalline product. Initially the m.p. was 107 – 125 °C, but a slow increase of the melting point was observed with time. After recrystallization from ethanol – light petroleum it was 165 – 166 °C (reported [20] for the dimeric form of 2,4-*O*-ethylidene-D-threose 163 – 165 °C), $[\alpha]_D^{20}$ −5° (*c* 2, chloroform) (lit.[20] −5,2°).

The obtained 2,4-*O*-ethylidene-D-threose (*7*) was treated with acetone – sulfuric acid as described for 2,4-*O*-benzylidene-D-threose (*3*), to give 1,2-*O*-isopropylidene-β-D-threofuranose, indistinguishable from the authentic sample (TLC, solvent C, and m.p.).

Oxidation of 4,6-O-ethylidene-D-galactopyranose (5) in methanol. 4,6-*O*-Ethylidene-D-galactopyranose (*5*, 300 mg) in methanol (60 ml) was stirred at reflux temperature with silver carbonate on Celite (6 g) for 2 h. The solution was filtered and water (30 ml) was added and the solution was treated with ion exchange resin as described above. Work-up in the usual way gave crystalline 2,4-*O*-ethylidene-D-threose (*7*). Yield 138 mg (65 %), m.p. 122 – 127 °C, after recrystallization from ethanol – light petroleum, crystals were obtained with m.p. 163 – 164 °C, $[\alpha]_D^{20}$ −6° (*c* 2, chloroform). The product was indistinguishable from the product obtained by periodate oxidation of 3,5-*O*-ethylidene-D-lyxose (*6*) by TLC (solvents B and C).

Oxidation of 4,6-O-benzylidene-D-glucopyranose (8) in benzene – DMF. 4,6-*O*-Benzylidene-D-glucopyranose (*8*, 250 mg) in DMF (2.5 ml) was added to stirred silver carbonate on Celite (2.5 g) in boiling benzene (75 ml). Stirring was continued for 3.5 h at reflux temperature, the solution was then filtered and somewhat concentrated. From the solution 4,6-*O*-benzylidene-D-glucono-1,5-lactone (*9*) crystallized. Yield 123 mg (49 %), m.p. 190 °C (after recrystallization from ethyl acetate), $[\alpha]_D^{20}$ +33° (*c* 1, acetone). Anal. $C_{13}H_{16}O_6$: C, H, IR (KBr): 1740 cm^{-1} (s), (C = O, 1,5-lactone), the product was detectable (TLC) with hydroxylamine – iron(III) chloride only.

Hydrolysis of the product in 50 % aqueous acetic acid at 90 °C for 3 h, gave a product indistinguishable from glucono-1,4-lactone by TLC (solvent A, R_F 0.33), small amounts of a component with mobility corresponding to that of the 1,5-lactone (R_F 0.27) were also present.

The mother liquor from the crystallization of 4,6-*O*-benzylidene-D-glucono-1,5-lactone (*9*) was evaporated under diminished pressure and the residue was hydrolyzed in aqueous acetic acid as above. Paper chromatography of the product showed, as the only reducing sugars,

a compound indistinguishable from glucose and traces of a component with a mobility corresponding to that of arabinose.

Oxidation of 4,6-O-ethylidene-D-glucopyranose (10) in benzene. 4,6-*O*-Ethylidene-D-glucopyranose (*10*, 100 mg) in boiling benzene (250 ml) was stirred with silver carbonate on Celite (3 g) for 3 h. Filtration and concentration of the solution gave a crystalline product; after addition of small amounts of light petroleum (b.p. 60–80 °C), the product was filtered off. Recrystallization from chloroform gave pure 4,6-*O*-ethylidene-D-glucono-1,5-lactone (*12*). Yield 70 mg (71 %), m.p. 136–141 °C, $[\alpha]_D^{20}$ +19° (*c* 0.5, chloroform). Anal $C_8H_{12}O_6$: C, H. IR (chloroform): 1750 cm^{-1} (s) (C = O, 1,5-lactone), detectable (TLC) with hydroxylamine–iron(III) chloride only.

Hydrolysis of a part of the product in 50 % aqueous acetic acid for 3 h at 90 °C and evaporation of the solvents gave a syrup, containing a mixture of the D-glucono-1,4- and -1,5-lactones, indistinguishable by TLC (solvent A) from authentic samples, $[\alpha]_D^{20}$ +6° (*c* 2, water, 48 h) (reported[21] for the 1,5-lactone +9° after 24 h), m.p. of the derived phenylhydrazide 196–198 °C (lit.[19] 195–200 °C).

The mother liquor after the crystallization of 4,6-*O*-ethylidene-D-glucono-1,5-lactone (*12*) was evaporated, and the residue was hydrolyzed in 0.5 M sulfuric acid at 90 °C for 1 h. The solution was neutralized with Dowex 1 (HCO_3^-) ion exchange resin and the solvent evaporated; the resulting syrup contained glucose as the major component, together with traces of arabinose, indistinguishable from authentic samples by paper chromatography and by GLC after conversion to *O*-isopropylidene derivatives.[22]

Oxidation of 4,6-O-ethylidene-D-glucopyranose (10) in methanol. 4,6-*O*-Ethylidene-D-glucopyranose (*10*, 300 mg) was oxidized at 55 °C in methanol (60 ml) with silver carbonate on Celite (6 g) for 1 h. Work-up as described for 2,4-*O*-ethylidene-D-threose (*7*) gave chromatographically homogeneous (TLC) amorphous 2,4-*O*-ethylidene-D-erythrose (*14*), the yield was 131 mg (61 %). $[\alpha]_D^{20}$ −36° (*c* 1, water) (lit.[23] −36.8°). The product was indistinguishable (TLC, solvents B and C) from an authentic sample.[23] Treatment of a part of the product with acetone–sulfuric acid as described above for the 2,4-*O*-alkylidenethreoses (*3* and *7*), and subsequent oxidation of the product with silver carbonate on Celite in benzene gave 2,3-*O*-isopropylidene-D-erythrono-1,4-lactone, m.p. 64–67 °C (lit.[24] 65–67.5 °C), $[\alpha]_D^{20}$ −85° (*c* 1, acetone) (lit.[24] −116°, *c* 0.3, water), indistinguishable from an authentic sample[8] by TLC (solvent C).

Oxidation of 4,6-O-isopropylidene-D-glucopyranose (11) in benzene. 4,6-*O*-Isopropylidene-D-glucopyranose[25] (*11*, 100 mg) was stirred with silver carbonate on Celite (3 g) in boiling benzene (250 ml) for 3 h. Filtration and concentration of the solution and addition of light petroleum (b.p. 60–80 °C) gave amorphous 4.6-*O*-isopropylidene-D-glucono-1,5-lactone (*13*), chromatographically (TLC, solvents B and C) homogeneous, detectable with hydroxylamine–iron(III) chloride only. Yield 71 mg (72 %), $[\alpha]_D^{20}$ +23° (*c* 2, chloroform). Anal. $C_9H_{14}O_6$: C, H. MS [IP 70 eV; *m/e* (% rel. int.)]: 203 (34), 160 (3), 143 (22), 131 (5), 125 (5), 115 (6), 101 (5), 97 (9), 85 (7), 73 (34), 59 (74), 43 (100). IR (chloroform): 1750 cm^{-1} (s), (C = O, 1,5-lactone).

Hydrolysis of a part of the product as described for 4,6-*O*-ethylidene-D-glucono-1,5-lactone (*12*) gave the D-glucono-1,4- and -1,5-lactones, indistinguishable from the authentic specimens by TLC (solvent A), $[\alpha]_D^{20}$ +10° (*c* 2, water, 48 h), m.p. of the derived phenylhydrazide 195–199 °C.

The concentrated solution obtained from separation of 4,6-*O*-isopropylidene-D-glucono-1,5-lactone (*13*) was taken to dryness under reduced pressure, the residue after hydrolysis contained glucose and small amounts of arabinose, as detected by GLC of their *O*-isopropylidene derivatives.

Comparison of the reactivities of the anomers of the 4,6-O-ethylidenehexoses (5 and 10). The 4,6-*O*-ethylidenehexose to be investigated (10 mg) was dissolved in DMF (2 ml), the solution was heated at 80 °C for 15 min. Benzene (20 ml) was added and the solution heated at reflux temperature for 20 min. To 20 ml of the solution was added silver carbonate on Celite (400 mg) and the mixture was stirred under reflux. Aliquots (50 µl) were withdrawn at intervals, immediately trimethylsilylated (trimethylchlorosilane–hexamethyldisilazane–pyridine 0.05:0.1:0.5 ml) and subjected to GLC. From the remaining part (2 ml) of the solution, which was kept at reflux temperature without oxidant, samples were also withdrawn, trimethylsilylated and investigated by GLC to ensure that no significant change in the anomeric ratio occurred in the absence of oxidant. Assignment of configuration to the anomers in the gas chromatogram was achieved by trimethylsilylation followed by GLC of the crystalline α-anomers of the 4,6-*O*-ethylidenehexoses, almost exclusively a single peak was observed in both cases.

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Regulation of Ornithine Decarboxylase and *S*-Adenosyl-L-Methionine Decarboxylase in Regenerating Rat Liver by Various Amines: Evidence for Translational Control

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The activity of ornithine decarboxylase in regenerating rat liver could be completely or partially inhibited *in vivo* by a single intraperitoneal injection of various amines. Unphysiological, 1,3-diaminopropane depressed most effectively the activity of ornithine decarboxylase. It depressed also the activity of adenosylmethionine decarboxylase, which was not inhibited by other amines. The activity of tyrosine aminotransferase was invariably stimulated by injection of the amines.

Cycloheximide caused a rapid decay of the activity of liver ornithine decarboxylase (half-life 15 min) and also a decay of the activity of adenosylmethionine decarboxylase (half-life 36 min). 1,3-Diaminopropane inhibited the activity of ornithine decarboxylase (half-life 13 min) and to lesser extent also the activity of adenosylmethionine decarboxylase (half-life 120 min). On the contrary, α-amanitin did not have any effect on the activity of the decarboxylases.

These experiments are consistent with the view that diamines and spermidine might conceivably control the activity of ornithine decarboxylase in regenerating rat liver *in vivo* at steps beyond transcription. It is also possible that 1,3-diaminopropane similarly controls the activity of adenosylmethionine decarboxylase thus suggesting that the synthesis of ornithine and adenosylmethionine decarboxylases may be coordinatively regulated in liver.

The first two enzymes in the biosynthetic pathway of putrescine and the higher polyamines, spermidine and spermine, are in vertebrate tissues: (i) a soluble ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) that catalyzes the decarboxylation of L-ornithine to yield putrescine and CO_2,[1,2] and (ii) a soluble *S*-adenosyl-L-methionine decarboxylase (*S*-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) that catalyzes the decarboxylation of *S*-adenosyl-L-methionine to yield *S*-adenosylmethylhomocysteamine (decarboxylated adenosylmethionine) and CO_2[3–6]

Ornithine decarboxylase (ODC) is the rate controlling enzyme in the synthesis of polyamines and may be associated somehow with the control of cell growth.[7,8] ODC possesses the shortest biological half-life of only 10 – 20 min known for enzymes in animal tissues.[9,10] The activity of the enzyme is greatly stimulated under conditions of rapid growth, such as in rat liver after partial hepatectomy [1,2] or after treatment with growth hormone.[11]

In bacteria the activity of ODC appears to be controlled directly by low molecular weight modulators [12,13] but in higher organisms such effector(s) have not yet been found.[7,14] It thus appears that the activity of eukaryotic ODC is largely regulated through changes in the rate of the synthesis and/or degradation of the enzyme protein.[7,15] There is considerable amount of indirect evidence suggesting that the activity of ODC in mammalian tissues is controlled *in vivo* by a repression type inhibition. In regenerating rat liver [16–18] and in cultured mammalian cells [19,20] exogenous putrescine, spermidine or 1,3-diaminopropane rapidly depressed ODC activity. It has been proposed that the control of ODC by polyamines occurs at some posttranscriptional, possibly at translational level.[17,21]

The enhanced activity of ODC rapidly results in a concomitant increase in liver put-

rescine [2] and an accumulation of spermidine somewhat later.[23,24] Adenosylmethionine decarboxylase (AMDC) in animal tissues and in yeast is strongly stimulated by putrescine and by other diamines.[3,5,6,25] Putrescine thus directly regulates the activity of the second enzyme of the biosynthetic pathway of polyamines. AMDC has also very short half-life (about 35 min) in regenerating rat liver [10] indicating that its activity is likely to be influenced by changes in protein synthesis.

In the present paper I have investigated the efficacy and specificity of various amines to inhibit ODC and AMDC *in vivo*. The most potent inhibitor seemed to be 1,3-diaminopropane which also depressed the activity of AMDC.

Based on comparison of the effect of diaminopropane, cycloheximide and α-amanitin on the activity of ODC and AMDC it is proposed that 1,3-diaminopropane controls the synthesis of ODC and AMDC at translational level.

MATERIAL AND METHODS

Chemicals. Unlabelled *S*-adenosyl-L-methionine was synthesized by the method originally described by Cantoni *et al.*[26] and modified by Pegg and Williams – Ashman.[3]

(Carboxyl-^{14}C)*S*-adenosyl-L-methionine (sp. radioactivity 60 mCi/mmol) was purchased from New England Nuclear Corp. (Dreieichenhain, West-Germany) and DL-ornithine-1-^{14}C (sp. act. 51 mCi/mmol) from the Radiochemical Centre (Amersham, Bucks, England). Putrescine, spermidine and spermine (as their hydrochlorides) were obtained from Calbiochem (San Diego, Calif., U.S.A.). 1,3-Diaminopropane was the product of Fluka AG (Buchs SG, Switzerland) and cadaverine hydrochloride was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.). Methylglyoxal bis(guanylhydrazone) was purchased from Aldrich Chemicals (Milwaukee, Wis., U.S.A.). Cycloheximide (Acti-Dione®) was from Nutritional Biochemicals Corp. and α-amanitin from Boehringer (Mannheim, West-Germany). Amines were neutralized before use. Cycloheximide and α-amanitin were dissolved in 0.9 % NaCl just before injections.

Experiments in vivo. Male rats of the Wistair strain weighing 65 – 150 g (95 – 105 g in Table 1; 130 – 150 g in Table 2; 65 – 85 g in Figs. 1 and 2) were used. The animals were partially hepatectomized 24 or 4 h before death. They received the compounds as an intraperitoneal injection 60 min before death (Table 1 and Table 2) or at the time points indicated in Figs. 1 and 2. The amount of amines was 75 μmol per 100 g of body wt., that of cycloheximide 0.8 mg per 100 g of body wt. and that of α-amanitin 100 μg per 100 g of body wt.

Partial hepatectomy was performed under light ether anaesthesia by the method of Higgins and Anderson.[27]

Preparation of liver extracts. After decapitation of the rats, the livers were removed and immediately homogenized with 2 volumes of cold 25 mM Tris-HCl buffer, pH 7.4, containing 0.1 mM EDTA and 5mM dithiothreitol. The homogenates were centrifuged at 105 000 g_{max} for 30 min at 2 °C. The enzyme activities were assayed as quickly as possible using undialyzed supernatant fractions as the source of enzymes.

Analytical methods. The activity of ODC was measured by the method of Jänne and Williams-Ashman,[14] that of AMDC as described by Jänne and Williams-Ashman,[4] and that of tyrosine aminotransferase (EC 2.6.1.5) by the method of Diamondstone.[28] The enzyme activities are expressed as nmol of product formed per mg protein per 30 min.

Quantitative polyamine measurements were carried out by the method of Raina and Cohen.[29] Protein was measured by the method of Lowry *et al.*[30] The decay lines of the enzyme activities were computed by the least-squares method.

RESULTS

The effect of various amines on ODC and AMDC activities. A single intraperitoneal injection (75 μmol/100 g body weight) of putrescine, spermidine, 1,3-diaminopropane, cadaverine, methylglyoxal bis(guanylhydrazone) (MGBG) or MGBG with putrescine into partially hepatectomized (24 h earlier) rats one hour before death clearly depressed the activity of ODC (Table 1). 1,3-Diaminopropane was the most potent inhibitor of ODC decreasing the enzyme activity by more than 90 %. 1,3-Diaminopropane also inhibited the activity of AMDC. The latter enzyme was unaffected by other amines although MGBG, which is the specific inhibitor of AMDC *in vitro*,[31] appeared to inhibit the enzyme activity in undialyzed cytosol fractions. In fact, putrescine, spermidine and cadaverine appeared to even slightly stimulate AMDC (Table 1).

The inhibition of ODC by MGBG might partly be due to the fact that the latter compound inhibits AMDC and hence prevents the conversion of putrescine to spermidine. Thus intracellular concentration of putrescine is expected to rise rapidly after the administration

Table 1. Effect of various amines on the activity of liver ODC, AMDC, tyrosine aminotransferase (TAT) and on the concentration of putrescine, spermidine and spermine in partially hepatectomized rats. The animals, partially hepatectomized 24 h earlier, were treated as described in the text. Four animals in each group. The percentages in the parentheses indicate the relative enzyme activities in comparison with controls.

Treatment	Enzyme activities ±S.D. ODC	AMDC	TAT	Polyamine concentrations (nmol/g of liver ±S.D.) Putrescine	Spermidine	Spermine
Controls	1.1(3) (100 %)	0.11(5) (100 %)	537(76) (100 %)	248(39)	869(139)	298(85)
Putrescine	0.4(3) (33 %)	0.18(9) (170 %)	805(112) (150 %)	410(85)	864(143)	278(7)
Spermidine	0.2(1) (19 %)	0.16(4) (146 %)	798(66) (148 %)	276(69)	1130(130)	255(10)
Diaminopropane	0.10(2) (7 %)	0.07(3) (63 %)	916(186) (170 %)	–	884(213)	275(23)
Cadaverine	0.5(3) (47 %)	0.17(7) (160 %)	864(78) (161 %)	222(44)	830	294(62)
MGBG + Putrescine	0.15(7) (14 %)	0.03(1) (32 %)	746(188) (140 %)	717(188)	851(77)	259(45)
MGBG	0.3(2) (31 %)	0.02(1) (17 %)	817(116) (152 %)	352(49)	853(49)	256(23)

of MGBG. The increased putrescine might, in turn, influence the activity of ODC.

Table 1 also illustrates that the concentrations of spermidine and spermine did not change after injection of the amines, except when spermidine was injected. MGBG alone appeared to elevate, as expected, the concentration of putrescine. After an injection of diaminopropane, it was impossible to measure the concentration of putrescine because the separation of putrescine from diaminopropane is very difficult or impossible by the electrophoretic method employed.

It also appears that the inhibition of ODC by amines and AMDC by diaminopropane is specific since the activity of tyrosine aminotransferase which was used as "reference" enzyme because its short half-life [32] was not inhibited by the amines. In fact, the activity of tyrosine aminotransferase was invariably stimulated after injection of these compounds (Table 1).

1,3-Diaminopropane also inhibited the activity of ODC and AMDC at 4 h after partial hepatectomy when injected 1 h before death. The inhibition of AMDC was in this particular experiment smaller at 4 h (18 %) than at 24 h (37 %) after partial hepatectomy (Table 2).

The inhibition of ODC and AMDC after partial hepatectomy at 4 and 24 h suggests that diaminopropane might influence the synthesis of both decarboxylases during early and later phases of liver regeneration in a coordinative manner.

Table 2. Comparison of the effect of 1,3-diaminopropane on the activity of liver ODC and AMDC. The animals, partially hepatectomized 4 h or 24 h earlier, were treated as described in the text. Other details as in Table 1.

Treatment	4 h regenerating liver ODC activity	AMDC activity	24 h regenerating liver ODC activity	AMDC activity
Controls	0.7(1) (100 %)	0.05(1) (100 %)	0.6(2) (100 %)	0.18(8) (100 %)
Diamino-propane	0.05(2) (7 %)	0.042(8) (82 %)	0.045(5) (12 %)	0.13(8) (63 %)

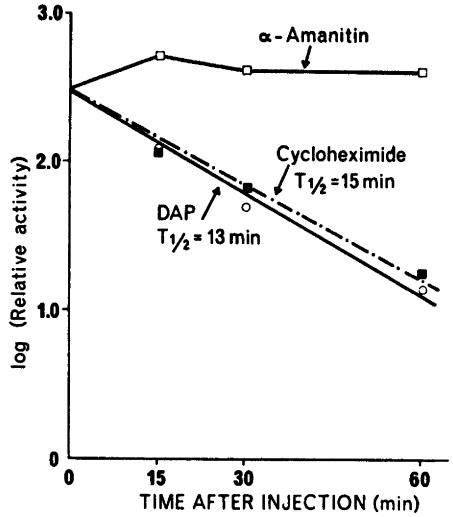


Fig. 1. Effect of 1,3-diaminopropane (DAP), cycloheximide and α-amanitin on liver ODC. The animals, partially hepatectomized 24 h earlier, were treated as described in the text. Three animals in each group.

Comparison of the effect of 1,3-diaminopropane, cycloheximide and α-amanitin on the activity of ODC and AMDC. When partially hepatectomized (24 h earlier) rats were treated with cycloheximide, which is a known inhibitor of eukaryotic protein synthesis,[33] or with diaminopropane, the activity of ODC rapidly decreased with an apparent half-life of 13 min after diaminopropane and 15 min after cycloheximide. α-Amanitin which is a specific inhibitor of nucleoplasmic RNA-polymerase,[34] did not have any effect on the enzyme activity during the whole period of observation (Fig. 1).

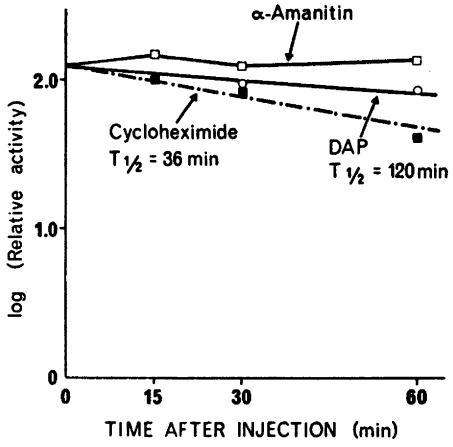


Fig. 2. Effect of 1,3-diaminopropane (DAP), cycloheximide and α-amanitin on liver AMDC. Details as in Fig. 1.

The activity of AMDC was also decreased after treatment with cycloheximide with an apparent half-life of 36 min and also diaminopropane caused an inhibition of AMDC, the half-life being now about 120 min. As in the case of ODC, α-amanitin did not have any effect on the enzyme activity, suggesting that also AMDC has a stable mRNA like ODC (Fig. 2).

It seems possible, even likely, that diaminopropane exerted its effect on the synthesis of ODC mainly at translational level because the enzyme activity was after diaminopropane administration decaying as rapidly as after cycloheximide (Fig. 1).

DISCUSSION

Various compounds have been tested to serve as specific inhibitors of polyamine biosynthesis in mammalian tissues in order to unravel the physiological function(s) of natural polyamines. MGBG was the first and probably the most extensively studied inhibitor of putrescine-activated AMDC and hence the synthesis of spermidine and spermine. It has been reported that millimolar concentrations of MGBG inhibited DNA and protein synthesis when added to cultures of activated human lymphocytes. However, it should be mentioned that these concentrations of MGBG were at least ten times higher than those needed for a complete inhibition of AMDC under these conditions.[35] MGBG, though being a specific inhibitor of putrescine-activated AMDC *in vitro*, appeared also to stabilize the enzyme against intracellular degradation *in vivo*[36] resulting in large increases in the enzyme activity which made it quite impossible to prevent the accumulation of spermidine after partial hepatectomy using sublethal doses of the compound.[37]

A few congeners of ornithine, inhibiting the activity of ODC *in vitro*, have also been tested for their capacity to stop the synthesis of polyamines *in vivo*. It has been reported that the administration of α-hydrazino-δ-valeric acid

depressed ODC activity, inhibited putrescine accumulation and resulted in a concomitant decrease in DNA synthesis in mouse parotid gland.[38] Moreover, Relyea and Rando showed that "unsaturated" ornithine (dehydroornithine) inhibited polyamine synthesis and cell division in chick embryo muscle cultures.[39]

In addition to the use of these kinds of inhibitors, structurally resembling more or less closely L-ornithine or *S*-adenosylmethionine, there is another approach to inhibit polyamine synthesis *in vivo*, namely by directly influencing the synthesis of ODC. This kind of inhibition is based on the fact that the activity of ODC *in vivo* appears to be regulated by polyamines *via* a repression-type mechanism.[17–19,22] 1,3-Diaminopropane, which is not found in mammalian tissues in any appreciable amounts, clearly inhibited the activity of ODC and also prevented the accumulation of spermidine in the regenerating liver remnant when given every 3 h after partial hepatectomy.[18] Interestingly, chronic treatment with diaminopropane resulted in a concomitant block of the synthesis of DNA which normally is greatly enhanced during the second day of liver regeneration.[19] Whether the prevention of the stimulation of DNA synthesis is causally related to the inhibition of polyamine synthesis by diaminopropane remains to be proved.

The present investigation shows that 1,3-diaminopropane is the most effective inhibitor among a number of other amines tested. A remarkable advantage of diaminopropane is the fact that the compound is unphysiological and cannot replace putrescine in the synthesis of higher polyamines.[18] It should be mentioned that it does not inhibit ODC in high concentrations *in vitro*.[18] So it is "fooling" by its structural resemblance, the control mechanisms responsible for the transcription and/or translation of the mRNA for ODC. The type of inhibition caused by diaminopropane resembles that exerted by isopropyl thiogalactoside (not attacked by β-galactosidase) on *E. coli* lac operon.[40]

This investigation also shows that diaminopropane might control the activity of ODC at steps beyond the transcription. The time dependence of the inhibition of ODC by diaminopropane was comparable to that observed after the injection of cycloheximide. Jänne and Hölttä [17] have shown that the decay rate of ODC activity after putrescine injection was of the same order of magnitude. α-Amanitin did not have any effect on the activity of ODC obviously indicating that mRNA of the enzyme is rather stable. The mRNA of ODC is most probably synthesized at the beginning of the liver regeneration which means that the control of ODC through transcription is not possible after the very early period of regeneration. By comparing the effect of polyamines and that of the inhibitors of transcription and translation on the activity of ODC in 3T3 cells, Clark and Fuller [22] likewise suggested that the control of polyamines could occur at translational level.

The physiological significance of the inhibition of ODC *in vivo* by various amines is not known yet, although it has been suggested that the typical phasic fluctuations [41] of ODC activity in regenerating rat liver could be due to putrescine affecting the enzyme activity after its concentration has risen to sufficient levels during the liver regeneration.[41] 1,3-Diaminopropane can also depress the activity of ODC but is not, in contrast to putrescine, converted to higher polyamines.[18]

The use of these compounds also appears to offer a convenient means to investigate the general regulatory mechanisms of protein synthesis in eukaryotic organisms using ODC and AMDC as marker enzymes. However, the most intriguing opportunity offered by a specific inhibitor of polyamine biosynthesis, like diaminopropane, is the possibility finally to solve the physiological function(s) of the natural polyamines, especially during periods of rapid growth.

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Dérivés Phloroglucinoliques d'*Hagenia abyssinica*. IV.* Résonance Magnétique Nucléaire du ^{13}C de la Kosotoxine, du Pseudo-aspidinol et de l'α-Kosine

MAURI LOUNASMAA

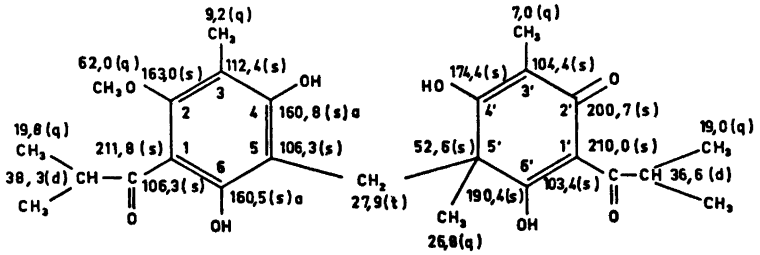
Laboratoire de Chimie des Substances Naturelles, c/o Centre National de la Recherche Technique, Laboratoire de Chimie, SF-02150 Espoo, Finlande

La RMN du ^{13}C a été utilisée pour confirmer la structure de la kosotoxine *1*. Les spectres de RMN du ^{13}C du pseudo-aspidinol *2* et de l'α-kosine *3* ont été également mesurés dans le but d' obtenir des spectres de référence.

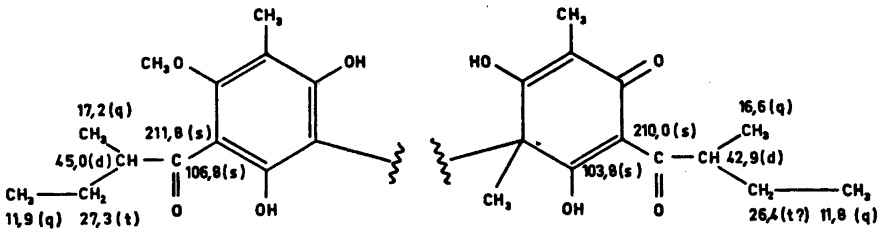
^{13}C NMR spectroscopy has been utilized to confirm the structure of kosotoxin *1*. The ^{13}C NMR spectra of pseudo-aspidinol *2* and α-kosin *3* have also been measured in order to obtain reference spectra.

Il y a peu de temps nous avons proposé la structure *1* pour la kosotoxine, qui est le dérivé phloroglucinolique le plus abondant d'*Hagenia abyssinica* (Bruce) Gmel. [1–3] La structure proposée a été établie surtout grâce à l'utilisation de techniques spectrales telles que résonance magnétique nucléaire du ^1H et spectrométrie de masse, ainsi que de clivages réductifs alcalins.

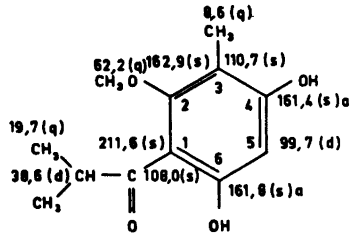
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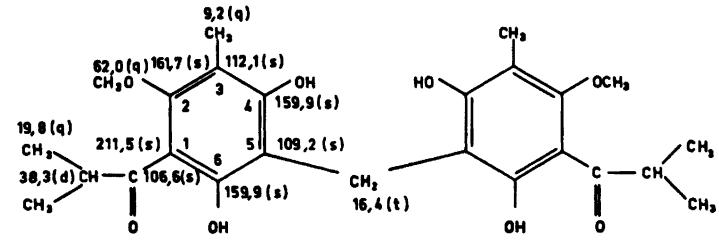
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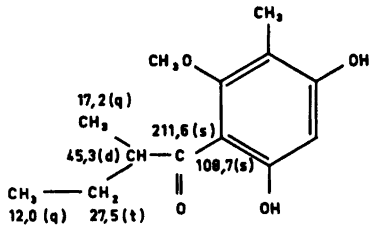
1a 1b



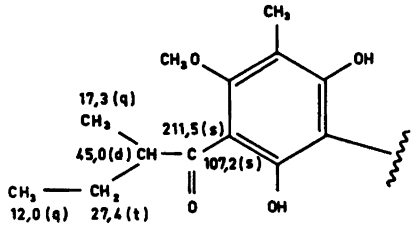
2



3



2a



3a

Pour confirmer la structure proposée nous avons examiné le spectre de résonance magnétique nucléaire du ^{13}C de la kosotoxine *1*. Comme produits de référence nous avons utilisé le pseudo-aspidinol *2* et l'α-kosine *3*, dont les structures étaient connues auparavant et confirmées par les synthèses.[4]

Les déplacements chimiques trouvés, relatifs au TMS utilisé comme référence interne, sont donnés dans les formules. Les multiplicités des signaux, trouvées par les découplages partiels des hydrogènes sont indiquées entre parenthèses. L'attribution des signaux marqués par un *a* peut être renversée dans la même formule.

Dans les travaux antérieurs [1–3] nous avons montré que les dérivés phloroglucinoliques isolés d'*Hagenia abyssinica* sont des mélanges des homologues isobutyryles (iB) (majeur), isovaléryles (iV) (mineur) et méthyl-2 butyryles (2-MeB) (mineur) des chaînes latérales. Les résultats présents montrent la prédominance de la chaîne latérale méthyl-2 butyryle (2-MeB) parmi les homologues mineurs. Le fait que les échantillons utilisés consistaient, en outre des homologues isobutyryles (iB), des quantités suffisantes des homologues méthyl-2 butyryles (2-MeB) facilitait l'identification univoque de certains signaux *(vide infra)*.

Le spectre du pseudo-aspidinol *2* est assez simple et se prête à l'identification facilement. Les signaux trouvés entre 8,6 ppm et 62,2 ppm sont identifiés sans difficulté par leurs déplacements chimiques et par leurs multiplicités. Ainsi, le signal à 8,6 (q) ppm représente le carbone du groupement méthyle attaché au noyau aromatique. Les signaux à 19,7 (q) et 38,6 (d) ppm peuvent être attribués aux carbones sp^3 de la chaîne latérale isobutyryle (iB) et ceux à 12,0 (q), 17,2 (q), 27,5 (t) et 45,3 (d) ppm aux carbones sp^3 de la chaîne latérale méthyl-2 butyryle (2-MeB). Le signal observé à 62,2 (q) ppm représente le carbone du groupement méthoxyle.

La multiplicité du signal à 99,7 (d) ppm permet son identification univoque au C-5. Le rapport caractéristique entre les intensités relatives des signaux* à 108,0 (s) et 108,7 (s) ppm rend possible l'attribution individuelle de ces signaux au C-1 dans *2* et *2a*, respectivement, et permet également l'identification univoque du signal à 110,7 (s) ppm au C-3.

Les signaux trouvés à 161,4 (s), 161,8 (s) et 162,9 (s) ppm représentent des carbones fortement déblindés. Ils peuvent être attribués aux C-2, C-4 et C-6, qui se trouvent en position α par rapport à un atome d'oxygène. Le carbone le plus déblindé est probablement le C-2 attaché au groupement méthoxyle. La distinction entre les deux autres signaux, non indispensable à la compréhension du présent travail, n'a pas été tentée.

Le signal à 211,6 (s) ppm représente le carbone du groupement carbonyle de la chaîne latérale dans les différents homologues (*vide supra*).

L'identification des signaux du spectre de l'α-kosine *3* suit en grande partie le même principe que celle du pseudoaspidinol *2* (*vide supra*). Ainsi, à titre d'example, le déplacement chimique permet l'attribution du signal à 112,1 (s) ppm au C-3 et le rapport caractéristique entre les intensités relatives des signaux à 106,6 (s) et 107,2 (s) ppm rend possible l'attribution individuelle de ces signaux au C-1 dans *3* et *3a*, respectivement. En conséquence, cela permet l'identification univoque du signal à 109,2 (s) ppm au C-5.

Le signal "supplémentaire" à 16,4 (t) ppm peut être attribué au carbone du groupement méthylène entre les deux noyaux aromatiques.

Le spectre de la kosotoxine *1*, interprété partiellement en le comparant avec ceux des produits de référence, confirme la structure proposée. Le signal à 9,2 (q) ppm est attribué au carbone du groupement méthyle attaché au noyau aromatique. Les signaux à 19,8 (q) et 38,3 (d) ppm peuvent être attribués par analogie avec les cas précédents (*vide supra*) aux carbones sp^3 de la chaîne latérale isobutyryle (iB) et ceux à 11,9 (q), 17,2 (q), 27,3 (t) et 45,0 (d) ppm aux carbones sp^3 de la chaîne latérale méthyl-2 butyryle (2-MeB) attachées au noyau aromatique. En conséquence, les signaux à 19,0 (q) et 36,6 (d) ppm sont attribués aux carbones sp^3 de la chaîne latérale isobutyryle (iB) et ceux à 11,8 (q), 16,6 (q), 26,4 (partiellement caché) et 42,9 (d) ppm aux carbones sp^3 de la chaîne latérale méthyl-2 butyryle (2-MeB) attachées au noyau non-aromatique. L'attribution des signaux à 19,0 et 36,6 ppm aux carbones sp^3 de la chaîne latérale isobutyryle (iB) attachée au noyau non-aromatique est donnée par le fait que les signaux correspondants dans le spectre de l'acide méthyl-3 isobutyryl-5 filicinique (Va dans Réf. 3) apparaissent à 19,0 et 35,4 ppm [5] et dans celui de la colupulone (*4* dans Réf. 6) à 18,9 et 35,3 ppm.[6]

Le signal à 7,0 (q) ppm représente le carbone du groupement méthyle attaché au C-3′ et celui à 26,8 (q) ppm le carbone du groupement méthyle attaché au centre chiral C-5′, qui est lui-même représenté par le signal à 52,6 (s) ppm. L'intensité et la multiplicité du signal à 27,9 (t) ppm permet son identification univoque au carbone du pont méthylene entre les deux noyaux. Le signal trouvé à 62,0 (q) ppm représente le carbone du groupement méthoxyle.

Le signal à 112,4 (s) ppm est attribué par analogie avec les cas précédents (*vide supra*) au C-3. Les deux signaux presque superposés à 106,3 (s) ppm représentant les C-1 (dans *1*) et C-5. Le faible signal à 106,8 (s) ppm est dû au C-1 (dans *1a*) portant une chaîne latérale méthyl-2 butyryle (2-MeB). Le rapport carac-

* Quoique dans la spectroscopie RMN utilisant la transformée de Fourier l'intensité des signaux perde beaucoup de sa valeur diagnostique pour compter les carbones, elle peut toujours être appliquée dans les cas où les carbones à comparer sont substitués par un nombre égal d'hydrogènes et qui, en première approximation, possèdent des temps de rélaxation similaires.

téristique entre les intensités relatives des signaux à 103,4 (s) et 103,8 (s) ppm permet l'attribution individuelle de ces signaux au C-1′ dans *1* et *1b*, respectivement, et rend possible l'identification univoque du signal à 104,4 (s) ppm au C-3′.

Les signaux trouvés à 160,5 (s), 160,8 (s) et 163,0 (s) ppm sont attribués par analogie avec les cas précédents (*vide supra*) aux C-2, C-4 et C-6, qui se trouvent en position α par rapport à un atome d'oxygène. Le carbone le plus déblindé est probablement le C-2 attaché au groupement méthoxyle. La distinction entre les deux autres signaux, sans importance pour la compréhension du présent travail, n'a pas été tentée.

Les signaux observés à 174,4 (s) et 190,4 (s) ppm peuvent être attribués aux C-4′ et C-6′, respectivement. La différenciation entre ces deux signaux est basée sur le fait que le C-6′ est le carbone β dans deux systèmes cétoniques α,β-insaturés, tandis que le C-4′ ne l'est que dans une. Le signal à 200,7 (s) ppm est celui du C-2′.

Les signaux à 211,8 (s) et 210,0 (s) ppm représentent les carbones des groupements carbonyles des chaînes latérales attachées aux noyaux aromatique et non-aromatique, respectivement.

PARTIE EXPÉRIMENTALE

Les spectres de RMN du ^{13}C ont été realisés dans $CDCl_3$ à 22,63 MHz sur un spectromètre Bruker HX 90 E.

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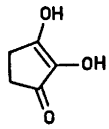
The Crystal Structure of Reductic Acid (2-Cyclopentene-2,3-diol-1-one)

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The crystal and molecular structure of reductic acid, $C_5H_6O_3$ (2-cyclopentene-2,3-diol-1-one) has been determined by X-ray methods, using 1919 observed intensities collected by counter methods. The crystals are monoclinic, space group $P2_1/c$ with unit cell dimensions $a = 7.172(1)$, $b = 8.680(1)$, $c = 10.328(2)$ Å, $\beta = 129.37(1)°$. The refinements yielded a conventional R-factor of 0.044; standard deviations in bond lengths and angles are 0.001 Å and 0.1°. The molecular geometry and the hydrogen bond system are discussed.

Reductic acid is a colourless, sublimable compound with a melting point of 214 °C. It is the simplest carbocyclic analogue of triose reductone (2-propen-2,3-diol-1-one), and has the formula (*1*). Being an acireductone the molecule



1

comprises the functional group $-COH=COH-CO-$, also found in ascorbid acid and triose reductone. The title compound is thus an organic acid of strength comparable to the carboxylic acids and possesses the characteristic reducing properties of the acireductones. The compound was first synthesized by the action of mineral acids on certain carbohydrates, as for instance pectins, and alginates.[1] Reductic acid is, however, accessible in high yields (70 %) from 3-bromo-2-hydroxy-2-cyclopentene-1-one by hydrolysis.[2]

The present X-ray study of reductic acid was undertaken in order to provide a comparison with the previous X-ray investigation of triose reductone [3] and to further examine the hydrogen bonding properties of the conjugated system common to both molecules.

EXPERIMENTAL

An extraordinarily pure sample of the compound, superior to those commercially available, was kindly supplied by prof.dr. G. Hesse. Suitable crystals showing the forms {100}, {010} and {001} were obtained by slow evaporation from formic acid solutions. Preliminary photographic investigations implied the space group to be $P2_1/c$. Unit cell dimensions were determined from measurement of the diffractometer setting angles of 17 reflections, using a manual Picker four-circle diffractometer (Cu$K\beta$ radiation, $\lambda = 1.3894$ Å). Intensity measurements were made on a SYNTEX P $\bar{1}$ computer controlled four-circle diffractometer with graphite monochromated Mo$K\alpha$ ($\lambda = 0.71069$ Å) radiation. With the crystal mounted in an arbitrary orientation, the intensities within one quadrant for $2\theta < 80°$ were measured with the $\omega - 2\theta$ scanning mode and a symmetrical scan range based on 1.8° varied to allow for spectral dispersion. The scan speed for each reflection was calculated from a fast preliminary $\omega - 2\theta$ scan of the reflection and varied between 1.0 and 6.0 °/min, so as to maintain satisfactory counting statistics even for reflections of moderate intensity. A rejection level was specified to avoid measurement of reflections with intensities less than the instrumental threshold value. Background counts were taken at each end of the scan, the ratio of the total background counting time to the scan time was 0.7. A total of 2171 independent reflections were recorded, of which 1919 had intensities greater than twice their standard deviations estimated from counting statistics. These were considered to be observed, the remaining were rejected from the structure

Table 1. Final fractional coordinates and thermal parameters with estimated standard deviations. Expression for anisotropic vibration is: $\exp[-2\pi^2(h^2a^{*2}U11 + \cdots + 2klb^*c^*U23)]$.

ATOM	X	Y	Z	U11	U22	U33	U12	U13	U23
O 1	.77841(13)	.18690(8)	.59151(10)	.0254(3)	.0266(3)	.0488(4)	.0007(3)	.0165(4)	.0065(3)
O 2	.26388(13)	.28569(9)	.48126(11)	.0260(3)	.0283(4)	.0589(5)	-.0014(3)	.0268(4)	.0078(3)
O 3	.28781(14)	.48545(9)	.21715(12)	.0238(3)	.0333(4)	.0494(5)	.0028(3)	.0156(3)	.0122(3)
C 1	.68193(16)	.22420(10)	.50891(12)	.0215(3)	.0235(4)	.0283(4)	-.0024(3)	.0140(3)	-.0022(3)
C 2	.43848(15)	.27314(11)	.48484(12)	.0200(3)	.0231(4)	.0304(4)	-.0038(3)	.0161(3)	-.0013(3)
C 3	.40583(16)	.40385(11)	.32066(12)	.0221(4)	.0246(4)	.0283(4)	-.0019(3)	.0134(3)	-.0014(3)
C 4	.63146(19)	.45705(13)	.35634(14)	.0289(5)	.0318(5)	.0366(5)	-.0029(4)	.0212(4)	.0041(4)
C 5	.81733(18)	.33508(13)	.47607(14)	.0235(4)	.0338(5)	.0399(6)	-.0022(4)	.0197(4)	.0037(4)

ATOM	X	Y	Z	B	ATOM	X	Y	Z	B
H 1	.2926(27)	.1103(19)	.4257(18)	4.9(3)	H 2	.0757(31)	.4381(24)	.1884(19)	5.9(4)
H 3	.6693(24)	.5636(15)	.4021(16)	3.5(2)	H 4	.6069(24)	.4642(16)	.2537(17)	4.1(3)
H 5	.9547(26)	.3758(16)	.5833(18)	4.4(3)	H 6	.8938(25)	.2833(16)	.4357(17)	4.5(3)

Table 2. Bond distances, bond angles, and hydrogen bond lengths and angles. Estimated standard deviations in bond lengths between heavy atoms are 0.001 Å, in angles 0.1° (0.01 Å and 1° in bonds and angles involving hydrogen atoms). Distances in parenthesis are corrected for librational thermal motion.

Bond distances (Å)		Bond angles (°)	
C1–O1	1.250 (1.253)	O1–C1–C2	125.6
C2–O2	1.362 (1.366)	O1–C1–C5	125.4
C3–O3	1.321 (1.324)	O2–C2–C3	124.1
C1–C2	1.420 (1.426)	O2–C2–C1	126.3
C2–C3	1.356 (1.360)	O3–C3–C4	118.7
C3–C4	1.487 (1.494)	O3–C3–C2	128.8
C4–C5	1.529 (1.534)	C1–C2–C3	109.5
C1–C5	1.500 (1.505)	C2–C3–C4	112.5
O2–H1	0.85	C3–C4–C5	103.8
O3–H2	0.89	C4–C5–C1	104.8
C4–H3	0.99	C5–C1–C2	109.4
C4–H4	0.96	C4–C5–H5	114.2
C5–H5	0.97	C4–C5–H6	114.9
C5–H6	0.98	C1–C5–H5	109.4
		C1–C5–H6	110.0
		C5–C4–H3	115.1
		C5–C4–H4	113.2
		C3–C4–H3	109.4
		C3–C4–H4	109.9

Hydrogen bond distances (Å)		Hydrogen bond angles (°)	
O1···O2	2.729	C2–O2–H1	111.4
O1···O3	2.641	C3–O3–H2	112.9
O1···H1	1.92	C1–O1···H1	139.2
O1···H2	1.78	C1–O1···H2	120.9
		O1···H1–O2	163.5
		O1···H2–O3	160.5

analysis. The intensities of three reference reflections measured at regular intervals showed no significant change. Corrections for Lorentz and polarization effects were applied to the intensities and their standard deviations and a 2 % uncertainty in diffractometer stability was included in the latter.

The structure was determined by the application of symbolic addition procedures and refined by Fourier and fullmatrix least-squares methods. The function minimized was $M = \sum w(F_o - F_c)^2$, $w = 1/\sigma^2(F_o)$. The calculations were performed on CD 6600 using the programs described in Ref. 4. The final residuals were $R_w = 0.048$ and $R = 0.044$. Weight analysis showed that weighting based on standard deviations from counting statistics was satisfactory.

No correction was made for possible sources of systematic error such as absorption, extinction, or multiple diffraction. Atomic scattering factors used during the calculations were those of Doyle and Turner [5] for the carbon and oxygen atoms. For the hydrogen atoms those of Stewart, Davidson and Simpson [6] were used.

The final positional and thermal parameters are given in Table 1, bond lengths and angles in Table 2; estimated standard deviations are calculated from the correlation matrix of the final least-squares refinement cycle.

A rigid-body motion analysis was carried out by the method of Schomaker and Trueblood.[7] There is good agreement between the observed and calculated vibration tensor elements; $\Delta U_{ij} = 0.0007$ Å^2, while the average estimated standard deviation in U_{ij} is 0.0010 Å^2, suggesting that the molecular motion may be regarded as that of an oscillating rigid body. The r.m.s. translational amplitudes are 0.17, 0.15, and 0.13 Å and the r.m.s. librational amplitudes are 5.5, 4.2, and 2.3°. Correction

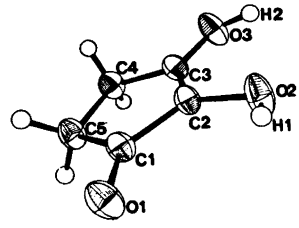


Fig 1. Perspective drawing of the molecule with atom numbering as indicated. Thermal ellipsoids are scaled to include 50 % probability.

Table 3. Bond distances in the conjugated system in reductic acid and some related compounds. Atom numbering as in Fig. 1.

	C1–O1	C2–O2	C3–O3	C1–C2	C2–C3	Ref.
L-Ascorbic acid	1.216(2)	1.361(2)	1.326(3)	1.452(3)	1.338(2)	10
D-Isoascorbic acid	1.200(5)	1.340(5)	1.329(5)	1.446(5)	1.331(5)	9
α-Methyltetronic acid	1.219(3)	–	1.326(3)	1.436(3)	1.335(3)	11
Dimedone (5,5-dimethyl-1,3-cyclohexanedione)	1.246(2)	–	1.326(2)	1.418(2)	1.351(2)	8
Triose reductone (2-propene-2,3-diol-1-one)	1.241(2)	1.351(2)	1.322(2)	1.414(2)	1.352(2)	3
Reductic acid (2-cyclopentene-2,3-diol-1-one)	1.250(1)	1.362(1)	1.321(1)	1.420(1)	1.356(1)	–

in bond lengths range from 0.003 Å to 0.006 Å. However, discussion of the structure is based on the uncorrected values.

CRYSTAL DATA

Reductic acid $C_5H_6O_3$, monoclinic; $P2_1/c$, $a = 7.172(1)$ Å, $b = 8.680(1)$ Å, $c = 10.328(2)$ Å, $\beta = 129.37$ (0.01)°, $\varrho_{obs} = 1.50$ g/cm³, $\varrho_{cal} = 1.52$ g/cm³, $Z = 4$, $V = 497.03$ Å³, M = 114.10 g/mol, $F(000) = 240$.

DISCUSSION

The molecular structure and atom numbering is shown in Fig. 1. Reductic acid is thus found to have the acireductone structure (*1*) with bond lengths in the alternating bond system which agree well with those previously found in triose reductone [3] and dimedone [8] (see Table 3). The values of these bond lengths are of intermediate character indicating a substantial amount of π-electron delocalization in the conjugated system. The formal single bond C3–O3 (1.321 Å) has considerably more doublebond character than the C2–O2 (1.362 Å) bond. It is therefore reasonable to assume that O3–H2 is the acidic group in the molecule, thereby forming a symmetrically delocalized anion on dissociation, which will account for the acidic properties of reductic acid. Similar differences (0.03–0.04 Å) between the central and terminal hydroxyl groups have been found in many other acireductone crystal structures. The only marginal exception known to the author is in the crystal structure D-isoascorbic acid, where the difference is only 0.01 Å and where the central hydroxyl group participates in an unusually strong hydrogen bond (2.585 Å).[9]

It is interesting to compare the bond lengths found in the present investigation with those found in the related 5-membered ring systems of D-isoascorbic-,[9] L-ascorbic-,[10] and α-methyltetronic acids.[11] Table 3 shows the relevant bond lengths observed in the investigation of these compounds together with those from the investigation of reductic acid and also those from the dimedone [8] and triose reductone [3] determinations. It is seen that the conjugation in reductic acid is higher than it is in the 5-membered lactone ring systems. With the exception of the hydroxyl bond length (C3–O3) the differences in bond lengths are significant, judged by the combined standard deviations from the individual investigations. The reason for the higher double bond fixation in the molecules containing the γ-lactone ring is probably that lone pairs on the ring oxygen

Table 4. Deviations (Å) from least-squares planes through parts of the molecule. For atom numbering see Fig. 1. Deviations of atoms not defining the planes in parenthesis.

O1	−0.019	(−0.002)	−0.006
O2	0.031	(0.069)	(−0.065)
O3	−0.022	(−0.019)	−0.005
C1	−0.006	0.001	0.004
C2	−0.013	0.004	0.007
C3	−0.009	−0.007	0.003
C4	0.028	0.007	(0.024)
C5	0.012	−0.005	(0.006)
H1	(−0.29)	(−0.27)	(−0.25)
H2	(−0.19)	(−0.17)	(−0.16)

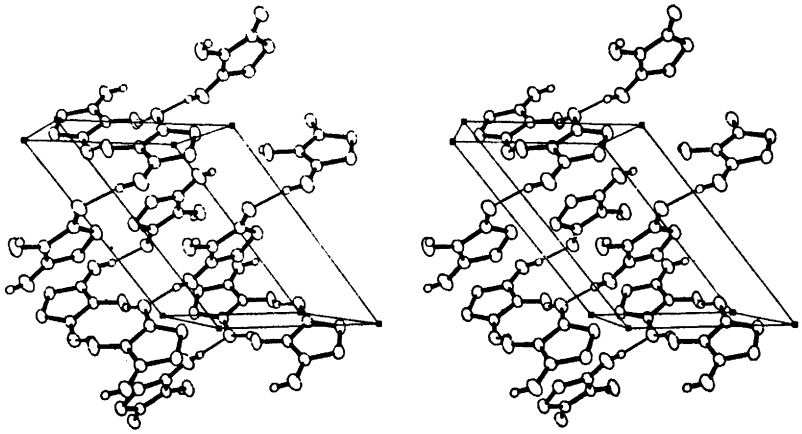


Fig. 2. Stereoscopic illustration of the crystal structure as viewed along the *b* axis. Thermal ellipsoids are scaled to include 50 % probability.

atom may to a certain extent also supply electron density to the neighbouring carbonyl group. In reductic acid, on the other hand, charge will essentially be transmitted through the conjugated system. The interior angles in the 5-membered ring vary from 103.8 to 112.5°. The average value is 108.0°, in good agreement with the angle in a regular pentagon. The larger deviations occur in the angles about the double bond. The external O–C–C angles vary from 118.7° to 128.8°, the larger angles being *cis* to the hydroxyl hydrogen atoms which are situated approximately in the molecular plane. This feature is also observed in many phenol crystal structures,[12,13] and in the crystal structure of squaric acid.[14]

Deviations from least-squares planes through various parts of the molecule are given in Table 4. Although the molecule as a whole is essentially planar, there are significant deviations from a least-squares plane through all heavy atoms. However, the atoms which make up the 5-membered ring and those which make up the conjugated system are separately planar. The deviations of O2 from these planes cause the enediol group in reductic acid to be slightly but significantly non-planar. The deviations from planarity mentioned above are not considered to be of chemical origin, but rather due to deformations from packing and hydrogen bonding forces.

The hydrogen bonding scheme deduced from the approach distances and hydrogen atom positions is shown in Fig. 2. The carbonyl group accepts two intermolecular hydrogen bonds, the shorter of which at 2.641 Å involves the terminal hydroxyl group, and is of a similar order of strength as the hydrogen bonds found in carboxylic acid crystal structures. The other hydrogen bond is somewhat weaker, 2.729 Å and comes from the central hydroxyl group. This is in keeping with the above conclusion that H2 is the acidic proton in the molecule.

The hydrogen bonding scheme is formally very similar to that found in the crystal structure of triose reductone, which contains the same number of donors and acceptors for hydrogen bonding per molecule as reductic acid. The hydrogen bond lengths (2.641, 2.729 Å) also correspond closely to those previously found in triose reductone structure (2.635, 2.744 Å). Although none of the hydrogen bonds in reductic acid lie exactly in the plane of the conjugated system, the stronger hydrogen bond deviates somewhat less from this plane. The crystal structure is built up from hydrogen-bonded dimers around the center of inversion through the longer hydrogen bonds. These dimers are tied together into pairs of chains *via* the shorter hydrogen bond (2.641 Å), running along [201].

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Short Communications

Barriers to Rotation of the Acetyl Groups in Acetylruthenocene and 1,1'-Diacetylruthenocene. A ^1H and ^{13}C NMR Study

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In a previous study [1] we measured the barriers to acyl group rotation in a number of acylferrocenes, including acetylferrocene and 1,1'-diacetylferrocene. It was observed that, due to the strong donor properties of the ferrocenyl group, the barrier to rotation of the formyl group is higher in ferrocenaldehyde than in benzaldehyde. The barrier raising effect of the ferrocenyl group was, however, lower than expected on basis of its Hammett σ^+ constant evaluated from protonation of acetylferrocene.[2] The barriers in acetylferrocene (35.6 kJ/mol) and in 1,1'-diacetylferrocene (32.6 kJ/mol) show a difference significantly larger than the experimental error. The lower barrier in 1,1'-diacetylferrocene was interpreted as due to a transannular effect.

The donor capacity of metallocenyl groups is manifested in ease of solvolysis of α-metallocenylcarbinyl acetates, which increases in the order ferrocenyl < ruthenocenyl < osmocenyl [3] and is related to the stabilities of the intermediate metallocenyl carbonium ions. Turbitt and Watts [4] have measured the optical stability of methylferrocenyl carbonium ion and its ruthenium analog and found barriers to racemization (rotation around the exocyclic bond) of 86.2 and > 130 kJ/mol. These large stabilizations, however, are not to be ascribed exclusively to the donor capacity of the substituted cyclopentadienyl ring. It is fairly generally agreed that part of the stabilization stems from a direct interaction between metal orbitals and the carbonium ion center.[4–6] The better stabilization of the ruthenocenyl carbonium ion is ascribed to better overlap between the carbonium ion center and the more extended ruthenium 4*d* orbitals.[4]

Other evidence points to a reversed order of donor effect, *i.e.* osmocenyl < ruthenocenyl < ferrocenyl. Such evidence is found in the carbonyl stretching frequency of metallocenylketones,[7] in the acidity of metallocenylcarboxylic acids,[7] in the rate of Friedel-Crafts acylation,[7] and possibly also in the basicity of metallocenyl ketones.[2]

This study was undertaken with two aims, to decide if the effect of the metal atom on the rotational barriers in acylmetallocenes followed the first or the second of the above sequences, and to decide if the transannular effect observed in the ferrocenes was present also in the ruthenocenes.

Experimental. Acetylruthenocene and 1,1'-diacetylruthenocene were prepared according to Ref. 7. The ^1H and ^{13}C NMR spectra were recorded as described in Ref. 1. The free energy

Table 1. ^1H NMR chemical shifts for acetylruthenocenes in $CHCl_2F$ at 30 °C.

Compound	δ (2,5) [a,b]	δ (3,4)	δ (C_5H_5)	δ (CH_3)
Acetylruthenocene	5.079	4.756	4.575	2.251
1,1'-Diacetylruthenocene	5.089	4.782		2.164

[a] In ppm downfield from TMS. [b] Center of multiplet.

Table 2. Free energy barriers from coalescence data.

	Compound	$\Delta\nu_0$/ Hz[a]	T_c/ K	$\Delta G^{\ddagger}$ (T_c)/ kJ mol^{-1}[b]
H	Acetylruthenocene	6.2[c]	152	33.1 ± 0.8 (7.9 ± 0.2)
^{13}C	Acetylruthenocene	64	173	34.4 ± 1.2 (8.2 ± 0.3)
^1H	1,1'-Diacetylruthenocene	5.3[c]	148	32.3 ± 0.8 (7.7 ± 0.2)
^{13}C	1,1'-Diacetylruthenocene	62	171	34.1 ± 1.2 (8.1 ± 0.3)

[a] $|\nu_2 - \nu_5|$. [b] In kcal mol^{-1} in parentheses. [c] Measured between centers of multiplets.

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Table 3. ^{13}C NMR chemical shifts for acetylruthenocenes in $CHCl_2F$ with 20 % (v/v) of $(CD_3)_2CO$ at 30 °C.

Compound	δ (1)[a]	δ (2,5)	δ (3,4)	δ (C_5)	δ (CO)	δ (CH_3)
Acetylruthenocene	84.9	71.4	74.0	72.5	200.5	26.8
1,1′-Diacetylruthenocene	86.5	73.1	75.6	–	199.1	26.9

[a] In ppm downfield from TMS.

barriers were obtained at the coalescence temperature only, using the expression (1), which is derived from the expression for the rate of exchange at the temperature of coalescence of a symmetrical doublet [8] in conjunction with the $\Delta G^{\ddagger}_{T_c} = 0.01913\ T_c(9.972 + \log T_c - \log \Delta\nu_0)$ (1) Eyring equation.[9] It was shown in Ref. 1, by comparison with data from complete bandshape analyses, that this approximation gives sufficiently precise free energy barriers, the temperature measurement still being the main source of error.

Results and discussion. The barriers obtained from ^1H spectra and those from ^{13}C spectra are found in Table 2. The deviations between these sets are somewhat larger than normally found but still within the experimental error. The main source of uncertainty is the temperature measurement, which is much more accurate for ^1H than for ^{13}C spectra, and consequently the data from the ^1H spectra are more reliable.

The barrier in acetylruthenocene is thus somewhat lower than that in acetylferrocene, though the region of uncertainty for the ^{13}C barrier of acetylruthenocene shows some overlap with the region for acetylferrocene. The donor effect on the barrier to acyl rotation follows the order ruthenocenyl < ferrocenyl. Furthermore, the barrier difference between acetylruthenocene and 1,1′-diacetylruthenocene is of the same order as the experimental error, *i.e.* no interannular effect has been demonstrated in ruthenocene as opposed to ferrocene.

It has been pointed out [2] that the donor order osmocenyl < ruthenocenyl < ferrocenyl follows the order of Pauling electronegativity of the metal atoms, Os (2.2), Ru (2.2), Fe (1.8),[10] *i.e.* an order of decreasing electron withdrawal from the cyclopentadienyl rings. This rationalization is in good agreement with the ^1H (Table 1) and ^{13}C chemical shifts (Table 3). In both cases the ruthenocene resonances appear downfield with respect to the analogous signals in the acetylferrocene and 1,1′-diacetylferrocene spectra. The ^1H resonances of the ring protons in acetylruthenocene appear 0.3 – 0.4 ppm downfield from those in acetylferrocene, and for the ring carbons the corresponding differences vary from 4.8 ppm for C-1 to 1.2 ppm for C-3, C-4, On the other hand, the ^1H and ^{13}C resonances of the methyl group and the ^{13}C resonance of the carbonyl carbon appear at higher field in acetylruthenocene. This points to a weaker interaction between the acetyl group and the metallocene ring in this molecule than in the ferrocene analogue, in agreement with the observed barriers.

The weaker transannular interaction in 1,1′-diacetylruthenocene is probably related to the inter-ring distances, 3.25 Å in ferrocene [11] and 3.68 Å in ruthenocene [12] or to different electronic transmission properties of the iron and ruthenium atoms.

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Anodic Oxidation of Benzenesulfinate Ion

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In methanol, benzenesulfinate ion gives an anodic polarographic wave.[1] The cathodic reduction of aromatic sulfinic acids has been studied in aprotic medium.[2] The sulfinate ions are not reduced under such conditions. In order to ascertain whether the anodic reactions of benzenesulfinate ion are useful in (1) the quantitative determination of benzenesulfinate ion and/or (2) the preparation of diphenyl disulfone we have investigated the anodic behavior of benzenesulfinate ion and benzenesulfinic acid in more detail. Voltammetry and coulometry at controlled potential were applied at the mercury electrode and the glassy carbon electrode (GCE) in acetonitrile (AN), dimethylformamide (DMF) and dimethyl sulfoxide (DMSO).

In all the three solvents an anodic peak appeared at 0.5 V in the voltammograms of benzenesulfinate ion at a stationary GCE (Fig. 1a). The peak current was proportional to the concentration and to the square root of the potential scan rate and corresponded to an oxidation by 1 F/mol. (The diffusion coefficient was estimated from the polarographic limiting current and the transfer coefficient (α) from the dependence of peak potential on potential scan rate.) In the cathodic scan of the cyclic voltammograms at GCE a small peak appeared at -1.1 V due to the reduction of diphenyl disulfone (Fig. 1a). The benzenesulfinate ion dissolved in AN decomposed slowly, and DMF and DMSO should be preferred for quantitative determinations. The anodic peak potential of 1 mM benzenesulfinic acid in AN was 1.5 V. In the coulometric experiments with benzenesulfinate ion only 10–20 % of the initial amount of sulfinate ion was converted to diphenyl disulfone ($PhSO_2SO_2Ph$, Table 1). The other products of the electrolysis were not identified. An attempt to detect benzenesulfonate ion was not successful. In AN at the oxidation potential $E = 0.5$ V an apparent value of $n = 0.6$ F/mol had been consumed in the electrode reaction, when the current dropped to the background value of the supporting electrolyte. When the oxidation potential was increased to $E = 1.7$ V (more positive than the oxidation potential of benzenesulfinic acid) 1.9 F/mol were exchanged with no formation of diphenyl disulfone (Table 1). Only 2 mmol (20 %) of diphenyl disulfone were isolated in a coulometric experiment, where 20 mmol of benzenesulfinic acid were oxidized in DMF at $E = 0.9$ V with $n = 1.5$ F/mol. 1 F/mol was consumed in the coulometric experiments with benzenesulfinate ion at the Hg pool (Table 1). Polarographic curves together with UV spectra of the electrolysis product and mercury(II) benzenesulfinate ($Hg(SO_2Ph)_2$) were identical, confirming that a mercury(II) compound is formed in the anodic process of benzenesulfinate ion at the mercury electrode. The presence of mercury in the product of the electrolysis was proved by atomic absorption spectroscopy too (Table 1). In all the three solvents the polarographic half-wave potential of benzenesulfinate ion was 0.1 V. The limiting current was proportional to the concentration and polarography as well as voltammetry at GCE should be convenient methods for the quantitative determination of benzenesulfinate ion in aprotic solvents. The voltammetric peak currents at the hanging mercury drop electrode and the polarographic limiting currents corresponded to a consumption of 1 F/mol in the anodic process of benzenesulfinate ion at the mercury electrode. For benzenesulfinate ion as well as mercury(II) benzenesulfinate the potential difference between the anodic and the cathodic peaks in the cyclic voltammograms at a hanging mercury drop (Fig. 1b) and the slope of the

Table 1. Coulometric experiments with sodium benzenesulfinate at the glassy carbon electrode (GCE) and the mercury electrode at constant potential (E) and determination of diphenyl disulfone and mercury(II) benzenesulfinate in the product of the electrolysis. n = the apparent number of electrons consumed in the electrode reaction.

$PhSO_2^-$/ μmol	Solvent	Electrode material	E/ V *vs.* SCE	n/ F mol^{-1}	$PhSO_2SO_2Ph$/ μmol	$Hg(SO_2Ph)_2$/ μmol
20	AN	GCE	0.5	0.62	1.2	–
20	AN	GCE	1.7	1.9	0	–
30	DMF	GCE	0.8	1.1	3.1	–
10	AN	Hg	0.3	0.95	–	[a]
32	DMF	Hg	0.3	0.97	–	15
16	DMF	Hg	0.3	0.99	–	8

[a] Not analyzed.

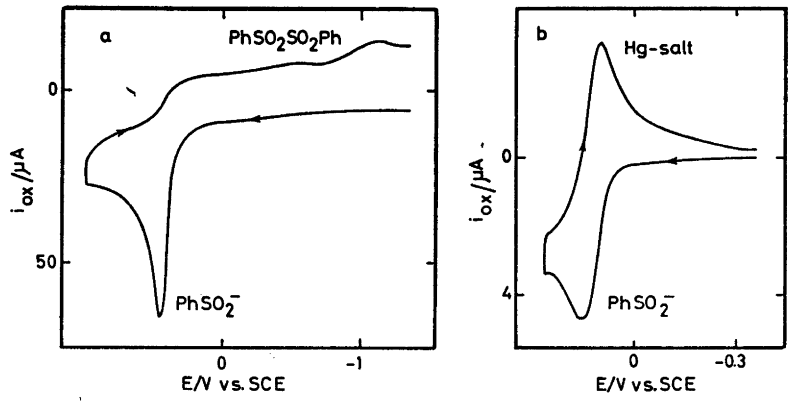


Fig. 1. Cyclic voltammograms of sodium benzenesulfinate. (a) 2.6 mM in DMF, 0.1 M TEAP, at a glassy carbon electrode and scan rate 0.2 V s^{-1}. (b) 1.0 mM in DMSO, 0.1 M TEAP, at a hanging mercury drop electrode and scan rate 0.05 V s^{-1}.

polarographic waves were consistent with a fast 1-electron process. The polarograms of mixtures of sodium benzenesulfinate and mercury(II) benzenesulfinate showed one composite anodic-cathodic wave indicating that benzenesulfinate ion – mercury(II) benzenesulfinate constitute a reversible redox system at the mercury electrode. Since the cyclic voltammograms at the hanging mercury drop electrode and the polarograms of benzenesulfinate ion as well as mercury(II) benzenesulfinate were characteristic of a reversible 1-electron process it may be assumed that the electron transfer proceeds over a mercury(I) compound. The intermediate formation of a mercury(I) compound followed by disproportionation to a mercury(II) compound has been suggested also for the anodic polarographic processes of dialkyldithiocarbamates in aqueous solutions.[3] In aprotic solvents another type of polarographic behavior is shown by the thiophenolate ion – mercury(II) thiophenolate system, which gives rise to two polarographic waves due to the formation of $Hg(SPh)_3^-$ as a stable intermediate.[4]

Experimental. Sodium benzenesulfinate (Fluka, *purum*) was used after drying in vacuum at 60 °C. Benzenesulfinic acid was precipitated from a sulfinate water solution by conc. sulfuric acid, washed and dried at room temperature. Diphenyl disulfone (m.p. 192 – 194 °C) was prepared according to the method of Hilditch.[5] Mercury(II) benzenesulfinate precipitated from an aqueous solution of mercury(II) acetate and sodium benzenesulfinate. The precipitate was washed with water and ethanol and contained 41.8 % Hg. The water content of the organic solvents under experimental conditions was 0.1 – 0.2 % as determined by gas chromatography.

A PAR Model 170, Electrochemistry System, was used for the electrochemical measurements. All potentials given refer to the SCE. The glassy carbon electrode (Chemtrix Inc.) had a geometrical area of 7 mm^2. The concentration range investigated in the voltammetric measurements was 0.10 – 3.0 mM and the solutions contained 0.1 M tetraethylammonium perchlorate (TEAP) as supporting electrolyte. The temperature was 25.0 ± 0.1 °C. The coulometric experiments were carried out in a volume of 5 – 10 ml in a glassy carbon beaker or over a Hg pool. Diphenyl disulfone was analyzed in the product of the electrolysis by polarography ($E_{1/2} = -0.8$ V) and mercury(II) benzenesulfinate by atomic absorption spectroscopy (Varian Techtron, Model 1000). The large scale electrolysis of benzenesulfinic acid (2.9 g) at GCE was performed in an undivided cell with a rotating platinum disk as a counter electrode. A white solid precipitated when the electrolyzed solution was diluted with water. The precipitate was dissolved in diethyl ether and washed with acid and neutral aqueous buffer solutions. The organic phase was evaporated and 0.56 g of electrolysis product was obtained. Melting points and also UV spectra (Pye Unicam SP 1800) of the isolated product and diphenyl disulfone were identical. In the coulometric experiments with sodium benzenesulfinate at the mercury electrode UV spectra (in AN) and polarograms were recorded without isolating the mercury(II) benzenesulfinate formed in the electrode reaction.

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Synthesis of Methanesulfonyl Derivatives of 2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE), Present in Seal from the Baltic

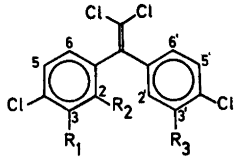
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The low and declining reproduction of the seal in the Baltic [1] has made urgent a thorough characterisation of foreign compounds accumulating in this species. Already known are representatives of compounds related to DDT [2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane] and PCB (polychlorinated biphenyls).[2] In connection with such studies Jensen and Jansson [3] recently detected a series of compounds which, from their physicochemical and mass spectral properties, were characterized as methyl sulfones derived from *p,p'*-DDE and from PCB, respectively.

This paper describes the synthesis and structural elucidation of three methanesulfonyl derivatives of *p,p'*-DDE. The work was performed in order to make available reference compounds for analytical and toxicological studies.

Simple aryl methyl sulfones are generally accessible by Friedel-Crafts type acylations of arenes, under mild conditions, with methanesulfonic anhydride.[4] In the case of *p,p'*-DDE it proved necessary to perform the reaction over an extended period in a melt of a large excess of the anhydride. Chromatography on silica gel of the crude product gave a fair yield of a methanesulfonyl derivative of DDE (*1*). Traces of an isomeric methyl sulfone (*2*) and larger amounts of a more polar bis-methanesulfonyl derivative (*3*) were also obtained.



1 $R_1=SO_2CH_3$ $R_2=R_3=H$

2 $R_1=R_3=H$ $R_2=SO_2CH_3$

3 $R_1=R_3=SO_2CH_3$ $R_2=H$

The structure of the main methyl sulfone was indicated by its oxidation by chromium(VI) oxide to the corresponding benzophenone; the latter compound proved to be identical to 4,4'-dichloro-3-methanesulfonylbenzophenone (*4*) as prepared from 4,4'-dichlorobenzophenone by reaction with methanesulfonic anhydride. The expected formation of the 3-methanesulfonyl derivative in the latter reaction was borne out by the reported formation of the 5,3'-dinitro derivative as a single bisnitration product from 2,4'-dichlorobenzophenone.[5]

From these results, the main methyl sulfone was formulated as 2-(4-chloro-3-methanesulfonylphenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene (*1*). The structure is also in accordance with observed ^1H NMR spectral properties.

The minor isomer was tentatively formulated as 2-(4-chloro-2-methanesulfonylphenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene (*2*). Due to very low amounts of the pure compound *2* the analytical data are in certain parts incomplete.

The two isomers gave characteristic mass spectra, which were qualitatively and partly quantitatively similar; the relative intensities of certain common fragments did, however, show pronounced differences. Both compounds thus gave rise to strong fragments $m/e=280$, interpreted as fluorene type ions.[3] This view was supported by the presence of a metastable ion at $m^*=201$, as expected in the case of simultaneous loss of CH_3SO_2+Cl (114 m.u.) from the molecular ion. Furthermore, in both spectra the ion $m/e=210$ was prominent, indicating a loss of 2 Cl from the fluorene ion. However, *1* showed the molecular ion (M) $m/e=394$ with the base peak $m/e=396$ whereas *2* exhibits the base peak $m/e=125$ which according to the high resolution mass spectrum was interpreted as a tropylium ion C_7H_6Cl. In the case of *1* the intensity of this fragment was only 8 % of the base peak. Further *2* gave an intense fragment at $m/e=359$ $(M-Cl)$ which was of almost negligible importance in the case of *1*. It is tempting to postulate that, in support of the proposed structure of sulfone *2*, these two major fragmentation routes both reflect the sterically crowded situation caused by the methanesulfonyl group in the *ortho*-position to the bridge carbon.

It is noteworthy that the isomerisation to a sulfinate with subsequent fragmentation, a route typical for alkyl aryl sulfones,[6] was observed only in the case of *2*, $m/e=331$ $(M-SOCH_3)$.

The above-mentioned bis-methanesulfonyl derivative of *p,p'*-DDE, should, from its formation in as much as *ca.* 20 % yield, be formulated as 2,2-bis(4-chloro-3-methanesulfonylphenyl)-1,1-dichloroethylene (*3*). This symmetrical structure was fully confirmed by its ^1H NMR spectrum which contained signals from three types only of aromatic protons.

The methyl sulfone *1* prepared here has recently been shown to have a retention time and mass spectral properties in full agreement with those of the sulfone obviously derived from DDE and detected as an essential metabolite in grey seal from the Baltic.[3] Likewise *2* is apparently identical with a trace metabolite

also derived from DDE in the same species.[3]

Experimental. ^{1}H NMR spectra (100 MHz) were obtained on a Varian XL-100 instrument in $CDCl_3$. High resolution mass spectrometry was carried out on a Varian-MAT SM 1 instrument. Combined gas chromatography-mass spectrometry (GLC-MS) was performed as described by Jensen and Jansson.[3] Chromatography was performed on silica gel plates (DC-Fertigplatten, Kiselgel 60 F-254, Merck). Hexane-ethyl acetate (2:1) was used as eluent.

Synthesis of mono- and bis-methanesulfonyl derivatives of p,p'-*DDE.* Freshly distilled methanesulfonic anhydride [7] (12.2 g, 70 mmol) was added to *p,p'*-DDE (0.45 g, 1.4 mmol) [synthesised from *p,p'*-DDT (Merck) and crystallised from ethanol until free from *o,p'*-DDE (GLC)].[8] The mixture was heated with magnetic stirring to 140 °C (bath temp.) for 38 h. The reaction mixture was dissolved in chloroform and stirred with ice water for 3 h. The water phase was extracted with chloroform and the combined chloroform phases were washed with water and evaporated to dryness. The crude product was fractionated on a silica gel column (3 × 45 cm) and the separation was followed by TLC. The appropriate fractions were combined to give compound *2* (R_F-value 0.46), *1* (0.36), *4* (0.22) and *3* (0.08). Unreacted starting material and traces of 4,4'-dichlorobenzophenone were eluted before the sulfones.

Compound *1*, 2-(4-chloro-3-methanesulfonylphenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene, yield 290 mg (52 %), was crystallised from methanol – water to give prisms, m.p. 160 – 162 °C. Anal. $C_{15}H_{10}O_2Cl_4S$: C, H, Cl, S. IR ($CHCl_3$): 1150, 1325 cm^{-1}. UV [abs. ethanol (ε)]: 243 (16 000) and 213 (23 000) nm. MS [EE 70 eV; *m/e* (rel.int %)]: 396 (100, M+2), 394 (78, M), 359 (3), 314 (36), 280 (56), 210 (74) and 125 (8). *m** 396 →282, obs. 200, calc. 201. ^{1}H NMR: δ 3.26 (3 H, s), 8.07 (H_2, d, *J* 2 Hz), 7.53 (H_5, d, *J* 8 Hz), 7.41 (H_6, dd, *J* 8 and 2 Hz), 7.18 ($H_{2',6'}$, dd, *J* 8.5 and 2 Hz), 7.36 ($H_{3',5'}$, dd, *J* 8.5 and 2 Hz).

Compound *2*, 2(4-chloro-2-methanesulfonylphenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene, yield 12 mg (2 %), was analysed by high resolution mass spectrometry: Mol.wt., obs. 395.9126, calc. for $C_{15}H_{10}O_2{}^{35}Cl_3{}^{37}ClS$ 395.9140. Base peak obs. 125.0157, calc. for $C_7H_6{}^{35}Cl$ 125.0159. Prisms from hexane, m.p. 144 – 145 °C. UV [abs. ethanol (ε)]: 256 (16 000) and 212 (26 000) nm. MS [EE 70 eV; *m/e* (rel.int %)]: 394, (32, M), 359 (44), 331 (12), 314 (2), 280 (56), 210 (62) and 125 (100). ^{1}H NMR: δ 2.48 (s), 7.88 (H_2, d, *J* 2 Hz), 7.53 (H_5, dd, *J* 8 and 2 Hz), 7.29 (H_6, d, *J* 8 Hz), 7.13 ($H_{2',6'}$, d, *J* 7.5 Hz), 7.22 ($H_{3',5'}$, d, *J* 7.5 Hz).

Compound *3*, 2,2-bis(4-chloro-3-methanesulfonylphenyl)-1,1-dichloroethylene, was crystallised from methanol – water to give two crystalline forms m.p. 183 – 185 and 208.5 – 209.5 °C, respectively. Anal. $C_{16}H_{12}O_4Cl_4S_2$: C, H, S. IR ($CHCl_3$): 1150, 1320 cm^{-1}. UV [abs. ethanol (ε)]: 260 (14 000) and 216 (26 000) nm. MS [EE 70 eV; *m/e* (rel.int %)]: 474 (100, M+2), 472 (75, M), 392 (7), 358 (8), 314 (40) and 244 (81). ^{1}H NMR: δ 3.28 (6H, s), 8.03 ($H_{2,2'}$, d, *J* 2 Hz), 7.58 ($H_{5,5'}$, d, *J* 8 Hz), 7.47 ($H_{6,6'}$, dd, *J* 8 and 2 Hz).

Synthesis of 4,4'-dichloro-3-methanesulfonylbenzophenone(4). 4,4'-Dichlorobenzophenone (200 mg, 0.80 mmol) was prepared from *p,p'*-DDE in analogy with the oxidation procedure described below. It was treated with methanesulfonic anhydride (7.1 g, 40.8 mmol) as above for 120 h. The crude reaction product, containing large amounts of starting material was purified on a silica gel column (3 × 35 cm). The sulfone was isolated and crystallised from methanol to give *4*, yield 29 mg, m.p. 192.5 – 193.5 °C. Anal. $C_{14}H_{10}O_3Cl_2S$: C, H, Cl, S. IR ($CHCl_3$): 1147, 1325, 1665 cm^{-1}. UV [abs. ethanol (ε)]: 262 (19 000) and 219 (20 000) nm. MS [EE 70 eV, *m/e* (rel. int. %)]: 328 (12, M), 293 (1), 217 (13), 139 (100) and 111 (37). ^{1}H NMR: δ 3.31 (3 H, s), 8.48 (H_2, d, *J* 2 Hz), 7.69 (H_5, d, *J* 8 Hz), 7.98 (H_6, dd, *J* 8 and 2 Hz), 7.72 ($H_{2',6'}$, dd, *J* 8.5 and 2 Hz), 7.48 ($H_{3',5'}$, dd, *J* 8.5 and 2 Hz).

Oxidation of the sulfone 1 *by chromium(VI) oxide.* The sulfone *1* (109 mg, 0.28 mmol) was treated with chromium(VI) oxide (Merck) (275 mg, 2.75 mmol) in acetic acid (14 ml) for 1 h at 100 °C. Water was added and the reaction product was isolated by chloroform extraction. The crude product (100 %) was crystallised from methanol to give scales, m.p. 192.5 – 193.5 °C. The identity of the oxidation product with 4,4'-dichloro-3-methanesulfonylbenzophenone (*4*) was proved by mixed m.p. determination and by comparison of IR, ^{1}H NMR and mass spectra.

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The Structure of *N,N,N''*-Trisubstituted Formamidrazones

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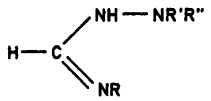
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In connection with the preparation of *N,N,N''*-trisubstituted formamidrazones from the reaction between isocyanides and *N,N*-disubstituted hydrazines [1] it was of interest to determine the structure of the formamidrazones.

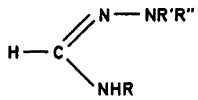
The structure of amidrazones with the possibility of tautomerism has recently been discussed,[3–5] mainly on basis of IR and ^{1}H NMR spectra. For *N''*-phenyl substituted formamidrazones these spectra do not always give conclusive evidence, whereas ^{13}C NMR spectra may be of some use.

This paper reports the application of ^{13}C and ^{1}H NMR spectroscopy for distinguishing between the amide hydrazone structure (B) and hydrazide imide form (A) in some trisubstituted amidrazones and a discussion of the number of conformers present on basis of IR and NMR measurements.

Results. The ^{13}C NMR spectra of the formamidrazones (*1–4*, Table 1) and of some model substances (*5–8*) were obtained in $CDCl_3$-solution.



(A)

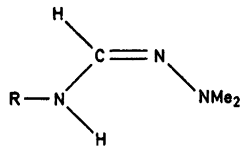


(B)

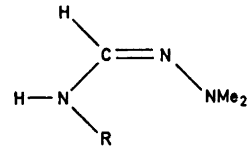
The chemical shift values of the quaternary ring carbon atom for R = phenyl, and the tertiary carbon atom in the CN double bond are those most influenced by the position of the NH hydrogen atom. From Table 1 it is evident that the chemical shift values for the carbon atom in the CN bond are 152 – 155 ppm for compounds with the CN bond in conjugation with the benzene ring. For the same compounds the quaternary ring carbon atom shows chemical shift values of approximately 152 ppm. For the formamidrazones *1* and *2* the corresponding signals are found at 146 – 148 ppm and 139 ppm, respectively, indicating a structure different from the hydrazide imide structure (A).

The signal pattern from the tertiary phenyl carbon atoms supports our assumption that the structure of compounds *1* and *2* is (B) and not (A). For compounds with the benzene ring in conjugation to the CN-double bond the chemical shift value of the *ortho*-carbon atoms was approximately 6 ppm low-field compared with the values for compounds *1* and *2*, while the values for *meta*- and *para*-carbon atoms were almost identical.

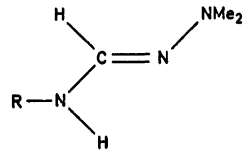
To estimate the position of the NH hydrogen atom in *3*, (R = cyclohexyl), the ^{1}H NMR (60 MHz) spectrum was used. At room temperature in $CDCl_3$ the CH = N proton signal was a broad doublet δ 6.88 (collapsed on shaking with D_2O), and the NH signal was broad δ 5.1 – 5.8 (disappeared on shaking with D_2O). At – 50 °C the NH-proton showed four signals of equal intensity due to coupling with both the cyclohexyl CH and the imino CH (J_{NHCH} 9 Hz resp. 12 Hz, δ 5.55). These data indicate that compound *3* exists in form (B).



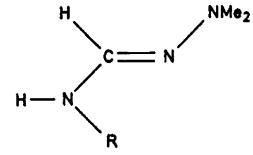
(C)



(D)



(E)



(F)

To distinguish between the possible forms (C – F) of tautomer (B) the IR and NMR data were compared. In chloroform solution the IR spectra of the formamidrazones showed weak to medium NH-stretching absorptions around 3365 and 3260 cm^{-1}, absorptions which did not change in relative intensity on dilution, indicating no intermolecular hydrogen bond.

The ^{13}C NMR spectra obtained in $CDCl_3$ at room temperature and – 55 °C showed only one set of signals for compound *1* and *3*, indicating the presence of only one isomer in accordance with IR data.

In tetrachloromethane and benzene solution the IR spectra were different from those in chloroform showing concentration dependence of the NH stretching absorption at lowest wavenumber. The absorption disappeared on dilution, indicating the presence of an intermolecular hydrogen bond, which seems impossible in form (C).

^{1}H NMR (270 MHz) of *1* (21 and – 20 °C) in CCl_4 showed two singlets for the methyl groups at δ 2.49 and 2.39 (intensity 1/5), the CHN proton was one broad singlet at δ 7.39, which sharpened on shaking with D_2O. The NH signal

Table 1. ^{13}C Chemical shift values (δ values).

Compound		−NH−CH=N− Carbon atom	Ring carbon atom			
			Quaternary	*meta*	*para*	*ortho*
$PhNHCHNNMe_2$	(*1*)	145.8	139.8	129.6	122.1	115.5
$PhNHCHNNEt_2$	(*2*)	148.2	139.2	129.0	121.5	114.5
$C_6H_{11}NHCHNNMe_2$	(*3*)	149.5				
$PhNCHN(Me)NMe_2$	(*4*)	154.7	151.8	128.9	122.6	121.3
$PhNCHNMe_2$ [6]	(*5*)	153.4	152.2	128.9	122.2	121.1
$PhNCHNEt_2$ [6]	(*6*)	152.2	152.2	128.8	122.1	121.1
$PhNC(NMe_2)_2$ [7]	(*7*)	159.0	152.0	128.4	121.5	119.6
PhNCHNHMe	(*8*)	151.6	151.6	129.0	122.7	121.1

was broad around δ 7.7. For compound *3* (21 °C, CCl_4) the methyl signal consisted of two singlets at δ 2.25 and 2.28 (intensity 3/1), the CHN proton was a doublet centered at δ 6.62 and the NH proton a broad signal around δ 5.4. On cooling to −20 °C the NH signal sharpened to four signals of equal intensity as in the $CDCl_3$ solution.

Discussion. The determination of the tautomer as (B) is in accordance with results on basis of IR measurements.[2] In chloroform solution it seems reasonable to conclude that the configuration is (C) because of the position of the NH-stretching absorption, its independence of dilution and the presence of only one set of lines in the ^{13}C NMR spectra. Other formamidrazones [3] have been shown to exist as equilibrium mixtures of (C) and (D) forms in solution, with coalescense temperature above 0 °C. The compounds investigated here show no doubling of NMR signals on cooling to −55 °C why the existence of an equilibrium mixture of (C) and (D) in chloroform solution seems less probable.

In CCl_4 solution the presence of two methyl signals in the ^1H NMR spectra for compounds *1* and *3* indicates the presence of two forms. According to the IR spectra this must be one form with intermolecular and one structure with intramolecular hydrogen bond. Since the presence of both an *E*- and a *Z*-isomer [*e.g.* forms (C) and (E)] possibly would have given rise to two CH=N signals in the NMR spectra (both in $CDCl_3$ and CCl_4) it seems reasonable to conclude that the two forms present in CCl_4 are the forms (C) and (D).

Experimental. ^1H NMR spectra (270 MHz) were recorded on a Bruker HX-270 S apparatus, concentration 100 mg/ml. ^{13}C NMR spectra were recorded on a Bruker WH 90 apparatus, concentration 300 mg/ml. For other experimental details see Ref. 1.

N^1-*Methyl*-N^2-*phenylformamidine* (*8*) was prepared by refluxing equimolar amounts (0.1 mol) of aniline hydrochloride, *N*-methylformamide and *p*-toluenesulfonyl chloride in 100 ml of benzene for 24 h. The solution was made alkaline with 50 % NaOH, extracted with benzene, the benzene layer evaporated to dryness and the residue recrystallized from benzene. Yield 60 %, m.p. 88−89 °C. Anal. $C_8H_{10}N_2$: C, H, N. ^1H NMR spin decoupling experiments at −50 °C proved the position of the NH hydrogen atom to be adjacent to the methyl group.

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Properties of the Sulfhydryl Groups of Three Amino Acid:Transfer RNA Ligases

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Some properties of the sulfhydryl groups of three amino acid:tRNA ligases have been investigated.** For the valine ligase from yeast SH-groups were found to be essential both for valine activation and aminoacylation of $tRNA^{Val}$. The lysine ligase from yeast required SH-groups for the amino acid activation reaction whereas the transfer of [^{14}C]-lysine from the lysyladenylate–enzyme complex to $tRNA^{Lys}$ was not inhibited by 5,5′-dithiobis(2-nitrobenzoic acid) (Nbs_2). The lysine ligase from *E. coli* was not sensitive to Nbs_2 neither with regard to lysine activation nor tRNA aminoacylation.

The interactions between amino acid:tRNA ligases and their substrate molecules can be fully understood only in the light of structural information from single crystal analyses now in progress in several laboratories.[1–5] Nevertheless it is useful to characterize these enzymes with respect to their properties in solution and the effect of substrate molecules on these properties. We have previously reported the purification of three amino acid ligases [6–8] as well as some of their molecular and crystallographic parameters.[2,9,10] In this paper we have attempted to study the properties of the sulfhydryl groups of the enzymes and the influence of substrates on enzyme conformation in solution as judged by such properties as accessibility of sulfhydryl groups for SH-reagents, sedimentation velocities and circular dichroism.

MATERIALS AND METHODS

Materials. The valine and lysine:tRNA ligases were prepared using previously described procedures,[6–8] which had been modified to allow the processing of 5 kg of yeast or 3 kg of *E. coli*. Highly purified $tRNA^{Lys}$ and $tRNA^{Val}$ were prepared from crude tRNA from *E. coli* (General Biochemicals) and brewer's yeast (Boehringer-Mannheim) by chromatography of the *N*-phenoxyacetylated aminoacyl-tRNA's on benzoylated DEAE-cellulose according to Gillam *et al.*[11,12] as described in Ref. 2.

Inorganic [^{32}P]pyrophosphate and ^{14}C-labelled amino acids were purchased from the Radiochemical Centre, Amersham, England. 5,5′-Dithiobis(2-nitrobenzoic acid), (Nbs_2), was obtained from Sigma Chemical Company. Guanidine hydrochloride, sequanal grade, was from Pierce Chemical Company. Other chemicals used were of the highest grade available.

Protein determinations. The protein concentrations were determined spectrophotometrically at 280 nm in 0.05 M potassium phosphate buffer, pH 7.0, using the following molar absorption coefficients for the enzymes: lysine:tRNA ligase (yeast) 92 500 M^{-1} cm^{-1}, lysine:tRNA ligase *(E. coli)* 66 600 M^{-1} cm^{-1} and valine:tRNA ligase (yeast) 171 000 M^{-1} cm^{-1}. All calculations in this paper are based on the molecular weights of the enzymes given in Ref. 2.

Reaction of sulfhydryl groups with Nbs_2. Prior to reaction with Nbs_2 the enzymes were incubated with 5 mM dithiothreitol. After 60 min at room temperature the reducing agent was removed by chromatography on Sephadex G-25 equilibrated with 0.5 M potassium phosphate buffer, pH 7.5, containing 0.1 mM EDTA and saturated with N_2. This treatment did not significantly change the specific activity of the enzymes. The reaction of the enzymes

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** *Abbreviations:* Nbs_2, 5,5′-dithiobis(2-nitrobenzoic acid); $tRNA^{Val}$, transfer ribonucleic acid specific for valine; $tRNA^{Lys}$, transfer ribonucleic acid specific for lysine. *Enzymes:* Lysine:tRNA ligase (EC 6.1.1.6); Valine:tRNA ligase (EC 6.1.1.9).

with Nbs_2 was carried out at room temperature in 0.1 M potassium phosphate buffer, pH 7.5, containing 0.1 mM EDTA. The release of 2-nitro-5-mercaptobenzoate was followed spectrophotometrically by measuring the absorbance at 412 nm with a Gilford 240 spectrophotometer against a blank without enzyme. A molar absorption coefficient of 13 600 M^{-1} cm^{-1} for 2-nitro-5-mercaptobenzoate [13] was used for the calculations in this paper.

Assay of enzyme activity. The activities of the enzymes were determined in two ways. The amino acid-dependent exchange of [^{32}P]-pyrophosphate with ATP was assayed essentially as described by Stern and Mehler.[14] The ability of the enzymes to catalyze the incorporation of ^{14}C-amino acids into tRNA was assayed as described earlier.[8,15]

Transfer of amino acid to tRNA. For the formation of the aminoacyladenylate – enzyme complexes 1 to 5 nmol of enzyme were incubated 10 min at 0 °C with 0.3 mM ^{14}C-L-amino acid (10 mCi/mmol), 1 mM ATP, 10 mM $MgCl_2$ and 0.05 M potassium phosphate buffer, pH 7.5, in a final volume of 0.2 ml. EDTA was then added to a concentration of 0.05 M, followed, in some reaction mixtures, by the addition of Nbs_2 to a final concentration of 15 mM. After 30 min at room temperature the aminoacyladenylate – enzyme complexes were isolated by chromatography on Sephadex G-25.[15] The isolated complexes were incubated at 0 °C with a 200 fold molar excess of unfractionated tRNA from yeast or *E. coli* in 2 to 3 ml of a solution containing 0.25 M ammonium sulfate, 0.01 M sodium cacodylate buffer, pH 7.0, and 0.05 M EDTA. Samples were withdrawn from the reaction mixture at different time intervals and analyzed for the formation of [^{14}C]aminoacyl-tRNA as described previously.[8,15]

Analytical sedimentation studies. A Spinco model E ultracentrifuge equipped with the RTIC temperature control unit was used for the sedimentation velocity experiments. The centrifuge was operated at 20 °C at a speed of 42 040 rpm. A plain and a wedge 12 mm double sector cell were used for the two-cell schlieren studies. The experiments were performed in 0.1 M potassium phosphate buffer, pH 7.0, at protein concentrations from 3 to 5 mg/ml.

Circular dichroism measurements were carried out in a Cary 60 recording spectropolarimeter with the Cary 6002 circular dichroism accessory attachment using a 1 mm cell. Instrument performance was checked by using the spectrum of camphorsulfonic acid as a reference.[16] The measurements were performed at room temperature in 0.1 M potassium phosphate buffer, pH 7.0, containing 0.1 mM dithiothreitol. Protein concentrations were from 1 to 2 mg/ml.

RESULTS

Reaction of enzymes with Nbs_2. The reactivity of the SH-groups of the enzymes under different conditions was examined by titration with Nbs_2, as described in Methods. The enzymes were analyzed in the presence and absence of substrates and under denaturing conditions. Details of the experimental procedures are given in the legends to Fig. 1 and Table 1.

In the native lysine:tRNA ligase from yeast 13 SH-groups (13.2 ± 0.4, mean value ± standard deviation) per enzyme molecule reacted with Nbs_2. The kinetics of the reaction suggested that 2 groups reacted rapidly followed by a slower reaction of the other SH-groups (Fig. 1). Under denaturing conditions 18 SH-groups (17.5 ± 0.3) per molecule were titratable. When the enzyme was titrated in the presence of ATP or under conditions where the lysylade-

Table 1. Reactivity to Nbs_2 of SH groups of three amino acid:tRNA ligases in the presence of substrates or under denaturing conditions. Determination of SH-groups was performed as described in Methods. The incubation mixtures contained in a final volume of 0.2 ml: potassium phosphate buffer pH 7.5 (0.1 M), EDTA (0.1 mM), enzyme (5.0 – 8.0 μM), Nbs_2 (1 – 2 mM) and the additions indicated in the table. The additions were present at the following concentrations: ATP (10 mM); $MgCl_2$ (10 mM); substrate amino acid (10 mM); cognate tRNA (about 0.1 mM); guanidine.HCl (6 M).

Additions	SH-groups/enzyme (mol/mol)		
	E^{Lys}(yeast)	E^{Val}(yeast)	E^{Lys}(*E. coli*)
None	13.2	9.7	1.2
ATP	13.6	6.2	0.8
Substrate amino acid	15.0	8.9	1.1
ATP, amino acid, Mg^{2+}	13.0	8.7	0.6
Cognate tRNA	8.7	7.4	0.6
Guanidine.HCl	17.5	11.7	2.6

nylate – enzyme complex was formed, there was no change in the number of accessible SH-groups compared to the reaction in the absence of substrates (Table 1). L-Lysine alone had a small effect on the accessibility of the SH-groups for Nbs_2. A pronounced effect was obtained when the enzyme was preincubated with $tRNA^{Lys}$ from yeast. Under these conditions about 5 SH-groups were protected from reaction with Nbs_2.

When the native valine:tRNA ligase from yeast was titrated with Nbs_2 10 SH-groups (9.7 ± 0.5) per molecule of enzyme reacted (Fig. 1). Two groups reacted rapidly followed by a slower reaction of the other SH-groups. Under denaturing conditions about 12 SH-groups (11.7 ± 0.2) per enzyme molecule reacted completely in less than 1 min. When the enzyme was titrated in the presence of ATP 4 SH-groups were protected from reaction with Nbs_2 as compared to the native enzyme in the absence of substrates (Table 1). Reactions performed in the presence of L-valine or under conditions where the valyladenylate – enzyme complex was formed, gave about the same number of SH-groups accessible to Nbs_2 as did the native enzyme. Preincubation of the enzyme with $tRNA^{Val}$ from yeast protected 2 SH-groups from reaction with Nbs_2.

In the native lysine:tRNA ligase from *E. coli* 1.2 SH-group per molecule reacted with Nbs_2 while 2.6 reacting SH-groups per molecule were

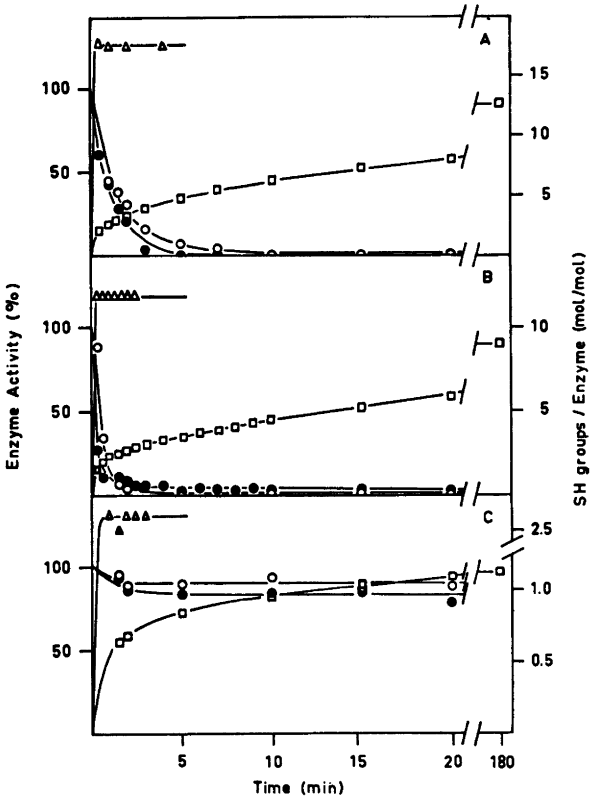


Fig. 1. Titration of SH-groups in three amino acid:tRNA ligases with Nbs_2 under native and denaturing conditions. (*A*) Lysine:tRNA ligase from yeast. (*B*) Valine:tRNA ligase from yeast. (*C*) Lysine:tRNA ligase from *E. coli*. During titrations of native enzymes ($4.0 - 9.0$ μM) with Nbs_2 ($0.05 - 0.9$ mM) aliquots were withdrawn at the times indicated and analysed for enzyme activity. □, titration under native conditions; △, titration in the presence of 6 M guanidine.HCl. Enzyme activity was determined as $[^{32}P]PP_i$ – ATP exchange (○), or incorporation of ^{14}C-amino acids into tRNA (●).

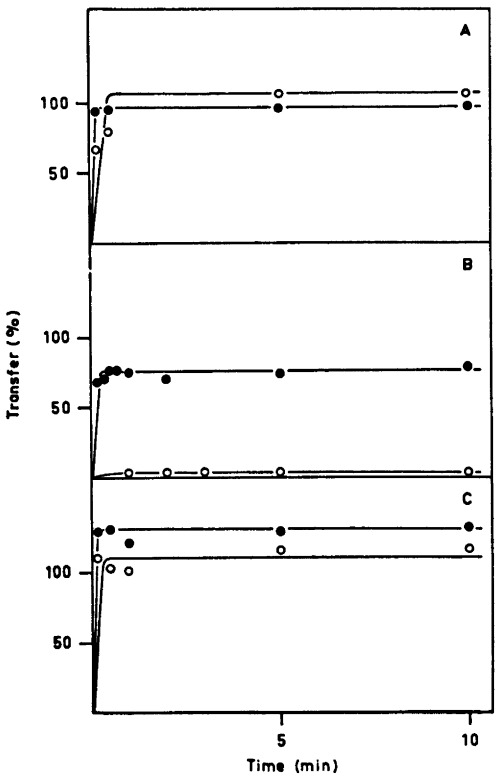


Fig. 2. Effect of Nbs_2 on the transfer of amino acid from aminoacyladenylate – enzyme complex to tRNA. Experimental procedure and conditions were as described in Methods. The transfer of amino acid to tRNA was studied with aminoacyladenylate – enzyme complexes which had been incubated with (○) or without (●) Nbs_2. (*A*) Lysine:tRNA ligase and tRNA from yeast. (*B*) Valine:tRNA ligase and tRNA from yeast. (*C*) Lysine:tRNA ligase and tRNA from *E. coli*.

found under denaturing conditions. Incubation with ATP or L-lysine did not significantly change the Nbs_2 titration curve from that obtained with the native enzyme. The presence of $tRNA^{Lys}$ from *E. coli* or incubation under conditions where the lysyladenylate – enzyme complex was formed prevented 0.6 SH-groups from reacting with Nbs_2.

Effect of Nbs_2 on enzyme activity. The amino acid:tRNA ligases were incubated with Nbs_2 as described in Methods and the legend to Fig. 1. At time intervals indicated in the figure aliquots of the reaction mixtures were removed and were assayed for enzyme activity using both the ATP – [^{32}P]pyrophosphate exchange and the tRNA acylation assay.

Lysine:tRNA ligase from yeast lost its activity somewhat faster than the rate at which 2-nitro-5-mercaptobenzoate was liberated (Fig. 1). When 5 SH-groups per molecule had reacted with Nbs_2 the enzymatic activity was completely lost.

Valine:tRNA ligase from yeast was very rapidly inactivated during the reaction with Nbs_2 (Fig. 1). A complete loss of activity was seen after blocking the two fast reacting SH-groups.

Lysine:tRNA ligase from *E. coli* retained 80 to 90 % of its activity after treatment with Nbs_2 in agreement with previous reports of its lack of sensitivity to SH-reagents.[17]

Effect of Nbs_2 on the transfer of amino acid to tRNA. The ligases were mixed with amino acid, ATP and Mg^{2+} to form their respective aminoacyladenylate – enzyme complexes. Nbs_2 was added to the reaction mixtures and the incubations were continued at room temperature for 30 min. The aminoacyladenylate – enzyme complexes were then isolated by chromatography on Sephadex G-25 as previously described.[15] Essentially the same recoveries were obtained as in parallel incubations without Nbs_2. The isolated complexes were incubated with unfractionated tRNA from yeast and *E. coli*, respectively, and the transfer of ^{14}C-amino acid from the complex to tRNA was studied (Fig. 2). The transfer of [^{14}C]valine from the complex to tRNA was completely inhibited by the incubation of the valyl-adenylate – enzyme complex with Nbs_2. The results obtained with the lysine:tRNA ligases from yeast or *E. coli*, on the other hand, showed no significant effect on the transfer reaction by the treatment of the lysyladenylate – enzyme complexes with Nbs_2.

Sedimentation properties of Nbs_2-treated enzymes. The methionine:tRNA ligase from *E. coli* and the tryptophan:tRNA ligase from beef pancreas have been shown to dissociate into subunits and at the same time lose their catalytic activities after reaction with Nbs_2.[18–20] When the lysine:tRNA ligases from yeast or *E. coli*, which also contain subunits, were reacted with Nbs_2 as described in the legend to Table 2, no dissociation into subunits occurred as shown by the lack of major changes

Table 2. Influence of Nbs_2 on sedimentation properties of the lysine:tRNA ligases from *E. coli* and yeast, with and without substrates. Sedimentations were as described in Methods. Nbs_2 and substrates, as indicated in the table, were added at the following concentrations: Nbs_2 (10 mM), $MgCl_2$ (10 mM), ATP (1 mM), L-lysine (1 mM). In the experiments with Nbs_2 the reactions were allowed to proceed for 30 min before centrifugation in order to insure complete titration of all available SH-groups.

Exp.	Enzyme	Additions	s_{obs} (10^{-13} s)
I	E^{Lys}(yeast)	–	6.0
		Nbs_2	5.5
II	E^{Lys}(yeast)	ATP, L-lysine, Mg^{2+}	5.9
		ATP, L-lysine, Mg^{2+}, Nbs_2	5.5
III	E^{Lys}(*E. coli*)	–	5.1
		Nbs_2	5.1
IV	E^{Lys}(*E. coli*)	ATP, L-lysine, Mg^{2+}	5.3
		ATP, L-lysine, Mg^{2+}, Nbs_2	5.3

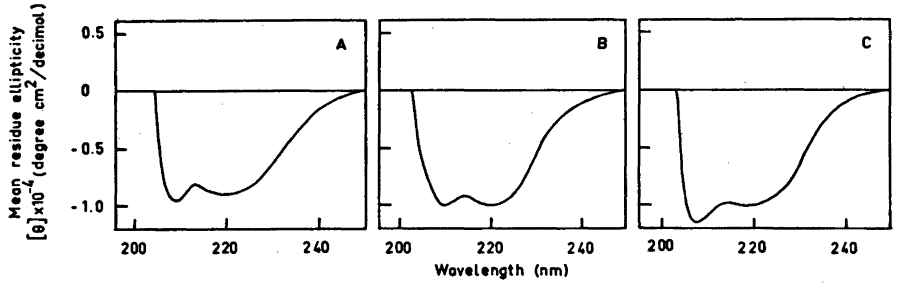


Fig. 3. Circular dichroism spectra of three amino acid:tRNA ligases. Experimental conditions were as described in Methods. The mean residue weights (MRW) were calculated from the amino acid compositions of the enzymes.[9] (*A*) Lysine:tRNA ligase (yeast), MRW = 115. (*B*) Valine:tRNA ligase (yeast), MRW = 114. (*C*) Lysine:tRNA ligase (*E. coli*), MRW = 113.

of the sedimentation coefficients of the enzymes (Table 2). Incubation of the enzymes under conditions where the lysyladenylate – enzyme complexes were formed, with or without subsequent treatment with Nbs_2, did not change the sedimentation behaviour of the enzyme – substrate complexes to the extent expected for a change of the quaternary structure of the enzymes.

Circular dichroism measurements. CD might be used to demonstrate conformational changes of enzymes caused by the interaction with substrates. Proteins in general exhibit negative circular dichroic bands around 208 and 222 nm corresponding to their helical content.[21] In the region around 220 nm tRNA shows scarcely any ellipticity.[22] This has been used by Ohta *et al.* to show a change in the helical content of tyrosine:tRNA ligase from yeast on binding its cognate tRNA.[23]

The CD of lysine:tRNA ligase from yeast and *E. coli* and valine:tRNA ligase from yeast displayed typical protein patterns with minima around 208 and 220 nm (Fig. 3). Since the circular dichroic contribution of all ligands to be tested was at a minimum at 220 nm this band was chosen to illustrate a possible change in the helical content of the enzymes when interacting with their substrates.

The enzymes were mixed with substrates at concentrations chosen so as to give a complete saturation of the enzymes. When the circular dichroism patterns were corrected for the contributions of the substrates the resultant patterns in all cases had the general appearance of that of the enzyme alone. Although, in

some cases, the difference between the ellipticity at 220 nm of the native enzyme and the ellipticity in the presence of substrate was statistically significant, the difference never exceeded 10 % of the value observed for the native enzyme.

DISCUSSION

When the three amino acid:tRNA ligases studied (lysine ligase from *E. coli* and yeast and valine ligase from yeast) were titrated with the sulfhydryl reagent Nbs_2 under denaturing conditions, the number of sulfhydryl groups found closely approximates the number of halfcystine residues determined with the amino acid analyzer.[9] This might indicate that the enzymes do not contain any disulfide bridges to stabilize their tertiary or quaternary structure. In agreement with this is the observation, that the lysine ligases dissociated into subunits when they were treated with 1 % SDS in the absence of a reducing agent (unpublished experiments).

When the three ligases were titrated with Nbs_2 under nondenaturing conditions, both the yeast enzymes were completely inactivated. The activity of the lysine ligase from *E. coli* was resistant to Nbs_2 in agreement with previous reports of its lack of sensitivity to sulfhydryl reagents.[17] There was a difference between the inactivation curves for the lysine and valine enzymes from yeast inasmuch as the valine enzyme lost its activity, measured both as ATP-pyrophosphate exchange and tRNA acylation, after the titration of the two SH-groups which reacted faster than the rest. The lysine ligase, on the other hand, showed a more gradual loss of activity, which did not parallel the titration of the two fast reacting groups, and which was not complete until about 5 SH-groups had reacted with Nbs_2. Furthermore, when the valyladenylate – enzyme complex was isolated by chromatography on Sephadex after treatment with Nbs_2, it had completely lost its ability to transfer valine to $tRNA^{Val}$, while treatment of the lysyladenylate – enzyme complexes of the two lysine enzymes had no effect on their ability to transfer lysine to $tRNA^{Lys}$. These results indicate, that for the valine ligase from yeast sulfhydryl groups are essential both for valine activation and aminoacylation of $tRNA^{Val}$. The two fast reacting sulfhydryl groups would seem to be of particular importance for the valine activation reaction.

The results indicate that the lysine ligase from yeast also requires SH-groups for the amino acid activation reaction. On the other hand, our data do not permit any conclusions regarding the possible role of SH-groups in the aminoacylation of $tRNA^{Lys}$, since one or more of the SH-groups, which were not available to Nbs_2 in the lysyladenylate – enzyme complex, could conceivably participate in the aminoacylation reaction Therefore, our results do not necessarily contradict the catalytic reaction mechanism proposed by McElroy *et. al.*[24] involving an intermediate thioacylenzyme.

One of the aims of this study was to find evidence for conformational changes of the enzymes upon binding of substrates. The results of the SH-titrations in the presence of substrates might indicate that the binding of a ligand in some cases induces conformational changes of the enzymes leading to changes in the reactivity of the sulfhydryl groups towards Nbs_2 (Table 1). The protective effect of some of the ligands could also be explained by a direct mechanism where an SH-group in close proximity of the substrate binding site is prevented from reacting with Nbs_2 by the presence of the substrate. The changes in hydrodynamic properties or circular dichroism recorded for these enzymes in the presence of substrates are too small to warrant any conclusions regarding possible alterations of their secondary or tertiary structure induced by the ligands.

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Interaction of Charged Nitroxyls with Some High-polymer Membranes. A Spin Label Study

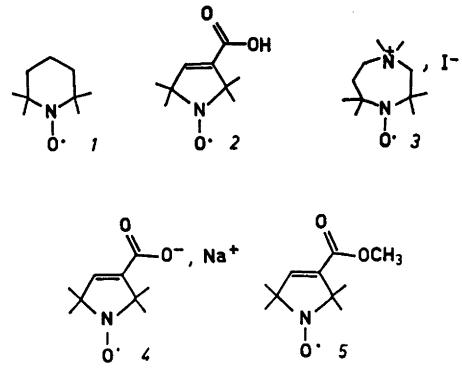
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The bonding and immobilization of a neutral, an anionic and a cationic nitroxyl radical to some neutral and negatively charged high-polymer membranes have been investigated. ESR measurements show that the mobility and the diffusion of the radicals in neutral cellulose membranes are related to the degree of swelling and to the nature of the radical. Additional hydrogen bonding, electrostatic interaction and stereochemical interaction between nitroxyls and cellulose may exist. The latter types of interaction are strong between cellulose and *4* in acetone, and exist also between *5* and cellulose in water. In the charged membranes the charge of the radical has a much greater influence on the form of the ESR spectra than has the degree of swelling. It was shown that sulfonated polysulfones bind the cation radical stronger than carboxymethylcellulose, even if the sulfonated polysulfone has larger pores. A neutral radical is also bound and immobilized in the sulfonated polysulfone. Increasing ionic strength in the membrane decreases the mobility of the radical. The possibility of stereochemical and electrostatic interactions between membranes and nitroxyls is discussed.

The purpose of this work is to extend previous results[1] on the non-covalent spin labelling of polycarboxylic acid membranes. The use of nitroxyl radicals has recently been introduced in the field of ion exchange membrane chemistry;[2–4] the aim in this case is to characterize the interactions inside a mechanically ordered matrix, which are dependent on the porosity of the support or specifically due to the nature of the functional groups of the radical and the support, with solvent effects taken into account.

Cellulose membranes were studied for two reasons: (1) because of its neutrality the membrane serves as model of the backbone of polymeric ion exchange membranes, and (2) to determine the adsorption and desorption of neutral, positively charged and negatively charged small molecules in the cellulose in water, acetone, and acetone/water, respectively. The choice of nitroxyl spin labels was made accordingly; the nitroxyls *1* – *5* are of similar molecular size, their spectroscopic behaviour is similar, the only variable is the nature of the non-paramagnetic part of the molecule.



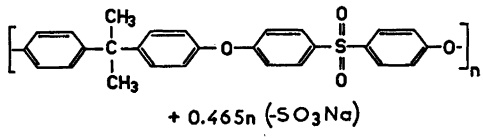
Cation exchange membranes of different chemical nature (polycarboxylic and sulfonated polysulfonic) and with varying porosity were chosen to determine the nature of the electrostatic attraction and repulsion in the membrane using the spin labels *1*, *3* and *4*. Provided there are no stereochemical interactions or hydrogen bonds present the neutral spin label would provide information on the electrostatic interaction between the membrane and the

counterion, and the anion spin label on the activity of the anions in the membrane, respectively.

EXPERIMENTAL

Nitroxyls. The syntheses of the radicals *1* – *5* have been described previously.[5–8] The radicals were dissolved in water or the appropriate solvent to a concentration of 1 mg/ml.

Membranes. The polymeric membranes consisted of regenerated cellulose, carboxymethylcellulose and of sulfonated polysulfone (structural unit *6*).



6

The films of regenerated cellulose are from a tube for dialysis manufactured by the Union Carbide Corporation, U.S.A., treated with a boiling solution of sodium bicarbonate and subsequently washed with water. The pore size for the water swollen cellulose membrane is 2 nm (estimated by the manufacturer).

The films of carboxymethylcellulose (CMC) were prepared as described in Ref. 9; they are polycarboxylic ion exchange membranes obtained by the reticulation of CMC with formaldehyde. Pore size in water swollen membranes is 0.9 nm.[10]

The membranes of the sulfonated polysulfone are manufactured by Rhône-Poulenc, France; they are membranes for hyperfiltration (dense film (PSS) or asymmetric membrane (TR) and for ultrafiltration (IRIS)). The pore size increases from PSS to IRIS.[11]

The degree of swelling, τ, of the membrane is expressed by the weight of solvent in the membrane to the weight of dry membrane.

Preparation of samples. The cellulose films were equilibriated in water, acetone, and water/acetone, the ionic membranes were equilibriated in water or in water solutions of high ionic strength (1 M with respect to NaCl or $CaCl_2$). The effect of the counterion was studied by stabilizing the membranes in Na^+ and Ca^{2+} forms, respectively.

2 – 5 mg of the membranes were equilibriated during at least 24 h in 1 ml of the solvent containing 0.1 ml of the solution of the radical. Before measuring the spectra the membranes were washed rapidly in an excess of the proper solvent to eliminate radicals absorbed to the surface of the membrane, and rapidly dried between sheets of filter paper.

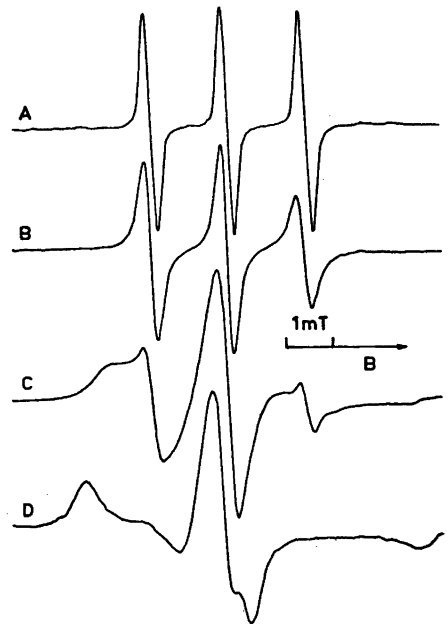


Fig. 1. ESR spectra of nitroxyl radicals. A, *3* in water solution; B, *3* in a Na-CMC membrane in water; C, *3* in a Ca-TR membrane in 1 N $CaCl_2$ water solution; D, *4* in a cellulose membrane after 2 h washing in acetone.

Mobility and diffusion of nitroxyls by ESR. The spectra were measured at 20 °C with an X-band Varian E 3 spectrometer; the films were placed in a quartz plate sample tube S806A from J. F. Scanlon & Co.

The different types of spectra obtained are collected in Fig. 1.

The hyperfine coupling constant of the nitrogen nucleus ^{14}N,a_N, expresses the separation between the lines in spectra of types A and B. It is supposed to be the isotropic value and is a measure of the polarity of the environment of the nitroxyl. Spectra of types A and B represent cases of rapid rotational diffusion; the width of such a spectrum, L_1, is determined from the first to the last maximum of the spectrum. When the nitroxyl is immobilized the spectrum is characterized by its total width, L_2, from the first maximum to the last minimum. L_2 gives an estimate of the anisotropic contributions to the linewidths in cases where molecular tumbling makes the angular components of the hyperfine tensor a function of time.

The mobility of the spin label is expressed by the ratio of the height of the high field line I_{-1} and the center line I_0 in the limit of rapid rotation ($10^{-11} - 5 \times 10^{-9}$ s).[13] In the range of slow diffusion ($> 5 \times 10^{-9}$ s) it is difficult to

Table 1. Data from ESR-spectra of *1*, *3* and *4* in water and acetone ($\sim 10^{-3}$ M) and of *4* in glycerol solution (10^{-3} M).

Radical	Solvent	t °C	a_N/mT	I_{-1}/I_0	L_1/mT	L_2/mT
1	Acetone	+20	1.60 ± 0.02	0.98	3.23	
	Water	+20	1.73 ± 0.02	0.94	3.21	
3	Acetone	+20	1.54 ± 0.02	0.95	3.05	
	Water	+20	1.54 ± 0.02	0.94	3.23	
4	Acetone	+20	1.53 ± 0.02	0.94	3.28	
	Water	+20	1.63 ± 0.02	0.95	3.36	
	Glycerol	+80	1.58 ± 0.02	0.68	3.11	
	Glycerol	+27	1.56 ± 0.02	0.39	3.12	
	Glycerol	0	1.55 ± 0.02	0.23	3.12	
	Glycerol	−35				6.68
	Glycerol	−70				7.10

estimate correlation times only from experimental spectra, since the little motion the spin labels have relative to the macromolecular structure is anisotropic. A complete lineshape analysis would yield details concerning the nature of the motion in addition to the apparent correlation time. Work on this matter is in progress.

The interaction between the membrane and the radical is evidenced by the change in mobility of the radical on introducing a portion of solvent into the sample cell. The spectra were measured from time to time in order to estimate the rate of diffusion of the radical from the membrane into the solvent. Finally the membrane was stored in pure solvent, and spectra of the films were measured after different periods of washing.

Radical *4* has been used as a model, and its spectra in glycerol solutions at different temperatures have been measured and compared to results of measurements with labelled membranes. The ESR characteristics of *1*, *2* and *4* are collected in Table 1.

RESULTS AND DISCUSSION

Interactions in cellulose membranes. The nitroxyls *1*, *3* and *4* are all incorporated into cellulose. However, the amount of adsorbed radical is different in the three cases. From the intensity of the spectra the conclusion is drawn that the anion is adsorbed in greatest concentration and the neutral radical is adsorbed in smallest concentration. Radicals *1* and *4* show similar spectral patterns both in glycerol solution and in the cellulose matrix, respectively. The spectral width L_1 for *1* is larger in cellulose than in solution. The environment of the radicals *1* in the cellulose is therefore to be considered very strongly polar. The radicals *3* and *4* are adsorbed in an environment resembling water solution since the spectral widths L_1 are equal in cellulose and in water. The mobility of *3* in cellulose is greater than that of *1* and *4*. The results of the measurements are collected in Table 2.

Of particular interest is the very strong interaction between the cellulose and *4* in acetone. In order to investigate the influence of chemical nature of the radical on the form of ESR spectrum, the results of measurements with *4* were compared to spectra of cellulose labelled with the corresponding free acid *2*, its methyl ester *5*[8] and with the anion *4* at pH = 9. The anion radical was prepared by neutralizing solutions of *2* in water and acetone to pH = 7 and to pH = 9 with a solution of sodium hydroxide in water.

From acetone *2* is incorporated to a smaller degree than the anion form *4*. The latter is very strongly associated and does not diffuse from the cellulose. At pH = 9, *4* is very easily incorporated from water, probably due to extensive swelling of the macromolecular structure at this pH value. The radical *5* is incorporated to a very low degree; in acetone a fraction of it stays immobilized in the membrane over a long period, probably due to hydrogen bond formation. The acid form of *2* and the anion form *4* are strongly associated to the cellulose in acetone, on addition of water to the samples they are very rapidly liberated.

The radicals *1*, *3* and *4* were kept in contact with samples of the cellulose membrane in

Table 2. Incorporation and diffusion of the radicals *1*–*5* in cellulose membranes.

Radical	Solvent	I_{-1}/I_0	a_N/mT	L_1/mT	L_2/mT	Effect of addition of solvent into sample tube	Ratio [a]	Ratio [b]
1	Acetone	0.33±0.03	1.68±0.2	3.48±0.02		+ Acetone: slow diffusion	3:1	0.34
	Water	0.55±0.05	1.75±0.02	3.48±0.02		+ Water: rapid diffusion	∞	
2	Acetone	Partly incorporated and completely immobilized			7.0±0.2	+ Acetone: partial diffusion		
	Water	0.33±0.03	1.58	3.19±0.02		+ Water:		
	+ water	0.99±0.03	1.61	3.15±0.02		rapid diffusion		
3	Acetone	0.48±0.01	1.53	3.02±0.05		+ Acetone: slow diffusion of free radical	2:1	0.33
	Water	0.86±0.02	1.60	3.23±0.05		+ Water: slow diffusion of free radical	20:1	0.77
4	Acetone	Very large fraction incorporated and strongly immobilized				+ Acetone: no diffusion	1:1	No change
		0.20			6.9±0.1	+ Water: rapid diffusion		
	Water	0.69±0.02	1.66	3.25±0.05		+ Water:		
	+ water	0.91±0.04	1.66	3.25±0.05		rapid diffusion	100:1	0.60
	Water/NaOH pH = 9	0.58±0.02	1.60	3.20±0.05		+ Water: rapid diffusion		
5	Acetone	Small fraction incorporated and immobilized			6.7±0.2	+ Acetone: no diffusion		
	Water	0.42±0.06	1.58	3.15±0.05		+ Water: partial diffusion		

[a] I_0(initial)/I_0(after 1.5 h). [b] I_{-1}/I_0(after 1.5 h).

mixtures of acetone and water. This allows for comparison with respect to the swelling of the membrane. In the solvent mixtures the spectra show the three line pattern of the diffusing radical, but the relative intensities of the lines are dependent on the degree of swelling of the cellulose in the same way as the spectra of *4* in glycerol are temperature dependent. The spectrum of *4* in cellulose is a superposition of the spectra of free and associated radicals in acetone-water mixtures; the spectra are of type C, Fig. 1. After washing with acetone an immobilized portion of the radical stays within the membrane (spectrum of type D in Fig. 1). This is probably explained by interaction between the anionic group of *4* and functional groups in the cellulose.

The mobility of the spin label is directly related to the porosity of the film, determined as the degree of swelling, τ, Fig. 2. To the value $\tau \equiv 0.3$ the mobility of the radical increases rapidly, at higher values the changes are small. At values $\tau > 0.3$ the radicals show a nearly constant I_{-1}/I_0 ratio which is supposed to be dependent only on the nature of the radical and its interaction with the support.

Interactions in the ion exchange membranes. The radicals *1*, *3* and *4* are incorporated in the ion exchange membranes, except for *1* in CMC-membranes, where the concentration of radical in the membrane is too low to give

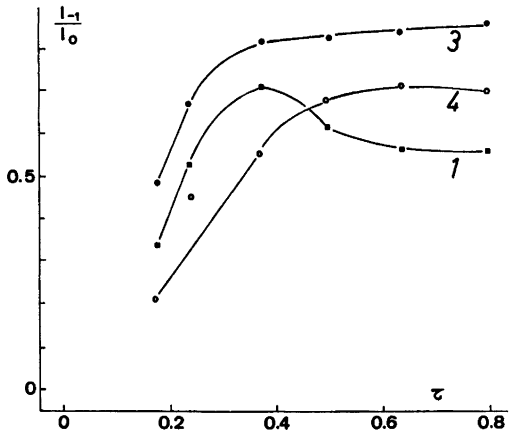


Fig. 2. The dependence of the ratio I_{-1}/I_0 for the nitroxyl radicals *1*, *3* and *4* on the degree of swelling τ of a cellulose membrane. The τ values correspond to the swelling of the membrane in (from left to right) pure acetone, acetone/water 80/20 (w/w), acetone/water 60/40 (w/w), acetone/water 40/60 (w/w), acetone/water 20/80 (w/w), and pure water, respectively.

Table 3. Characteristics of the interaction of *1*, *3* and *4* with ion exchange membranes from ESR-spectra. A, type of incorporation; B, spectrum.

Membrane	τ	*1*[a] A	B	L_2/mT[d]	*3*[a] I_{-1}/I_0[e]	A	B	(L_1 or L_2)/mT	*4*[c] I_{-1}/I_0	L_1/mT
NaCMC/H_2O	0.758	N			0.6			$L_1 = 3.3$	0.35	3.27
NaCMC/NaCl	0.576	N			0.4			$L_1 = 3.3$	0.25	3.27
CaCMC/H_2O	0.468	N			0.4				Very low incorporation	
CaCMC/$CaCl_2$	0.410	[b]			[b]					
NaPSS/H_2O	0.160						D	$L_2 = 6.2$		
NaPSS/NaCl	0.154	L	D	6.3		S	D	$L_2 = 6.3$	0.83	3.27
CaPSS/H_2O	0.154	L	D	6.3		S	D	$L_2 = 6.2$	Very weak signal	
CaPSS/$CaCl_2$	0.150	S	D	6.3		S	D		Very weak signal	
NaIRIS/H_2O	1.91	F	C			F	C		0.91	3.27
NaIRIS/NaCl	1.65	F	C	6.4		F	C		0.86	3.27
CaIRIS/H_2O	1.81	F	C			F	C		0.88	3.27
CaIRIS/$CaCl_2$	1.66	F	C	6.2		F	C		0.84	3.27
NaTR/H_2O	1.64	S	D			S			0.94	3.27
NaTR/NaCl	1.62	S	D			F	C		0.88	3.27
CaTR/H_2O	1.52	S	D			S	D		0.88	3.27
CaTR/$CaCl_2$	1.49	S	D			F	C		0.69	3.27

[a] N, no incorporation; L, low incorporation; S, strong association; F, fixation of two types. [b] Too low incorporation to give significant results. [c] Spectra of type B. [d] ± 0.2 mT. [e] ± 0.1 mT.

significant results. The radical concentration in membranes in sodium form is lower than in membranes in calcium form. Results of the ESR measurements are collected in Table 3.

The sulfonated polysulfonic membranes immobilize *1* and *3*, the measured spectra are of type C or D, Fig. 1. The immobilization of *3* is explained by interactions with negatively charged groups of the membrane. However, this explanation is not valid for *1* which shows a spectrum of type D also for membranes with a high degree of swelling (IRIS and TS). This may be taken as evidence for electrostatic interaction between *1* and PSS. Furthermore, *1* is not incorporated in the CMC membrane in spite of the relatively high porosity. Likewise, *3* is less immobilized in CMC than in IRIS and TR with a higher degree of swelling.

These results permit the following conclusions of the influence of the charged $-SO_3H$ and $-COOH$ groups on the fixation of *3* and hence the form of the spectra; the $-SO_3^-$ group binds *3* more strongly than the $-COO^-$ group. The above conclusion is valid also when stereochemical interactions between the radical and the polysulfone and CMC matrices are taken into account.

The role of the ionic strength and the nature of the cation on the mobility of the radical can be estimated by the use of *4*. It is notable that the ionic strength which decreases the degree of swelling also diminishes the mobility of the radical. Consequently, in the Ca-form the swelling is the least which is reflected in a low value of the ratio I_{-1}/I_0 in the spectra.

Spectra of *4* in the three sulfonated polysulfone membranes indicate the difference in the morphology of the three membranes (PSS, TR and IRIS): (1) in the dense film (PSS) there is a low degree of incorporation and the radical is totally immobilized, (2) in the asymmetric membranes (TR)[11] and the ultrafiltration membranes (IRIS) the measured I_{-1}/I_0 ratios are almost identical. In the asymmetric membrane the influence of the skin is not measurable; this shows that the macroporous phase is modified by the influence of the conditions in the environment.

Diffusion of the radicals from cellulose. The radical labelled, washed and dried films were kept in contact with the pure solvent during 1 h 30 min after which they were dried and spectra measured. The results are collected in Table 2.

The diffusion rate from the membrane into water decreases for the radicals in the order *1* > *4* > *3*. On acetone treatment the radicals stay largely immobilized in the membranes; the diffusion into water is rapid in all the cases except for a small portion of radical which probably is immobilized in pores of minor dimension in the membrane. The diffusion of *1*, which is very easily soluble in water and has somewhat smaller dimensions than the other radicals, is very rapid on comparison with the more polar radicals.

The electrostatic interaction between *4* and the membrane in acetone solution is confirmed as *4* does not diffuse into acetone. The spectrum of this complex is comparable to the spectrum of dry covalently labelled cellulose.[12]

The distribution of radicals between pores of varying sizes gives a possibility to explain the results of Fig. 2 in view of the diffusion from the membrane: in the cases of *3* and *4* the contribution of a small immobilized fraction of radical does not change the ratio I_{-1}/I_0 significantly, whereas for *1* the mobility decreases while the radical still shows a line spectrum of type B; consequently the ratio I_{-1}/I_0 diminishes more than for *3* and *4*.

Diffusion of the radicals from ion exchange membranes. In all the cases the diffusion of *4* into solvent is total in one hour. The radical anion is thus excluded from the negatively charged membrane.

It was not possible to measure the diffusion from the CMC membranes for *1*; the fraction of radical incorporated was too small. From the sulfonated polysulfone membranes the diffusion of *1* is very slow. After 2 h of washing 4/5 of the radical remain incorporated in the membrane, and the spectral pattern remains the same (type D). Neither the presence of salt, nor the elevated swelling of the IRIS and the TR membranes alter this result. It is probable that there is a stereochemical or electrostatic interaction in these cases.

The diffusion of *3* from the CMC membrane is more rapid for the membrane in calcium form than in sodium form. The amplitude of the center line has decreased to 1/10, and 1/2 respectively, during 2 h washing. The same phenomenon is observed for the sulfonated polysulfone membranes, although the difference

is smaller. Thus, after 2 h washing the amplitude of the signal from the TR membrane in its sodium form has not changed while the signal from the membrane in its Ca^{2+} form has decreased to 2/3. This difference was not found in the presence of sodium chloride or calcium chloride; after 2 h washing the signal had decreased with 1/3 in both cases. The presence of salt suppresses the difference between the sodium and the calcium forms by rapid exchange between bound cations and those unbound in the membrane.

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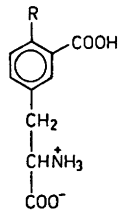
Acta Chemica Scandinavica B 31 (1977) 109—113

Chemistry of *m*-Carbon-substituted Aromatic Amino Acids

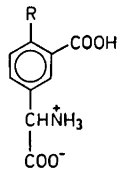
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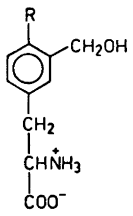
Syntheses are reported for 3-(3-carboxy-4-hydroxyphenyl)-DL-alanine, DL-3′-carboxyphenylglycine, DL-3′-carboxy-4′-hydroxyphenylglycine, 3-(3-hydroxymethylphenyl)-DL-alanine, and 3-(3-aminomethylphenyl)-DL-alanine, all naturally occurring amino acids, and also for 3-(4-amino-3-carboxyphenyl)-DL-alanine, hitherto undescribed but a potential metabolite of chorismic acid. 3-(3-Cyanophenyl)-DL-alanine, DL-3′-carbamidophenylglycine, 3-(3-chloromethylphenyl)-DL-alanine, and 3-(3-carboxy-4-nitrophenyl)-DL-alanine have been isolated as by-products or intermediates. A synthesis is also reported for [2-^{14}C]3-(3-carboxy-4-hydroxyphenyl)-DL-alanine, and contents of crystal water, pK-values, UV-spectra, and R_F-values in PC have been determined for the amino acids.



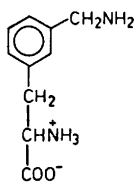
1a : R=H
1b : R=OH



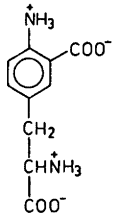
2a : R=H
2b : R=OH



3a : R=H
3b : R=OH



4



5

m-Carbon-substituted aromatic acids with the formulas *1a*, *1b*, *2a*, *2b*, *3a*, *3b*, and *4* are occurring in various higher plants.[1–4] We have studied their distribution and biosynthesis (Refs. 1, 2, and 5 and references cited there). The studies have been used as basis for a coherent representation of all derivations of amino acids from chorismic acid (2-(5-carboxy-1,2-dihydro-2-hydroxyphenoxy)acrylic acid).[6] These studies have depended on the availability of the amino acids produced by chemical syntheses and in special cases labelled with radioactive isotopes and on knowledge of their chemical and analytical properties. The present paper reports new syntheses for a number of these amino acids and also presents details of their analytical and chemical properties. Furthermore, since the representation of possibilities for transformation of chorismic acid implies 3-(4-amino-3-carboxyphenyl)alanine (*5*) as a potential chorismic acid metabolite we also report the synthesis and properties of this hitherto undescribed compound.

Traditional methods have previously been described for the synthesis of 3-(3-carboxyphenyl)-DL-alanine (*1a*),[7] 3-(3-carboxy-4-hydroxyphenyl)-L- and -DL-alanine (*1b*)[8,9] DL-3′-carboxyphenylglycine (*2a*)[10] D-2a,[11] and DL-3′-carboxy-4′-hydroxyphenylglycine (*2b*).[12,13] We have now repeated the synthesis of *1a* and obtained the hitherto undescribed 3-(3-cyanophenyl)-DL-alanine (*6*) as a byproduct. Furthermore, we have developed a new synthesis of DL-*1b* and ^{14}C-labelled DL-*1b* by the azlactone method, a new synthesis of DL-*2a* *via* *m*-cyanobenzaldehyde and 5-(*m*-cyanophenyl)hydantoin, and a new synthesis of DL-*2b* from salicylic acid and benzoylaminoglycolic acid (*cf.* Ref.

14). During the synthesis of *2a* a small amount of the hitherto undescribed DL-3-carbamidophenylglycine (*7*) was isolated.

3-(3-Hydroxymethylphenyl)alanine (*3a*) has been isolated from two different sources [3,15] but synthetic material has not been available. Synthesis of 3-(3-aminomethylphenyl)-DL-alanine (*4*) by catalytic reduction of DL-*6* mentioned above has been reported although without experimental details.[4] By use of the diethyl acetamidomalonate ester route we have now obtained DL-*3a*, DL-*4* as the dihydrochloride, and 3-(4-amino-3-carboxyphenyl)-DL-alanine (*5*). In the first of these syntheses the hydrochloride of 3-(3-chloromethylphenyl)-DL-alanine (*8*) was isolated as a by-product and characterised, whereas in the last of the syntheses the intermediate 3-(3-carboxy-4-nitrophenyl)-DL-alanine (*9*) was characterised.

For all analytical work with the amino acids a knowledge of molecular weights and contents of crystal water as a function of drying conditions is essential. However, a number of references contain no or conflicting information on these matters. We have therefore collected, verified, and supplemented the available information as described under Experimental. Special attention is called to the behaviour of the dihydrate of DL-*2b* which by drying over P_2O_5 at 50 °C loses the water of crystallization. The anhydrous sample by reequilibration in the atmosphere forms a hemihydrate.[13] X-Ray powder diagrams. have now shown that this procedure involves a definite change in crystal structure. Also the monohydrate of DL-*1a* after prolonged drying over P_2O_5 at 120 °C does not regain all the water when exposed to the atmosphere, but here X-ray powder diagrams show no differences.

In the experimental section is also given UV-data for all the amino acids in their various ionization forms. The pK-values of the amino acids have been determined by microtitrations. The observed values, reported in Experimental, are in good agreement with the protolytic properties of the amino acids as exhibited in ion-exchange and paper chromatography and in paper electrophoresis. R_F-values from paper chromatography are reported under Experimental.

The syntheses reported here and those previously described in the literature have made the racemates of *1a*, *1b*, *2a*, *2b*, *3a*, 3-(4-hydroxy-3-hydroxymethylphenyl) alanine (*3b*),[16] *4*, and *5* and L-*3b* [17,18] available. Also convenient methods are available for the resolution of *1b*,[19] *2a*,[11] and *2b* [13] and for the production of some of the amino acids labelled with ^{14}C. On the other hand methods for the direct synthesis of the optically active amino acids and preferentially methods which could be adopted for microscale synthesis of labelled materials would be desirable. Presently experiments are undertaken here with this aim by two approaches. First it is attempted to use the enzyme β-tyrosinase, which catalyses the formation of tyrosine from phenol and serine and of 3-(3,4-dihydroxyphenyl)alanine from resorcinol and serine,[20] for the production of *1b* from salicylic acid and serine and of *5* from anthranilic acid and serine. Secondly the recently described production of *N*-acetyl-3-(3-formyl-4-methoxyphenyl)-L-alanine ethyl ester, from *N*-acetyl-*O*-methyl-L-tyrosine ethyl ester, dichloromethyl methyl ether, and $TiCl_4$,[18] is used as a basis for the synthesis of L-*1b*, and a similar reaction route is envisaged from 3-(4-aminophenyl)-L-alanine to L-*5*.

EXPERIMENTAL

UV-spectra were measured on a Zeiss DMR-21 instrument at 25 °C. ^1H NMR spectra were measured on a JEOL C-60 HL instrument. Melting points are uncorrected. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Microanalyses were performed by Mr. G. Cornali and his staff. H_2O determinations were made by drying weighed samples under the conditions indicated for each compound. When not otherwise stated the water was regained when the sample was exposed to the atmosphere. pK-Values are uncorrected and were determined by microtitrations of solutions in 1 N HCl with 1 N NaOH or of solutions in 0.1 or 1 N NaOH with 0.1 N HCl.

DL-*1a* was prepared as described in the literature [7] with an overall yield from *m*-cyanobenzylbromide and acetamidomalonic ester (0.33 mol) of 58 %. The final isolation of the amino acid was performed by adsorption to a strongly basic ion-exchange resin (Dowex 1 × 8, 200–400 mesh) in the acetate form with subsequent elution with 1 M AcOH. Anal. (air-dried $C_{10}H_{11}NO_4.H_2O$: C, H, N, H_2O (P_2O_5, 120 °C, 100 h; only 41 % of the water was regained when the sample was exposed to the atmosphere). Ref. 7: No crystal water. UV [0.1 M HCl (ε)]: 278 (1000), 233 (9000), 209 (6000) nm

(*cf.* Ref. 21). [phosphate pH 7, 0.05 M]: 272 (560), 226 (7500), 202 (22 000) nm. [0.1 M NaOH]: 274 (960), 217 (11 000) nm. Lit. 275 nm (H_2O).[7] p*K*-values: 2.4; 4.0; 9.4 (*cf.* Ref. 7).

3-(3-Cyanophenyl)-DL-*alanine*, (*6*), was obtained from the effluent from the ion-exchange resin used in the purification of DL-*1a* by evaporation and recrystallization from water. Yield 2.53 g (4 %). Found after drying over $CaCl_2$: C 57.13; H 6.01; N 13.32; H_2O 8.57 (P_2O_5, 50 °C, 11 h). Calc. for $C_{10}H_{10}H_2O_2.H_2O$: C 57.69; H 5.81; N 13.45; H_2O 8.65. The IR showed a sharp peak at 2220 cm^{-1} (CN). UV [0.1 M HCl (ε)]: 282 (1050), 274 (1090), 227 (13 000), 202 (18 000) nm. [phosphate pH 7, 0.05 M]: 282 (990), 274 (1050), 227 (12 000), 197 (45 000) nm. [0.1 M NaOH]: 284 (1100), 276 (1200), 228 (11 000) nm. p*K*-values: 2.6; 8.7.

L-*1a* was isolated from seeds of *Lunaria annua* L.[22] Found after air drying: C 49.94; H 6.01; N 5.80; H_2O 12.16 ($CaCl_2$, 20 °C, 48 h). Calc. for $C_{10}H_{11}NO_4.1\frac{1}{2}H_2O$: C 50.85; H 5.97; N 5.93; H_2O 11.44. Ref. 7: No crystal water. Ref. 23: One mol of crystal water.

DL-*1b*. A mixture of 4-(4-acetyloxy-3-carbomethoxybenzylidene)-2-methyl-2-oxazolin-5-one[24] (19 g), HI (19 ml, *d* 1.7), red P (17 g) and acetic anhydride (50 ml) was refluxed for 4 h. After cooling, filtration, and washing with glacial acetic acid the solution was taken to dryness. 4 M HCl (20 ml) was added twice to the residue with subsequent evaporation. The residue was then mixed with ethanol (30 ml) and 4 M HCl (125 ml) and refluxed for 10 h. After removal of the ethanol the solution was decolourised with charcoal and after cooling the crystals of the hydrochloride of *1b* were collected. Yield 9.3 g (57 %). The hydrochloride was dissolved in water (100 ml), and pH adjusted to 3 with NaOH. After 24 h. at 0 °C the crystals were collected. Yield 6.8 g (46 %). Anal. (dried over $CaCl_2$) $C_{10}H_{11}NO_5.\frac{1}{2}H_2O$: C, H, N, H_2O (P_2O_5, 50 °C, 60 h). Ref. 9: No crystal water. p*K*-values 2.4; 3.5; 9.5; 12.4 (*cf.* Ref. 8).

DL-*2a*. 5-(*m*-Cyanophenyl)hydantoin[25] (50 g) was refluxed with 100 g of $Ba(OH)_2.8H_2O$ in 1700 ml of water for 10 days. 700 ml of 6 % $(NH_4)_2CO_3$ was added and after 15 min of boiling the mixture was filtered. The filtrate was taken to dryness, dissolved in 100 ml of water and applied to a strongly basic ion-exchange resin in the acetate form. After flushing with water the column was eluted with 1 M AcOH. Evaporation and recrystallization from water gave 14.5 g (27 %) of DL-*2a*.H_2O (identified by IR and PC). Anal. (air-dried) $C_9H_8NO_4.H_2O$: C, H, N, H_2O (P_2O_5, 100 °C, 16 h). Ref. 10: No crystal water. UV [0.1 M HCl (ε)]: 275 (820), 229 (10 000), 200 (29 000) nm. [phosphate pH 7, 0.05 M]: 272 (490), 200 (26 000) nm. [0.1 M NaOH]: 273 (670), 217 (10 000) nm. Lit. 275 nm (H_2O),[26] 275, 228, 205 nm (0.05 M HCl).[13] p*K*-values: 2.4; 3.8; 9.1.

DL-*3'-Carbamidophenylglycine*, (*7*), was obtained from the effluate of the ion-exchange resin used in the purification of DL-*2a* by evaporation and recrystallization from water. Yield 0.15 g. Found (after drying over P_2O_5): C 54.67; H 5.27; N 14.10. Calc. for $C_9H_{10}N_2O_3$: C 55.67; H 5.19; N 14.43. IR showed the absence of a nitrile group. UV [0.1 M HCl (ε)]: 271 (560), 200 (30 000) nm. [phosphate pH 7, 0.05 M]: 271 (590), 197 (36 000) nm. [0.1 M NaOH]: 275 (750), 217 (12 000) nm.

N-*Benzoyl*-DL-*3'-carboxy-4'-hydroxyphenylglycine*, (*10*). 23.6 g of benzoylaminoglycolic acid[27] was dissolved in 230 ml 20 % H_2SO_4 in glacial acetic acid and mixed with a solution of 16.7 g of salicylic acid in 330 ml of the same solvent. After 18 h at room temperature the reaction mixture was diluted with 5 volumes of water, cooled and filtrated to give 12.3 g (32 %) of material, m.p. 232–235 °C. Two recrystallizations from water-ethanol, m.p. 250–251.5 °C. Anal. (dried over $CaCl_2$) $C_{16}H_{13}NO_6$: C, H, N.

DL-*2b*. 8.4 g of *10* was refluxed with 500 ml of 20 % HCl for 48 h. The solution was extracted with diethyl ether to remove benzoic acid and evaporated. The residue was suspended in 100 ml of water and pH adjusted to 2.3 with ammonia. After cooling and filtration 5.8 g (88 %) of the dihydrate of DL-*2b* was obtained, identified by IR and PC. p*K*-values: 3.1; 9.2; 12 (pK_1 undetermined).

Diethyl (3-cyanobenzyl)acetamidomalonate, (*11*). A solution of Na (1.92 g), diethyl acetamidomalonate (17.4 g) and *m*-cyanobenzyl bromide (15.7 g) in 130 ml of absolute ethanol was refluxed for 8 h with stirring. After addition of water (1000 ml) and cooling, the crystalline material was collected by filtration. Yield 18 g (68 %), m.p. 132–135 °C. Recrystallization from ethanol-water, m.p. 142–143 °C. Anal. $C_{17}H_{20}N_2O_5$: C, H, N.

Diethyl (3-aminomethylbenzyl)acetamidomalonate.HCl, (*12*). 8 g of *11* was hydrogenated at atmospheric pressure and room temperature for 8 h in 96 % ethanol (500 ml) and conc. HCl (6 ml) with Pd/C as catalyst. After filtration the solution was taken to dryness. Water (50 ml) was added to the residue, and unreacted material removed by filtration. The filtrate was again concentrated to dryness, and after addition of acetone (500 ml) and cooling the crystals were collected. Yield 7 g (78 %), m.p. 198–200 °C. Recrystallization from ethanol-acetone, m.p. 200–201 °C. Anal. $C_{17}H_{24}N_2O_5.HCl$: C, H, N.

Diethyl (3-hydroxymethylbenzyl)acetamidomalonate, (*13*). A solution of *12* (7.45 g), $NaNO_2$ (1.75 g) and water (10 ml) was heated for 2 h on a water bath, cooled and extracted with ethyl acetate. The extract was washed with 1 M HCl, water, 5 % $NaHCO_3$, and water, dried over $MgSO_4$, and taken to dryness. The solid residue was recrystallized from benzene-light-petroleum (b.p. 100–120 °C). Yield 5.2 g

(77 %), m.p. 78 – 79 °C. Recrystallization from cyclohexane, m.p. 83 – 85 °C. Anal. $C_{17}H_{23}NO_6$: C, H, N. For deamination conditions, *cf.* Ref. 28.

DL-*3a*. A mixture of *13* (2.7 g), Dowex 50W X 8 (50 – 100 mesh, H^+) (50 g) and water (50 ml) was refluxed for 18 h with stirring. The resin was transferred to a column and washed with water to neutral reaction. *3a* was then eluted with 100 ml 1 M NH_3, aq. The eluate was evaporated and the residue recrystallized from water. Colourless crystals of *3a* were obtained. Yield 1.03 g (66 %), m.p. 215 – 217 °C (decomp.) Anal. (dried over $CaCl_2$ at 50 °C) $C_{10}H_{13}NO_3$: C, H, N. UV [0.1 M HCl (ε)]: 260 (280), 201 (9500) nm. [H_2O]: 260 (280), 193 (41 000) nm. [0.1 M NaOH]: 271 (225), 261 (350), 215 (7800). Lit. 262 nm (without indication of solvent).[3] p*K*-values: 2.5; 9.2.

3-(3-Chloromethylphenyl)-DL-*alanine.HCl*, (DL-*8*). *12* (3.7 g) in water (50 ml) and conc. H_2SO_4 (0.4 ml) was mixed at 0 °C with a solution of $NaNO_2$ (4 g) in water (40 ml). Dilute H_2SO_4 was added dropwise until N_2 evolution started. After 2 h at room temperature (pH 4) the reaction mixture was extracted with ethyl acetate. The extract was washed with water, 10 % $NaHSO_3$, and water, dried over $MgSO_4$, and taken to dryness. The residue was refluxed with 20 % HCl (100 ml) for 2 h. After cooling the crystals of *8* were collected. PC of the mother liquor demonstrated the presence of *3a*. Recrystallization of the hydrochloride from ethanol produced an analytical sample. Yield 1.2 g (48 %), m.p. 208 – 214 °C (decomp). Anal. $C_{10}H_{12}ClNO_2.HCl$: C, H, N, Cl. UV [0.1 M HCl (ε)]: 262 (250), 202 (14 000) nm. [phosphate pH 7, 0.05 M]: 262 (270), 202 (13 000) nm. [0.1 M NaOH]: 263 (310), 215 (7800) nm. p*K*-values: 2.5; 9.3.

DL-*4*.2HCl.H_2O. *12* (2 g) in 8 M HCl (30 ml) was refluxed in a N_2 atmosphere for 3 h. The solution was then taken to dryness, and the residue was evaporated twice with water. The residue was dissolved in water and *4* absorbed on a column of Dowex 50W X 8 (50 – 100 mesh) in the ammonium form. After washing with water the column was eluted with 1 M NH_3,aq. The eluate was taken to dryness, and after evaporation twice from water, 2 M HCl was added, and the solution again evaporated to dryness to give a crystalline residue of DL-*4*.2HCl.H_2O. Yield 1.36 g (89 %). Recrystallization from 96 % ethanol, m.p. 153 – 155 °C (decomp). Anal. $C_{10}H_{14}N_2O_2.2HCl.H_2O$: C, H, N, Cl. UV [0.1 M HCl (ε)]: 270 (160), 260 (250), 208 (9600) nm. [phosphate pH 7, 0.05 M]: 270 (180), 260 (260), 208 (9800) nm. [0.1 M NaOH]: 271 (170), 262 (260), 215 (8000) nm. p*K*-values: 2.3; 8.7; 9.9.

3-Carboxy-4-nitrobenzylbromide, (*14*). Br_2 (8 ml) was added dropwise over a period of 4 h to a boiling solution of 5-methyl-2-nitrobenzoic acid (18.1 g) in $CHBr_3$ (100 ml). The reaction mixture was refluxed for additional 6 h and the $CHBr_3$ removed by distillation. The remaining oil was dissolved in CCl_4 (300 ml) and the solution decolourised with charcoal. After cooling the crystals were collected. Yield 17.4 g of impure material. The crystals were extracted with boiling cyclohexane to remove unreacted starting material and afterwards recrystallized several times from CCl_4 to give an analytical sample, m.p. 104 – 106 °C. Anal. $C_8H_6BrNO_4$: C, H, N. ^1H NMR (60 MHz, DMSO-d_6): δ 4.52 (2 H, s, CH_2), 7.2 – 7.8 (3 H, m, aromatic protons).

Diethyl (3-carboxy-4-nitrobenzyl)acetamidomalonate, (*15*). To a solution of Na (2.3 g) in absolute ethanol (300 ml) was added diethyl acetamidomalonate (11 g). After stirring for 30 min at 60 °C, *14* (13 g) was added. The reaction mixture was refluxed for 12 h, evaporated to dryness, and the residue dissolved in water. pH was adjusted to 1 with HCl, and the solution extracted with $CHCl_3$. The extract was washed with water and extracted with a saturated $NaHCO_3$-solution. The extract was acidified with HCl, and after cooling the crystals were collected. Two recrystallizations from ethanol produced an analytical sample. Yield 7.65 g (39 %), m.p. 209 – 211 °C. Anal. $C_{17}H_{20}N_2O_9$: C, H, N.

3-(3-Carboxy-4-nitrophenyl)-DL-*alanine*, (*9*). *15* (3.96 g) in 10 M HCl (25 ml), water (10 ml) and ethanol (20 ml) was refluxed with stirring under N_2 for 8 h. The ethanol was then removed, 10 M HCl (25 ml) was added, and reflux continued for 10 h. After filtration and cooling overnight the crystals of the hydrochloride were collected. Yield 2.3 g (79 %), m.p. 180 – 215 °C. The crude hydrochloride was dried at 60 °C and boiled with water (50 ml) for 2 h. The hot solution was filtered and ethanol (50 ml) was added. The crystalline precipitate of *9* was isolated. Yield 1.9 g (75 %). Recrystallization from water, m.p. 260 °C (decomp). Anal. $C_{10}H_{10}N_2O_6$: C, H, N. UV [0.1 M HCl (ε)]: 270 (7000), 201 (18 000) nm. [phosphate pH 7, 0.05 M]: 275 (7000), 202 (13 000) nm. [0.1 M NaOH]: 282 (8000), 214 (11 000) nm. p*K*-values: 1.8; 2.5; 8.7.

DL-*5*. *9* (314 mg) was hydrogenated at atmospheric pressure in 0.5 M AcOH (50 ml) with PtO_2 (40 mg) for 2 h at 50 °C. After filtration the solution was taken to dryness. The residue was dissolved in water and *5* absorbed to a column of Dowex 1 × 8 (200 – 400 mesh) in the acetate form. After washing with water, *5* was eluted with 0.5 M AcOH. The eluate was taken to dryness, and recrystallization was performed twice from water-ethanol. Yield 210 mg (76 %), m.p. 275 – 277 °C (decomp). Anal. $C_{10}H_{12}N_2O_4$: C, H, N. UV [1 M HCl (ε)]: 278 (1100), 227 (8500), 203 (13 000) nm. [phosphate pH 7, 0.05 M]: 314 (2700), 245 (15 000), 211 (27 000) nm. [0.1 M NaOH]: 314 (2800), 243 (sh, 10 000), 214 (27 000) nm. p*K*-Values: 1.5; 2.1; 4.2; 9.1. p*K*-Values for *p*-aminophenylalanine: 2.2; 4.3; 9.1.

[2-^{14}C]DL-*1b*. ^{14}C-4-(4-Acetoxy-3-carbomethoxybenzylidene)-2-methyl-2-oxazolin-5-one was prepared as described previously [29] from [2-^{14}C]-glycine (250 μCi) *via* [2-^{14}C]*N*-acetylglycine. The azlactone (20 mg) was refluxed with HI (0.035 ml, *d* 1.7), red P (25 mg) and acetic anhydride (0.15 ml) for 4 h. After cooling, filtration, and washing with glacial acetic acid (2 ml), the solution was taken to dryness. 4 M HCl (2 ml) was added to the residue and the solution taken to dryness again. After repetition of this step, 4 M HCl (2 ml) was added, and the solution was refluxed for 10 h, filtered hot, and taken to dryness. Water was added twice with subsequent evaporation, the residue was dissolved in water and applied to a column of Dowex 1 × 8, 200 – 400 mesh, in the acetate form. The column was washed with water and 0.5 M AcOH and eluted with 1 M formic acid. The fractions containing *1b* (found by the fluorescence in UV) were taken to dryness, and water was added twice with subsequent evaporation to give a crystalline residue of *1b*. Yield 12.8 mg, 7 μCi/mg. 36 % radiochemical yield. The purity was ascertained by PC followed by autoradiography.

Paper chromatography. The following R_F-values were determined for the new amino acids by descending chromatography on Whatman Paper No. 1 at 22 °C in (1) butanol-acetic acid-water (12:3:5), and (2) phenol-water-conc. ammonia (120:30:1) (w/v/v):

Amino acid	(1)	(2)
3-(3-Hydroxymethylphenyl)-alanine (*3a*)	0.48	0.76
3-(3-Aminomethylphenyl)alanine (*4*)	0.22	0.87
3-(4-Amino-3-carboxyphenyl)-alanine (*5*)	0.29	0.31
3-(3-Cyanophenyl)alanine (*6*)	0.53	0.83
3′-Carbamidophenylglycine (*7*)	0.24	0.64
3-(3-Chloromethylphenyl)alanine (*8*)	0.73	0.95
3-(3-Carboxy-4-nitrophenyl)-alanine (*9*)	0.24	0.29

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The Crystal Structure of Phenylmalondialdehyde at —162 °C

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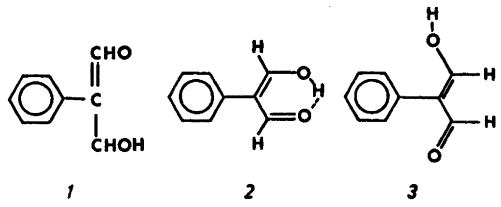
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The crystal structure of 2-phenylmalondialdehyde (2-phenyl-2-propen-3-ol-1-one, $C_9H_8O_2$) has been determined at −162 °C. The crystals are orthorhombic with space group $Pna2_1$, cell dimensions $a=7.523(2)$, $b=17.165(3)$, $c=5.552(2)$ Å, and four molecules in the unit cell. The structure was solved by direct methods and refined by the full-matrix least-squares methods to $R=0.032$ ($R_w=0.035$) for 1012 reflections with sin $\theta/\lambda>0.5$ Å^{-1}. The compound crystallizes in the *trans*-enol form. The crystal packing consists of asymmetrically hydrogen bonded chains of molecules in a pseudo *Pnab* cell. The polar axis of the space group may therefore be reversible and the crystals may show ferroelectric properties.

Phenyl malondialdehyde was first prepared by reduction of hydroxymethylenebenzyl cyanide followed by mild acidic hydrolysis of the intermediate aldimine.[1] More recently it has been prepared in high yield by hydrolysis of 2-phenyl-3-(dimethylamino)acrolein.[2] It was originally assigned the structure (*1*) but NMR studies in solution suggest the presence of an intramolecular hydrogen bond (*2*).[2,3] Since no structural analysis has so far been reported for pure β-dialdehyde compounds, it was considered of interest to perform a structural analysis of phenylmalondialdehyde. During the structure analysis it turned out that the compound in the solid state assumes a *trans* enol arrangement (*3*), similar to that found in dimedone (5,5-cyclohexane-1,3-dione) [4] and triose reductone (2-propene-2,3-diol-1-one).[5]

EXPERIMENTAL

A sample of the compound was kindly provided by Dr. G. M. Coppola and Dr. G. E. Hardtmann. White, transparent crystals suitable for X-ray work were grown by slow sublimation at 50 °C and 0.1 mmHg. The identity of the sublimed sample was confirmed by infrared spectroscopy (KBr). X-Ray photographs showed the following systematic absences ($0kl$): $k+l=2n+1$ and ($h0l$): $h=2n$ indicating the space group to be either $Pna2_1$ or *Pnam*. However, it was noted that in addition, most $hk0$ for $k=2n+1$ were weak or absent, suggesting that the space group might be pseudo *Pnab*. Data collection was carried out with a crystal of approximate dimensions 0.3 × 0.4 × 0.35 mm on a SYNTEX P$\bar{1}$ diffractometer with graphite monochromatized Mo$K\alpha$ radiation ($\lambda=0.71069$ Å) equipped with an Enraf-Nonius liquid nitrogen gas-flow cooling device (modified by H. Hope). The temperature at the crystal position was −162 °C. The cell parameters (at −162 °C) were determined by least squares refinements from 20 high angle reflections. One octant of the reciprocal lattice was examined for which $2\theta<70°$, and data were collected in the $\omega-2\theta$ scanning mode. For 2θ below 45° a constant scan speed of 4°/min in 2θ was used, while for $45<2\theta\leq 70°$ a variable scan speed (2−4°/min) and a rejection level were applied. A symmetrical scan range of 1.8° corrected for spectral dispersion was specified and the ratio of background time to time of integration was 0.6. Three reference reflections were monitored every 50 measurement cycles and showed no significant variation. A total of 1559 reciprocal lattice points including all space group absences were examined, of which 1430 had intensities larger than $2\sigma(I)$. The remainder, including all symmetry absent reflections for $Pna2_1$ were rejected, while most reflections for $hk0$ for k odd



1 *2* *3*

were found to be observable at −162 °C. Corrections for Lorentz and polarization effects were applied to the intensities and their standard deviations and a 2 % uncertainty due to instrument instability was included in the latter.

CRYSTAL DATA (AT −162 °C)

Phenylmalondialdehyde $C_9H_8O_2$
Space group $Pna2_1$, orthorhombic
$a = 7.523(2)$, $b = 17.165(3)$, $c = 5.552(2)$ Å
$V = 716.9$ Å^3; $F.W. = 148.16$; $F(000) = 312$; $Z = 4$.
D_{obs}(flotation 20 °C) = 1.36 g cm^{-3}; $D_{calc} = 1.373$ g cm^{-3}.

STRUCTURE SOLUTION AND REFINEMENTS

Statistical tests strongly indicated a centric distribution. The space group *Pnam* was, however, considered unlikely for chemical reasons since it requires either a mirror plane or a centre of inversion in the molecule. Direct methods [6] were therefore applied to $Pna2_1$ and positions of all non-hydrogen atoms in a non-planar, chemically reasonable arrangement were obtained. Least-squares refinements [7]* of these positions resulted in divergence. However, preliminary refinement by the minimum residual method [8] resulted in a model which was then successfully refined by conventional least-squares procedure to $R = 0.09$ and $R_w = 0.12$ (hydrogen atoms and anisotropic parameters included). Unfortunately the resulting model was found to contain a number of unreasonable bond lengths in both the benzene ring and the enol moiety. Least-squares refinements with unit weights and only reflections with $\sin\theta/\lambda < 0.5$ did not improve the model, but reduced the residuals to 0.08. An acceptable model for the structure was obtained from the initial coordinates after several cycles of Fourier refinements followed by least-squares refinements yielding the residuals $R = 0.040$, $R_w = 0.045$ and $S = 2.27$.** All hydrogen atoms were located in a difference Fourier map, which did not contain other density fluctuations larger than ±0.3 e Å^{-3}. A comparison of the atomic positions finally arrived at and those of the former false minimum showed that the average shift in the x coordinates was about 0.3 Å, while only small changes in the y and z coordinates were found. Refinements of the heavy atoms parameters with 1012 reflections for which $\sin\theta/\lambda > 0.5$ were also performed, resulting in the residuals $R = 0.032$, $R_w = 0.035$ and $S = 1.38$. Positional parameters were shifted by amounts up to 4 estimated standard deviations (e.s.d.), the average shift being of the order of one e.s.d., while all U_{ij} were reduced by amounts ranging between 0.5 to 4 e.s.d.'s by exclusion of the low angle data. Atomic parameters for non-hydrogen atoms obtained in the latter refinement are listed in Table 1 along with the parameters for the hydrogen

* Descriptions of all programs used (except those for the phase determination) are included in this reference.

** Standard deviations of unit weight $S = [\sum W(F_o^2 - F_c^2)^2/(m-n)]^{\frac{1}{2}}$

Table 1. Final fractional coordinates and thermal parameters with estimated standard deviations, from the final refinement of high angle data. Expression for anisotropic vibration is exp $[-2\pi^2(h^2a^{*2}U11 + \cdots + 2klb^*c^*U23)]$. Hn is bonded to Cn and HO to O2.

ATOM	X	Y	Z	U11	U22	U33	U12	U13	U23
O1	.41117(15)	.78881(7)	0.88888(8)	.0224(4)	.0219(4)	.0149(5)	-.0066(3)	.0050(4)	-.0001(4)
O2	.85582(14)	.69734(6)	.69528(25)	.0188(4)	.0191(4)	.0143(4)	.0035(3)	.0049(4)	.0007(4)
C1	.32362(18)	.73617(7)	.16698(41)	.0173(4)	.0164(5)	.0126(6)	-.0036(4)	.0002(4)	.0018(4)
C2	.14788(16)	.73164(7)	.52388(42)	.0157(4)	.0142(4)	.0138(6)	.0022(3)	-.0002(4)	-.0004(5)
C3	.23727(16)	.69847(6)	.35888(45)	.0137(4)	.0133(3)	.0116(3)	-.0005(3)	.0002(3)	.0011(6)
C4	.24588(16)	.66481(5)	.34986(48)	.0122(3)	.0136(3)	.0101(3)	-.0006(4)	.0003(3)	-.0005(6)
C5	.32366(19)	.64484(8)	.54882(42)	.0151(4)	.0156(5)	.0132(7)	.0015(3)	-.0014(4)	.0002(4)
C6	.33271(18)	.68271(8)	.53786(45)	.0197(5)	.0155(5)	.0156(8)	.0031(3)	.0006(5)	.0020(5)
C7	.25866(22)	.44132(6)	.34677(55)	.0216(5)	.0142(4)	.0171(4)	.0000(4)	.0038(4)	.0006(6)
C8	.17687(20)	.48878(8)	.15734(46)	.0214(5)	.0165(5)	.0154(8)	-.0038(4)	-.0005(5)	-.0027(5)
C9	.17245(17)	.86286(8)	.15641(42)	.0162(4)	.0169(5)	.0184(6)	-.0013(4)	-.0014(4)	-.0002(4)

ATOM	X	Y	Z	B	ATOM	X	Y	Z	B
H1	.3896(27)	.7922(11)	.1715(52)	2.1(4)	H2	.1445(26)	.7887(10)	.4974(53)	2.9(3)
H6	.3745(25)	.6933(11)	.6759(56)	2.1(3)	H6	.3948(27)	.4571(11)	.6758(54)	2.2(4)
H7	.2662(27)	.3846(12)	.3689(77)	3.2(4)	H8	.1225(29)	.4519(11)	.0281(70)	2.7(4)
H9	.1184(27)	.5876(11)	.9279(62)	2.4(4)	HO	.8102(34)	.7388(14)	.7944(58)	4.1(6)

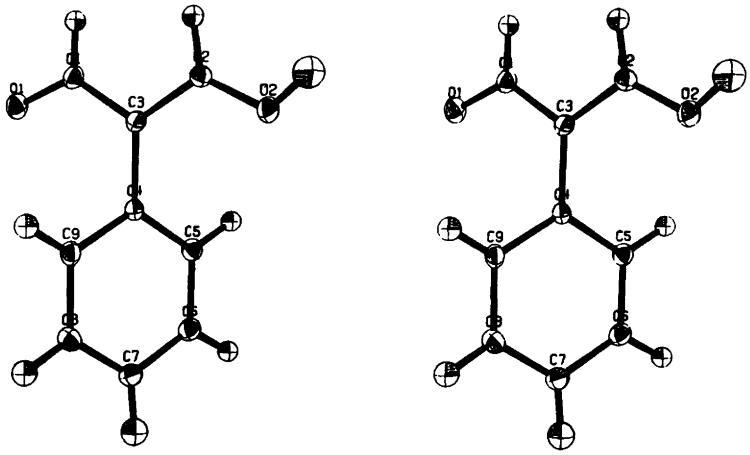


Fig. 1. Stereoscopic illustration of the molecule with atom numbering as indicated. Ellipsoids indicate amplitudes of vibration and enclose 50 % of the probability.[9]

atoms from the refinements including all observed reflections. The average and maximum parameter shifts as fractions of the e.s.d.'s in the final cycle of computation were 0.15 and 0.3, respectively. The function minimized during refinements was $\sum w(F_o - F_c)^2$, $w = \sigma(F_o)^2$. The atomic form factors were those of Doyle and Turner[9] except for hydrogen.[10] Lists of observed and calculated structure factors are available from the author upon request.

DISCUSSION

Bond lengths and angles are given in Table 2, and Fig. 1 shows a drawing of the molecule indicating the atom numbering scheme. The compound crystallizes in the *trans*-enol form (3) as previously has been found for other derivatives of malondialdehyde in the solid state.[5,11] The stability of the *trans*-form (*3*) relative to the *cis*-form in these compounds is mainly due to the small size of the terminal substituents (H1 and H2), facilitating an essentially planar conjugated system with intermolecular hydrogen bonds in the solid state. The non-bonded intra-molecular distance between H1 and H2 is 2.2(1) Å in phenylmalonaldehyde and longer than the sum of the van der Waals radii (2.0 Å) between these atoms.[12,13] A *trans*-enol arrangement corresponding to that observed in the crystal structure of phenylmalondialdehyde has not been found for any of the acyclic β-diketones so far investigated by diffraction methods.[14] This is no surprise in view of the increase in steric hindrance to a planar structure where larger substituents are incorporated at the β-positions. The *trans* arrangement does, however, occur in cyclic homologues as has been demonstrated in the structure investigation of dimedone.[14]

The average C–C bond length in the benzene ring is 1.396 Å in perfect agreement with the r_α^0 value of benzene [1.396(2) Å] obtained from spectroscopic measurements.[15] The largest

Table 2. Interatomic distances, bond angles and hydrogen bond parameters, with estimated standard deviations, from the final refinement of high angle data.

Distance	(Å)	Distance	(Å)
O1 - O2	2.577(1)	O1 - C1	1.238(2)
O2 - C2	1.316(2)	C1 - C3	1.431(2)
C2 - C3	1.372(2)	C3 - C4	1.465(1)
C4 - C5	1.398(2)	C5 - C6	1.398(2)
C6 - C7	1.395(3)	C7 - C8	1.392(3)
C8 - C9	1.396(2)	C4 - C9	1.400(2)
C1 - H1	.968(0)	C2 - H2	.990(0)
C5 - H5	.980(0)	C6 - H6	.996(0)
C7 - H7	.978(0)	C8 - H8	.964(0)
C9 - H9	.931(0)	O2 - HO	.867(0)
O1 - HO	1.712(0)		

Angle	(°)	Angle	(°)
C1 - O1 - O2	119.0(1)	C2 - O2 - O1	114.7(1)
O1 - HO - O2	175.6(0)	C1 - O1 - HO	120.4(0)
C2 - O2 - HO	111.8(0)	O1 - C1 - C3	124.4(1)
O2 - C2 - C3	122.4(1)	C1 - C3 - C2	115.7(1)
C1 - C3 - C4	121.8(1)	C2 - C3 - C4	122.4(1)
C3 - C4 - C5	120.3(1)	C3 - C4 - C9	120.1(1)
C4 - C5 - C6	120.1(1)	C5 - C6 - C7	119.9(1)
C6 - C7 - C8	120.2(1)	C7 - C8 - C9	120.0(2)
C4 - C9 - C8	120.1(1)	C5 - C4 - C9	119.6(1)

Table 3. Deviations (Å) of atoms from least-squares planes through the benzene ring (I) and enol arrangement (II). Atoms in parenthesis are not included in the calculations.

I		II	
C4	0.001	O1	−0.006
C5	−0.011	O2	−0.014
C6	0.010	C1	−0.008
C7	0.006	C2	0.015
C8	−0.010	C3	0.019
C9	0.009	(C4)	0.044
(C3)	0.003	(C7)	0.082
		(HO)	0.024
		(H1)	−0.074
		(H2)	−0.069

discrepancy from the mean value is about 0.004 Å, only slightly more than the average combined standard deviation in these bonds (0.003 Å). The agreement is taken to indicate that the bond lengths derived for the other heavy atoms are physically meaningful. The bond lengths found in the enol arrangement show considerable conjugation, although single and double bonds are clearly recognizable. This is in qualitative agreement with the findings in the previous study of dimedone. However, the C−C bonds in the enol fragment in the present study appear to be somewhat longer than those determined in dimedone [1.418(2) and 1.351(2) Å], whereas the C−O bonds are slightly shorter [1.246(2) and 1.326(2) Å in dimedone]. It is tempting to correlate these small changes with the negative inductive effect exerted by the phenyl ring. Since the phenyl ring makes an angle of 63.2° with the enol system, it seems unlikely that the enol system is influenced through conjugation across the central bond C3−C4. The length of this bond [1.485(1) Å] compares well with other carbon sp^2-sp^2 single bonds determined in similar systems.[14]

Deviations of the atoms from least-squares planes through the enol system and the benzene ring planes are given in Table 3. Although the benzene ring and enol system separately are essentially planar, the deviations are significant in terms of the estimated standard deviations in atomic positions. These deviations are, however, not considered to be of chemical origin.

The O---O distance [2.575(2) Å] shows the presence of a strong asymmetric hydrogen bond, similar to those found in dimedone [2.593(2) Å] and α-methyltetronic acid [2.600(3) Å].[16] The hydrogen bond formed by the *trans*-enol arrangement thus appear to be somewhat stronger than those formed by carboxylic acids (2.63−2.64 Å).[17] The hydrogen atom (HO) is

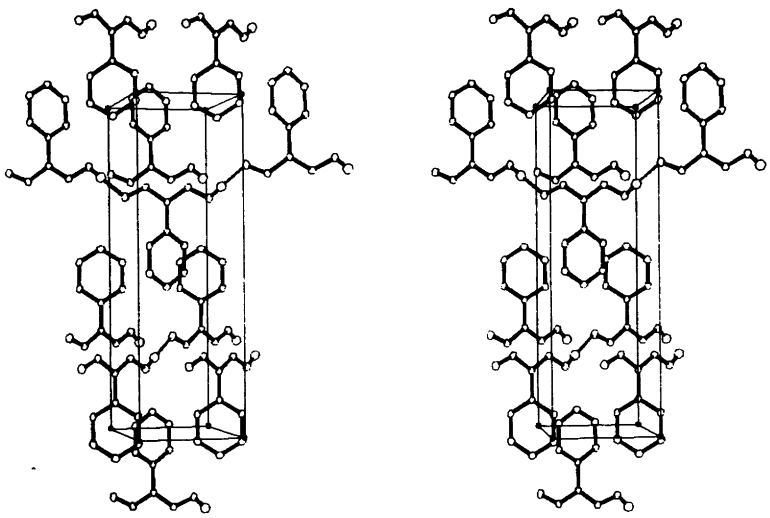


Fig. 2. Stereoscopic illustration of the crystal structure of phenylmalondialdehyde, as viewed along the *a* axis.

close to the mean squares planes through the donor and acceptor groups at distances 0.87 and 1.71 Å from O2 and O1, respectively. The bond is nearly linear (O−H···O = 175.6°) and the observed asymmetries in the heavy atom arrangement and the proton position form a consistent chemical pattern. No evidence of disorder in the HO proton could be seen from the final difference Fourier map.

The packing of the molecules is shown in Fig. 2. The hydrogen bonds link the molecules together along the [102] and [10$\bar{2}$] directions into infinite chains. The long dimension of the molecule nearly coincides with the [010] direction, and the structure is built up from alternating regions containing phenyl rings and hydrogen bonded enol arrangements around the twofold screw axes and *a* glides, respectively.

Crystallographically the structure of phenylmalondialdehyde is very interesting. Referring to the origin at the twofold screw axis there is a pseudo twofold axis through the molecule at $y \simeq 0.25$, and a pseudo centre of inversion at the centre of the hydrogen bond (0,0.25,0.15). The crystal structure is therefore pseudo *Pnab*, in agreement with the observed pseudo extinctions in the *hk*0 zone for $k = 2n+1$. The space group $Pna2_1$ is polar, and the structure arrived at may possibly show cooperative properties such as reversibility of the sense of the polar axis and a phase-transition to *Pnab*. However, no ferroelectric twinning was observed in the crystalline material utilized for this investigation, nor could any phase-transition be inferred from difference scanning calorimetric measurements in the temperature region from 25 to 95 °C (the melting point of the substance). The entropy of such a transition may, however, be so small that it is not detectable in a routine measurement of this kind.

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The Crystal and Molecular Structure of DL-2-Hydroxyphenylalanine (*o*-Tyrosine) Hydrochloride

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The crystal structure of the title compound has been determined by X-ray methods using 2524 reflections obtained by counter methods. The crystals are orthorhombic, space group $Pca2_1$ with unit cell $a = 16.667(5)$ Å, $b = 7.438(2)$ Å, and $c = 16.659(5)$ Å, $Z = 8$. The structure was solved by Patterson methods and refined to a conventional R-value of 0.035. The estimated standard deviations in bond lengths not involving hydrogen atoms are 0.003 – 0.004 Å and in angles 0.2°; when hydrogen atoms are involved e.s.d. are about 10 times those given above.

There are only minor differences in the geometry of the two nonequivalent ions and the molecular structure agrees well with those found for salts of several other phenylalanine analogues. In the present structure neither all potential hydrogen donors nor acceptors have actually been used for hydrogen bonding.

The crystal structure of DL-2-hydroxyphenylalanine showed the molecule to exist in a conformation different from that usually found for amino acids with aromatic β-substituents.[1] The exceptional conformation is caused by an intramolecular hydrogen bond between the α-amino group and the hydroxyl group on the phenyl ring. In order to examine the influence on the molecular conformation from a different crystalline environment we have determined the crystal structure of the corresponding hydrochloride.

EXPERIMENTAL

Crystals were formed by slow diffusion of diethyl ether into a solution of racemic 2-hydroxyphenylalanine hydrochloride in 96 % ethanol at 0 °C. The crystal used for the X-ray experiments was cut to approximate dimensions $0.2 \times 0.4 \times 0.4$ mm³.

Oscillation and Weissenberg photographs showed the crystals to be orthorhombic. Reflections were systematically absent for $(0kl)$, l odd and $(h0l)$, h odd indicating one of the space groups $Pca2_1$ or $Pbcm$ of which the former turned out to be correct.

Unit cell dimensions were calculated from diffractometer measurements of 30 reflections using Cu$K\beta$ radiation ($\lambda = 1.3922$ Å). The intensity data were recorded on an automatic Picker four-circle diffractometer with graphite crystal monochromated Mo$K\alpha$ radiation. The $\omega - 2\theta$ scanning mode was employed with a 2θ scan speed of 1° min⁻¹; the scan range was from 0.6° below $2\theta(\alpha_1)$ to 0.6° above $2\theta(\alpha_2)$. The background counts were taken for 30 s at each of the scan range limits. Three standard reflections were measured after every 100 reflections; they showed no significant change in intensity during the data collection. The estimate of the standard deviation for the intensities was based on counting statistics with an additional term of 2 % of the net intensity. A total of 2968 unique reflections with $2\theta < 60°$ were measured; of these 2524 were considered observed with intensities larger than $2.5\sigma(I)$ and used for the structure determination. Absorption corrections were applied ($\mu = 3.55$ cm⁻¹).

Atomic form factors used were those of Doyle and Turner [2] for the chlorine ion and the oxygen, nitrogen, and carbon atoms and of Stewart, Davidson and Simpson [3] for hydrogen atoms. Calculations for the structure investigation were performed by means of computer programs described in Ref. 4. The full-matrix least-squares refinement program minimizes the quantity $\sum w(\Delta F)^2$ where $w = \sigma^{-2}(Fo)$.

CRYSTAL DATA

DL-2-Hydroxyphenylalanine hydrochloride (*o*-tyrosine hydrochloride) $C_9H_{11}NO_3.HCl$, orthorhombic, $a = 16.667(5)$ Å; $b = 7.438(2)$

Table 1. Fractional atomic coordinates and thermal parameters ($\times 10^5$) with estimated standard deviations. The temperature factor is given as exp $-(B11h^2+B22k^2+B33l^2+B12hk+B13hl+B23kl)$.

Atom	x	y	z	$B11$	$B22$	$B33$	$B12$	$B13$	$B23$
O1	29306(11)	33952(25)	55155	250(6)	1049(32)	594(11)	55(25)	−82(15)	−162(33)
O2	−9(11)	24072(25)	41963(19)	315(7)	1822(43)	329(8)	−548(28)	−175(14)	106(29)
O3	6358(12)	11062(26)	52235(20)	378(8)	1319(38)	598(12)	−499(28)	−420(16)	482(35)
N1	9735(13)	51891(31)	41118(19)	339(8)	1598(48)	230(8)	−525(33)	21(14)	48(35)
C1	20405(14)	58473(32)	55534(21)	199(8)	1115(43)	228(9)	−172(32)	− 12(14)	−21(33)
C2	28301(15)	52188(35)	55394(22)	244(8)	1125(44)	262(10)	−13(33)	−33(16)	−80(39)
C3	34657(15)	64103(40)	55810(24)	202(8)	1553(54)	369(12)	−172(36)	−24(17)	−74(45)
C4	33224(17)	82329(39)	56253(23)	314(11)	1425(54)	316(12)	−608(39)	−10(18)	4(41)
C5	25520(20)	88813(31)	56225(25)	400(11)	927(40)	369(13)	−185(42)	1(19)	−9(42)
C6	19135(16)	76831(35)	55890(22)	244(8)	1239(46)	316(11)	104(35)	−18(16)	−109(40)
C7	13429(14)	45450(34)	55303(22)	196(8)	1136(42)	284(10)	−80(31)	11(15)	35(39)
C8	11966(14)	37576(33)	47040(21)	184(8)	1199(46)	289(10)	−144(30)	58(15)	−177(36)
C9	5416(14)	23454(35)	46742(23)	212(8)	1117(45)	304(10)	−95(31)	49(15)	−205(37)
O11	55600(10)	115580(25)	24230(17)	223(6)	1088(34)	632(13)	−73(24)	83(16)	−85(36)
O12	26124(12)	124069(27)	35984(21)	284(7)	1951(40)	474(10)	439(28)	293(14)	226(34)
O13	33128(14)	139117(29)	26865(23)	444(9)	1433(43)	792(14)	689(31)	559(19)	749(40)
N11	36424(12)	97269(30)	37073(18)	242(7)	1394(41)	253(9)	245(29)	−2(13)	70(32)
C11	46717(14)	91152(30)	22663(21)	208(8)	1077(41)	196(8)	53(29)	−13(14)	3(32)
C12	54624(14)	97388(35)	23320(23)	235(8)	1039(44)	296(11)	−0(31)	74(17)	29(40)
C13	60958(15)	85555(38)	23065(24)	206(8)	1577(53)	389(12)	237(35)	41(18)	34(45)
C14	59566(17)	67353(37)	22285(23)	344(11)	1511(53)	292(11)	595(39)	40(19)	59(42)
C15	51863(19)	60909(34)	21739(23)	416(12)	993(44)	299(12)	153(37)	20(19)	−95(39)
C16	45504(15)	72862(34)	21933(21)	289(9)	1224(43)	234(10)	−55(34)	0(15)	4(37)
C17	39819(14)	104198(36)	22930(21)	207(8)	1258(45)	246(19)	126(31)	5(15)	37(39)
C18	38473(13)	111742(30)	31262(21)	163(7)	1081(41)	297(10)	−36(26)	−29(16)	−89(36)
C19	31776(14)	125595(33)	31738(22)	224(8)	1134(48)	322(11)	91(30)	42(16)	−128(38)
Cl1	46720(3)	22303(9)	49496(15)	241(2)	1145(11)	280(3)	148(8)	−75(4)	−260(12)
Cl2	22694(3)	71820(8)	30385(15)	222(2)	1091(10)	460(3)	8(7)	26(4)	159(12)

Table 2. Fractional atomic coordinates (x10^3) and B-values with estimated standard deviations for hydrogen atoms.

Atom	x	y	z	B (Å^2)
HC3	402(2)	592(4)	558(2)	4.1(0.7)
HC4	374(2)	904(5)	562(2)	5.5(1.0)
HC5	248(2)	1005(3)	571(2)	3.5(0.6)
HC6	139(2)	807(4)	562(2)	3.5(0.7)
H1C7	144(2)	371(4)	583(2)	3.0(0.6)
H2C7	87(1)	519(3)	567(1)	1.7(0.5)
HC8	168(2)	331(4)	445(2)	2.6(0.6)
H1N1	78(2)	465(4)	370(2)	4.1(0.7)
H2112	144(2)	599(5)	397(2)	6.3(0.8)
H3N1	56(2)	607(6)	434(3)	8.0(1.2)
HO1	344(2)	324(5)	539(3)	6.7(1.4)
HO3	29(2)	29(6)	506(2)	7.4(1.6)
HC13	660(2)	894(4)	237(2)	4.3(0.7)
HC14	636(2)	596(5)	220(2)	3.3(1.0)
HC15	507(2)	496(3)	219(2)	6.3(0.6)
HC16	401(2)	692(4)	218(2)	3.4(0.7)
H1C17	347(2)	985(4)	210(2)	3.3(0.6)
H2C17	409(1)	1139(3)	199(1)	2.6(0.5)
HC18	428(2)	1171(4)	332(2)	1.9(0.6)
H1N11	353(2)	1015(4)	419(2)	4.0(0.7)
H2N11	323(2)	908(5)	355(2)	4.7(0.8)
H3N11	402(2)	917(6)	377(3)	4.1(1.2)
HO11	606(2)	1172(5)	235(3)	3.5(1.4)
HO13	295(2)	1471(6)	272(2)	6.1(1.6)

Table 3. Interatomic distances (Å), bond angles and dihedral angles (°).

Bond	(A) Distance	(A) Corrected	(B) Distance	(B) Corrected
C1–C2	1.397(3)	1.405	1.401(3)	1.409
C2–C3	1.383(4)	1.387	1.375(4)	1.379
C3–C4	1.379(4)	1.386	1.379(4)	1.387
C4–C5	1.372(4)	1.379	1.373(4)	1.380
C5–C6	1.389(4)	1.393	1.384(4)	1.388
C1–C6	1.383(4)	1.389	1.381(3)	1.387
O1–C2	1.367(3)	1.372	1.371(3)	1.376
C1–C7	1.514(3)	1.518	1.505(3)	1.509
C7–C8	1.516(4)		1.514(4)	
C8–C9	1.516(3)		1.521(3)	
N1–C8	1.498(4)		1.488(3)	
O2–C9	1.201(3)		1.183(3)	
O3–C9	1.308(3)		1.312(4)	
C3–HC3	1.00		0.90	
C4–HC4	0.92		0.88	
C5–HC5	0.89		0.86	
C6–HC6	0.92		0.94	
C7–H1C7	0.82		1.00	
C7–H2C7	0.95		0.90	
C8–HC8	0.98		0.89	
N1–H1N1	0.86		0.87	
N1–H2N1	1.01		0.89	
N1–H3N1	1.02		0.76	
O1–HO1	0.88		0.84	
O3–HO3	0.88		0.84	

Angle	(A)	(B)
C1–C2–C3	120.4	120.5
C2–C3–C4	120.0	120.1
C3–C4–C5	120.5	120.4
C4–C5–C6	119.5	119.5
C5–C6–C1	121.2	121.5
C6–C1–C2	118.4	118.1
O1–C2–C1	116.6	116.5
O1–C2–C3	122.9	123.0
C2–C1–C7	120.6	120.2
C6–C1–C7	121.0	121.7
C1–C7–C8	113.2	112.3
C7–C8–C9	114.4	114.1
C7–C8–C1	111.3	111.3
C8–C9–O2	122.4	123.4
C8–C9–O3	112.3	111.2
O2–C9–O3	125.4	125.5
C6–C1–C7	121.1	121.6
N1–C8–C9	106.9	106.7
Dihedral angles (positive for a right-hand screw)		
C2–C1–C7–C8	−75.9	−72.1
C1–C7–C8–N1	−62.9	−61.7
C1–C7–C8–C9	175.7	177.5
N1–C8–C9–O2	+5.1	−1.2

Hydrogen bonds

D–H···A		D–A	H···A	∠D–H···A	∠D–D(H)···A
O3–HO3···Cl1	$(x-\frac{1}{2},-y,z)$	2.99	2.15	161	114
O1–HO1···Cl1	(x,y,z)	3.17	2.30	168	113
N1–H3N1···Cl1	$(x-\frac{1}{2},1-y,z)$	3.22	2.20	174	108
N1–H2N1···Cl2	(x,y,z)	3.17	2.26	150	122
O13–HO13···Cl2	$(x,y+1,z)$	3.06	2.23	164	113
O11–HO11···Cl2	$(x+\frac{1}{2},2-y,z)$	3.17	2.47	141	116
N11–H2N11···Cl2	(x,y,z)	3.17	2.29	173	112
N11–H3N11···O2	$(x,\frac{1}{2},1-y,z)$	2.88	2.13	168	114

Å; $c=16.659(5)$ Å. ($t=18$ °C) $V=2065.1$ Å^3; $M=217.65$; $F(000)=912$; $Z=8$; $D_{calc}=1.400$ g cm^{-1}; $\mu=3.55$ cm^{-1}.

Absent reflections: $(0kl)$ for l odd, $(h0l)$ for h odd. Space group $Pca2_1$.

STRUCTURE DETERMINATION

Statistical tests gave no definite conclusions as to the presence of a crystallographic center of symmetry. Space group $Pca2_1$ was adopted for the structure determination; this turned out to be the correct choice. The structure was solved by Patterson methods and refined by successive Fourier syntheses and least-squares calculations. All hydrogen atoms were localized from a difference Fourier map.

All positional parameters, anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for hydrogen atoms were varied; the overdetermination ratio was 7.2, the final conventional R-factor was 0.035 ($R_w=0.033$), and the goodness of fit $S=[\sum w\Delta^2/(n-m)]^{\frac{1}{2}}=1.8$.

Final parameters for non-hydrogen atoms are listed in Table 1 and for hydrogen atoms in Table 2. The structure factor list may be obtained from the authors upon request. In-

teratomic distances and bond angles are given in Table 3; estimated standard deviations were calculated from the correlation matrix and found to be 0.003 – 0.004 Å for distances and 0.2° for angles involving oxygen, nitrogen, and carbon atoms. A rigid-body analysis showed that the thermal motion of the hydroxyphenyl part of the molecules to a fair approximation could be interpreted in terms of translational and librational oscillations (r.m.s.ΔU of 0.0023 Å^2 and 0.0017 Å for the two cations, respectively). The corrected bond lengths are also given in Table 3.

DESCRIPTION OF THE STRUCTURE

The crystal packing of DL-2-hydroxyphenylalanine hydrochloride is illustrated in Fig. 1 in which the two independent molecules are labelled A and B, respectively. A stereoscopic view of an asymmetric unit is given in Fig. 2.

The structure may be described as bimolecular layers parallel to (001). The aromatic rings are nearly parallel to the layer planes, the phenyl ring of A molecules lying on one of the layer surfaces and that of B molecules on the other. Between the layers there appear

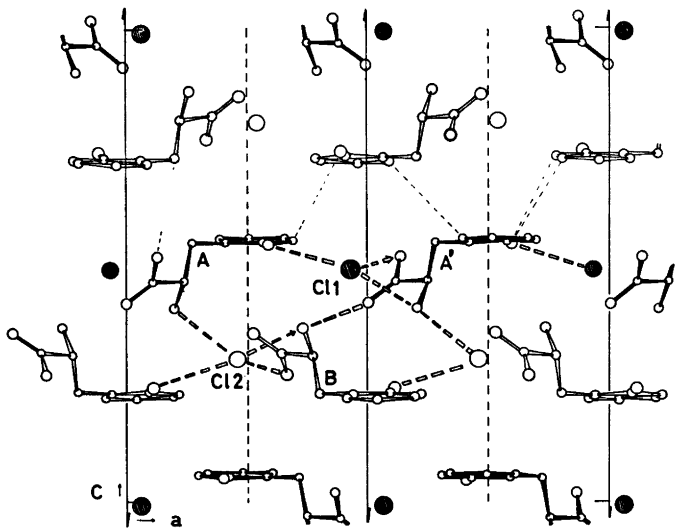


Fig. 1. The crystal structure of *o*-tyrosine hydrochloride as viewed down the *b* axis.

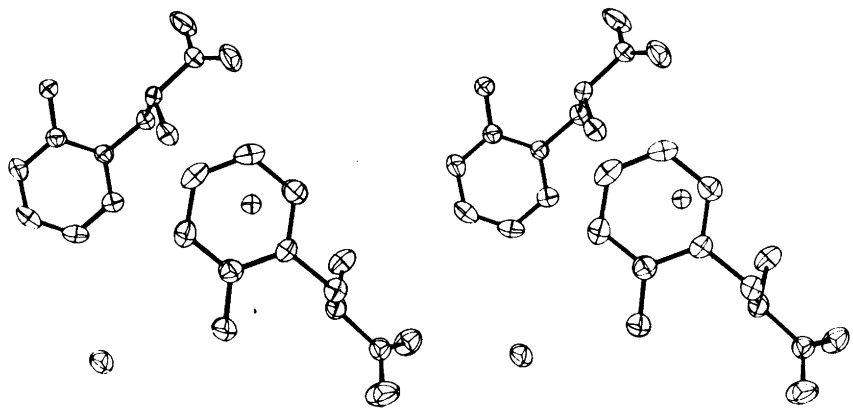


Fig. 2. Stereoscopic illustration of *o*-tyrosine hydrochloride.

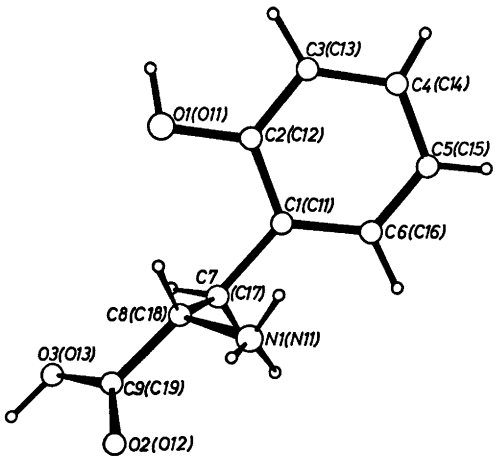


Fig. 3. Numbering of the atoms in the A and B molecules. The numbering of the B molecules in parentheses.

to be only van der Waals forces, the shortest interatomic distances being 3.40 Å (O1 – C14), 3.69 Å (O1 – C13), 3.54 Å (O3 – C17), 3.53 Å (O11 – C4) and 3.63 (C6 – C16). In this way each molecule in one layer is in contact with four molecules in an adjacent layer. Pseudo centers of symmetry are situated between the layers giving symmetry relations between A molecules in one layer and B molecules in the next. Within the layers molecules are closely connected through hydrogen bonds also involving chloride ions. The formally independent A and B molecules within a layer are related through a pseudo glide plane.

However, the environment of the A molecules differ from that of the B molecules, as may be seen from the hydrogen bonding. A molecules are involved in five hydrogen bonds whereas the B molecules are engaged in only four. There is only one hydrogen bond between the two types of molecules (O2 – N11); the others are directed to chloride ions. Accordingly there are differences also in the environments of the two chloride ions, one being coordinated to four of the hydrogen donor atoms in a distorted tetrahedral arrangement, the other to only three in an almost planar trigonal arrangement. The N1 atom has two of its hydrogen atoms directed to chlorine ions whereas N11 has one hydrogen bond to a chloride ion and one to an oxygen atom (O2). It is interesting to note that both of the ammonium groups are involved in only two hydrogen bonds.

In the A molecules both oxygen atoms of the carboxyl group take part in hydrogen bonding, O3 as a donor to a chloride ion and O2 as an acceptor from N11. In the B molecules only one oxygen atom of the carboxyl group participates in hydrogen bonding, *i.e.* O13 as a donor to a chloride ion.

A drawing of the molecules is given in Fig. 3 where also the numbering of the atoms may be found. The drawing displays the *S*-form and the conformational angles are given accordingly in Table 3 where also bond lengths and angles are presented. In Table 3 only the numbering of molecule A appears. The respective values for molecule B are listed in column B.

DISCUSSION

The lack of a conclusion from the intensity statistical tests may be explained by the presence of the pseudo symmetry elements. It may be noted that such pseudo symmetry elements also exist in the structure of *o*-tyrosine itself.[1] In both cases a racemate crystallizes in a noncentric space group, and it may

Table 4. The C – COO bond length (Å) for various α-amino acids and some corresponding salts.

Compound	C_α – COO	Ref.	Compound	C_α – COO	Ref.
L-Tyrosine	1.529(2)	6	L-Tyrosine.HCl	1.520(2)	11
DL-Tyrosine	1.530(3)	7			
o-Tyrosine	1.534(2)	1	*o*-Tyrosine.HCl	1.519(2)	(mean value)
m-Tyrosine	1.534(2)	8			
L-DOPA	1.539(2)	9	L-DOPA.HCl	1.522(2)	12
α-Methyl tyrosine	1.538(2)	10			

not be uncommon to find pseudo symmetry in such cases.

A rather unusual feature in the present structure is that the protonated amino group in both A and B molecules is involved in only two hydrogen bonds. It seems possible that for both molecules a comparably small rotation about the C–N bond could lead to the formation of another contact without breaking the existing hydrogen bonds, but apparently a strictly staggered conformation about the C–N bonds is preferred.

In the crystals of *o*-tyrosine hydrochloride the molecules have a conformation close to that found in other phenylalanines but quite different from that of *o*-tyrosine in which the conformation is dominated by the intramolecular hydrogen bond between the ammonium group and the *ortho* hydroxyl group. In the structure of the hydrochloride, however, the conformational angle about the C1–C7 bond is rotated 180° bringing the *ortho* hydroxyl group away from the ammonium group and thus excluding any interaction between the groups.

As may be seen from Table 3 there is good agreement in bond lengths and angles found in A and B molecules; the agreement also applies to the conformational angles. The only significant difference in bond lengths namely between C9–O2 and C19–O12 may probably be explained by the difference in hydrogen bonding. The torsional angle N1–C8–C9–O2 is positive (+5.1°); this angle has usually a small negative value in amino acids.

According to Sundaralingam [5] the C_α–COO bond length is 1.530 Å as a mean value for protonated as well as zwitterionic α-amino acids. In the present structure determination this bond was found to be 1.516 and 1.521 Å for the A and B molecules, respectively. It appears from studies of related molecular species that there may be a difference in this bond length for protonated and ionized carboxyl groups. The values found for this bond length in various structures determined with an e.s.d. of 0.003 or less are listed in Table 4. For protonated amino acids the C_α–COOH bond length has a mean of 1.520(3) Å and for ionized compounds the mean C_α–COO^- distance is 1.533(4) Å.

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UDPglucuronic Acid Pyrophosphatase Assay with the Aid of Alkaline Phosphatase

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A simple and sensitive method has been described for the determination of UDPglucuronic acid pyrophosphatase activity. Pyrophosphatase-free alkaline phosphatase preparation is added to the reaction mixture in order to hydrolyze the phosphate esters (UMP and α-D-glucuronic acid 1-phosphate) produced by pyrophosphatase. The inorganic phosphate liberated is measured by a modification of Fiske and SubbaRow's method. The phosphatase coupled method is time saving, easy to perform and accurate. It can also be used for pyrophosphatase assays with other nucleotide substrates like UDPglucose, UDP-*N*-acetylglucosamine, NAD^+, NADH, $NADP^+$ and NADPH.

The presence of UDPglucuronic acid pyrophosphatase activity in hepatic microsomal fractions has been reported by a number of authors.[1–4] This enzyme and α-D-glucuronic acid 1-phosphate hydrolyzing enzymes are of importance in the production of free D-glucuronic acid needed in the biosynthesis of D-glucaric acid, L-ascorbic acid and L-xylulose.[1,4,5] UDPglucuronic acid pyrophosphatase activity is low in the liver of the guinea pig,[4,6] which is unable to synthetize L-ascorbic acid.[7] UDPglucuronic acid pyrophosphatase may also have a role in the control of the production of β-D-glucopyranosiduronic acids (glucuronides), since it and UDPglucuronosyltransferase share a common substrate and pyrophosphatase activity is much higher than the transferase activity, *e.g.* in rat liver microsomal fractions.[5]

In spite of the obvious importance of UDPglucuronic acid pyrophosphatase, the enzyme and its regulation are rather poorly known, since the currently used methods in the determination of UDPglucuronic acid pyrophosphatase activity are not satisfactory for routine use. The enzyme activity has been measured by determining uronic acid by the carbazole [8] or naphthoresorcinol [3,9] reactions after removal of unhydrolyzed UDPglucuronic acid by charcoal adsorption or by back titration methods, in which the remaining UDPglucuronic acid has been determined with the aid of UDPglucuronosyltransferase.[4,10] Also radiochemical methods have been developed for UDPglucuronic acid pyrophosphatase assay, but they are time consuming, since they require chromatography.[4,5] A method based on the determination of phosphate released by phosphatase has also been shortly described.[2] It appears that it has been rarely used. Reasons for this have probably been the pyrophosphatase impurities in commercial alkaline phosphatase preparations and a release of endogenous phosphate from the microsomal fractions during incubation. Furthermore, problems are encountered with some methods commonly used in phosphate determinations.

In the present report we have studied in detail the conditions for the use of purified alkaline phosphatase in the determination of UDPglucuronic acid pyrophosphatase activity.

EXPERIMENTAL

Animals

Male albino rats (*Rattus norvegicus*) of Wistar/Af/Han/Mol/(Han 67) strain were used as experimental animals. The strain was purchased five generations ago as specific pathogen-free

from Møllegaard Avlslaboratorier A/S (Ejby, Denmark). The rats were outbred by a rotational mating system and kept under conventional circumstances in the Laboratory Animal Center of the University of Kuopio.

The rats were stunned by a blow on the head and were bled by cutting the cervical vessels. Livers were immersed in ice-cold 0.25 M sucrose and homogenized in three volumes of cold isotonic sucrose with five strokes of a Potter-Elvehjem homogenizer (300 rpm). After a precentrifugation for 10 min at 10 000 *g* the microsomal fraction was separated from the cytosol by centrifuging for 1 h at 105 000 *g*. The pellets were washed once and resuspended in ice-cold 0.25 M sucrose to give about 25 mg of microsomal protein per ml. The protein content was determined by Lowry's method [11] using bovine serum albumin as a standard.

Special reagents

Alkaline phosphatase (EC 3.1.3.1, No. 15 436 Boehringer GmbH, Mannheim, Fed. Republic of Germany) purified from calf intestine was daily diluted to give an activity of 6000 IU/l. Four different portions of alkaline phosphatase preparations made by Boehringer have been obtained and all of them worked satisfactorily. In preliminary studies other alkaline phosphatase preparations (No. P 3877 Sigma Chemical Co., St. Louis Mo., U.S.A. and No. 79 393 Fluka AG Chemische Fabrik, Buchs, Switzerland) were also tested but they hydrolyzed UDPglucuronic acid very rapidly due to pyrophosphatase impurities.

Trichloroacetic acid-ascorbic acid reagent, ammonium heptamolybdate reagent and sodium metaarsenite-trisodium citrate reagent were prepared as described earlier.[12] The chemicals used were *p.a.* products purchased from E. Merck AG, Darmstadt, Federal Republic of Germany.

Measurement of UDPglucuronic acid pyrophosphatase activity

UDPglucuronic acid pyrophosphatase activity was determined by measuring the production of inorganic phosphate. It was quantitated by the method of Fiske and SubbaRow as described by Baginski *et al.* [12] with slight modifications.

The incubations were carried out at 38 °C in a final volume of 150 µl in the presence of 67 mM Tris-HCl buffer, pH 8.9, containing 2 mM UDPglucuronic acid (or other nucleotide substrates studied), 25 mM $MgCl_2$ and 2000 IU/l of alkaline phosphatase. The reaction was started by adding microsomes (corresponding to about 25 µg of microsomal protein) diluted in 2.5 % albumin. The incubation was terminated after 5 – 10 min by adding 1.0 ml of trichloroacetic acid-ascorbic acid reagent. After spinning down the denatured proteins (5 min at 5000 *g*) the supernatants were pipetted into new tubes and 0.25 ml of ammonium hepta-molybdate reagent was added and mixed. Thereafter 0.5 ml of arsenite-citrate reagent was added. The solutes were thoroughly mixed and after 15 min the colour was measured at 700 nm in a Hitachi-Perkin Elmer UV-Vis 139 spectrophotometer against a water blank. The measurements were carried out as duplicates and every microsome sample had its own blank lacking substrate. For every alkaline phosphatase preparation, reagent blanks were also made by omitting the microsomes in the reaction mixture.

Potassium phosphate references (0 – 2.0 mM) were used to standardize the method.

In all stages acid washed glassware was used.

RESULTS

In the beginning of the work inorganic phosphate was determined as described by Lowry and Lopez.[13] This method was, however, abandoned due to instability of the colour and high absorbance in blank samples. In the method adopted the colour was stable for several hours after full development (10 – 15 min) and only traces (0.005 – 0.010 absorbance units) of colour were found in reagent blanks.

Some alkaline phosphatase preparations appeared to contain plenty of UDPglucuronic acid pyrophosphatase activity as impurity. Therefore they cannot be used for the determination of the UDPglucuronic acid pyrophosphatase in tissue samples. The preparation used also contained traces of UDPglucuronic acid pyrophosphatase activity (2 – 6 % of the pyrophosphatase activity in microsomes in the usual reaction mixture), but this could be eliminated by blank samples. The amount of alkaline phosphatase added to the reaction mixture (2000 IU/l) instantaneously hydrolyzed α-D-glucuronic acid 1-phosphate and uridine monophosphate produced by UDPglucuronic acid pyrophosphatase under the conditions used. According to calculations, the phosphate esters produced during a 10 min incubation with microsomes were split in about a couple of seconds.

The production of inorganic phosphate in the reaction mixture was linearly proportional to the incubation time in the method described (Fig. 1A). Due to the high sensitivity of the method, highly diluted microsomal samples

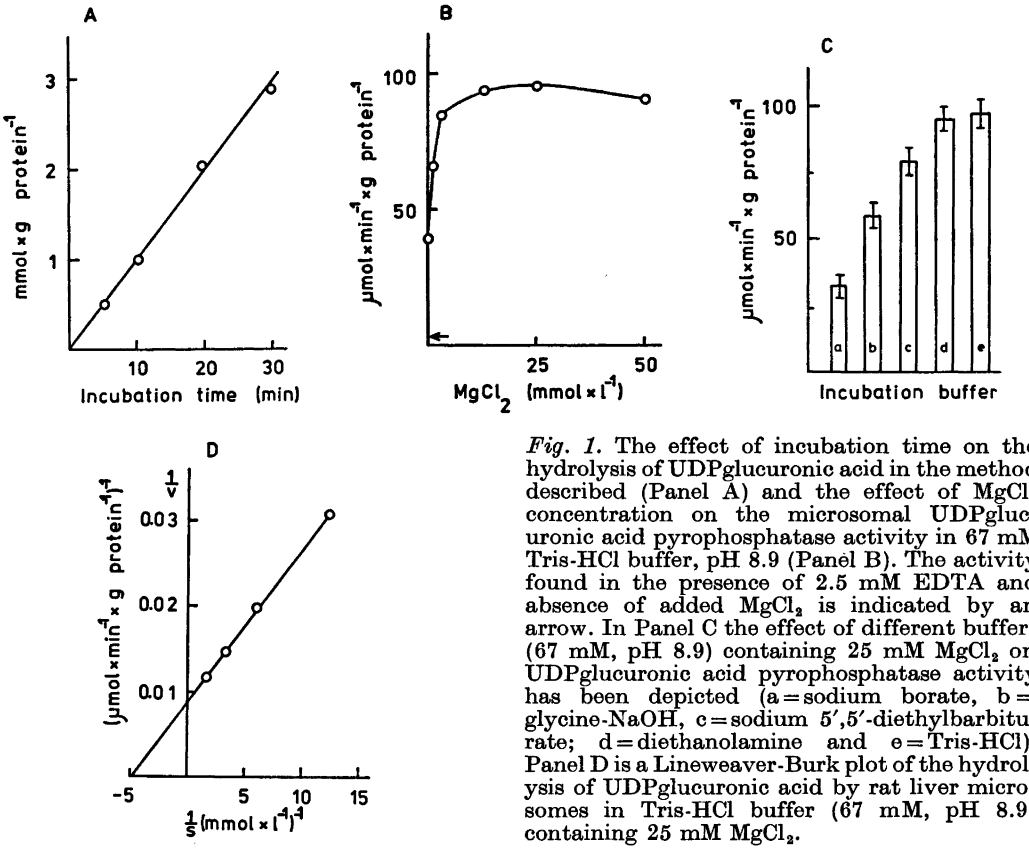


Fig. 1. The effect of incubation time on the hydrolysis of UDPglucuronic acid in the method described (Panel A) and the effect of $MgCl_2$ concentration on the microsomal UDPglucuronic acid pyrophosphatase activity in 67 mM Tris-HCl buffer, pH 8.9 (Panel B). The activity found in the presence of 2.5 mM EDTA and absence of added $MgCl_2$ is indicated by an arrow. In Panel C the effect of different buffers (67 mM, pH 8.9) containing 25 mM $MgCl_2$ on UDPglucuronic acid pyrophosphatase activity has been depicted (a = sodium borate, b = glycine-NaOH, c = sodium 5',5'-diethylbarbiturate; d = diethanolamine and e = Tris-HCl). Panel D is a Lineweaver-Burk plot of the hydrolysis of UDPglucuronic acid by rat liver microsomes in Tris-HCl buffer (67 mM, pH 8.9) containing 25 mM $MgCl_2$.

could be used as enzyme source. The dilution of microsomes also diminished the background release of endogenous phosphate from microsomes to the reaction mixture [up to 10 µmol min^{-1} (g protein)$^{-1}$]. Added alkaline phosphatase appeared to increase the hydrolysis of microsomal phosphate esters. Of the diluting media used 2.5 % albumin gave the best linearity with dilution factor. If 0.15 M KCl or 0.25 M sucrose were used, a slight autoactivation was found upon dilution. The cytosol and also denatured microsomes were unsuitable as diluting media, since they contained much phosphate and its esters and produced an intense background colour.

The recovery and precision of the method described was tested by adding different amounts of potassium dihydrogen phosphate (the final concentrations being up to 1.5 mM) to the reaction mixture after incubation. It was found that the increase in the intensity of the developed colour was linearly proportional to the amount of phosphate added. The accuracy of the method was tested by measuring absorbance of 25 samples. The mean was 0.665 ± 0.0098 (± S.D.) absorbance units and the variation coefficient 1.5 %.

The pH optimum of UDPglucuronic acid pyrophosphatase was found to be 8.9. The optimal concentration of $MgCl_2$ was 25 mM (Fig. 1B). The chelator EDTA was a powerful inhibitor of the production of inorganic phosphate from UDPglucuronic acid in the method used. It inhibited both microsomal pyrophosphatase and alkaline phosphatase used as reagent (Fig. 1B).

The measurable activity of UDPglucuronic acid pyrophosphatase was found to be markedly dependent on the buffer in the reaction mixture. The highest activities 96.2 and 94.1 µmol α-D-glucuronic acid 1-phosphate produced min^{-1} (g protein)$^{-1}$ were measured at optimal pH and

$MgCl_2$ concentration in Tris-HCl and diethanolamine buffers, respectively. The activities were lower in other buffers tested (sodium 5',5'-diethylbarbiturate, glycine-NaOH and sodium borate) (Fig. 1C). The amount of alkaline phosphatase was not limiting the reaction rate either in Tris-HCl or sodium borate buffers, in which the highest and lowest activities were measured.

A *K*m value of 0.21 mM was determined for UDPglucuronic acid in Tris-HCl buffer, pH 8,9, containing 25 mM $MgCl_2$ by the method described, when rat liver microsomes were used as enzyme source (Fig. 1D). A slight substrate inhibition was observed if UDPglucuronic acid concentrations higher than 2 mM were used.

UDPglucuronosyltransferase is often measured in the presence of surfactants which enhance the transferase activity manifold. Therefore the UDPglucuronic acid pyrophosphatase assay was studied also under these conditions. Results obtained indicate that surfactants Triton X-100 and sodium deoxycholate (up to a concentration of 2 %) do not disturb the assay and the method developed can be successfully used in measurements of UDPglucuronic acid pyrophosphatase activity in their presence. These surfactants appeared to increase the measurable UDPglucuronic acid pyrophosphatase activity up to 2 – 3-fold.

In addition to UDPglucuronic acid, a number of other nucleotides could be used as pyrophosphatase substrates in the method described. The relative hydrolysis of UDPglucuronic acid, UDPglucose, UDP-*N*-acetylglucosamine, NAD+, NADH, NADP+, and NADPH have been listed in Table 1. It appears that rat liver microsomes hydrolyze UDPglucuronic acid most efficiently.

DISCUSSION

The use of alkaline phosphatase in the determination of UDPglucuronic acid pyrophosphatase activity has been studied. Such nucleotide hydrolyzing activity can be found, *e.g.*, in liver nuclei,[2] microsomes,[3,11] and plasma membranes.[14,15] The method described does not need two successive incubations as do the back titration methods commonly used in UDPglucuronic acid pyrophosphatase assays.[4,10] Also standardization with inorganic phosphate is easier and more accurate to perform than with the substrate UDPglucuronic acid in these methods. The radiochemical methods require labeled substrate and they are expensive and time consuming in routine work. In the methods based on the condensation reaction of carbazole[8] or naphthoresorcinol [3,8] with uronic acid and the acid hydrolysis of D-glucuronic acid 1-phosphate it means extra steps. The sensitivity of these procedures is also poorer than that of the method described here. Two mol of inorganic phosphate are produced from one mol of UDPglucuronic acid, which increases the sensitivity.

The UDPglucuronic acid pyrophosphatase activities obtained in the present study are in rather good agreement with results reported earlier in the literature. In our experiments the enzyme activity in rat liver microsomes was 96.2 ± 5.0 μmol α-D-glucuronic acid 1-phosphate produced min^{-1} (g microsomal protein)$^{-1}$ in Tris-HCl buffer, pH 8.9. Ogawa *et al.*[3] have reported activities 79.5 and 156 μmol min^{-1} (g protein)$^{-1}$ using the method based on the measurement of nucleotide-free glucuronic acid. Lau and Wong [16] have found an activity of 58.3 μmol min^{-1} (g protein)$^{-1}$ in veronal buffer, pH 9.4, using the back titration method and harmol as glucuronic acid acceptor in subsequent glucuronidation. In veronal buffer the method described here gave an activity of 79

Table 1. The relative hydrolysis of different nucleotides by microsomal UDPglucuronic acid pyrophosphatase in 67 mM Tris-HCl buffer, pH 8.9, containing 25 mM $MgCl_2$ as measured by the phosphatase coupled method and compared to the activities reported by Ogawa *et al.*[3] The value 100 in the present method corresponds to the activity of 96 μmol α-D-glucuronic acid 1-phosphate produced min^{-1} (g protein)$^{-1}$.

Substrate	Relative hydrolysis. Present method	Ogawa *et al.*[3]
UDPglucuronic acid	100	100
UDPglucose	85	88
UDP-*N*-acetylglucosamine	78	71
NAD+	48	30
NADH	87	110
NADP+	41	26
NADPH	82	–

μmol min^{-1} (g protein)$^{-1}$. We have previously reported that a radiochemical method based on the production of ^{14}C-labeled α-D-glucuronic acid 1-phosphate from UDP[U-^{14}C]glucuronic acid [5] gives UDPglucuronic acid pyrophosphatase activity of 72 μmol min^{-1} (g protein)$^{-1}$ in 0.3 M Tris-HCl buffer, pH 8.9. The slightly higher activity found using the new method is probably due to the differences in the ion concentrations of the buffers used.

Schliselfeld *et al.*[2] have also determined UDPglucuronic acid pyrophosphatase with the aid of phosphatase coupling, but they have obtained considerably higher UDPglucuronic acid pyrophosphatase activity [142 μmol min^{-1} (g protein)$^{-1}$ in liver microsomes] in their experiments than observed in the present study.

In the present study it was found that pyrophosphatase is common as an impurity in alkaline phosphatase preparations and only a properly purified enzyme can be used. The release of endogenous phosphate from the microsomal membranes can also be quite considerable [1 – 10 μmol min^{-1} (g protein)$^{-1}$], and corrections should be made for its effect on the assay by using suitable controls. Furthermore, it appears that certain methods commonly used in phosphate determinations (*e.g.* Ref. 13) cannot be used in this assay. The addition of arsenite-citrate reagent stabilizes, however, the final colour.[12] The phosphatase coupled method can also be used in pyrophosphatase assays with other nucleotide substrates containing a pyrophosphate bond, since a coupling of the pyrophosphatase reaction with the unspecific alkaline phosphatase reaction makes the assay widely applicable.

The activities obtained in the present study with UDPglucose and UDP-*N*-acetylglucosamine as substrates were in good agreement with the results of Ogawa *et al.*[3] who measured the hydrolysis of UDPglucuronic acid, UDPglucose and UDP-*N*-acetylglucosamine by a naphthoresorcinol method. It is easier to measure the hydrolysis of NAD$^+$, NADH, NADP$^+$, and NADPH by the present method than by coupling the reactions with isocitrate or alcohol dehydrogenases and by following the changes in absorption at 340 nm.[3]

The method described here is reproducible, easy to perform and time-saving. Furthermore, it is sensitive and applicable to the determination of nucleotide pyrophosphatase activity with several substrates. It should, however, be kept in mind that the inhibition of pyrophosphatases by EDTA [2,5,17] or by other chelating agents, cannot be studied with the aid of the phosphatase coupled method, because the chelators inhibit also alkaline phosphatase in the reaction mixture.

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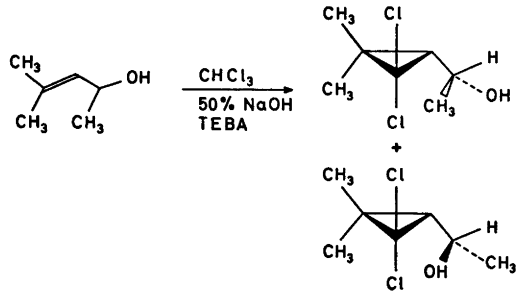
The Crystal Structure of 1-(2,2-Dichloro-3,3-dimethylcyclopropyl)ethanol

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The crystal structure of one of the diastereomers of the title compound has been determined by X-ray methods using 1120 observed reflections. The crystals are trigonal, space group $R\bar{3}$ with hexagonal axes $a = 26.773(3)$ Å, $c = 7.093(1)$ Å, 18 molecules in the unit cell. The structure was solved by use of the program assembly MULTAN and refined by full-matrix least-squares methods to a conventional R-factor of 0.042. Estimated standard deviations are 0.003 – 0.006 Å in distances and 0.2 – 0.3° in angles not involving hydrogen atoms. The (corrected) bond lengths in the cyclopropane ring are found to be 1.515, 1.498, and 1.527 Å. The conformation of the molecule is discussed.

When 4-methyl-3-penten-2-ol (*1*) is reacted with dichlorocarbene under two-phase conditions 1-(2,2-dichloro-3,3-dimethylcyclopropyl)ethanol (*2*) is formed in high yield as a 90:10 mixture of diastereomers [1] (Scheme 1). From a mechanistic point of view it was of interest to know the structure of the predominant diastereomer. It was also of interest to continue the study of the influence of the substituents on the bond lengths in the cyclopropane ring because substitution has turned out to cause changes in the geometry of the ring.[2,3]



Scheme 1.

EXPERIMENTAL AND STRUCTURE DETERMINATION

The title compound was prepared as described in Ref. 1. After two recrystallizations of the mixture of diastereomers from pentane, the most abundant was isolated pure, m.p. 71 – 72 °C. A crystal of approximate dimensions $0.25 \times 0.25 \times 0.65$ mm³ was used for the X-ray experiments which had to be carried out with the specimen sealed in a capillary because of rapid sublimation.

Oscillation and Weissenberg photographs showed the crystals to be trigonal. Determination of unit cell dimensions and collection of intensity data were carried out on a SYNTEX $P\bar{1}$ four-circle diffractometer at 18 °C, using graphite crystal monochromated Mo$K\alpha$ radiation. In the least-squares refinement of the cell dimensions the coordinates of fifteen symmetry independent reflections were used. The $\theta/2\theta$ scanning mode was employed with a scan speed (2θ) of 4° min^{-1}; the scan range was from 1° below $2\theta(\alpha_1)$ to 1° above $2\theta(\alpha_2)$. Background was counted for 0.35 times the scan time at each of the scan range limits. After every 97 reflections three standard reflections were measured; they showed no systematic fluctuation. 2026 reflections with $2\theta < 50°$ were measured; of these 1120 were found to have intensities larger than 2.5 times the estimated standard deviation and were regarded as observed. The remaining reflections were excluded from the refinement procedure. The standard deviations were taken as $\sigma(I) = (C_T + (0.03\ C_N)^2)^{\frac{1}{2}}$ where C_T is the total number of counts and C_N is the net count. The data set was corrected for Lorenz and polarization effects; no absorption correction was carried out.

The atomic form factors used were those of Doyle and Turner [4] for chlorine, oxygen, and carbon atoms and of Stewart, Davidson, and Simpson [5] for hydrogen. The full-matrix least-squares program employed minimizes the quantity $\sum w \Delta F^2$ where w is the inverse of the variance of the observed structure factors.

Table 1. Fractional atomic coordinates and thermal parameters ($\times 10^4$) with estimated standard deviation for the heavy atoms. The temperature factor is given by exp $-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)$. For numbering of atoms, see Fig. 1.

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl1	2276(1)	2507(1)	1864(2)	27(1)	30(1)	444(4)	22(1)	1(2)	−128(2)
Cl2	2569(1)	1610(1)	1808(2)	30(1)	34(1)	297(3)	32(1)	−43(2)	26(2)
O	1154(1)	766(2)	6(6)	17(1)	16(1)	386(9)	16(1)	17(4)	14(4)
C1	2321(1)	1991(1)	472(5)	18(1)	18(1)	224(9)	18(1)	−6(4)	−26(4)
C2	2495(1)	2120(1)	−1562(5)	18(1)	16(1)	236(10)	16(1)	6(5)	6(4)
C3	1880(2)	1686(1)	−1010(5)	17(1)	17(1)	245(9)	21(1)	−5(5)	1(5)
C4	2606(2)	2689(2)	−2381(8)	31(1)	20(1)	414(15)	17(2)	2(8)	53(7)
C5	2881(2)	1916(2)	−2392(7)	24(1)	29(1)	312(12)	26(2)	54(7)	−6(7)
C6	1594(2)	1052(2)	−1361(5)	20(1)	17(1)	252(11)	21(2)	1(5)	−8(5)
C7	1356(3)	904(3)	−3316(8)	47(2)	34(1)	315(14)	28(3)	−61(8)	−79(8)

Positions of the heavy atoms were determined using the program assembly MULTAN [6] and refined by full-matrix least-squares methods.[7] For the hydrogen atoms not involved in hydrogen bonding the positions were postulated from stereochemical consideration. Preliminary results showed that the distances from the oxygen atom to the oxygen atoms in two neighbouring molecules correspond to that of a hydrogen bond. Hydrogen atoms were therefore postulated to be situated in the direction of both of these and the population factor was refined for both. The resulting population factors were insignificantly different from 0.5 and we conclude that the structure is disordered with respect to these hydrogen atoms.

Final refinements including all positional parameters, anisotropic thermal parameters for the heavy atoms, one common isotropic thermal parameter for the methyl hydrogen atoms and one for the others yielded a conventional R-factor of 0.042, $R_w=0.040$, and goodness of fit $S=(\sum w\Delta F^2/n-m)^{\frac{1}{2}}=1.43$. The corresponding atomic parameters are listed in Tables 1 and 2; interatomic distances and selected bond angles and dihedral angles are listed in Table 3; the numbering of the atoms is shown in Fig. 1. Upon request the structure factor list may be obtained from the authors.

The thermal parameters were analysed in terms of rigid-body motion of the molecule. When the oxygen and C7 atoms were excluded from the analysis, the r.m.s. ΔU decreased from 4.9×10^{-3} to 2.1×10^{-3} Å^2. It is therefore obvious that these atoms do not belong to the rigid body; consequently a system consisting of the cyclopropyl group and the atoms directly bonded to it served as the basis for correction of the corresponding bond lengths for libration. The C6−C7 and C6−O bonds were corrected for riding motion. The corrected bond lengths as well as selected bond angles are given in Fig. 1.

Table 2. Fractional atomic coordinates ($\times 10^3$) and isotropic thermal parameters with estimated standard deviations for hydrogen atoms.

Atom	x	y	z	B
H3	163(1)	181(1)	−118(4)	2.9(4)
H41	233(2)	281(2)	−168(6)	9.2(4)
H42	254(2)	265(2)	−371(6)	9.2
H43	297(2)	297(2)	−209(6)	9.2
H51	281(2)	155(2)	−177(6)	9.2
H52	327(2)	217(2)	−208(6)	9.2
H53	284(2)	189(2)	−377(6)	9.2
H6	188(1)	93(1)	−119(4)	2.9
H71	118(2)	51(2)	−344(7)	9.2
H72	168(2)	103(2)	−420(6)	9.2
H73	107(2)	103(2)	−349(7)	9.2
HO1	100(3)	41(5)	15(7)	2.9
HO2	93(5)	88(5)	13(14)	2.9

CRYSTAL DATA

1-(2,2-Dichloro-3,3-dimethylcyclopropyl)ethanol $C_7H_{12}Cl_2O$, trigonal, space group $R\bar{3}$ (No. 148). Axes (hexagonal): $a=26.773(3)$ Å; $c=7.093(1)$ Å. $V=4404.3$ Å^3 ($t=18$ °C); $M=183.08$; $Z=18$; $D_{calc}=1.24$ g cm^{-3}; $\mu(\text{Mo}K\alpha)=6.4$ cm^{-1}.

DISCUSSION

The Newman projection along the C3−C6 bond (see Fig. 2) shows that the cyclopropane alcohol exists in a staggered conformation with the hydroxyl group and the dichloro-substituted

Table 3. Inter-atomic distances, selected bond angles, and dihedral angles. For numbering of atoms, see Fig. 1.

Bond distances (Å)

Cl1 – C1	1.750(3)	C6 – O	1.420(5)	C5 – H53	0.98
Cl2 – C1	1.747(4)	C6 – C7	1.495(6)	C6 – H6	0.99
C1 – C2	1.502(5)	C3 – H3	0.91	C7 – H71	0.93
C1 – C3	1.484(5)	C4 – H41	1.06	C7 – H72	0.98
C2 – C3	1.517(5)	C4 – H42	0.95	C7 – H73	0.98
C2 – C4	1.516(6)	C4 – H43	0.92	O – HO1	0.83
C2 – C5	1.508(5)	C5 – H51	1.01	O – HO2	0.80
C3 – C6	1.493(5)	C5 – H52	0.94		

Bond angles (°)

Cl1 – C1 – Cl2	110.5(2)	C4 – C2 – C1	118.8(4)
Cl1 – C1 – C2	119.8(3)	C4 – C2 – C3	117.4(3)
Cl1 – C1 – C3	119.0(3)	C5 – C2 – C1	118.8(3)
Cl2 – C1 – C3	119.5(2)	C5 – C2 – C3	120.0(3)
Cl2 – C1 – C2	119.4(3)	C3 – C6 – O	108.3(4)
C2 – C1 – C3	61.1(2)	C7 – C6 – O	111.1(4)
C1 – C2 – C3	58.9(2)	C7 – C6 – C3	111.4(4)
C2 – C3 – C1	60.0(2)	C6 – C3 – C2	124.8(3)
C4 – C2 – C5	113.0(4)	C6 – C3 – C1	124.0(3)

Dihedral angles (°) (positive for a right-hand screw)

C1 – C3 – C6 – O	79.5(4)
C1 – C3 – C6 – C7	– 158.1(4)
C1 – C3 – C6 – H6	– 38.0(1.7)
C2 – C3 – C6 – O	154.1(3)
C2 – C3 – C6 – C7	– 83.4(5)
C2 – C3 – C6 – H6	36.7(1.7)

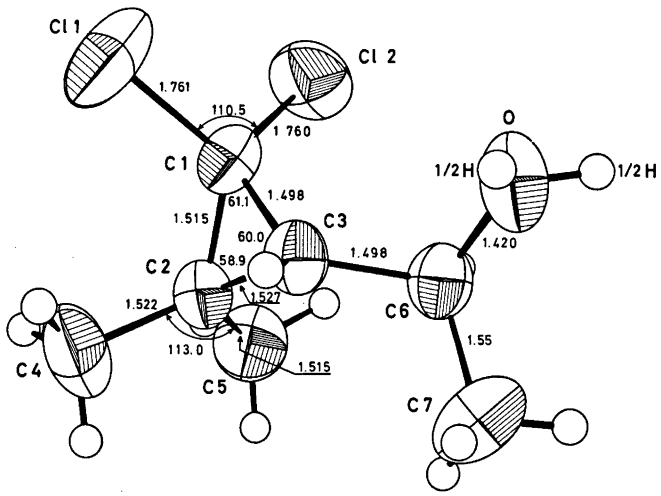


Fig. 1. Structure of the molecule with the numbering of the atoms, corrected bond lengths (Å), and selected angles (°). Non-hydrogen atoms are presented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 50 % probability.

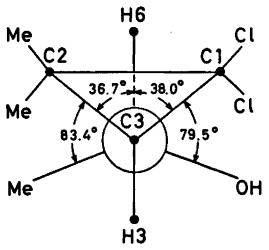


Fig. 2. Newman projection of the molecule viewed along the C3 – C6 bond.

carbon atom adjacent to each other ($\angle$Cl – C3 – C6 – O = 79.5°). The consequences for the understanding of the mechanism of addition of dihalocarbenes to allylic alcohols under two-phase conditions will be discussed elsewhere.[1]

The $C_{methyl} - C_{ring}$ distance (mean value 1.519 Å) as well as the C3 – C6 bond length (1.498 Å) correspond approximately to the sum of single bond radii for sp^2 and sp^3 hybridized carbon atoms (1.505 Å). The $Cl - C_{ring}$ bond length (mean value 1.761 Å) is found to be the same as in 2,2,2′,2′-tetrachloro-3,3,3′,3′-tetramethylbicyclopropyl (*3*) (1.763 Å)[3] and, within the accuracy of the determinations, as in 1,1-dichloro-2,2-diphenylcyclopropane (*4*) (1.754 Å)[2] whereas it is slightly longer than the Cl – C bond in 1,1-dichlorocyclopropane (*5*) (1.734 Å).[8] The $C_{methyl} - C_{ring} - C_{methyl}$ and Cl – C – Cl angles are 113.0(4) and 110.5(2)°, respectively, and these values are as found for tetrachloride *3* (113.1(2) and 110.2(1)°, respectively);[3] the corresponding angles in dichloride *4* are 113.9 and 110.9°, respectively.[2]

A comparison of bond lengths observed in substituted cyclopropanes is given in Ref. 2. The ring bond lengths are obviously influenced by the type of substituents, the observations ranging from 1.44 to 1.56 Å. According to Ibers the main reason for this variation is a difference between substituents which can interact through a π-orbital system, *e.g.* phenyl, and substituents which can only interact through σ orbitals, *e.g.* halogens and which will affect all bond lengths equally. In 1-(2,2-dichloro-3,3-dimethylcyclopropyl)ethanol the cyclopropane ring has substituents of the latter type and consequently the ring should be an equilateral triangle. This, however, is not the case; the bond lengths and the angles of the ring are similar to those found for 1,1-dichloro-2,2-diphenylcyclopropane [2] and nearly the same as those found for 2,2,2′,2′-tetrachloro-3,3,3′,3′-tetramethylbicyclopropyl [3] (Table 4). However, a discussion of this finding must await further investigations of similar compounds.

Correction for riding motion did not change the C6 – O bond, which is of normal length. However, the C6 – C7 bond distance increased from 1.495 to 1.551 Å; this indicates that this bond may in fact be of normal length.

Each molecule is hydrogen bonded to two neighbouring molecules; the length of the hydrogen bonds is 2.72 Å. Other intermolecular

Table 4. A comparison of bond lengths (Å) and angles (°) within substituted cyclopropane rings.

Compound	$R_2C - CCl_2$	$Cl_2C - CHR'$	$R_2C - CHR'$	Internal angles $\overline{C(Cl_2)}$	Internal angles $\overline{C(R_2)}$	Ref.
1-(2,2-Dichloro-3,3-dimethylcyclopropyl)-ethanol (*2*) [a]	1.515	1.498	1.527	61.1	58.9	This work
1,1-Dichloro-2,2-diphenylcyclo-propane (*4*) [b]	1.520	1.490	1.529	61.1	58.5	2
2,2,2′,2′-Tetrachloro-3,3,3′,3′-tetramethyl-bicyclopropyl (*3*) [a]	1.512	1.507	1.541	61.4	59.2	3

[a] R = Me. [b] R = Ph.

separations are as normal for van der Waals contact.

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A New Synthesis of [2.2.2.2]Cyclophanetetraenes

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[2.2.2.2]Cyclophanetetraenes and some related compounds have been prepared in moderate yields, in a single step Wittig reaction performed at low temperature, from aromatic dialdehydes and bistriphenylphosphonium salts of bis(halomethyl)arenes. Some spectroscopic characteristics of the cyclophanes and their stereochemistry are briefly discussed.

Cyclophanes or more generally phanes [1] are the common names for large ring compounds in which an aromatic nucleus is a part of the ring. The numbers in brackets give the length and number of bridges between the aromatics.

A large number of cyclophanes have been synthesised and studied for various reasons. Much of the work has been concerned with the stereochemistry and dynamic behaviour of small cyclophanes, interactions and deformations of benzene rings in small and strained cyclophanes [2–4] and photoisomerisation of unsaturated cyclophanes to annulenes.[5] Larger cyclophanes have properties as potential cage compounds or multidentate ligands if properly substituted. Size, shape and other properties of the cavity in the center of larger cyclophanes also depend on their stereochemical and conformational behaviour.

Previously we have reported that 1,4-benzenedicarbaldehyde and the bistriphenylphosphonium salt of 1,4-bis(bromomethyl) benzene gave [2.2.2.2]paracyclophanetetraene, *1*, (15 %) in a Wittig reaction at −40 °C in dimethylformamide (DMF) with lithium ethoxide as base.[6] In order to get a total yield of 15 %, statistically 60 % of the double bonds formed must have *cis* configuration.

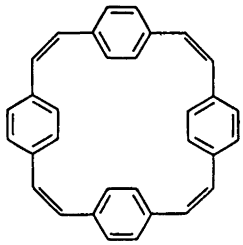
In this paper we describe the application of the above method to the synthesis of some new [2.2.2.2]cyclophanetetraenes and related compounds.

RESULTS AND DISCUSSION

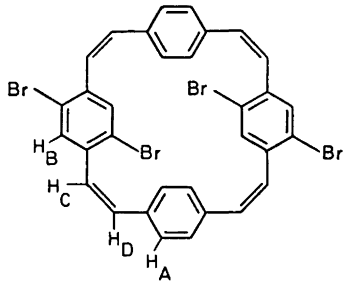
The Wittig reaction has been successfully used for the preparation of a variety of large ring compounds.[7] However, this reaction has some inherent limitations for the synthesis of cyclic compounds. The initial reaction between the ylid and the carbonyl group to form a betain is reversible. Thus the linear unstrained products are favoured over cyclic strained ones. It is also of prime importance to control the *cis*/*trans* ratio. Selective formation of *cis* double bonds, which in part can be achieved by careful control of the reaction conditions, favours the ring formation over open-chain oligomerisation. The yield, however, will always suffer from incomplete *cis*-selectivity.

Substituents in either the dialdehyde or the bisphosphonium salt appear to decrease the yield of [2.2.2.2]paracyclophanetetraenes when prepared by the Wittig reaction. Thus, 1,4-benzenedicarbaldehyde and the bisphosphonium salt of 1,4-bis(bromomethyl)-2-iodobenzene gave diiodo[2.2.2.2]paracyclophanetetraene, *3*, in 10 % yield.[8] The same dialdehyde and the bisphosphonium salt of 1,4-bis(bromomethyl)-2,5-dibromobenzene gave a 10 % yield of tetrabromo[2.2.2.2]paracyclophanetetraene, *2*, whereas 2,5-dibromobenzene-1,4-dicarbaldehyde and the bisphosphonium salt of 1,4-bis(bromomethyl)benzene gave a 9 % yield of the same product, *2*.

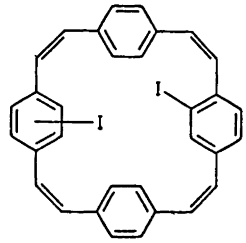
Heterocyclic dialdehydes also react with the bisphosphonium salt to form [2.2.2.2]cyclo-



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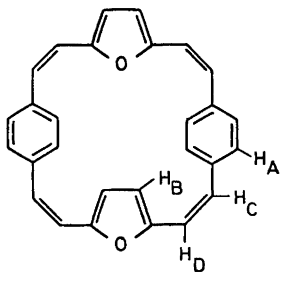


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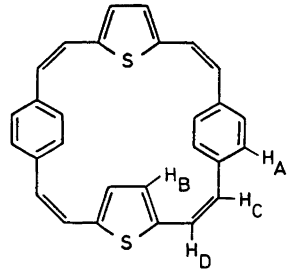


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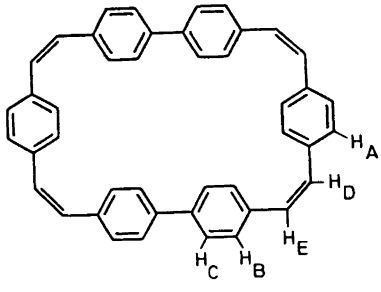
phanetetraenes under the same reaction conditions. 2,5-Furandicarbaldehyde reacts with bisphosphonium salt of 1,4-bis(bromomethyl) benzene to give [2](2,5)furano[2]paracyclo[2]-(2,5)furano[2]paracyclophanetetraene, *4*, in 10 % yield and some larger cyclophanes with three furan and three benzene nuclei bridged by six double bonds in 15 % yield. 2,5-Thiophenedicarbaldehyde reacts analogously to give 4 % of [2](2,5)thiopheno[2]paracyclo[2](2,5)-thiopheno[2]paracyclophanetetraene, *5*, together with some larger cyclophanes. 1,4-Benzenedicarbaldehyde and the bisphosphonium salt of 2,5-bis(chloromethyl)thiophene give a somewhat better yield, 10 %, of the same cyclophane, *5*, under similar conditions. The cyclophanetetraenes *4* and *5* are light-sensitive and must be protected from light. The relatively poor yields of the cyclophanetetraenes *4* and *5* may be due in part to their decomposition



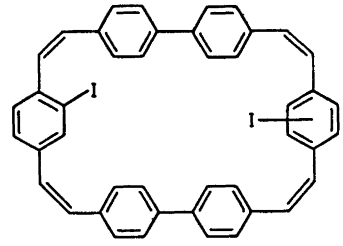
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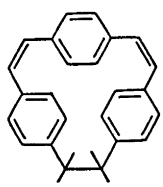
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during workup.

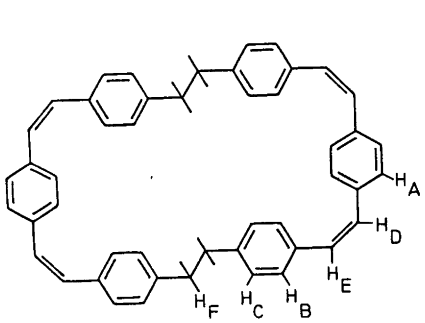
The method has also been extended to the synthesis of larger cyclophanes. 4,4′-Biphenyldicarbaldehyde reacts with the usual bisphosphonium salt of 1,4-bis(bromomethyl)benzene under the standard conditions to give 15 % of [2.2.0.2.2.0]paracyclophanetetraene, *6*. The product shows an intense fluorescence on irradiation with UV light. The corresponding reaction with the bisphosphonium salt of 1,4-bis-(bromomethyl)-2-iodobenzene gave a lower yield (4.5 %) of diiodo[2.2.0.2.2.0]paracyclophanetetraene, *7*.[9]

In an attempt to prepare smaller cyclophanes we reacted 4,4′-bibenzyldicarbaldehyde with the bisphosphonium salt of 1,4-bis(bromomethyl)benzene. No [2.2.2]paracyclophanediene, *8*, was observed among the products. Instead a high-melting solid which showed the simple NMR spectrum expected from [2.2.2]paracyclophanediene, was isolated in 9 % yield. Combined information from mass and NMR spectra showed it to be [2.2.2.2.2.2]paracyclophanetetraene, *9*, with four *cis* double bonds. The cyclophane *9* was hydrogenated to [2.2.2.2.2.2]-paracyclophane, *10*. This route to [2.2.2.2.2.2]-paracyclophane gives an improved yield over the previous method [10b] and should allow some variation of substituents in the product. Known methods for the synthesis of [2_n]cyclophanes ($n \geq 3$) use a bis(chloromethyl)benzene and sodium in the presence of tetraphenylethylene.[10] The isolation of the products involves a tedious separation of cyclophanes of different ring sizes. The method is also of limited use for the preparation of substituted cyclophanes. A few attempts to prepare [2.2.2.2]cyclophanetetraenes by the Wittig reaction from simple starting materials have also been reported.[11–13]

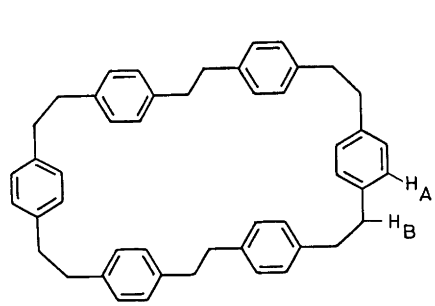
Planarity and stereochemistry. [2.2.2.2]Paracyclophanetetraene, *1*, can be regarded as a cyclooctatetraene with benzene rings inserted into the single bonds. Simple molecular models of [2.2.2.2]paracyclophanetetraene show the molecule to be non-planar as is cyclooctatetraene. However, because of the size of the molecule, small distortions in bond angles and bond lengths could flatten the molecule. If the molecule is close to planar and exposed to a strong magnetic field, an induced paramagnetic shielding of the outer protons and deshielding of the inner protons would be expected.[14] As the outer and inner aromatic protons are equivalent on the NMR time scale, the net effect of an induced paramagnetic "ring current" over the entire molecule would only affect the chemical shift for the olefinic protons. These are expected to be located outside the ring.



8



9



10

The experimental value for the chemical shift, δ 6.45, is quite close to that of the olefinic protons in *cis*-stilbene, δ 6.53.[15] The UV spectrum of [2.2.2.2]paracyclophanetetraene does not give any conclusive information about planarity, although the maximum shows a red shift of 23 nm relative to that of *cis*-stilbene.[16] A full solution of the problem must await a complete structural determination. This is being done by an X-ray method.

A non-planar structure of [2.2.2.2]paracyclophanetetraene with rapidly rotating benzene rings, still has two mirror planes and three perpendicular C_2-axes of symmetry, one of which coincides with an S_4-axis. The non-planar molecule thus belongs to symmetry point group D_{2d}. The cyclophanes *2*, *4*, *5*, and *6* in which the aromatic rings are pairwise identical have no mirror planes of symmetry of S_4-axes but still three perpendicular C_2-axes. They all belong to the rare symmetry point group D_2.

The hetero[2.2.2.2]cyclophanetetraenes *4* and *5* can adjust towards more planar conformations than cyclophane *1*, with the heteroatoms pointing outwards from the center of the ring. This is even more pronounced in [2.2.2.2](2,5)-thiophenophanetetraene and [2_4(2,5)furano[2]-(2,5)thiopheno[2] (2,5)furano[2] (2,5)thiophenophanetetraene.[17] The NMR spectra of these cyclophanes indicate that a small paramagnetic shielding of the outer protons and deshielding of the inner protons occur in a strong magnetic field and thus the molecules can attain near planar conformations. The UV spectra show a red shift of the maxima on changing the benzene rings for thiophene or furane rings in [2.2.2.2]paracyclophanetetraenes. This is also consistent with existence of near planar conformations with delocalisation of π-electrons in hetero[2.2.2.2]cyclophanetetraenes.

Mass spectra. The mass spectra of [2.2.2.2]-cyclophanetetraenes and related compounds show the high stability of the ring systems on ionisation. The typical fragmentation reactions are the loss of hydrogens and halogens, if present. This could occur accompanied by the formation of new six-membered rings, equivalent to the photochemical ring closure of *cis*-stilbenes. Doubly charged and sometimes triply charged ions are frequent and contribute substantially to the total ion current.

Conclusions. The new method described above for the synthesis of [2.2.2.2]cyclophanetetraenes and related compounds from the readily available dialdehydes and bisphosphonium salts is simple. It can conveniently be employed for the synthesis of many cyclophanes which are interesting intermediates for the preparation of circulenes, annulenes, helicenes, and aromatic cage compounds. Some cyclophanes may also be used as ligands for transition metals.

EXPERIMENTAL

Melting points were determined on a Reichert hot stage apparatus. UV spectra in cyclohexane were recorded with a Beckman DK-2A, MS with a AEI MS 902, IR spectra in KBr with a Beckman IR 9 and NMR spectra in $CDCl_3$ with a Varian A 60 or a Bruker WH 270.

Phosphonium salts. The bistriphenylphosphonium salt from 1,4-bis(bromomethyl)benzene was prepared by the standard procedure.[18] The bistriphenylphosphonium salt from 1,4-bis(bromomethyl)-2,5-dibromobenzene was prepared from triphenylphosphine and 1,4-bis-(bromomethyl)-2,5-dibromobenzene. The latter was prepared by bromination of 2,5-dibromo-1,4-dimethylbenzene with bromine in tetrachloromethane (30 %, m.p. 161–162 °C). ^{1}H NMR: δ 7.75 (2 H, s) and 4.55 (4 H, s). Analysis: C, H and Br. The bisphosphonium salt from 2,5-bis(chloromethyl)thiophene was prepared by the standard procedure from triphenylphosphine and 2,5-bis(chloromethyl)-thiophene [19] in dry dimethylformamide (DMF) at 110 °C. All phosphonium salts were carefully dried under vacuum at 110 °C before use.

Aldehydes. Commercial 1,4-benzenedicarbaldehyde was recrystallised before use. 2,5-Furandicarbaldehyde was prepared from sucrose and hydrochloric acid which gave 5-chloromethylfuran-2-carbaldehyde. A Sommelet reaction gave the desired 2,5-furandicarbaldehyde.[20] 2,5-Thiophendicarbaldehyde was prepared from 2,5-dibromothiophene, butyllithium and DMF or conveniently from 2,5-bis(chloromethyl)thiophene.[21] 2,5-Dibromobenzene-1,4-dicarbaldehyde was prepared by tetrabromination of 2,5-dibromo-1,4-dimethylbenzene with *N*-bromosuccinimide in tetrachloromethane to give 1,4-bis(dibromomethyl)-2,5-dibromobenzene which on mild hydrolysis gave 2,5-dibromobenzene-1,4-dicarbaldehyde (m.p. 170 °C). 4,4′-Biphenyldicarbaldehyde [22] was prepared from 4,4′-dibromobiphenyl,[23] butyllithium and DMF (55 %, m.p. 142–144 °C). 4,4′-Bibenzyldicarbaldehyde was prepared from 4,4′-dibromobibenzyl [23], butyllithium and DMF (62 %, m.p. 125–127 °C).

Wittig reaction, general procedure. The Wittig reactions were run in a three-necked flask equipped with a mechanical stirrer, a nitrogen inlet tube and a dropping funnel with a pressure-equalising side arm. The flask was immersed into a thermostated cold bath. The aldehyde and the phosphonium salt were dissolved or suspended in dimethylformamide, purified by azeotropic distillation with 10 % benzene and then from calcium hydride. The reaction vessel was flushed with nitrogen and a slow stream of nitrogen was maintained during the reaction which was usually conducted at −35 or −40 °C. A freshly prepared solution of lithium ethoxide in ethanol (0.2–0.3 M) was added dropwise in such a way that the coloured ylid was allowed to be consumed between successive additions. The total reaction time varied from a few hours to several days. When no colour change was observed on addition of the base, the reaction mixture was warmed to room temperature and diluted with an equal volume of water. A yellow sticky precipitate was usually formed. The mixture was extracted with diethyl ether three times and the ether extract was then washed with water several times, dried over sodium sulfate and the solvent distilled. The yellow to red residue contained, besides the desired cyclophanes, a large proportion of triphenylphosphine oxide which in a few cases was removed by extraction with warm ethanol. The cyclophanes were separated by chromatography on silica gel (Merck Kieselgel 60, 70–230 mesh ASTM) with tetrachloromethane as eluent. The first fractions usually gave pure [2.2.2.2]cyclophanetetraenes on evaporation and recrystallisation from tetrachloromethane/methanol. The subsequent fractions gave a mixture of *cis* and *trans* isomers of [2.2.2.2.2.2]cyclophanehexaenes as apparent from their simple MS and complex ^{1}H NMR spectra.

Tetrabromo[2.2.2.2]paracyclophanetetraene, 2. To a suspension of 2,5-dibromobenzene-1,4-dicarbaldehyde (3 mmol) and the bistriphenylphosphonium salt from 1,4-bis(bromomethyl)-benzene (3 mmol) in DMF (250 ml) at −40 °C, was added lithium ethoxide in ethanol. The addition required *ca.* 24 h. Subsequent to work-up and purification as described under general procedure, there was obtained tetrabromo-[2.2.2.2]paracyclophanetetraene, *2*, (100 mg, 9 %, m.p. 284–286 °C) as the first yellow compound. IR: cm^{-1} 1503 (m), 1460 (m), 1410 (m), 1340 (m), 1052 (s), 960 (m), 890 (s) and 738 (m). ^{1}H NMR: δ 7.43 (4 H, s), 6.96 (8 H, s), 6.59 (4 H, d) and 6.50 (4 H, d, J 12 Hz). MS (70 eV): *m/e* 728 (20 %), 727 (22), 726 (70), 725 (35), 724 (100), 723 (25), 722 (67), 566 (15), 564 (30), 562 (15), 484 (12), 482 (12), 405 (25), 404 (73), 403 (18), 402 (22), 401 (18), 400 (20), 399 (12), 398 (15), 202 (40), 201.5 (15), 201 (30), 200.5 (20), 200 (40), 199.5 (12) and 199 (25). UV: nm 295 (log ε = 4.71).

The analogous reaction between 1,4-benzenedicarbaldehyde (3 mmol) and the bis triphenylphosphonium salt from 2,5-dibromo-1,4-bis-(bromomethyl)benzene (3 mmol) in DMF (250 ml) at −40 °C gave the same product, tetrabromo[2.2.2.2]paracyclophanetetraene, *2*, (110 mg, 10 %) after the usual work-up procedure.

[2](2,5)Furano[2]paracyclo[2](2,5)furano[2]-paracyclophanetetraene, 4. To a suspension of 2,5-furandicarbaldehyde (35 mmol) and the bis triphenylphosphonium salt from 1,4-bis-(bromomethyl)benzene (35 mmol) in DMF (600 ml) at −40 °C was added lithium ethoxide in ethanol. The addition required *ca.* 55 h. The usual work-up followed by purification by column chromatography gave cyclophane *4* (710 mg, 10.5 %, m.p. 242 °C) as the first yellow compound. IR: cm^{-1} 1182 (m), 1030 (s), 960 (s), 800 (s). ^{1}H NMR: δ 7.08 (4 H, s, H_A), 6.41 (2 H, s, H_B), 6.34 (2 H, d, H_C) and 6.21 (2 H, d, H_D, J_{CD} 12.7 Hz). MS (70 eV): *m/e* 388 (100 %), 196 (12.5). UV: nm 385 (sh), 345 (sh), 320 (log ε = 4.68) and 250 (4.60). Analysis: C, H, O.

Later fractions from the column gave an orange solid (1.04 g, 15 %) believed to be a mixture of larger cyclophanes, mainly isomers of [2](2,5)furano[2]paracyclo[2](2,5)furano[2]-paracyclo[2](2,5)furano[2]paracyclophanehexaene. The ^{1}H NMR spectrum was very complex but the MS showed the base and parent peak at *m/e* 582. The IR spectrum was similar to that of cyclophane *4* with broadened peaks at 1022 (s), 958 (s), 810 (sh) and 795 (s) cm^{-1}.

[2](2,5)Thiopheno[2]paracyclo[2](2,5)thiopheno[2]paracyclophanetetraene, 5. To a suspension of 1,4-benzenedicarbaldehyde (10 mmol) and the bistriphenylphosphonium salt from 2,5-bis(chloromethyl)thiophene (10 mmol) in DMF (250 ml) at −35 °C was added a solution of lithium ethoxide in ethanol over a period of 75 h. After the usual isolation and separation operations, cyclophane *5* (160 mg, 8 %, m.p. 257–259 °C) was obtained as a yellow compound. The corresponding Wittig reaction between 2,5-thiophenedicarbaldehyde (5 mmol) and the bis triphenylphosphonium salt from 1,4-bis(bromomethyl)benzene (5 mmol) in DMF (200 ml) at −40 °C with lithium ethoxide as base gave, after the usual isolation and separation, the same cyclophane *5* (40 mg, 4 %). IR: cm^{-1} 1617 (m), 1402 (m), 1392 (m), 1200 (m), 882 (s) and 797 (s). ^{1}H NMR: δ 7.24 (4 H, s, H_A), 6.80 (2 H, s, H_B), 6.55 (2 H, d, H_C or H_D) and 6.38 (2 H, d, H_D or H_C, J_{CD} 11.8 Hz). MS (70 eV): *m/e* 420 (100 %). UV: nm 335 (log ε = 4.49) and 276 (4.59). Analysis: C, H, S.

[2.2.0.2.2.0]Paracyclophanetetraene 6. To a suspension of 4,4′-biphenyldicarbaldehyde (5 mmol) and the bistriphenylphosphonium salt from 1,4-bis(bromomethyl)benzene (5 mmol) in DMF (250 ml) at −40 °C was added a solution of lithium ethoxide in ethanol. The addition required *ca.* 50 h. After the usual isolation, the solvent was distilled and the pale yellow residue

was extracted with warm ethanol to remove triphenylphosphine oxide. The residue was recrystallised from tetrachloromethane to give [2.2.0.2.2.0]paracyclophanetetraene *6* (208 mg, 15 %, m.p. 246–248 °C). IR: cm^{-1} 1600 (m), 1495 (s), 1412 (m), 892 (s), 830 (s), 748 (s). ^1H NMR: δ 7.50 (4 H, d, H_B or H_C), 7.39 (4 H, d, H_C or H_B, J_{BC} 8 Hz), 7.24 (4 H, s, H_A) and 6.60 (4 H, s, H_D and H_E). MS (70 eV): *m/e* 560 (100 %), 559 (2), 281 (4), 280.5 (4), 280 (8), 279 (4), 278 (4), 277 (4) and 276 (4). UV: nm 312 (log $\varepsilon = 4.79$).

[*2.2.2.2.2.2*]*Paracyclophanetetraene* 9. To a suspension of 4,4'-bibenzyldicarbaldehyde (10 mmol) and the bistriphenylphosphonium salt from 1,4-bis(bromomethyl)benzene (10 mmol) in DMF (250 ml) at −40 °C was added a solution of lithium ethoxide in ethanol. The addition required *ca.* 30 h. The usual isolation and separation gave the pale yellow all-*cis* [2.2.2.2.2.2]paracyclophanetetraene *9*, (280 mg, 9 %, m.p. 262–264 °C) as the first compound. ^1H NMR: δ 7.21 (4 H, d, H_C or H_B), 7.17 (4 H, s, H_A), 7.08 (4 H, d, H_B or H_C, J_{BC} 8 Hz), 6.57 (2 H, d, H_D or H_E), 6.52 (2 H, d, H_E or H_D, J_{DE} 12.5 Hz) and 2.85 (4 H, s, H_F). MS (70 eV): *m/e* 616 (100 %), 512, 308, 205. UV: nm 337 (log $\varepsilon = 4.50$), 280 (4.29, sh) and 237 (4.43). Analysis: C, H.

The cyclophane *9* was quantitatively hydrogenated over palladium on charcoal in benzene (1 atm., 25 °C, 24 h) to give [2.2.2.2.2.2]-paracyclophane,[10b] *10*, (m.p. 211–213 °C). ^1H NMR: δ 6.83 (1 H, s, H_A) and 2.85 (1 H, s, H_B).

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Preparation of an Active Soluble Lactate Dehydrogenase—Nicotinamide Adenine Dinucleotide Complex Using Glutaraldehyde

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The cofactor analogue N^6-[(6-aminohexyl)-carbamoylmethyl]-NAD was bound to beef heart lactate dehydrogenase by the glutaraldehyde coupling method and the enzyme-coenzyme complex subsequently separated from excess of non-coupled cofactor by gel filtration chromatography. The number of cofactor molecules bound per enzyme molecule could be varied between 0 and 8 by altering the coupling time. The enzyme-coenzyme preparation could catalyze the reaction lactate → pyruvate in the absence of externally added free cofactor although with low efficiency.

The enzyme-coenzyme preparation was also immobilised on Sepharose in such a way that possible contact between complexes was minimized (low substitution of the gel) and the activity of the immobilised complex was found to be partly retained, indicating that a cofactor molecule can interact with the enzyme molecule to which it is bound.

One of the major problems to be overcome if enzymes are to be used to their full potential in industrial, analytical and medical applications is that of cofactor dependance. Many enzymes of interest in this field depend on the presence of a cofactor (in stoichiometric amounts) for their activity. Such cofactors are expensive, and because of their relatively small size are difficult to retain in a reaction vessel by use of a membrane, for example, in the same way as enzymes.

This report presents one approach to this problem by covalently binding a cofactor (in this case an analogue of nicotinamide adenine dinucleotide (NAD)) to the enzyme lactate dehydrogenase (LDH:E.C. 1.1.1.27). The method should also be applicable to other NAD-dependant enzymes, and possibly also to other classes of enzymes and other cofactors.

EXPERIMENTAL

Materials and methods. LDH (beef heart type III, 10 mg/ml suspension in $(NH_4)_2SO_4$), NAD (type III), NADH (type III), phenazine ethosulfate (PES), and 2,6-dichlorophenolindophenol (DCPIP) were obtained from Sigma (St. Louis, Mo., U.S.A.). Glutaraldehyde was obtained from BDH (Poole, England). Sephadex G-50 medium and Sepharose 4B CL were obtained from Pharmacia (Uppsala, Sweden), and N^6[(6-aminohexyl)-carbamoylmethyl]-NAD (N^6NAD) was prepared as described earlier.[1] Other chemicals were of analytical grade.

Coupling. LDH suspension (2.5 ml, 25 mg) was dialysed against 2 × 1 l standard buffer (potassium phosphate buffer, 0.1 M, pH 7.5) for 12 h. Eight mg of the dialysed LDH (60 nmol in 1.6 ml) were then equilibrated with N^6NAD (4.4 mg, 5 μmol) at 0 °C for 30 min. Glutaraldehyde (200 μl 0.25 % solution, 5 μmol) was then added and the volume was made up to 2.0 ml with standard buffer and the solution gently mixed. The coupling proceeded for up to 12 h at 4 °C. In a blank experiment, ε-aminocaproic acid (εACA) replaced the N^6NAD. After the reaction period, ethanolamine.HCl (50 μl 4 M solution, 200 μmol) was added to quench the reaction. After 1 h the mixture was separated on a column of Sephadex G-50 medium 1.0 × 100 cm using standard buffer as running buffer. Fractions were collected (1.3 ml; 1.0 ml/min) and subsequently analysed spectrophotometrically at 290, 280, and 266 nm. From these values the LDH and N^6NAD concentrations in the fractions could be determined using two simultaneous equations, with the absorption coefficients for the glutaraldehyde-treated LDH and N^6NAD as follows:

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LDH $A_{290nm} = 1.17$ mg ml^{-1} cm^{-1}
$A_{266nm} = 1.05$ mg ml^{-1} cm^{-1}

N^{6}NAD $A_{290nm} = 1750$ M^{-1} cm^{-1}
$A_{266nm} = 22500$ M^{-1} cm^{-1}

These values were calculated from the spectra of glutaraldehyde-treated LDH and N^{6}NAD preparations of known concentrations using suitable references.

Assay. The pooled protein fractions were assayed for activity, and their activities in the presence of excess NAD were also measured. The reaction assayed was the simultaneous conversion of lactate to pyruvate and NAD to NADH. The NADH formed was reoxidised, using PES, to NAD, and the reduced PES was reoxidised to PES by DCPIP. The decrease in concentration of oxidised DCPIP was followed at 610 nm, where it has an absorption maximum.[2,3]

The fraction to be assayed (10 – 100 µl) was pipetted into a 1 ml cuvette. DCPIP (50 nmol), PES (2 µmol), and standard buffer were added to a final volume of 950 µl. The blank rate was measured, and then lactate (50 µl, 50 µmol) was added to start the reaction. In the cases when NAD was added, its final concentration was 2 mM (2 µmol added).

When the immobilised preparation was assayed, 3 ml cuvettes were used (with magnetic stirring) with a final volume of 2 ml. The immobilised preparation (5 – 100 mg wet weight was weighed into the cuvette, and otherwise the assay mixture concentrations were identical to those of the free solution assay.

Immobilisation. The preparations were immobilised on Sepharose 4B CL (cross linked) by the cyanogen bromide method.[4] Sepharose 4B CL was washed extensively with water on a glass filter, excess water sucked off, and 20 g was then suspended in water (20 ml). Cyanogen bromide (1 g) was dissolved in acetonitrile (5 ml) and added to the stirred, cooled Sepharose suspension. The pH was raised, and maintained at 11.0 ± 0.2 by the careful addition of 1 M NaOH. After 8 min the activated gel was washed with $NaHCO_3$ (0.1 M, 500 ml), and 3.6 g sucked gel (equivalent to 5 ml of settled gel) was added to tubes containing 2.5 mg each of LDH, LDH-N^{6}NAD, or LDH-εACA in $NaHCO_3$ (0.1 M, 10 ml). After coupling overnight at 4 °C, the gels were exhaustively washed with $NaHCO_3$, standard buffer, NaCl (0.5 M), water and standard buffer again, and then assayed. The concentration of gel-bound protein was estimated from spectra of the settled gel in 2 mm light-path cells, using CNBr-treated blank gel as reference.

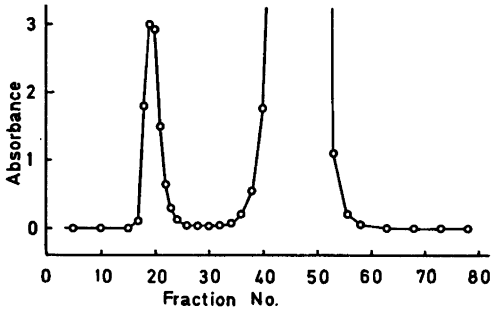


Fig. 1. Gel chromatography separation of N^{6}NAD and LDH-N^{6}NAD obtained from a 6 h coupling. The absorbance is measured at 280 nm (fractions 1 – 32) or 266 nm (fractions 33 – 80).

RESULTS AND DISCUSSION

A typical separation of the coupling mixture is shown in Fig. 1, in which it can be seen that the protein-containing fractions (first peak) are well separated from the fractions containing

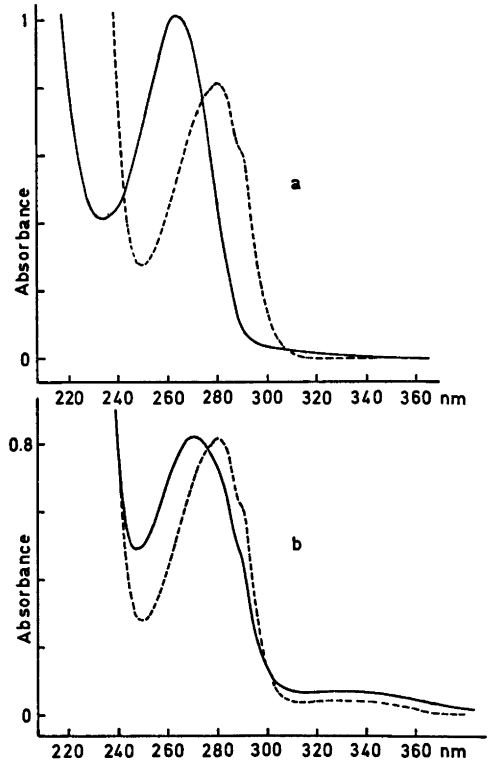


Fig. 2. (a) Spectra of N^{6}NAD (–) and LDH (---). N^{6}NAD 46 µM, LDH 530 µg/ml. (b) Spectra of LDH-N^{6}NAD (–) and LDH-εACA (---) from a 6 h coupling. LDH-N^{6}NAD 390 µg/ml, LDH-εACA 520 µg/ml.

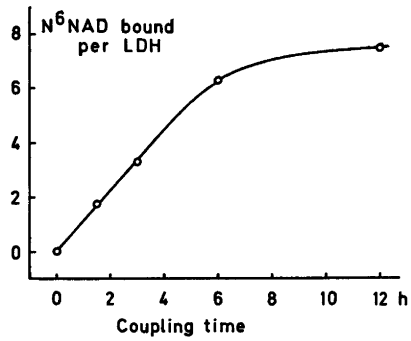


Fig. 3. Dependence on the reaction time of the number of N⁶NAD molecules bound per LDH molecule. Reaction conditions: LDH, 4 mg/ml; N⁶NAD, 2.5 mM; glutaraldehyde, 2.5 mM. After reacting up to 12 h, the reaction was quenched (ethanolamine.HCl 0.1 M, 1 h) and separated.

uncoupled N⁶NAD. A separation of the ε-aminocaproic acid-LDH (LDH-εACA) reaction mixture shows the same protein peak, and a separation of the LDH-εACA mixture to which N⁶NAD had been added after quenching also showed protein and N⁶NAD peaks in identical positions to those found for the separation of the LDH-N⁶NAD mixture. ε-Aminocaproic acid was chosen for the blank substituent because of the similarity it has to the "arm" of the N⁶NAD.

The spectra of LDH, N⁶NAD, LDH-εACA and LDH-N⁶NAD (Fig. 2) show that the wavelengths 290 and 266 nm are suitable for the determination of the concentrations of N⁶NAD and LDH in the coupled preparations. LDH-N⁶NAD shows a peak between 266 and 280 nm, whose exact location depends on the degree of substitution (higher N⁶NAD substitution shifts the peak towards 266 nm). The spectrum of LDH-εACA is almost identical to that of native LDH at wavelengths lower than 300 nm. Between 300 and 360 nm, on the other hand, the spectra of both LDH-εACA and LDH-N⁶NAD have a shoulder, which probably is caused by the glutaraldehyde treatment. This is substantiated by the fact that this shoulder increases with reaction time. Glutar-aldehyde itself shows no absorption in this region. After separation, the spectrum of LDH-εACA to which N⁶NAD was added, after quenching and before separation, is identical to that without N⁶NAD added; that is no N⁶NAD could be detected bound in any way to the LDH-εACA. This was substantiated by activity measurements (see below).

Fig. 3 shows the relationship between the coupling time and the calculated number of N⁶NAD molecules bound per LDH molecule. As can be seen, the coupling reaction is time-dependant, and this indicates that a covalently bound derivative is formed; the longer reaction time, the more N⁶NAD molecules bound per enzyme molecule. At higher concentrations of glutaraldehyde the coupling time must be shortened to avoid too high a substitution or protein denaturation.

In some cases a slight precipitation occurred during separation of the LDH-N⁶NAD preparations on the G-50 column. This never occurred with the LDH-εACA preparations. The pre-

Table 1. Activity of LDH derivatives.

Preparation	Activity[a] Intrinsic	+2 mM NAD
LDH-εACA	0.00(0.00)	530(100)
LDH-εACA[b]	0.06(0.01)	500(94)
LDH-εACA immobilised	0.00(0.00)	220(42)
LDH-εACA immobilised[c]	0.05(0.01)	–
LDH-N⁶NAD	1.7 (0.32)	200(38)
LDH-N⁶NAD immobilised	0.40(0.07)	180(34)

[a] Measured as ΔA min^{-1} mg^{-1} protein. Figures in brackets are percentages of the activity of the LDH-εACA preparation in the presence of 2 mM NAD. [b] N⁶NAD was added to the LDH-εACA reaction mixture after quenching, and after 30 min the mixture was separated. [c] The immobilised LDH-εACA preparation was steeped in N⁶NAD (2.5 mM) for 24 h and then washed (see text).

cipitate was not simply denatured protein, since it could be redissolved on addition of NADH, and this suggests that it was a complex of the LDH-N^{6}NAD which self-aggregated when excess, competing, N^{6}NAD was removed on the column. When this occurred, the pooled protein fractions were centrifuged (20 000 *g*, 10 min) and filtered (Millipore, 0.45 μm) to remove the precipitate. After this treatment, the precipitate did not reappear.

Table 1 summarizes the activities of the various preparations, both in the presence of excess NAD (2 mM), and in the absence of added NAD (the intrinsic activity). For these experiments, we used a preparation that had been coupled for 6 h, and which had 6 N^{6}NAD molecules bound per enzyme molecule. This preparation showed an activity of 1.7 Δ A min^{-1} mg^{-1} protein.

That the LDH and the N^{6}NAD are covalently bound by this procedure is suggested by the following facts:

1. LDH to which N^{6}NAD had been added in the absence of glutaraldehyde showed no activity in the protein peak fractions obtained on separation unless external NAD was added.

2. The activity shown by the LDH-εACA preparation to which N^{6}NAD had been added after quenching but prior to the separation is very low, more than twenty times lower than that of the LDH-N^{6}NAD preparations. This means that less than 5 % of the measured activity of such preparations is due to non-covalently bound N^{6}NAD which is not completely separated on the column.

3. The immobilised LDH-N^{6}NAD preparations retain activity despite rigorous washing conditions (identical to those described earlier), which are sufficient to remove non-covalently bound N^{6}NAD, as was demonstrated when N^{6}NAD was added to the immobilised LDH-εACA preparation, followed by the same washing conditions.

4. The relation between coupling time and substitution, which points to covalent binding.

The fact that the immobilised preparation is also active (although the activity was low) indicates that the reaction assayed is that between an N^{6}NAD molecule and the enzyme molecule to which it is bound ("internal" activity) rather than between one N^{6}NAD and another LDH molecule ("external" activity). The preparation was immobilised at a concentration of 430 μg/ml settled gel, at which concentration there is only a very small probability of external activity because the protein molecules are so far apart. This probability was calculated according to the method of Green.[5]

Although the activities reported here are low, the method is a step towards the solution of the problems of cofactor dependance and retention. The reason for the low activities is partly that the glutaraldehyde treatment is detrimental to the enzyme, considerably affecting its catalytic activity. This, we believe, can be overcome by the use of milder and more suitable coupling agents, and by using techniques that ensure that the coupled cofactor is in the most suitable position on the enzyme. We have found that the intrinsic activity is dependant on the degree of substitution of cofactor on the enzyme to a certain extent, and this indicates that only a proportion of the bound cofactor molecules are available to the active site. With more specific positioning of the N^{6}NAD molecule in the active site before coupling, higher intrinsic activities should be obtained. Preliminary results using bifunctional coupling reagents of differing lengths also indicate, as would be expected, that there is an optimum length.

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Methyl-substituted Semicarbazides

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The entire series (17 compounds) of semicarbazides substituted with one or more methyl groups has been prepared. The 1-methylsemicarbazides unsubstituted in the 2-position were prepared from 1-*tert*-butoxycarbonyl-1-methylhydrazine. The 1-methylsemicarbazide prepared by this unambiguous method proved to be identical with a methylsemicarbazide obtained earlier from the methyl derivative of camphorquinone cyanohydrazone. In some attempts to prepare 1,4,4-trimethylsemicarbazide the main product was 1,5-dimethylcarbonohydrazide.

In connection with NMR and IR spectroscopic studies of semicarbazides, thiosemicarbazides and selenosemicarbazides [1] the complete series derived from semicarbazide (*1*) by substitution with one or more methyl groups has been prepared. Among the 17 possible compounds of this type only the following were known with certainty: 2-methylsemicarbazide,[2] 4-methylsemicarbazide,[3–5] 1,2-dimethylsemicarbazide,[6] 2,4-dimethylsemicarbazide,[7] and 4,4-dimethylsemicarbazide.[7] 1,1-Dimethylsemicarbazide has been described as an intermediate [8] but no melting point or other details were given. A compound alleged to be 1-methylsemicarbazide was obtained by Foster and Saville [9] in a complicated reaction from the methyl derivative of camphorquinone cyanohydrazone but there has been some doubt as to the identity of the product; the compound is marked with a question mark in Beilstein's handbook.

$$\overset{4}{H_2N}-CO-\overset{2}{NH}-\overset{1}{NH_2} \qquad 1$$

Some of the methods we have used for the preparation of hitherto inaccessible thiosemicarbazides [10] and selenosemicarbazides [11] have also been used for the preparation of the missing methyl-substituted semicarbazides. Thus, 1-methylsemicarbazide was prepared in an unambiguous way analogous to one of the methods used for the preparation of 1-methylthiosemicarbazide:[10]

$$H^2N-N(CH_3)CO_2Bu^t + Bu^tNCO \longrightarrow \underset{2}{}$$

$$Bu^tNH-CO-NH-N(CH_3)CO_2Bu^t \xrightarrow{HCl}$$

$$H_2N-CO-NH-NHCH_3$$

By means of mixed melting point, IR and NMR spectra the semicarbazide prepared in this way proved identical with the semicarbazide prepared from camphorquinone cyanohydrazone. Thus Foster and Saville's assignment of the structure of 1-methylsemicarbazide is confirmed.

1,4-Dimethylsemicarbazide and 1,4,4-trimethylsemicarbazide were prepared by reaction of 1-methyl-1-*tert*-butoxycarbonylhydrazine [10] (*2*) with methyl isocyanate and dimethylcarbamoyl chloride, respectively. The *tert*-butoxycarbonyl group was eliminated by treatment with concentrated hydrochloric acid.

The other methyl-substituted semicarbazides were prepared in more conventional ways by reaction of hydrazine or methylsubstituted hydrazines with cyanic acid, methyl isocyanate, or dimethylcarbamoyl chloride.

An attempt was made to prepare semicarbazides unsubstituted in the 4-position by elimination of the *tert*-butyl group from 4-*tert*-butylsemicarbazides. The latter are obtained in almost quantitative yields from hydrazines and *tert*-butyl isocyanate. However, with the exception of 1-methylsemicarbazide mentioned above, none of these semicarbazides could be obtained by this method. The corresponding selenosemi-

carbazides [11] and thiosemicarbazides [10] were obtained in good and low yields, respectively, by elimination of a *tert*-butyl group in the 4-position. This difference in behaviour is not unexpected because the higher basicity of the CO group as compared to the CSe and CS groups render the CO-NH group more susceptible to acid catalyzed cleavage.

1,1,2-Trimethylsemicarbazide, 1,1,2,4- and 1,2,4,4-tetramethylsemicarbazides, and pentamethylsemicarbazide form colourless liquids which were distilled *in vacuo*. The other methyl-substituted semicarbazides form colourless crystals. Great difficulties were, however, encountered in preparing 1,4,4-trimethylsemicarbazide. In our first attempts to prepare this semicarbazide it was only possible to isolate 1,5-dimethylcarbonohydrazide (*3*) together with tetramethylurea. These compounds originate in a disproportionation reaction of the *tert*-butoxycarbonyl derivative of 1,4,4-trimethylsemicarbazide:

$$2Me_2N-CO-NH-NMeCO_2Bu^t \longrightarrow$$

$$Me_2N-CO-NMe_2 + OC(NH-NMeCO_2Bu^t)_2$$

On addition of concentrated hydrochloric acid to the reaction product the dihydrochloride of the hitherto unknown carbonohydrazide (*3*) crystallized.

$$CH_3NH-NH-CO-NH-NHCH_3 \qquad 3$$

All the methyl-substituted semicarbazides are readily soluble in alcohols and in water (some of them are very hygroscopic). They all form blue Cu(II) complexes with aqueous copper(II) salts but differ when the solution is made alkaline: 1-methyl-, 1,4-dimethyl-, and 1,4,4-trimethylsemicarbazide reduce Cu(II) (yellow precipitate of Cu_2O, aq); those disubstituted in the 4-position (or monosubstituted with *tert*-butyl) do not form complexes in alkaline solution [precipitate of $Cu(OH)_2$]; the others give a violet solution (biuret reaction). Unlike 1-methylsemicarbazide, 1,5-dimethylcarbonohydrazide forms a Cu(II) complex with a very intense purple colour in alkaline solution.

The 1-methylsemicarbazides unsubstituted in the 2-position become faintly yellow when exposed to air, possibly because of oxidation to the corresponding azo compounds. The 1,4,4-derivative decomposes rapidly, even when kept in a refrigerator.

EXPERIMENTAL

Satisfactory C, H, N, and Cl analyses have been obtained for all compounds described. The melting points are corrected.

1-Methylsemicarbazide, $C_2H_7N_3O$. A solution of *tert*-butyl isocyanate (4.0 g) and *2* [10] (7.3 g) in ether (50 ml) was kept at room temperature overnight. On addition of light petroleum and cooling 6.5 g of colourless crystals of 1-*tert*-bytoxycarbonyl-4-*tert*-butylmethylsemicarbazide, $C_{11}H_{23}N_3O_3$, separated. M.p. 130 °C after recrystallization from ether/light petroleum.

On addition of 3 ml of conc. hydrochloric acid to 3.0 g of this product it dissolved with the evolution of CO_2. When the effervescence had stopped 1 ml of conc. hydrochloric acid was added and the solution heated to boiling for 2 min. The solution was cooled in ice and made alkaline with solid Na_2CO_3. Ethanol (10 ml) was added and the mixture was heated to boiling and filtered. The filtrate was evaporated to dryness *in vacuo*, the residue was extracted with abs. ethanol, and the ethanol solution was evaporated to dryness and the residue extracted with boiling benzene. On cooling the benzene solution yielded 0.20 g (20 %) of colourless crystals of the title compound, m.p. 94 – 95 °C (chloroform). This semicarbazide was also prepared from camphorquinone cyanohydrazone following the procedure described by Foster and Saville.[9]

The other 4-unsubstituted semicarbazides were prepared by the following general procedure: The appropriate hydrazine was dissolved in the equivalent amount of 4 M hydrochloric acid and the equivalent amount of aqueous NaOCN or KOCN was added. The solution was heated to boiling for 15 min, maintaining a pH value of 8 – 9, and then evaporated to dryness. The residue was carefully dried *in vacuo* and extracted with abs. ethanol. The solvent was evaporated and the residue was recrystallized from the solvents indicated below.

1,1-Dimethylsemicarbazide, $C_3H_9N_3O$, yield 62 %, m.p. 141 – 142 °C (benzene).

1,1,2-Trimethylsemicarbazide, $C_4H_{11}N_3O$, yield 74 %, m.p. 50 – 51 °C (ether/light petroleum).

1,4-Dimethylsemicarbazide, $C_3H_9N_3O$. Methyl isocyanate (3.5 ml) and *2* (9.0 g) in ether/light petroleum yielded 10.6 g of 1-*tert*-butoxycarbonyl-1,4-dimethylsemicarbazide, $C_8H_{17}N_3O_3$ (m.p. 114 – 115 °C, recryst. ether/pentane). The hydrochloride of 1,4-dimethylsemicarbazide, $C_3H_{10}ClN_3O$ (m.p. 125 °C) crystallized by addition of ethanol and ether to a solution of the *tert*-butoxycarbonyl derivative (10.0 g) in conc. hydrochloric acid (10 ml). An additional crop was obtained by evaporating the mother liquor to dryness and recrystallizing the residue from ethanol/ether. Total yield 86 %. The free base was prepared by adding the equivalent amount of aqueous KOH to the hydrochloride, evaporating to dryness and extracting the residue with ether, yield 60 %, m.p. 83 – 84 °C (ether). In

our first preparation of this semicarbazide it could not be obtained crystalline in this way. It was distilled *in vacuo* (b.p. 130 – 131 °C/0.35 mmHg) but still formed a glass on cooling. However, when the latter was heated at 50 °C *in vacuo* for 1 h it was transformed into a crystalline mass.

The other 4-monosubstituted semicarbazides were prepared from the appropriate hydrazine and methyl isocyanate. For the preparation of 2,4-dimethylsemicarbazide and 1,1,4-trimethylsemicarbazide a solution of methyl isocyanate (0.05 mol) in ether was added slowly with stirring to a solution of the hydrazine (0.05 mol) in ether. The reaction was instantaneous and exothermic, the semicarbazides separating as crystalline compounds in almost quantitative yields when light petroleum was added. For the preparation of 1,2,4-trimethylsemicarbazide and 1,1,2,4-tetramethylsemicarbazide it was found advantageous to use benzene as solvent. After evaporation of the benzene these semicarbazides were isolated by distillation *in vacuo*. For the preparation of 4-methylsemicarbazide a solution of methyl isocyanate in benzene was added to a solution of anhydrous hydrazine in ethanol. An insoluble precipitate of 1,2-bis(methylcarbamoyl)hydrazine [7] (m.p. 260 °C) separated. The semicarbazide was isolated from the filtrate by evaporation of the solvent.

1,1,4-Trimethylsemicarbazide, $C_4H_{11}N_3O$, yield 95 %, m.p. 118 – 119 °C (benzene). *1,2,4-Trimethylsemicarbazide*, $C_4H_{11}N_3O$, yield 75 %, b.p. 100 – 101 °C/0.3 mmHg. *1,1,2,4-Tetramethylsemicarbazide*, $C_5H_{13}N_3O$, yield 80 %, b.p. 68 – 69 °C/1 mmHg.

The following 4-*tert*-butylsemicarbazides were prepared from the appropriate hydrazine and *tert*-butyl isocyanate in ether. The reaction is slower than with methyl isocyanate and the solutions were kept for 24 h at room temperature. The residues obtained by evaporation of the ether were recrystallized from pentane.

4-tert-*Butyl-2-methylsemicarbazide*, $C_6G_{15}N_3O$, m.p. 92 – 93 °C. 4-tert-*Butyl-1,2-dimethylsemicarbazide*, $C_7H_{17}N_3O$, m.p. 60 – 61 °C. 4-tert-*Butyl-1,1,2-trimethylsemicarbazide*, $C_8H_{19}N_3O$, m.p. 36 – 37 °C. The *tert*-butyl group was eliminated as *tert*-butyl chloride when these compounds were heated with conc. hydrochloric acid but the expected semicarbazide unsubstituted in the 4-position could not be isolated.

1,4,4-Trimethylsemicarbazide, $C_4H_{11}N_3O$. After some unsuccessful attempts (see below) this semicarbazide was obtained in the following way: Dimethylcarbamoyl chloride (2.2 g; 23 mmol) was added to a solution of *2* (6.0 g; 44 mmol) in ether (50 ml). On standing a precipitate of the hydrochloride of the hydrazine, $C_6H_{15}ClN_2O_2$ (m.p. *ca* 130 °C, decomp.) slowly separated. After 1 month (shorter reaction time resulted in correspondingly lower yields) the filtered solution was purified by column chromatography (Aluminium Oxide W 200 neutral). The main fraction yielded 1.2 g (24 %) of colourless crystals of 1-*tert*-butoxycarbonyl-1,4,4-trimethylsemicarbazide, $C_9H_{19}N_3O_3$, with m.p. 107 – 108 °C (ether/pentane). Treatment of 500 mg of this compound with 0.5 ml conc. hydrochloric acid, addition of ethanol and benzene, filtration and evaporation yielded 300 mg (85 %) of the hydrochloride of 1,4,4-trimethylsemicarbazide, $C_4H_{12}ClN_3O$. M.p. 154 – 157 °C (ethanol/ether). To obtain the free base the equivalent amount of methanolic KOH was added to the hydrochloride, the filtered solution was evaporated to dryness and the residue extracted with ether. Concentration of the ether solution to initiate crystallization followed by the addition of pentane gave a 62 % yield of the semicarbazide. M.p. 79 – 81 °C.

In the first attempts to prepare this semicarbazide equivalent amounts of *2* and dimethylcarbamoyl chloride were used together with an excess of triethylamine in ether. In this case the reaction was much faster. After 3 – 4 days the calculated amount of triethylammonium chloride had separated. The oily product, remaining after removal of ether and excess triethylamine, could not be induced to crystallize and was therefore treated with conc. hydrochloric acid in the usual way. On evaporation of an ethanol/benzene solution to remove water, followed by addition of ethanol/ether a colourless crystalline compound was obtained. This was identified by analysis and its NMR spectrum (D_2O, δ 3.0, s) as the dihydrochloride of 1,5-dimethylcarbonohydrazide, $C_3H_{12}Cl_2N_4O$ (m.p. 154 – 156 °C). Yields corresponded to up to 50 % conversion of dimethylcarbamoyl chloride. The same compound was formed from *2* and carbonyl chloride. The mother liquor from the hydrochloride was neutralized and distilled with ethanol/benzene until water had been removed and the filtered solution was then distilled *in vacuo*. A fraction boiling at 46 – 47 °C/0.35 mmHg was identified as tetramethylurea. A fraction boiling at 110 – 120 °C/0.25 mmHg contained a significant amount of 1,4,4-trimethylsemicarbazide, according to its NMR spectrum.[1] However, attempts to purify it to give a crystalline semicarbazide failed.

The free *1,5-dimethylcarbonohydrazide*, $C_3H_{10}N_4O$, was prepared by addition of the equivalent amount of ethanolic KOH to the hydrochloride, evaporation of the filtered solution and recrystallization of the residue from benzene. Yield 80 %. M.p. 100 – 101 °C. NMR spectrum ($CDCl_3$): δ 2.60 (s, CH_3); 3.84 (s, N^1-H); 7.00 (s, N^2-H).

The other 4,4-dimethylsemicarbazides – except 4,4-dimethylsemicarbazides which was prepared according to Vogelesang [7] – were prepared by the following procedure: A solution of dimethylcarbamoyl chloride (0.05 mol) in ether (50 ml) was added with cooling and stirring to a solution of the hydrazine (0.05 mol) and triethylamine (20 ml) in ether (50 ml). The mixture was kept for 2 h at room temperature or, for the preparation of pentamethylsemi-

carbazide, heated at reflux for 2 h. The solutions were filtered from Et_3NHCl; ether and excess triethylamine were removed *in vacuo*. The residue was recrystallized, or when liquid, distilled *in vacuo*.

2,4,4-Trimethylsemicarbazide, $C_4H_{11}N_3O$, yield 80 %, m.p. 47–48 °C (ether). *1,1,4,4-Tetramethylsemicarbazide*, $C_5H_{13}N_3O$, yield 62 %, m.p. 81–82 °C (ether). *1,2,4,4-Tetramethylsemicarbazide*, $C_5H_{13}N_3O$, yield 60 %, b.p. 48–50 °C/1 mmHg. *1,1,2,4,4-Pentamethylsemicarbazide*, $C_6H_{15}N_3O$, yield 40 %, b.p. 80–82 °C/12 mmHg.

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Comparison of the Proteolytic Specificities of Bovine and Porcine Pepsin A

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Bovine pepsin A inactivates pancreatic ribonuclease through a limited proteolysis the same way as does porcine pepsin A. The specific activity is lower and more dependent on the ionic strength.

The proteolytic specificity of bovine pepsin A has been investigated with B-chain of oxidized porcine insulin as the substrate. The specificity resembles qualitatively that of porcine pepsin but with quantitative differences.

A large majority of all studies on the enzymatic properties of the pepsins from mammalian gastric juice has been carried out with porcine pepsin A (EC 3.4.23.1, reviewed by Fruton [1]). Investigations on substrate specificity have shown that in addition to the amino acid residues adjacent to the peptide bond hydrolysed, there are important interactions with amino acid side chains at secondary binding sites up to four residues from this bond.[2]

Determination of the primary structure of bovine pepsin A is in progress.[3] The results show a high degree of homology between porcine and bovine pepsin A, but also that some 10 to 15 % of the amino acid residues are substituted. The question is now to which extent such substitutions may influence the binding of substrate peptide chains to the enzymes.

Anfinsen [4] observed that porcine pepsin would inactivate ribonuclease through a limited proteolysis during which the *C*-terminal tetrapeptide Asp-Ala-Ser-Val was cleaved off.* Berger *et al.*[5] suggested that this reaction could be used as a general assay for pepsin. We have found that bovine pepsin also inactivates ribonuclease, but that the reaction is more dependent on ionic strength.

The B-chain of oxidized insulin has been widely used for testing the specificities of acidic proteinases (reviewed by Hofmann [6]). Since the degradation of ribonuclease was dependent on ionic strength we have tested the degradation of the B-chain at pH 2 and ionic strengths of 0.01 and 0.2. The results show that porcine and bovine pepsin A attack the same peptide bonds but quantitative differences are observed.

EXPERIMENTAL

Materials

The porcine pepsin was a twice crystallized preparation from Sigma (lot 95B-1270) further purified by ion-exchange chromatography on DEAE-cellulose in 0.1 M sodium acetate (pH 5.1), with a linear gradient 0.2 – 0.5 M NaCl.

The bovine pepsin was prepared from activated bovine pepsinogen by ion-exchange chromatography on DEAE cellulose[7] or by affinity chromatography on polylysine-Sepharose.[8]

Bovine pancreatic ribonuclease was from Miles Seravac Ltd. (lot 36 – 512).

Oxidized insulin B-chain was prepared from swine insulin Novo (lot No. MC-S-169 Ac) by the procedure described by Bang-Jensen *et al.*[9]

Ribonucleic acid (Yeast RNA, Fluka) was freed from interfering low molecular weight components by dialysis against 50 volumes of 0.1 M sodium acetate (pH 5.0) for 20 h and twice against 50 volumes of water for 24 h.

* Abbreviations of amino acids and derivatives as listed in *J. Biol. Chem. 247* (1972) 997 with the addition of Cya = cysteic acid.

Pyridine (Merck, *p.a.*) was distilled over ninhydrin (2 g/l of pyridine) and over potassium permanganate (1 g/l of pyridine). Other reagents were of analytical grade and used without further purification.

Methods

Peptic digestion of B-chain from oxidized insulin. In all experiments reported in this paper 2.4 μmol of B-chain were digested with 3 nmol of bovine or porcine pepsin in 0.01 and in 0.20 M ammonium formate (pH 2.0). The reaction took place at 37 °C for 3 h and was stopped by addition of a few drops of 25 % ammonia to a final pH of 9.0 – 9.5. The digests were lyophilized and submitted to ion-exchange chromatography.

Ion-exchange chromatography. The lyophilized digests were dissolved in 525 μl of 25 % formic acid and 500 μl were applied to a cation exchange column by means of a sample loop valve. The ion-exchange resin (Aminex A-5, Bio-Rad Laboratories) was packed in a 0.9 × 24 cm glass column with a water-jacket. Elution took place at 50 °C with 30 ml of 0.01 M pyridine acetate (pH 1.90), followed by a linear gradient from this buffer to 2.0 M pyridine acetate (pH 4.90), total volume 500 ml. The flow-rate was 30 ml/h and fractions of 3.0 ml were collected. From the effluent 10 % were removed for automatic detection with a Technicon Auto-analyzer system. The peptides were detected by the ninhydrin reaction after alkaline hydrolysis with 10 % of NaOH (100 °C for *ca.* 70 min) and neutralization to pH 5.4 with 5.5 M acetic acid. The ninhydrin colour was developed at 95 °C for 5 min and was measured in a colorimeter fitted with a 570 nm interference filter.

Paper electrophoresis and paper chromatography. Whatman chromatography paper No. 3 MM was used for electrophoresis and chromatography. High voltage paper electrophoresis was carried out in liquid cooled tanks (coolants and buffers as described by Ambler,[10] but with addition of 8 % pyridine to the toluene coolant used for pH 6.5 electrophoresis). As internal marker a mixture of Dns-OH and Dns-Arg was used. The electrophoretic mobilities at pH 6.5 were calculated relative to Dns-OH (– 1.0); at pH 2 the electrophoretic mobilities were calculated relative to Dns-Arg (+ 1.0). Butanol-acetic acid-water-pyridin (15:3:12:10; v/v) was used as solvent for paper chromatography. Peptides were located on the paper sheets by staining with cadmium-ninhydrin reagent.

Characterization of peptides. The *N*-terminal amino acid determination was performed by the dansylation method described by Hartley[11] (5 cm × 5 cm polyamide sheets were used). Amino acid analysis of peptides were performed with a Durrum D-500 amino acid analyzer after hydrolysis with redistilled 6 M HCl in sealed, evacuated tubes at 110 °C for 20 h.

Peptic digestion of ribonuclease. Pepsin solution (100 μl) was added to 250 μl of a solution of ribonuclease (3 mg/ml). Reaction took place for 40 min at 30 °C, and was stopped by mixing 100 μl of the reaction mixture with 2 ml of 0.1 M phosphate buffer (pH 8.0). The pepsin was denatured by continued incubation at pH 8.0 for 15 min at 30 °C, before the ribonuclease assay was carried out.

To identify the degradation product after digestion with bovine pepsin, 200 nmol of ribonuclease were digested with 1 nmol of bovine pepsin in 0.10 M sodium citrate (pH 2.0) for 40 min at 30 °C. After inactivation of the pepsin at pH 8 the mixture was applied to a column of Sephadex® G-25 fine (0.9 cm × 60 cm).

Assay of ribonuclease. Solutions of ribonuclease were diluted by mixing 100 μl with 1.4 ml of 0.1 M sodium acetate (pH 5.0). To this 1 ml of a 0.8 % solution of ribonucleic acid in the same buffer was added. After incubation for 30 min at 30 °C the ribonuclease and high molecular weight RNA was precipitated with 0.5 ml of 0.75 % uranyl acetate in 25 % perchloric acid. The mixture was allowed to stand at room temperature for 20 min. After centrifugation, 100 μl of the clear supernatant were diluted with 5.0 ml of water and the absorption at 260 nm was measured.

To facilitate the comparison of the results we define one ribonuclease inactivation unit (RIU) as the proteolytic activity that will lower the extinction at 260 nm by 0.1 extinction unit under the present experimental conditions for the ribonuclease test. For this calculation we use only the linear part of the curves describing the inactivation of ribonuclease.

RESULTS

Degradation of ribonuclease. Experiments were performed in NaCl/HCl and citric acid/sodium citrate solutions (pH 2.0) with ionic strengths of 0.02 and 0.10. The degradation of ribonuclease relative to pepsin concentration is shown in Fig. 1. The curves show that the two pepsins have different specific activities towards ribonuclease, the bovine enzyme having only 10 % of the activity of the porcine pepsin in 0.02 M chloride. Furthermore it is clear that an increase in ionic strength from 0.02 to 0.10 has an effect depending on the anion present. In chloride solutions increasing ionic strength results in increasing activity. This effect is more pronounced with the bovine enzyme, which in 0.10 M chloride shows 20 % of activity relative to the porcine pepsin. In contrast to this, the activity of both enzymes decreases 10 – 15 % with increasing ionic strength in citrate solutions.

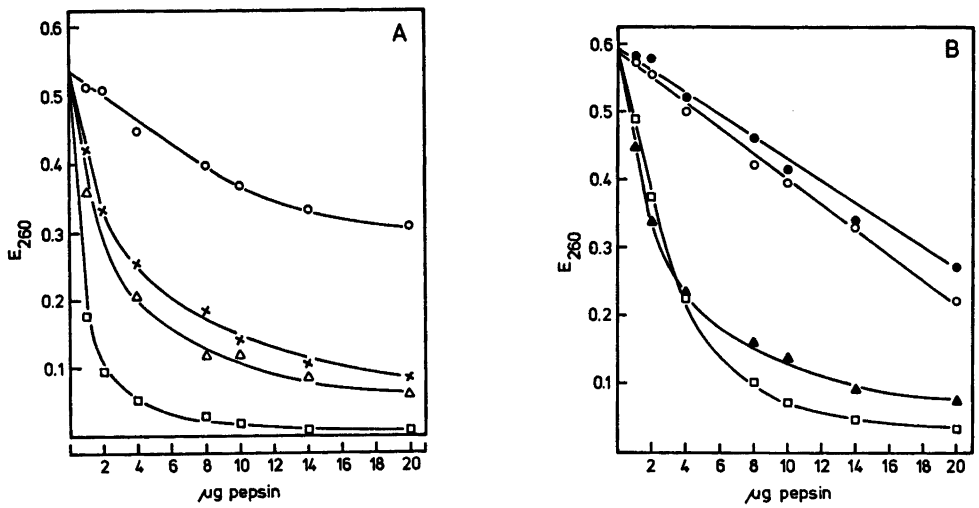


Fig. 1. The degradation of ribonuclease with porcine and bovine pepsins. pH 2.0.

Ionic strength	Specific activity of pepsin, RIU/mg In NaCl (A)		In Na-citrate (B)	
	Porcine pepsin	Bovine pepsin	Porcine pepsin	Bovine pepsin
0.02	1260(△)	120(○)	1270(▲)	200(○)
0.10	3960(□)	820(×)	1120(□)	165(●)

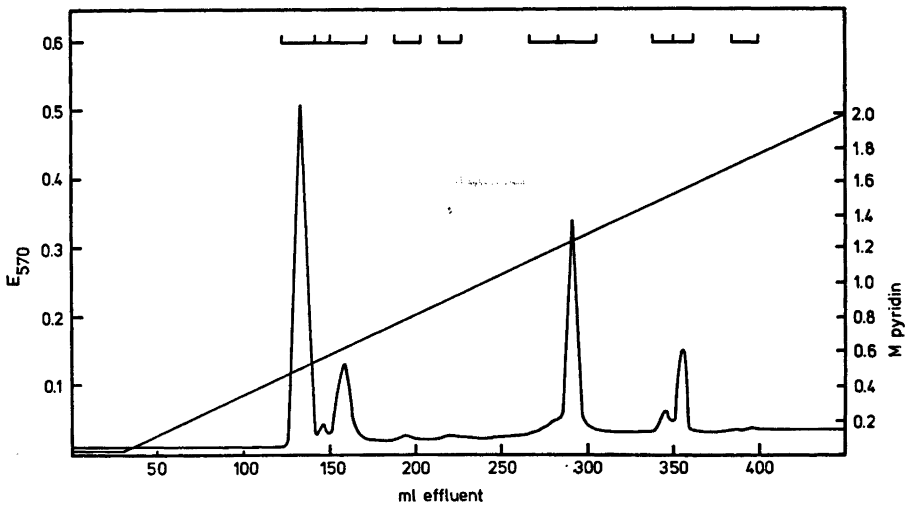


Fig. 2. Ion exchange chromatography of peptides from B-chain of oxidized insulin. The digestion was performed with porcine pepsin in 0.20 M ammonium formate for 3 h.

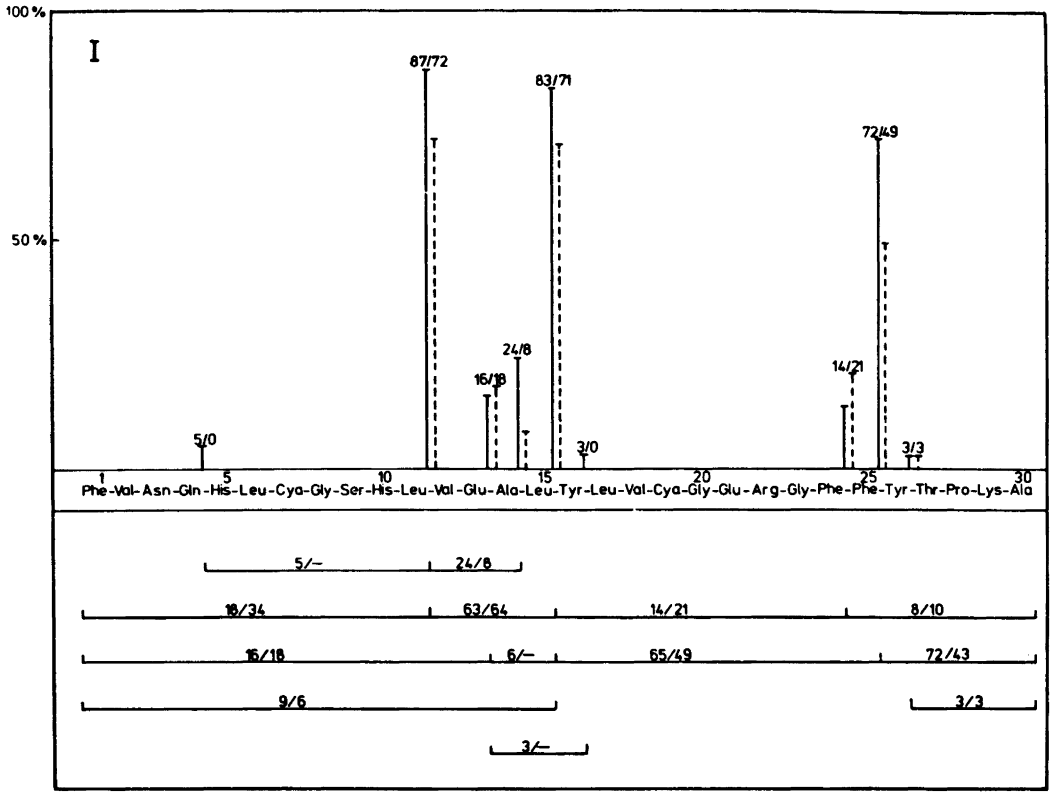


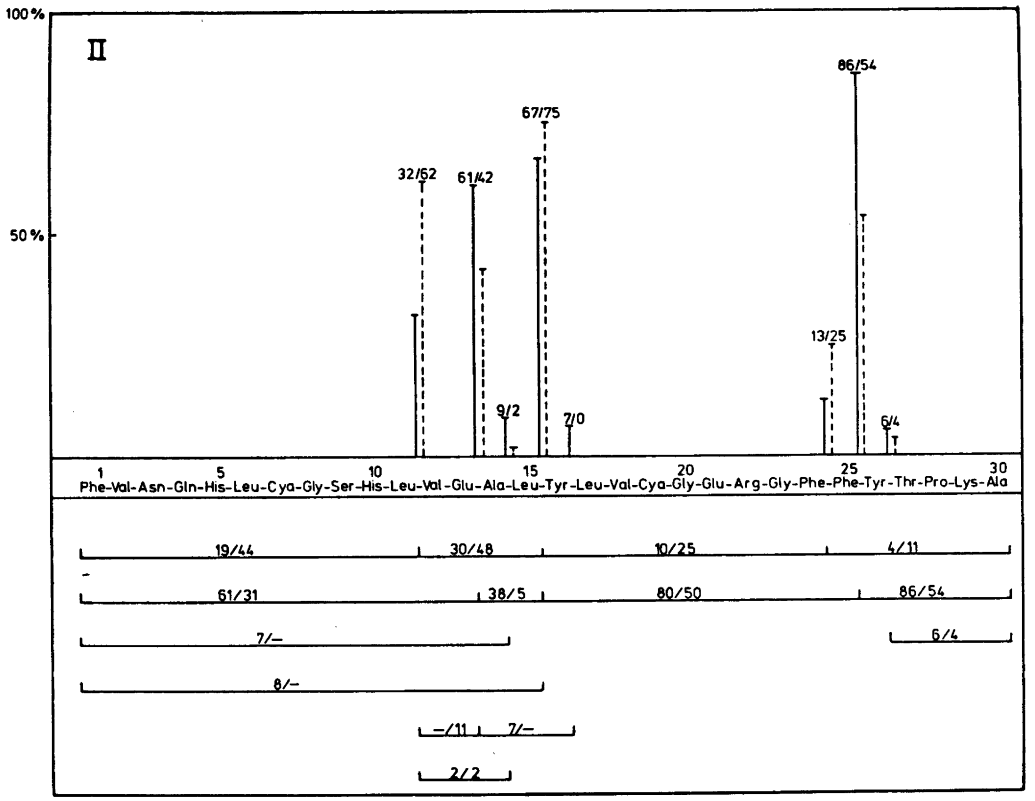
Fig. 3. Yields of peptides and degrees of cleavage of peptide bonds in insulin B-chain after digestion 0.01/0.2 M buffer. Above B-chain sequence: Degrees of cleavage of susceptible bonds in 0.01 M

Table 1. Distribution of peptides in peaks from ion-exchange chromatography on peptic digests of insulin B-chain.

Pool	Relative mobility at pH 6.5	at pH 2	Peptide
I	−0.34		Phe_1-Glu_{13}
I	−0.48		Tyr_{16}-Phe_{24}
I	−0.45		Tyr_{16}-Phe_{25}
I	−0.75		Val_{12}-Leu_{15}
II	−0.75		Val_{12}-Leu_{15}
II	−0.89		Val_{12}-Ala_{14}
II	−1.00		Val_{12}-Glu_{13}
III	−0.27		Phe_1-Leu_{15}
III	0	0.57	Phe_1-Leu_{11}
IV	0	0.76	Ala_{14}-Leu_{15}
V	0	0.54	Ala_{14}-Tyr_{16}
VI	−0.14		His_5-Leu_{11}
VII			No peptide, NH_3
VIII	+0.34		Phe_{25}-Ala_{30}
IX	+0.43		Tyr_{26}-Ala_{30}
X	+0.47		Thr_{27}-Ala_{30}

The elution profile from the experiment, in which ribonuclease digested by bovine pepsin was submitted to gel-filtration, consisted of two peaks only, one at the exclusion volume showing an amino acid composition approximately corresponding to that of total ribonuclease, and a second just preceding the salt volume, absorbing at 220 nm but not at 280 nm. The latter peak had an amino acid composition of 1.04 Asp, 0.82 Ser, 0.96 Ala and 0.99 Val, proving that the same tetrapeptide is released from ribonuclease by degradation with bovine and porcine pepsin. The yield was 150 nmol corresponding to 75 %.

Digestion of B-chain from oxidized insulin. In all four experiments in this series, the elution profile after ion-exchange chromatography was similar to that of Fig. 2, the peptide content of the pools being that listed in Table 1.



with porcine (I) and bovine (II) pepsins for 3 h. Below B-chain sequence: Yields of peptides in (solid line) and in 0.2 M buffer (dotted line).

Some of the pools contained a mixture of peptides. From aliquots of such pools the individual peptides were purified by paper electrophoresis/paper chromatography. Each peptide was then identified by amino acid composition and *N*-terminus. However, it is a general experience that purification of peptides on paper most often gives rather poor yields; so for quantitative determinations of recovery other aliquots of the same pools were hydrolysed and subjected to amino acid analysis. Knowing the peptide composition of each pool (see Table 1) it was in all cases possible to quantify the peptides from the recovery of amino acids characteristic for any given peptide. To compress the presentation of results only one example of detailed calculation is presented in Table 2. In pool I, the yield of the peptide Phe_1-Glu_{13} was calculated from the histidine and aspartic acid content. The sum of the yields of peptides Tyr_{16}-Phe_{24} and Tyr_{16}-Phe_{25} was calculated from the amount of arginine. From the residual amount of phenylalanine the separate yields of these two peptides were calculated, knowing that Tyr_{16}-Phe_{24} was to contain only one phenylalanine, whereas Tyr_{16}-Phe_{25} contains two. The yield of the peptide Val_{12}-Leu_{15} was based on the alanine content. In pool II the yield of the peptide Val_{12}-Leu_{15} was calculated from the leucine content, and in pool III the yield of the peptide Phe_1-Leu_{15} was calculated from the amount of alanine.

In Fig. 3 the yields of peptides in the four experiments are shown below the sequence of insulin B-chain. A dash indicates, that the peptide was present in yields smaller than 2 % of the amount of B-chain originally present, and the yield has not been calculated. The degrees of cleavage of the susceptible bonds are calculated by adding the yields of contributing

Table 2. Recoveries of peptides from a peptic digest of B-chain from oxidized insulin (same experiment as shown in Fig. 2). The first column of pools I, II and III show the total amino acid composition of the pool in nmol, as determined by amino acid analysis after acid hydrolysis. The amounts of material in pools IV, V and VI were less than 2 % of the starting material, and the recoveries of peptides present in these pools have not been calculated.

Peptide	Pool I					Pool II			Pool III			Pool VIII	Pool IX	Pool X
		Phe_1 -Glu_{13}	Tyr_{16} -Phe_{25}	Tyr_{16} -Phe_{24}	Val_{12} -Leu_{15}		Val_{12} -Leu_{15}	Val_{12} -Ala_{14}		Phe_1 -Leu_{15}	Phe_1 -Leu_{11}	Phe_{25} -Ala_{30}	Tyr_{26} -Ala_{30}	Thr_{27} -Ala_{30}
Cya	1 930	435	1 120	500	–	50	–	–	890	150	790	–	–	–
Asp	460	435	–	–	–	–	–	–	980	150	790	–	–	–
Thr	60	–	–	–	–	–	–	–	60	–	–	270	1 020	55
Ser	540	435	–	–	–	–	–	–	1 050	150	790	20	80	10
Glu	3 820	870	1 120	500	1 400	320	110	180	1 120	300	790	–	–	–
Pro	–	–	–	–	–	–	–	–	–	–	–	235	1 025	70
Gly	3 610	435	2 240	1 000	–	55	–	–	900	150	790	15	60	10
Ala	1 380	–	–	–	1 400	270	110	180	130	150	–	220	1 020	60
Val	3 700	870	1 120	500	1 400	300	110	180	1 050	300	790	–	–	–
Leu	3 940	870	1 120	500	1 400	110	110	–	2 180	450	1 580	–	–	–
Tyr	1 420	–	1 120	500	–	–	–	–	–	–	–	230	1 015	–
Phe	3 170	435	2 240	500	–	40	–	–	960	150	790	230	–	20
His	830	870	–	–	–	–	–	–	1 940	300	1 580	–	–	–
Lys	–	–	–	–	–	–	–	–	–	–	–	240	1 030	70
Arg	1 640	–	1 120	500	–	–	–	–	–	–	–	–	–	–
nmol of peptide		435	1 120	500	1 400		110	180		150	790	230	1 025	65
% Recovery		18.5	48.8	21.1	59.3		4.7	7.8		6.3	33.6	9.7	43.4	2.7

peptides on each side of the actual bond and using the higher of the two values. These figures are indicated above the sequence. (Additional experiments with digestion for 24 h did not show alteration in the cleavage pattern).

DISCUSSION

Bovine and porcine pepsin A will inactivate ribonuclease through the same limited proteolysis, the activity of the bovine pepsin being 10 – 20 % of that of the porcine enzyme (depending on the ionic strength). The result is consistent with the investigations of Lang and Kassell,[12] who tested the activity of the two enzymes towards synthetic di- and tripeptides as well as hemoglobin and found that bovine pepsin was a far less effective enzyme than was porcine pepsin.

Previous investigations on the specificity of porcine pepsin with the B-chain of oxidized insulin as substrate have been qualitative although Keil and Keilova[13] attempted a quantitative evaluation. To compare the specific activities of porcine and bovine pepsins a strictly quantitative treatment was required.

The yields of peptides in the present work show that some 80 % of the starting material has been recovered in the identified peptides and no indication of transpeptidation has been observed. The recovery is close to the expected value with the kind of chromatography used, thus assuring that no significant amount of peptide material has gone undetected due to low colour yields in the ninhydrin reaction or otherwise.

The absence of significant tailing in the ion exchange chromatography is illustrated by the low content of cysteic acid, glycine and phenylalanine in pool II as well as the total absence of phenylalanine in pool IX of Table 2.

The results of this work clearly demonstrate that qualitatively the two pepsins show the same kind of specificity, but quantitatively a few significant differences are observed. The present results largely confirm the quantitative figures of Keil and Keilova concerning porcine pepsin with one exception: The Tyr_{16}-Leu_{17} bond, while hardly being cleaved in the present experiments, is a medium cleavage point in the experiments of these authors. Consequently the adjacent Leu_{15}-Tyr_{16} bond exhibits a lower susceptibility in their experiments than in ours. Sanger and Tuppy[14] and Etherington and Taylor[15] on a qualitative basis determined Tyr_{16}-Leu_{17} to be a major cleavage point and Leu_{15}-Tyr_{16} to be a minor if any cleavage point at all. Furthermore these two authors have found cleavage at the bonds Phe_1-Val_2 and Gly_{23}-Phe_{24}, in contrast to the findings of Keil and Keilova and us (even after 24 h digestion). In view of our results showing the profound dependence of the peptic cleavage of a specific peptide bond on ionic strength and the anion present it is not surprising that different experimental conditions as for instance the kind of buffer and buffer concentration used will cause differences in the observed cleavage pattern.

One remarkable finding common to all investigations on the cleavage of the B-chain with porcine pepsin is the differences in susceptibility of the two identical bonds Leu_{11}-Val_{12} and Leu_{17}-Val_{18}, the former being cleaved rather efficiently while the latter is completely resistant. As the oxidized B-chain is supposed to exist in a random chain conformation, we may rule out structural factors as being responsible for the observed differences and assign them to the effect of secondary interaction.

The investigations of Inouye and Fruton[16] on the activity of porcine pepsin towards peptide substrates of the A-Phe-Phe-OEt type, where A included histidine, led them to conclude that a histidine in neighbour position to the sensitive bond might play a specific role in enhancing the susceptibility of this bond. This may be part of the explanation of the effective cleavage of the Leu_{11}-Val_{12} bond, which does not contain any of the amino acids normally considered to make a highly sensitive bond such as phenylalanine, tyrosine and tryptophan. The cumulative effect of absence of a histidine and presence of an adjacent cysteic acid (carrying a negative charge at pH 2, which is known to considerably inhibit the pepsin activity) may contribute to an explanation of the total resistance of the Leu_{17}-Val_{18} bond towards peptic activity.

The comparison with porcine pepsin shows that bovine pepsin follows the same pattern of specificity, but its significantly lower affinity to the Leu_{11}-Val_{12} bond suggests that the possible effect of histidine on the susceptibility

of this bond is less in bovine pepsin than in porcine pepsin.

As the histidine is thought to interact specifically with a subsite on the enzyme with consequent change in catalytic efficiency as mediated through induced conformational changes, it seems most probable that amino acid substitutions in this subsite are responsible for the altered sensitivity to secondary interaction (for the subsite model, see Ref. 17).

As a consequence of the fast cleavage of the Leu_{11}-Val_{12} bond by porcine pepsin, which liberates an α-amino group on Val_{12} this enzyme cleaves the nearby Glu_{13}-Ala_{14} bond only to a limited extent. By contrast, bovine pepsin clearly shows similar affinity for these two peptide bonds.

The differences in extent of cleavage of the bonds Phe_{24}-Phe_{25} and Phe_{25}-Tyr_{26} observed with both enzymes most certainly must be ascribed to some as yet undetermined secondary interaction, as the two types of bonds, when situated in peptide-substrates of the general structure Z-His-X-Y-OEt, are cleaved at comparable rates by the porcine pepsin.[16]

It is interesting to observe that the degrees of cleavage of peptide bonds in the B-chain with both enzymes are strongly dependent on the ionic strength. Most susceptible bonds show less cleavage in the high buffer concentration, but some show the opposite effect, *e.g.* the Phe_{24}-Phe_{25} bond. Some bonds even show different kinds of ionic strength dependence with the two enzymes as for instance the Leu_{11}-Val_{12} bond.

The studies on substrate specificity of proteolytic enzymes have shown that in many cases extended binding areas with a number of subsites contribute to the kinetic specificity of the enzyme. A satisfactory explanation of the observed specificities must account for the nature of such subsites and the way they interact with the side chains of the peptide substrate. One way of attacking the problem is to look at homologous enzymes which show substitutions in one or more subsites and to study the specificity consequences of such alterations. The elucidation of the three dimensional structure of porcine pepsin is near completion.[18] Such a model may enable us to localize the amino acid residues responsible for subsite interaction in this enzyme. We may then look for similarities and differences in the known sequences of homologous enzymes of mammal and microbial origin with the aim of correlating possible substitutions in the subsites with altered proteolytic specificity.

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On the Crystal Structure of an Analgetic Metabolite, Norpropoxyphene Maleate

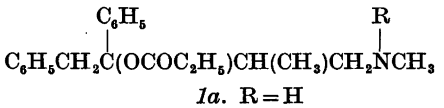
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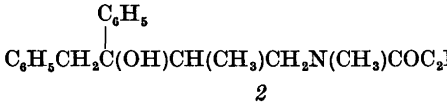
The crystal and molecular structure of norpropoxyphene maleate has been determined by X-ray methods. The crystals are orthorhombic, space group $P2_12_12_1$ with $a=9.365(3)$, $b=9.348(3)$, $c=27.639(6)$ Å. The structure was determined by direct methods and refined by the method of full-matrix least-squares to $R=0.049$ for 1224 observed reflections. The e.s.d.'s for non-hydrogen atoms are (in average) 0.01 Å and 0.5° in bond lengths and angles, respectively.

The *N*-methylpropylamino chain is nearly fully *extended* with the pertinent dihedral angle of $-162.0°$. The present metabolite conformation closely resembles that of its congener, dextropropoxyphene. The three-dimensional structure consists of chains parallel to (100) with alternating norpropoxyphene and maleate ions, formed by $N-H^+\cdots O$ hydrogen bonds of 2.70 Å and 2.76 Å, respectively. The intramolecular $O6-H\cdots O4$ hydrogen bond of the maleate ion is 2.40 Å, both oxygen atoms being displaced by 0.2 Å to the same side of the planar carbon skeleton.

Norpropoxyphene (*1a*) is known to be the principal metabolite of the weak analgetic dextropropoxyphene [1] (*1b*). In alkaline solution (pH > 11) (*1a*) is converted to (*2*) by an intramolecular rearrangement which has to involve a cyclic intermediate.[2]



1a. R = H
1b. R = CH_3



2

Being subjected to such a reaction norpropoxyphene is of interest for structure-activity studies of morphine-like analgetics.*

Some of the present results have been reported earlier.[3]

EXPERIMENTAL

A single crystal of dimensions $0.1\times0.1\times0.4$ mm was selected from commercial norpropoxyphene maleate and utilized for the experiments. Rotational and Weissenberg photographs indicated orthorhombic symmetry and systematic absences are compatible with space group $P2_12_12_1$. The 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}). The scan range was from 0.8° below $2\theta(\alpha_1)$ to 0.8° above $2\theta(\alpha_2)$ and the backgrounds were counted for 0.35 times the scan time at each end of the scan range. Three standard reflections were measured periodically during the data collection. They did not show any systematic variation. The e.s.d.'s of the intensities were taken as the square root of the total counts with a 2 % addition for instrumental instability.

Out of 2489 independently recorded reflections with $\sin\theta/\lambda\leq0.60$, only 1224 had $I\geq2.5\sigma(I)$. The low fraction of observed intensities to the total number (0.48) is partially caused by a small crystal volume (4×10^{-3} Å^3) and partially by large thermal motion of the atoms.

All calculations were performed on a CYBER-74 computer with the programs in Ref. 4, except for the phase determinations.[5] Atomic form factors were those of Hanson *et al.*[6] for O, N, C and of Stewart *et al.*[7] for H.

* Previous part in this series: Bye, E. *Acta Chem. Scand. B30* (1976) 549.

Table 1. Positional and thermal parameters with e.s.d.'s in parentheses. The anistropic temperature factors are given by $\exp(-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+U_{12}hka^*b^*+U_{13}hla^*c^*+U_{23}klb^*c^*)]$.

ATOM	x	y	z	U11	U22	U33	U12	U13	U23
O1	.4503(6)	.3228(5)	.4176(2)	.0815(36)	.0637(32)	.0600(31)	.0149(31)	.0084(31)	-.0142(30)
O2	.3551(5)	.4832(4)	.3708(1)	.0520(28)	.0468(26)	.0419(25)	.0039(25)	-.0004(24)	-.0019(22)
O3	.7761(6)	.4049(6)	.2078(2)	.0518(33)	.0694(34)	.1362(51)	.0051(29)	.0085(35)	.0156(38)
O4	.6073(6)	.2432(6)	.2143(2)	.0713(38)	.0520(34)	.1492(56)	.0048(31)	.0056(37)	-.0152(38)
O5	.1849(6)	.3772(6)	.1856(2)	.0491(35)	.0844(43)	.1463(52)	-.0094(32)	.0026(34)	.0057(39)
O6	.3527(6)	.2311(6)	.2047(3)	.0702(38)	.0501(34)	.1954(69)	-.0043(33)	-.0139(47)	.0007(45)
N	.0359(6)	.6843(5)	.2965(2)	.0494(31)	.0534(33)	.0532(32)	-.0061(31)	.0056(30)	.0013(32)
C1	.6954(10)	.3437(13)	.3562(4)	.0710(63)	.1421(94)	.1419(85)	-.0132(71)	-.0149(62)	.0274(81)
C2	.5596(9)	.3946(8)	.3418(3)	.0616(52)	.0629(50)	.0926(58)	-.0012(50)	-.0175(47)	-.0012(45)
C3	.4438(8)	.3954(8)	.3820(3)	.0502(44)	.0455(43)	.0629(52)	-.0029(44)	.0091(42)	.0050(40)
C4	.2169(8)	.5044(7)	.4042(2)	.0615(41)	.0394(38)	.0369(35)	.0025(38)	.0020(35)	-.0012(34)
C5	.1221(7)	.6134(7)	.3772(2)	.0466(40)	.0448(38)	.0474(41)	.0043(36)	.0013(37)	.0015(34)
C6	-.0118(8)	.6561(8)	.4048(3)	.0716(52)	.0609(45)	.0657(46)	.0133(47)	-.0055(42)	.0025(39)
C7	.0853(7)	.5625(6)	.3263(2)	.0595(43)	.0389(36)	.0568(40)	.0007(33)	.0072(36)	-.0100(36)
C8	.2013(10)	.6467(8)	.2461(3)	.0889(56)	.0700(47)	.0613(44)	.0067(52)	.0169(42)	-.0063(43)
C9	.2746(8)	.5692(7)	.4523(2)	.0663(45)	.0546(41)	.0414(39)	.0081(41)	.0116(37)	.0009(37)
C10	.3678(9)	.7005(8)	.4467(2)	.0692(54)	.0590(52)	.0383(39)	-.0033(47)	.0117(42)	.0013(37)
C11	.5126(10)	.6857(9)	.4418(3)	.0675(58)	.0671(52)	.0854(57)	-.0016(52)	.0118(48)	.0220(46)
C12	.5988(10)	.6030(15)	.4349(3)	.0705(63)	.1154(86)	.0964(71)	-.0231(71)	-.0023(58)	.0360(71)
C13	.5403(14)	.9368(14)	.4324(3)	.0957(84)	.1178(96)	.0691(59)	-.0538(81)	.0120(59)	.0074(61)
C14	.3967(13)	.9551(8)	.4386(3)	.1286(88)	.0393(47)	.0880(59)	-.0178(56)	.0369(61)	-.0104(43)
C15	.3196(8)	.8375(8)	.4453(3)	.0725(53)	.0492(48)	.0776(54)	-.0037(47)	.0217(47)	-.0041(43)
C16	.1382(7)	.3619(7)	.4122(2)	.0642(44)	.0374(37)	.0389(37)	.0048(39)	.0002(39)	-.0081(35)
C17	.0567(7)	.3399(8)	.4536(2)	.1168(43)	.0602(43)	.0606(40)	-.0180(39)	-.0262(39)	.0045(38)
C18	-.0216(9)	.2155(8)	.4591(3)	.1235(54)	.0756(44)	.0659(53)	-.0232(47)	-.0413(47)	-.0101(41)
C19	-.0182(10)	.1136(8)	.4245(3)	.1042(68)	.0518(47)	.0798(57)	-.0132(53)	-.0144(57)	-.0099(49)
C20	.0600(11)	.1301(9)	.3841(3)	.0810(76)	.0497(57)	.0639(51)	-.0044(64)	.0086(55)	.0020(50)
C21	.1379(10)	.2545(8)	.3776(3)	.0547(67)	.0535(50)	.0433(58)	.0027(58)	.0004(52)	.0019(44)
C22	.6486(9)	.3694(9)	.2089(3)	.0600(53)	.0575(52)	.0659(49)	.0029(48)	.0003(44)	.0144(43)
C23	.5406(9)	.4846(7)	.2039(3)	.0596(52)	.0351(39)	.1291(69)	-.0009(44)	.0088(56)	.0103(47)
C24	.4085(8)	.4790(9)	.1989(4)	.0452(48)	.0533(49)	.1476(88)	.0001(42)	.0064(52)	-.0052(58)
C25	.3061(9)	.3551(10)	.1955(3)	.0480(52)	.0713(62)	.0906(60)	-.0016(49)	-.0092(46)	.0131(50)

ATOM	x	y	z	B	ATOM	x	y	z	B
H1C1	.760	.347	.327	8.2	H2C1	.735	.407	.382	8.2
H3C1	.689	.243	.368	8.2	H1C2	.560	.495	.329	5.1
H2C2	.514	.331	.316	5.1	HC5	.181	.702	.375	3.0
H1C6	.015	.589	.438	4.8	H2C5	-.061	.736	.387	4.8
H3C6	-.077	.572	.407	4.8	H1C7	.172	.519	.311	4.8
H2C7	.008	.489	.328	4.0	H1C8	-.031	.734	.228	5.5
H2C8	.088	.607	.230	5.5	H3C8	-.076	.574	.246	5.5
H1N	.105	.752	.296	4.0	H2N	-.043	.722	.310	4.0
H1C9	.191	.596	.473	3.8	H2C9	.332	.494	.469	3.8
HC11	.557	.588	.443	5.2	HC12	.704	.790	.432	7.3
HC13	.603	1.021	.426	6.1	HC14	.355	1.053	.438	5.5
HC15	.205	.851	.449	4.7	HC17	.055	.414	.488	5.6
HC18	-.081	.202	.489	6.3	HC19	-.074	.028	.429	5.6
HC20	.062	.054	.359	4.6	HC21	.195	.267	.347	3.2
HC23	.581	.584	.205	5.5	HC24	.351	.574	.197	5.8
HN6	.459	.236	.209	8.0					

Table 2. Bond lengths (Å) and angles (°) of norpropoxyphene maleate with e.s.d.'s in parentheses.

DISTANCE	(Å)	DISTANCE	(Å)	DISTANCE	(Å)
C1 - C2	1,498(12)	C2 - C3	1,496(11)	C3 - O1	1,194(8)
C3 - O2	1,346(8)	O2 - C4	1,455(8)	C4 - C5	1,544(9)
C5 - C6	1,521(10)	C5 - C7	1,526(9)	N - C7	1,488(8)
N - C8	1,472(9)	C4 - C9	1,558(9)	C9 - C10	1,514(10)
C10 - C11	1,371(11)	C11 - C12	1,375(12)	C12 - C13	1,367(14)
C13 - C14	1,366(14)	C14 - C15	1,376(11)	C15 - C10	1,389(10)
C4 - C16	1,538(10)	C16 - C17	1,393(9)	C17 - C18	1,383(10)
C18 - C19	1,370(10)	C19 - C20	1,348(12)	C20 - C21	1,384(11)
C21 - C16	1,385(9)	C22 - O3	1,237(8)	C22 - O4	1,251(9)
C22 - C23	1,484(11)	C23 - C24	1,320(10)	C24 - C25	1,461(12)
C25 - O5	1,221(8)	C25 - O6	1,264(10)		

ANGLE	(°)	ANGLE	(°)
C1 - C2 - C3	114,3(7)	C2 - C3 - O1	125,1(7)
O1 - C3 - O2	125,0(6)	C2 - C3 - O2	109,7(6)
C3 - O2 - C4	120,9(5)	O2 - C4 - C5	102,7(4)
O2 - C4 - C9	109,3(5)	O2 - C4 - C16	109,7(5)
C5 - C4 - C9	110,8(5)	C5 - C4 - C16	111,4(5)
C9 - C4 - C16	112,4(5)	C4 - C5 - C6	113,9(5)
C4 - C5 - C7	111,7(5)	C6 - C5 - C7	111,0(5)
C5 - C7 - N	110,2(5)	C7 - N - C8	114,3(5)
C4 - C9 - C10	115,4(5)	C9 - C10 - C11	119,9(7)
C9 - C10 - C15	121,9(7)	C10 - C11 - C12	120,9(9)
C11 - C12 - C13	120,1(8)	C12 - C13 - C14	120,1(9)
C13 - C14 - C15	119,6(9)	C14 - C15 - C10	120,9(8)
C15 - C10 - C11	118,2(8)	C4 - C16 - C17	120,5(5)
C4 - C16 - C21	122,0(6)	C16 - C17 - C18	120,3(6)
C17 - C18 - C19	120,9(7)	C18 - C19 - C20	119,6(7)
C19 - C20 - C21	120,5(7)	C20 - C21 - C16	121,4(8)
C21 - C16 - C17	117,3(6)	O3 - C22 - O4	123,2(7)
O3 - C22 - C23	117,8(7)	O4 - C22 - C23	119,0(7)
C22 - C23 - C24	131,2(8)	C23 - C24 - C25	129,8(8)
C24 - C25 - O5	117,5(8)	C24 - C25 - O6	120,3(7)
O5 - C25 - O6	122,1(8)		

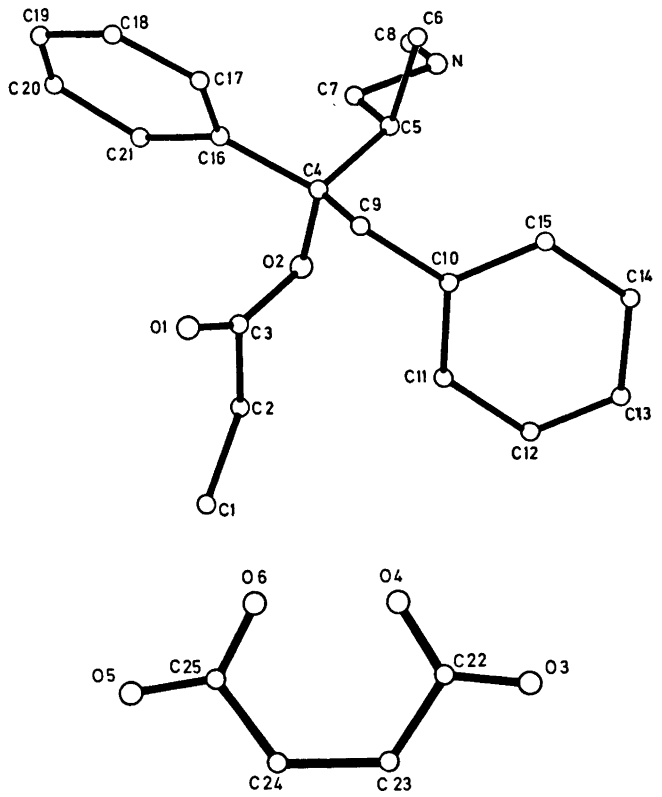


Fig. 1. The asymmetric unit of norpropoxyphene maleate with the atomic numbering.

Table 3. Torsional angles (°) of norpropoxyphene maleate (I) together with those of the hydrochloride [8] (II) and the free base [9] (III) of propoxyphene.

Atoms	Torsional angles I	II	III
C1–C2–C3–O1	−23.5	−5.4	−3.5
C2–C3–O2–C4	−179.3	175.1	180.0
C3–O2–C4–C5	178.9	64.5	−178.6
O2–C4–C5–C6	−179.7	174.5	−178.5
O2–C4–C5–C7	53.6	49.4	57.4
C4–C5–C7–N	−162.0	−176.7	−174.2
C5–C7–N–C8	178.5	173.6	−160.3
C3–O2–C4–C10	61.2	−56.3	63.6
C3–O2–C4–C17	−62.5	−176.4	−59.7
O2–C4–C10–C11	51.2	−42.1	49.8
C4–C10–C11–C12	−90.4	−76.2	−90.6
O2–C4–C17–C18	153.3	−26.1	154.5
C11–C10–C4–C17	173.2	71.8	172.1
C10–C4–C17–C18	31.5	−144.0	32.9
C6–C5–C7–N	69.7	57.8	57.4

CRYSTAL DATA

Nordextropropoxyphene* maleate, $C_{21}H_{27}NO_2 \cdot C_4H_4O_4$, orthorhombic.
$a = 9.365(3)$ Å, $b = 9.348(3)$ Å, $c = 27.639(5)$ Å.
$V = 2419.6$ Å^3, $M = 441.5$, $Z = 4$.
$D_o = 1.22$ g cm^{-3} (flotation), $D_c = 1.21$ g cm^{-3}.
Systematic absences: $h00$, $0k0$, $00l$ for odd indices, space group $P2_12_12_1$.

STRUCTURE DETERMINATION

A preliminary scale factor and an overall isotropic temperature factor ($B = 3.2$ Å^2) were determined by Wilson's statistical method and used to calculate normalized structure factors. The phase determination was carried out with MULTAN [5] utilizing the 320 highest E's

* The dextro-form of norpropoxyphene is studied.

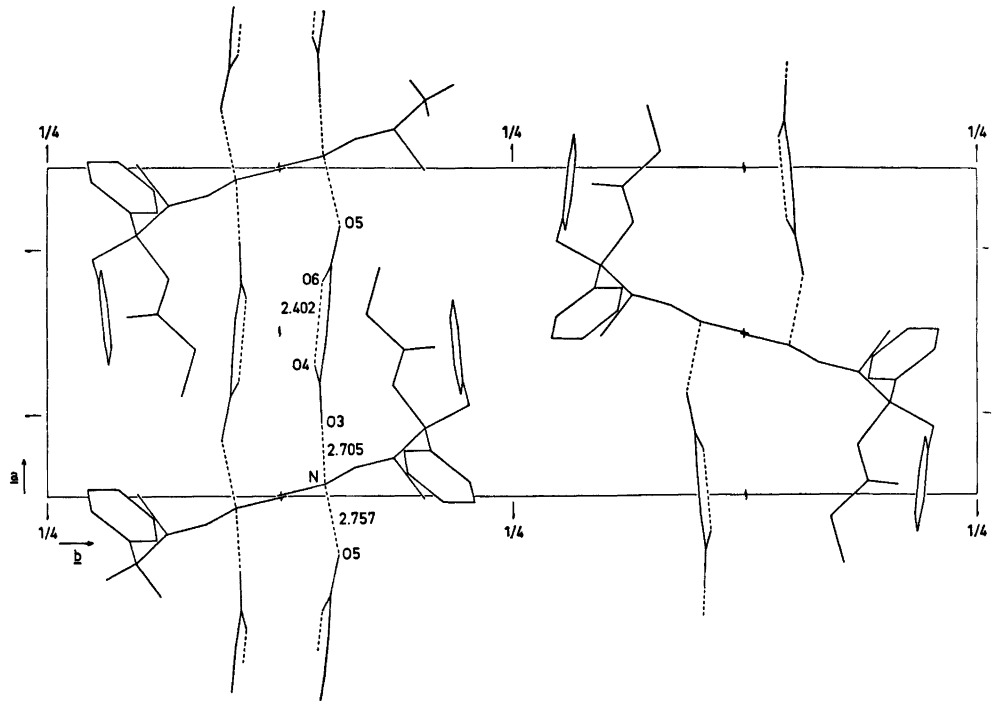


Fig. 2. The crystal structure as viewed down the *b*-axis.

Table 4. Deviations (Å) of individual atoms from a least-squares plane of the maleate ion. Atoms with an asterisk do not define the plane.

C22	0.002	O3*	−0.159
C23	−0.005	O4*	0.178
C24	0.005	O5*	−0.140
C25	−0.002	O6*	0.182

Table 5. Distances of the hydrogen bonds. The hydrogen atoms are excluded as their positional parameters have been calculated and not refined.

Atoms	Distance	Symmetry code
N..O3 (i)	2.707	(i) $x-\frac{1}{2},y+\frac{1}{2},\frac{1}{2}-z$
N..O5 (ii)	2.760	(ii) $\bar{x},y+\frac{1}{2},\frac{1}{2}-z$
O6..O4	2.402	Reference atom = x,y,z

(> 1.25), and all the non-hydrogen atoms were localized in the best *E*-map, except for one single atom. This atom was included after one Fourier synthesis and three cycles of full-matrix least-squares refinement gave $R=0.13$, including isotropic temperature factors. Anisotropic temperature factors were introduced and all the hydrogen atom positions were calculated from stereochemical considerations, taking reasonable hydrogen bonds into account. The hydrogen atoms were ascribed the *B*-value of the heavy atom they are bonded to and only included in the structure factor calculations. The refinement converged at $R=0.049$ ($R_w=0.052$) for the 1224 observed reflections, and the final atomic parameters are listed in Table 1.

A list of observed and calculated structure factors may be obtained from this Department upon request.

DISCUSSION

Bond distances and interbond angles are listed in Table 2 and the asymmetric unit may be seen in Fig. 1.

The norpropoxyphene ion. The bond lengths and angles found in the present compound are

normal. Large atomic thermal vibrations may explain the short terminal C1–C2 distance of 1.490 Å and the C–N$^+$ distances of 1.472 and 1.480 Å, respectively, which are slightly shorter than expected for charged amino groups.

C4 bridges four large groups and the lengthening of the bond distances and distortions of the angles involving this atom indicate the significance of non-bonded interactions in the molecule. The observed values of bond lengths and angles are consistent with those reported for dextropropoxyphene.HCl [8] and the free base.[9]

The intramolecular strain is evident as C9 and C4 are displaced by 0.07 and 0.09 Å from least-squares planes of the two phenyl rings A and B, respectively (see Fig. 1). The interplanar angle between plane A and B is 59.9°, close to 61.3° reported for dextropropoxyphene.[9]

In Fig. 1 it may be seen that the propylamino chain is *extended* in the present case. The pertinent dihedral angle C4–C5–C7–N is −162.0°, close to the values −176.7 and −174.2° found in the hydrochloride and the free base of dextropropoxyphene,[8,9] respectively. Some of these torsional angles are listed in Table 3.

There are only minor differences in the overall conformations of the three compounds, particularly between I and III (see Table 3). The present *extended* propylamine chain seems to confirm earlier suggestions about a reduced conformational flexibility for compounds with the methyl group at C5, as compared to those with C6–Me substituents.[10]

The maleate ion. Ionization of only one carboxyl group is evident from the two equal C–O bond distances C22–O3 [1.237(8) Å] and C22–O4 [1.251(9) Å], whereas the other end of the acid is non-symmetrical. O6 and O4 are engaged in a short intramolecular hydrogen bond of 2.402 Å, close to the values listed by James *et al.*[11] In the present ion, however, the oxygen atoms O4 and O6 are displaced to the same side by 0.18 Å from the planar carbon skeleton C22–C23–C24–C25 (see Table 4). This situation is somewhat different as compared with that normally encountered for maleic acid/maleates,[11] in which the two oxygen atoms most currently are found at opposite sides. This may be due to crystal packing effects (see below).

Molecular packing. The crystal structure is illustrated in Fig. 2 which shows the chains running along the *a*-axis with alternate norpropoxyphene and maleate ions. Each norpropoxyphene unity is linked to two different maleate ions through N–H$^+$···O hydrogen bonds of 2.707 and 2.760 Å. Some of the hydrogen bond parameters are given in Table 5. The molecular chains are connected through v. d. Waals forces only. The slightly short contacts O3···C8 (3.27 Å) and O5···C8 (3.34 Å) may probably force the oxygen atoms O3 and O5 (or O4 and O6) to the same side of the planar backbone of the maleate ion.

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The Crystal and Molecular Structure of Tyramine Hemihydrate

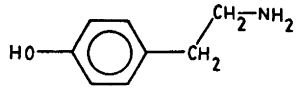
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The crystal structure of tyramine hemihydrate has been determined by X-ray diffraction techniques. The title compound crystallizes in the orthorhombic space group $Pca2_1$ with two tyramine molecules and one molecule of water in the asymmetric unit. Unit cell dimensions are $a = 10.445$ Å, $b = 18.226$ Å, and $c = 8.221$ Å. Refinement by least-squares methods yielded a conventional R-factor of 0.051 for 904 observed reflections. Standard deviations in bond lengths are 0.007 – 0.012 Å and in bond angles 0.7 – 0.9°. The independent tyramine molecules are approximately related by pseudo-centers of symmetry, and are connected by rather short O···O and N···N hydrogen bonds, thereby forming infinite chains along the b-axis. Neighbouring chains are linked through hydrogen bonds to build double molecular layers in the crystals, and water molecules are situated between these double layers.

Tyramine is an indirectly acting sympathomimetic drug, causing the release of endogenous catecholamines from storage sites.

The present work was undertaken as part of a structural study of the free base form of sympathomimetic amines, in order to gain information of the molecular characteristics of this class of drugs. In this compound it is of particular interest to study the nature of the phenolic hydroxyl and amino group, since former structure investigations have demonstrated a zwitterionic character of the directly acting amines (−)adrenaline,[1] (−)noradrenaline,[2] and (−)phenylephrine.[3]



EXPERIMENTAL

Thin crystal plates of the title compound were grown from a hot aqueous solution of tyramine by slow evaporation at room temperature. The crystals appeared to contain two tyramine molecules and one molecule of water per asymmetric unit. A crystal of dimensions 0.30 × 0.17 × 0.05 mm³ was used for the intensity measurements.

The systematic absences of reflections were consistent with space group $Pmca$ and $Pca2_1$ with two tyramine molecules in the asymmetric unit. The latter was chosen from considerations of the molecular packing in the crystals, and was confirmed by the structure analysis.

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 $P\bar{1}$ diffractometer with graphite crystal monochromated Mo$K\alpha$ radiation, using $\omega - 2\theta$ scan technique. The scan speed varied between 1 and 1.3° min⁻¹, the scan range was 1.6°, and the total background counting time was equal to the scan time. The measurements included 1251 unique reflections with $2\theta \leqslant 55°$. Of these, 347 reflections had $I < 2.5\sigma(I)$ and were classified as unobserved. 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[4] for oxygen, nitrogen, and carbon atoms, and of Stewart *et al.*[5] for hydrogen atoms.

Computers employed during the present study are described in Refs. 6 and 7.

CRYSTAL DATA

Tyramine hemihydrate, $C_8H_{11}NO.\frac{1}{2}H_2O$;
Space group $Pca2_1$, orthorhombic;
$a = 10.445(3)$ Å; $b = 18.226(6)$ Å, $c = 8.221(2)$ Å;
$V = 1565.12$ Å³; formula weight 146.19; $F(000) = 632$; $Z = 8$;
D_{obs} (flotation) = 1.20 g cm⁻³; $D_{calc} = 1.241$ g cm⁻³.

STRUCTURE DETERMINATION

The structure was determined by direct methods[6] and refined by full-matrix least-squares technique.

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations for non-hydrogen atoms. The anisotropic temperature factors are given by exp $-2\pi^2(U_{11}a^{*2}h^2+U_{22}b^{*2}k^2+U_{33}c^{*2}l^2+U_{12}a^*b^*hk+U_{13}a^*c^*hl+U_{23}b^*c^*kl)$.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
OW	.2331(6)	.2654(2)	.3545(18)	.077(4)	.066(3)	.099(5)	.005(3)	.023(4)	−.011(4)
OA	.4157(4)	.2083(2)	.0966(11)	.067(4)	.033(2)	.066(5)	−.009(3)	−.024(4)	.009(3)
NA	.3975(6)	−.2147(3)	.2647(11)	.094(5)	.035(3)	.052(5)	.019(4)	.008(4)	−.001(3)
C1A	.3717(8)	−.0050(4)	.2759(13)	.049(5)	.036(5)	.023(4)	.004(4)	.008(4)	−.000(4)
C2A	.4724(8)	.0399(4)	.3274(15)	.048(5)	.038(4)	.044(7)	.005(4)	−.002(6)	.007(4)
C3A	.4875(8)	.1101(4)	.2720(14)	.040(5)	.048(5)	.045(6)	−.015(4)	−.019(5)	−.001(5)
C4A	.4013(8)	.1398(4)	.1555(12)	.054(5)	.039(4)	.033(6)	.005(4)	−.001(6)	−.004(4)
C5A	.3008(8)	.0958(4)	.1041(15)	.037(5)	.034(4)	.052(7)	.002(4)	−.016(5)	−.008(5)
C6A	.2866(8)	.0258(3)	.1615(13)	.045(5)	.036(4)	.042(7)	.004(4)	−.010(5)	−.011(5)
C7A	.3579(9)	−.0826(4)	.3358(15)	.042(5)	.038(4)	.046(7)	.002(4)	.004(5)	.002(4)
C8A	.4129(9)	−.1377(4)	.2165(15)	.073(6)	.038(4)	.047(6)	.008(4)	.016(6)	−.001(5)
OB	.5922(5)	.2806(2)	.2210(11)	.060(3)	.036(3)	.056(4)	.009(3)	−.004(4)	.002(3)
C1B	.6152(7)	.4965(4)	.0510(15)	.037(5)	.033(4)	.051(6)	.002(4)	.007(5)	.006(4)
C2B	.5203(8)	.4496(5)	.0012(14)	.045(5)	.056(6)	.041(7)	−.008(4)	−.009(5)	.006(5)
C3B	.5104(8)	.3784(4)	.0527(14)	.053(6)	.031(4)	.047(7)	−.005(4)	−.003(5)	.000(4)
C4B	.5980(7)	.3509(3)	.1618(13)	.034(4)	.026(4)	.049(6)	.005(3)	.006(5)	.001(4)
C5B	.6935(7)	.3969(4)	.2185(14)	.047(5)	.038(4)	.040(6)	.003(4)	−.004(5)	−.008(4)
C6B	.7033(8)	.4686(4)	.1597(14)	.036(5)	.039(4)	.056(8)	.010(4)	.001(5)	.014(5)
C7B	.6209(10)	.5742(5)	−.0056(15)	.054(6)	.044(5)	.039(7)	−.000(5)	.008(5)	−.004(5)
C8B	.5374(13)	.6239(5)	.0935(17)	.077(7)	.031(4)	.062(7)	−.004(5)	.018(8)	−.001(5)
NB	.5311(6)	.7020(3)	.0328(11)	.071(4)	.040(3)	.053(5)	−.006(3)	−.004(4)	−.002(3)

Table 2. Fractional atomic coordinates and isotropic thermal parameters with estimated standard deviations for hydrogen atoms.

Atom	x	y	z	B
H2A	.531(7)	.014(4)	.401(10)	6.7(25)
H3A	.550(5)	.135(2)	.294(7)	2.0(14)
H5A	.234(6)	.119(2)	.043(8)	3.0(17)
H6A	.200(6)	−.004(2)	.127(8)	4.2(18)
H71A	.276(3)	−.094(3)	.332(12)	4.8(24)
H72A	.419(7)	−.090(4)	.449(11)	6.8(25)
H81A	.535(5)	−.122(2)	.230(7)	1.5(11)
H82A	.354(6)	−.139(3)	.104(11)	6.9(21)
HNA1	.287(5)	−.236(2)	.288(8)	9.3(12)
HNA2	.397(7)	−.226(3)	.377(12)	9.3(21)
HNAB	.462(7)	.743(3)	.149(11)	9.3(17)
HOAB	.502(8)	.229(5)	.143(14)	9.3(32)
H2B	.466(6)	.467(3)	−.081(8)	3.3(19)
H3B	.440(8)	.332(4)	.013(12)	12.3(34)
H5B	.749(6)	.384(2)	.310(8)	2.3(14)
H6B	.771(6)	.501(2)	.207(7)	2.3(12)
H71B	.716(5)	.591(2)	−.020(9)	2.7(16)
H72B	.607(5)	.579(2)	−.108(8)	1.5(15)
H81B	.488(10)	.612(4)	.166(13)	8.0(37)
H82B	.584(7)	.632(3)	.189(10)	4.5(21)
HNB1	.491(5)	.702(3)	−.063(8)	9.3(15)
HNB2	.618(8)	.721(5)	.050(16)	9.3(42)
HOW1	.189(11)	.272(6)	.274(21)	9.3(49)
HOW2	.201(8)	.228(4)	.379(13)	9.3(23)

An E map computed with 379 reflections having $|E| > 1.0$ revealed fragments of the two independent tyramine molecules. After two successive Fourier refinement cycles the positions of the remaining non-hydrogen atoms could be established. The two molecules appeared to be aproximately related by a pseudo-center of symmetry located at $x = 0.50$, $y = 0.25$, $z = 0.16$. Three cycles of least-squares refinement with isotropic temperature factors yielded an R-factor of 0.22. A difference Fourier map calculated at this stage showed an isolated peak of height 2.5 e Å^{-3}, which was assumed to be an oxygen atom of a water molecule. Subsequent anisotropic refinement of all non-hydrogen atoms, including isotropic refinement of hydrogen atoms bonded to carbon in calculated positions, lowered the R-factor to 0.067. In a difference Fourier map there was observed electron density corresponding to one phenolic and five amino hydrogen atoms among the largest peaks. Of these, the phenolic and one amino hydrogen atom were situated near pseudo-centers of symmetry between the two independent oxygen and nitrogen atoms, respectively. Also peaks corresponding to water hydrogen atoms could be located. These hydrogen atoms were included in the last least-squares cycle with a common isotropic temperature factor. The final conventional R-factor is 0.051 and the weighted R-factor is 0.041. A total difference Fourier map showed no electron density exceeding 0.20 e Å^{-3} in magnitude.

The final parameters for non-hydrogen atoms are listed in Table 1 and for hydrogen atoms in Table 2. Bond lengths and angles are given in Table 3. The numbering of the atoms is indicated in Fig. 1, which also presents the molecular arrangement in the crystals.

The structure factor list may be obtained from this institute upon request.

DESCRIPTION OF THE STRUCTURE

The independent tyramine molecules can both be described as consisting of two nearly planar parts: a phenol part and a fully extended ethylamine side chain. The deviations of the ring carbon atoms from a least-squares plane defined by these atoms are less than 0.007 Å in molecule A, and in molecule B less than 0.017 Å. The deviations of the exocyclic atoms from the corresponding least-squares planes are 0.024 Å(OA), 0.037 Å(C7A), −0.007 Å(OB), and −0.042 Å(C7B). The dihedral angles C1−C7−C8−N and C6−C1−C7−C8 are −177.5 and 80.6°, respectively, for molecule A and −175.2 and −94.2° for molecule B. This is the conformation normally observed for phenylethylamine derivatives in the crystals.[1-3, 8]

Table 3. Bond lengths (Å) and bond angles (°) with estimated standard deviations in parentheses.

	Molecule A	Molecule B
Bond length		
C1−C2	1.398(12)	1.372(10)
C2−C3	1.367(11)	1.369(11)
C3−34	1.421(11)	1.376(11)
C4−C5	1.388(10)	1.383(10)
C5−C6	1.369(10)	1.397(10)
C6−C1	1.410(11)	1.380(11)
C1−C7	1.505(10)	1.492(12)
C7−C8	1.518(11)	1.498(12)
C8−N	1.468(9)	1.511(11)
C4−O	1.348(8)	1.372(7)
Bond angle		
C1−C2−C3	122.3(9)	123.6(9)
C2−C3−C4	120.5(9)	119.8(8)
C3−C4−C5	117.7(7)	118.6(7)
C4−C5−C6	121.1(9)	120.2(9)
C5−C6−C1	122.2(9)	121.3(8)
C6−C1−C2	116.3(7)	116.5(8)
C2−C1−C7	121.5(8)	121.8(9)
C6−C1−C7	122.1(8)	121.7(8)
C3−C4−O	121.6(8)	122.8(7)
O−C4−C5	120.7(8)	118.6(8)
C1−C7−C8	112.0(8)	112.4(8)
C7−C8−N	114.7(8)	114.5(9)

None of the chemically equivalent bond lengths of the two independent tyramine molecules can within the error of the experiment be regarded as significantly different. The greatest discrepancy observed is between the two C−N bonds, which differ by more than 3σ. The C−N bond of molecule A (1.468 Å) corresponds to a normal C−NH_2 bond length, and that of molecule B (1.511 Å) agrees with the value usually found for a C−NH_3^+ bond. However, as there is a certain degree of correlation between the two molecules, it is question-

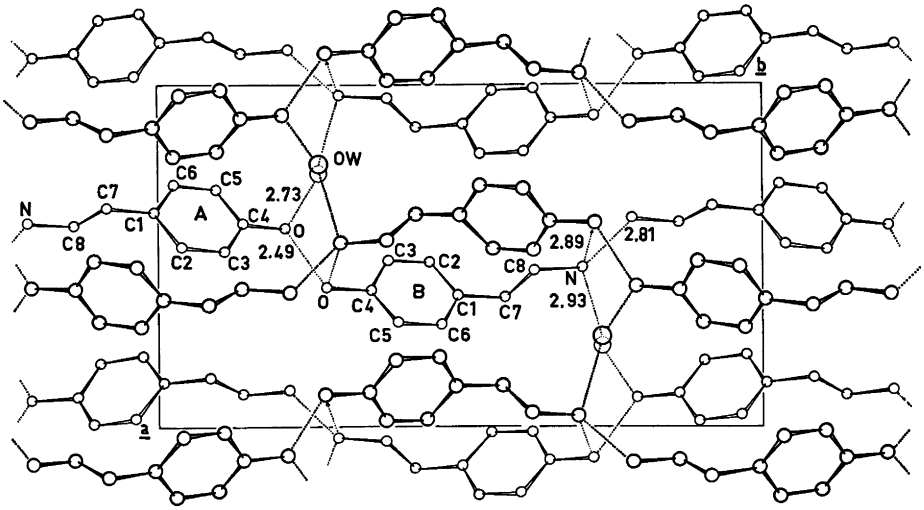


Fig. 1. The structure as viewed down the *c*-axis with hydrogen bonds (Å) indicated.

able if this difference can be regarded as real. Thus, the quality of the experiment does not allow any explicit conclusion regarding the characteristics of the tyramine molecules as compared with that of the free bases formerly investigated.[1–3] The latter molecules are observed to exist as zwitterions in the crystals having a protonated amino group and a negatively charged *meta* oxygen atom. There are, however, differences in the molecular packing arrangement of tyramine from that of the compounds just mentioned indicative of some disparities in molecular properties. The crystal structures of the free bases existing in a zwitterionic form [1–3] are all characterized by chains of ions, which are arranged head to tail with short contacts between positively charged nitrogen atoms and negatively charged oxygen atoms. In the tyramine crystals on the other hand, the crystallographic independent molecules are connected by rather short O···O (2.49 Å) and N···N (2.81 Å) hydrogen bonds across pseudocenters of symmetry. The crystals are thus consisting of chains of molecules connected head to head and tail to tail, as illustrated in Fig. 1. Although the hydrogen positions are uncertain, as may be seen from the temperature factors in Table 2, the electron density observed near the pseudocenters of symmetry indicates that one could describe the tyramine molecules as existing in the crystals in a state intermediary that of neutral molecules and zwitterions. Thus, it seems that the tyramine molecules differ somewhat in character from that of adrenaline.[1] noradrenaline,[2] and phenylephrine,[3] for which an obvious zwitterionic state is observed.

A common feature for all of the free bases hitherto investigated is the presence of double molecular layers in the crystals. For tyramine the double layers run parallel to (100) and are produced by N···O hydrogen bonds between adjacent molecular chains. Specific for the tyramine crystals is the presence of water molecules, which are situated between molecular double layers and are linking these through hydrogen bonds. As the pseudosymmetry observed does not apply to the water molecules, the crystals also contain cavities across which there are only van der Waals contacts between the double layers of tyramine molecules. The hydrogen bond distances are given in Table 4a and are also indicated in Fig. 1. Short intermolecular contacts are presented in Table 4b. The water molecule is probably involved in two hydrogen bonds; one as a donor (to OA) and one as an acceptor (from NB). Furthermore, the tyramine molecule A is linked by hydrogen bonds to two neighbouring B molecules, whereas molecule B is hydrogen bonded to two A molecules and two B molecules within a double layer.

Table 4.

a. Hydrogen bond lengths (Å)	
OW···OA $(-x+\frac{1}{2},y,z+\frac{1}{2})$	2.73
NB···OW $(-x+1,-y+1,z-\frac{1}{2})$	2.93
OA···OB (x,y,z)	2.49
NA···NB $(x,y+1,z)$	2.81
NB···OB$(-x+\frac{1}{2},y,z-\frac{1}{2})$	2.89
b. Other intermolecular contacts (Å)	
OW···OA (x,y,z)	3.04
OW···NB (x,y,z)	3.44
OW···C4A (x,y,z)	3.32
OA···NA $(-x+1,-y,z-\frac{1}{2})$	3.36
OA···C4B (x,y,z)	3.27
OA···C3B (x,y,z)	3.27
OB···C3A (x,y,z)	3.32
OB···C4A (x,y,z)	3.30
NA···OB $(x-\frac{1}{2},-y,z)$	3.43
C1A···C6A $(-x+\frac{1}{2},y,z+\frac{1}{2})$	3.62

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Synthesis and Structure Determination of Thiazolo- and Thiazinopteridinones

ERIK FALCH

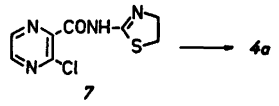
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A method for structure determination of dihydro derivatives of thiazolo- and thiazinopteridinones based on the UV and mass spectra of the hydrolysis products is described.

Previously we described the synthesis of isomeric 2,3-dihydro-thiazolo[3,2-*a*]pyrimidinones,[1,2] identified on the basis of UV and mass spectra of the hydrolysis products. In the present paper we extend the synthesis to the following dihydro derivatives of thiazolo- and thiazinopteridinones, representing the novel ring systems: 5*H*-thiazolo[2,3-*b*]pteridine (*3a* and *3c*), 4*H*,6*H*-thiazino[2,3-*b*]pteridine (*3b* and *3d*), 5*H*-thiazolo[3,2-*a*]pteridine (*4a*), and 3*H*,6*H*-thiazino[3,2-*a*]pteridine (*4b* and *4d*). Again, UV and mass spectra of the products formed by hydrolysis of the ring systems permit unequivocal structure assignment by reasoning similar to that previously employed.[1,2]

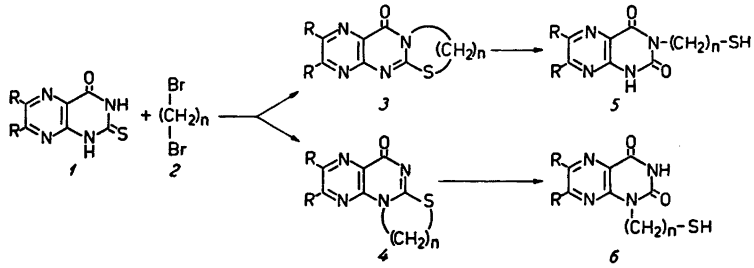
Reaction of the sodium salt of a 2-thiolumazine (*1*) with an α,ω-dibromoalkane in 2-propanol, in the presence of sodium hydrogen carbonate, afforded dihydrothiazolo- or dihydrothiazinopteridinones (Scheme 1). With 2-thiolumazine (*1*, R = H) only one isomer, *3a* or *3b*, could be detected, whereas 6,7-dimethyl-2-thiolumazine (*1*, R = CH_3) afforded a major (*3c* or *3d*) and a minor (*4c* or *4d*) isomer.

Reaction of 3-chloropyrazinoyl chloride with 2-amino-2-thiazoline in chloroform gave the 3-chloropyrazinecarboxamide (*7*) in high yield and as the sole product. Heating of 7 in DMF resulted in ring closure to *4a* (Scheme 2).

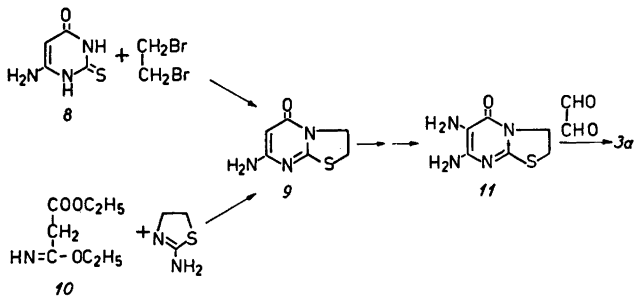


Scheme 2.

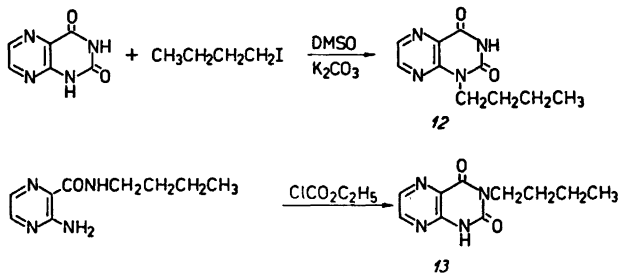
Analogously, reaction of 6-amino-2-thiouracil (*8*) with 1,2-dibromoethane gave solely the 2,3-dihydro-7-amino-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (*9*) (Scheme 3), formed also as the only product in the reaction of ethyl 2-carbethoxyacetimidate (*10*) and 2-amino-2-thiazoline. Nitrosation of *9*, followed by reduction, afforded 2,3-dihydro-6,7-diamino-5*H*-thi-



Scheme 1. *3 – 6 a*: R = H, *n* = 2; *b*: R = H, *n* = 3; *c*: R = CH_3, *n* = 2; *d*: R = CH_3, *n* = 3.



Scheme 3.



Scheme 4.

azolo[3,2-*a*]pyrimidin-5-one (*11*) convertible to *3a* upon reaction with glyoxal in hydrochloric acid.

Hydrolysis of *3* and *4* in aqueous alkali or acid resulted in opening of the thiazoline ring and formation of the 1- or 3-(ω-mercaptoalkyl)-lumazines (*5* and *6*) (Scheme 1). As model compounds 1- and 3-butyllumazine (*12* and *13*) were synthesized, the latter by an unambiguous route (Scheme 4).

Mass spectrometry. In a search for means of distinguishing between compounds of type *3* and *4*, their mass spectra were compared (Table 1). Conspicuously characteristic is the different character of the base peaks, consisting for compounds *3* of the molecular ions, and for compounds *4* of the $M^+ - CO$ ions.

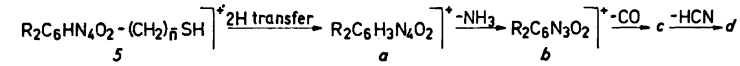
Mass spectrometric data for the hydrolysis products, *5* and *6*, and for the synthetic analogues, *12* and *13*, are listed in Table 2.

The typical fragmentation pattern of compounds of type *5* and of *13* analogous to the fragmentation pattern of 3-alkyluracils,[1,2] is shown in Scheme 5.

An alternative path for the 3-(3-mercaptopropyl)lumazines (*5b* and *5d*) produces ions one mass unit higher. A possible course of this fragmentation is outlined in Scheme 6. The unusual fragmentation, *i.e.* loss of the side chain with transfer of three hydrogen atoms and formation of the ion *e* was confirmed by high resolution mass spectrometry * on *5b*.

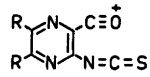
Contrary to the uracil series, the mass spectra of compounds *6* and *12* exhibit ions of the same type as outlined in Scheme 5 for compounds *5*. Significantly, however, *6* and *12* also fragment in a manner characteristic for type *6* compounds

* The author is indebted to Dr. Gustav Schroll, Department of General and Organic Chemistry, University of Copenhagen, for the high resolution mass spectral data.



Scheme 5.

Table 1. Relative intensities (in %) of the most abundant ions in the mass spectra of *3* and *4*.

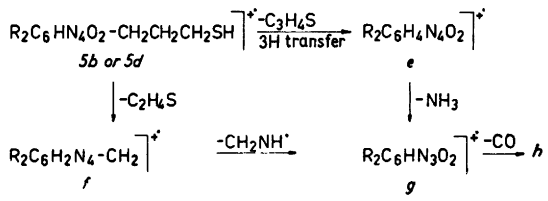


Y

Comp.	M+	M+−CH_3	M+−CO	M+−CHO	M+−SH	Y	Y−CO	M+−CH_3CN	M+−CH_3CN−CO
3a	100		3	14		6	7		
3b	100	26	3	5	16	13	12		
3c	100					3	4	77	28
3d	100	24	4		15	16	10	39	56
4a	36		100	15					
4c	75		100	11			3		
4d	83		100	7	5	9		3	4

Table 2. Relative intensities (in %) of the most abundant ions in the mass spectra of *5*, *6*, *12*, and *13*.

Comp.	M+	M+−SH	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h/n*	*i*	*j*	*k*	*l*	*m*
5a	2		100	24	33	12									
5b	34	15	67	38	52	26	100	95	28	35	5			11	3
5c	12		100	15	36	4									
5d	55	23	80	19	49	9	39	100	21	51	3			9	9
13	46		100	42	44	21	11	48	15	19	7			8	3
6a	4		100	6	6	10					3	22	13	38	3
6c	29		100		6	4					3	31	21	22	20
6d	25	100	13	12	10	7	4	23		9	4	33	18	37	13
12	93		100	25	21	22	12	43	8	57	9	65	28	60	80

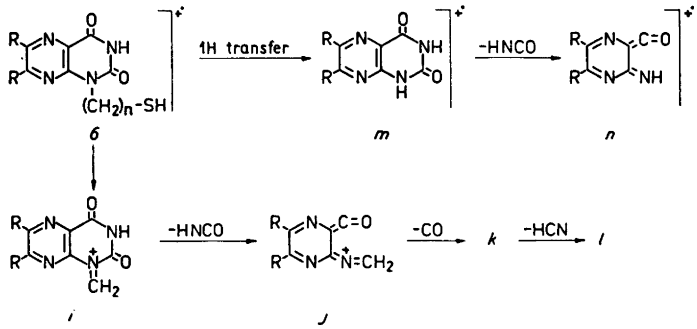


Scheme 6.

(Scheme 7). Thus, the ions *i*−*l*, particularly *j* and *k*, clearly identify them as originating from 1-substituted lumazines. Hence, the tricyclic compounds whence they derive must be thiazolo- or thiazino[3,2-*a*]pteridinones.

UV spectroscopy. The UV spectra of compounds *3* and *4* were recorded (see Experimental); a distinction between the two types of compounds on the basis of their UV spectra is hardly possible.

In Table 3, the UV spectra of the hydrolysis products *5* and *6* and of the two *N*-butyllumazines, *12* and *13*, in ethanol and in 0.1 N sodium hydroxide, respectively, are listed. The maxima at 330 nm are bathochromically displaced in base for both types of compounds, but in the 1-alkyllumazine series the displacement is only 5−10 nm, while the 3-alkyllumazines exhibit an alkali shift of 30−40 nm. These results are in agreement with previously reported data for



Scheme 7.

Table 3. UV spectra of the 1- or 3-substituted lumazines. $\lambda_{max}[\lambda_{infl}]$ nm (log ε).

Comp.	In EtOH				In 0.1 N NaOH			
5a	207(3.99)	234(4.12)		327(3.83)	219(4.44)	243(4.29)	270 (4.02)	364(3.79)
5b	206(4.00)	235(4.14)		325(3.48)	218(4.34)	243(4.26)	[267](4.01)	360(3.76)
5c	209(4.14)	236(4.13)		330(3.85)	220(4.48)	243(4.27)	272 (4.05)	364(3.86)
5d	209(4.18)	237(4.11)		331(3.96)	221(4.26)	245(4.31)	[270](4.09)	363(3.87)
13	206(3.96)	235(4.17)		327(3.84)	220(4.14)	248(4.22)	[270](3.94)	365(3.74)
6a	206(3.99)	237(4.11)		329(3.80)	218(4.34)	241(4.27)		338(3.75)
6c	210(4.15)	235(4.06)	250 (4.02)	332(3.92)	219(4.40)	243(4.32)		341(3.93)
6d	211(4.09)	235(4.03)	251 (3.98)	332(3.90)	224(4.17)	245(4.34)		341(3.96)
12	206(3.96)	236(4.12)	[250](3.96)	331(3.81)	218(4.38)	243(4.20)		338(3.80)

N-alkyluracils,[1,2] where a bathochromical shift in base was exhibited only by the 3-alkyl derivatives. Clearly, UV spectroscopy of the products formed by hydrolysis of the ring systems constitutes an efficient tool for structure assignment of compounds of type *3* and *4*.

EXPERIMENTAL

Melting points are uncorrected. Mass spectra were recorded with an LKB 9000 mass spectrometer fitted with an all-glass heated inlet system; an ionizing potential of 70 eV was used. UV spectra were recorded on a Unicam s.p. 820 recording spectrophotometer. Thin layer chromatography (TLC) were performed on silica gel (Merck) without fluorescence indicator; solvent system: butanol – acetic acid – water (4:1:5). Spots were visualized in UV light.

2,3-Dihydro-5H-thiazolo[2,3-b]pteridin-5-one (3a). To a refluxing suspension of $NaHCO_3$ (25.9 g) and 1,2-dibromoethane (87.2 g) in 2-propanol (210 ml) was added a solution of 13.9 g of 2-thiolumazine (*1*, R = H) and 3.1 g of NaOH in 750 ml of 60 % 2-propanol. The mixture was refluxed for 1 h and evaporated. The residue was suspended in 150 ml of water and the insoluble compound filtered off. Yield 7.71 g (50 %). After recrystallizations from DMF the m.p. was > 300 °C. Anal. $C_8H_6N_4OS$: C, H, N, S. UV [EtOH (log ε)]: 231(3.91), 261(4.05), 280(4.05), 232(3.80) nm.

2,3-Dihydro-1H,6H-thiazino[2,3-b]pteridin-6-one (3b). Reaction of 13.0 g of 2-thiolumazine and 44.1 ml of 1,3-dibromopropane in the same manner as described above for *3a* yielded, after treatment with water, a sticky compound. One recrystallization from DMF gave 4.77 g (30 %). After two recrystallizations from DMF the m.p. was 293 – 294 °C (dec.). Anal. $C_9H_8N_4OS$: C, H, N, S. UV [EtOH (log ε)]: 243(3.98), 295(4.15), 337(3.76) nm.

2,3-Dihydro-7,8-dimethyl-5H-thiazolo[2,3-b]-pteridin-5-one (3c) and 1,2-Dihydro-7,8-dimethyl-5H-thiazolo[3,2-a]pteridin-5-one (4c). To a refluxing suspension of 28.2 g of $NaHCO_3$ and 43.7 ml of 1,2-dibromoethane in 260 ml of 2-propanol was added a solution of 17.11 g of 6,7-dimethyl-2-thiolumazine [4] (*1*, R = CH_3) and 3.29 g of NaOH in 935 ml of 60 % 2-propanol. The mixture was refluxed for 1 h. The precipitate was filtered off and the filtrate evaporated. The residue was extracted with boiling EtOH. The EtOH was evaporated yielding 17.81 g (92 %) of a crude product, shown by TLC to consist of two compounds. The mixture was

recrystallized three times from DMF without achieving separation of the two components. Part of the recrystallized compound (3.80 g) was fractionated on an alumina column. Elution with $CHCl_3$ afforded 2.85 g of *3c* (m.p. 269–271 °C). Further elution with MeOH-$CHCl_3$ (1:3) gave 0.62 g of *4c*, m.p. > 300 °C. *3c*. Recrystallization from ethanolic DMF afforded pure *3c*, m.p. 271–272 °C (dec). Anal. $C_{10}H_{10}N_4OS$: C, H, N, S. TLC: $R_F = 0.33$. UV [EtOH (log ε)]: 248(4.04), 284(4.11), 327(388) nm. *4c*. Compound *4c* was recrystallized from ethanolic DMF; m.p. > 300 °C. Anal. $C_{10}H_{10}N_4OS$: C, H, N, S. TLC: $R_F = 0.17$. UV [EtOH (log ε)]: 225(4.12), 261(4.21), 334(4.01), 343(sh, 3.97) nm.

2,3-Dihydro-8,9-dimethyl-4H,6H-thiazino[2,3-b]pteridin-6-one (3d) *and 1,2-dihydro-8,9-dimethyl-3H,6H-thiazino[3,2-a]pteridin-6-one* (4d). To a refluxing suspension of 50.4 g of $NaHCO_3$ and 61.5 ml of 1,3-dibromopropane in 350 ml of 2-propanol was added, over a period of 3 h, a solution of 53.2 g of the sodium salt of 6,7-dimethyl-2-thiolumazine (*1*, R = CH_3) in 1250 ml of 50 % 2-propanol. The mixture was refluxed for 2 h, and cooled. The precipitate was filtered off, and the filtrate was evaporated. Water (200 ml) and chloroform (250 ml) were added to the residue. The layers were separated, and the aqueous phase was extracted with 150 ml of chloroform. The combined chloroform extracts were dried and evaporated. The residue was triturated with 200 ml of acetone yielding 19.1 g. The crude product was shown by TLC to consist of two compounds. R_F = 0.49 and $R_F = 0.35$. *3d*. Three recrystallizations from ethanol yielded *3d*, m.p. 227–229 °C (dec.) (68 % of the crude product). Anal. $C_{11}H_{12}N_4OS$: C, H, N, S. TLC: $R_F = 0.49$. UV [EtOH (log ε)]: 246(4.06), 295(4.18), 333(3.83) nm. *4d*. The crude product (14.7 g) was extracted with two 750 ml portions of boiling ethyl acetate yielding 1.74 g of an insoluble compound. The latter was fractionated on an alumina column. The column was eluted with ethyl acetate, mixtures of ethyl acetate and chloroform, and chloroform, in this order. The last fractions contained the isomer with $R_F = 0.35$. After recrystallisation from ethanol, 0.90 g of pure *4d* was obtained. M.p. 297–300 °C (dec.). Anal. $C_{11}H_{12}N_4OS$: C, H, N, S. UV [EtOH (log ε)]: 222(4.13), 261(4.16), 295(sh, 3.96), 325(4.06), 339(sh, 3.99) nm.

3-Chloro-N-(2-thiazolinyl)pyrazinecarboxamide (*7*). A suspension of 14.0 g of 3-hydroxypyrazinecarboxylic acid [5] in 100 ml of thionyl chloride and 0.5 ml of DMF was refluxed for 1 h. The dark solution obtained was evaporated. The residue was dissolved in 70 ml of dry $CHCl_3$ and added, over a period of 1 h, to a suspension of 25.5 g of 2-amino-2-thiazoline in 150 ml of $CHCl_3$. The temperature was kept at 10 °C during the addition. The mixture was stirred at room temperature for 2 h and the precipitate was collected, suspended in 100 ml of water, and stirred for 1 h at room temperature. The insoluble compound (10.3 g) was collected and washed with water. When the reaction mixture was left at room temperature for 2 days an additional crop (10.7 g) separated. The two portions were combined and recrystallized twice from ethanolic DMF yielding 15.1 g (62 %), m.p. 177–178 °C (dec.). Anal. $C_8H_7ClN_4OS$: C, H, Cl, N, S.

1,2-Dihydro-5H-thiazolo[3,2-a]pteridin-5-one (*4a*). A solution of 11.2 g of *7* in 50 ml of DMF was refluxed for 4 h. After cooling, the precipitate (8.14 g, 86 %) was collected and recrystallized twice from DMF. M.p. 258–260 °C. Anal. $C_8H_6N_4OS$: C, H, N, S. UV [EtOH (log ε)]: 221(4.11), 261(4.14), 288(sh, 3.76), 328(3.91), 345(sh, 3.81) nm.

2,3-Dihydro-7-amino-5H-thiazolo[3,2-a]pyrimidin-5-one (*9*). (*a*) In the same manner as described above for *3a*, 6-amino-2-thiouracil (*8*) (21.5 g) was reacted with 1,2-dibromoethane (80 ml). After treatment with water 14.0 g (56 %) was obtained. After two recrystallizations from ethanolic DMF, the m.p. was 274–277 °C. Anal. $C_6H_7N_3OS$: C, H, N, S. UV [EtOH (log ε)]: 223(4.19), 273(3.72) nm.

(*b*) A mixture of 3.45 g of 2-amino-2-thiazoline and 7.80 g of ethyl 2-carbethoxyacetimidate [6] in 50 ml of ethanol was stirred at room temperature for 2 h and then refluxed for 3 h. The mixture was cooled and the precipitate (2.28 g, 40 %) was collected. The filtrate was evaporated to half its volume and left at room temperature for several days when a further amount of compound had separated (2.41 g, 34 %). The two crops were combined and recrystallized from ethanolic DMF, yielding *9* m.p. 272–275 °C. The identity with the compound prepared above was established by IR, UV, and TLC.

2,3-Dihydro-6-nitroso-7-amino-5H-thiazolo-[3,2-a]pyrimidin-5-one. To a 35 °C warm solution of *9* (8.45 g) in 300 ml of 10 % acetic acid was added a solution of 3.80 g of $NaNO_2$ in 20 ml of water. The mixture was stirred at room temperature for 5 h. Yield 9.11 g (92 %). Recrystallizations of a small sample from DMF yielded a pure compound, m.p. 239–242 °C (dec.). Anal. $C_6H_6N_4O_2S$: C, H, N, S.

2,3-Dihydro-6,7-diamino-5H-thiazolo[3,2-a]-pyrimidin-5-one (*11*). To a suspension of 0.99 g of the nitroso compound in 30 ml of boiling water, sodium dithionite was added in small portions until the blue colour disappeared. After refluxing for a further 5 min the solution was cooled. Yield 0.54 g (58 %), m.p. 204–207 °C (dec.) (from water). Anal. $C_6H_6N_4OS$: C, H, N, S. UV [EtOH (log ε)]: 223(4.20), 295(3.85) nm.

2,3-Dihydro-5H-thiazolo[2,3-b]pteridin-5-one (3a) *from* 11 *and glyoxal*. Compound *11* (0.46 g) was dissolved in 4 ml of boiling 3 N HCl. The heating was discontinued, and 0.97 ml of a 30 % aqueous solution of glyoxal was added. The mixture was cooled, the precipitate (0.42

g, 82 %, m.p. > 300 °C) was collected and identified by IR, UV, and TLC as the previously synthesized *3a*.

3-(2-Mercaptoethyl)lumazine (5a). A suspension of 3.83 g of *3a* in 50 ml of 3 N NaOH was refluxed for 2 h. The solution obtained was acidified with HCl and the precipitate collected. Yield after one recrystallization from 40 % aqueous EtOH: 2.95 g (57 %), m.p. 212–213 °C. Anal. $C_8H_8N_4O_2S$: C, H, N, S.

3-(3-Mercaptopropyl)lumazine (5b). A suspension of 0.44 g of *3b* in 5 ml of 3 N NaOH was kept at 50 °C for 10 min. The mixture was stirred at room temperature for 2 h and the resulting solution was acidified. The precipitate (0.41 g, 86 %) was collected and recrystallized from EtOH. M.p. 192–194 °C. Anal. $C_9H_{10}N_4O_2S$: C, H, N, S.

3-(2-Mercaptoethyl)-6,7-dimethyllumazine (5c). The compound was prepared by hydrolysis of *3c* by the general procedure described above for *5a*. The yield was 93 %. After recrystallizations from aqueous EtOH, the m.p. was 258–262 °C. Anal. $C_{10}H_{12}N_4O_2S$: C, H, N, S.

3-(3-Mercaptopropyl)-6,7-dimethyllumazine (5d). A suspension of 0.30 g of *3d* in 10 ml of 3 N hydrochloric acid was refluxed for 1 h. The precipitate (0.28 g, 88 %, m.p. 221–223 °C) was recrystallized from ethanol without change in m.p. Anal. $C_{11}H_{14}N_4O_2S$: C, H, N, S.

1-(2-Mercaptoethyl)lumazine (6a). Hydrolysis of *4a* with 3 N NaOH yielded after acidification, 80 % of *6a*. After recrystallizations from EtOH the m.p. was 213–215 °C. Anal. $C_8H_8N_4O_2S$: C, H, N, S.

1-(3-Mercaptoethyl)-6,7-dimethyllumazine (6c). Hydrolysis of *4c* with 3 N NaOH yielded 84 %, m.p. 214–216 °C (from EtOH). Anal. $C_{10}H_{12}N_4O_2S$: C, H, N, S.

1-(3-Mercaptopropyl)-6,7-dimethyllumazine (6d). Hydrolysis of *4d* with 3 N NaOH yielded 77 %, m.p. 193–194 °C (from EtOH). Anal. $C_{11}H_{14}N_4O_2S$: C, H, N, S.

1-Butyllumazine (12). To a solution of 4.15 g of lumazine in 50 ml of DMSO was added 3.3 g of K_2CO_3, followed by 1.47 g of butyl iodide. The mixture was stirred at 90 °C for 3 h, cooled, and poured onto ice (100 g). The precipitate was removed and the filtrate acidified with HCl. Extraction with chloroform and evaporation of the chloroform yielded an oil, from which a solid compound could be isolated after trituration with water. The compound (1.13 g, 64 %) was recrystallized twice from acetonitrile. M.p. 174–176 °C (dec.). Anal. $C_{10}H_{12}N_4O_2$: C, H, N.

3-Amino-N-butylpyrazinecarboxamide. A suspension of 6.0 g of methyl 3-aminopyrazinecarboxylate [7] in 30 ml of butylamine was refluxed for 2.5 h. The solution was evaporated and the residue was recrystallized from ligroin (b.p. 80–110 °C). Yield 6.2 g (82 %), m.p. 56–57 °C. Anal. $C_9H_{14}N_4O$: C, H, N.

3-Butyllumazine (13). A solution of 6.2 g of 3-amino-*N*-butylpyrazinecarboxamide in 120 ml of ethyl chloroformate was refluxed for 7 h. The solution was evaporated, and the oily residue was added to a solution of 6.0 g of sodium in 300 ml of EtOH. The solution was refluxed for 4 h and evaporated. The residue was dissolved in 50 ml of water and acidified. The precipitate (6.1 g, 89 %) was recrystallized from EtOH. M.p. 214–215 °C (dec.). Anal. $C_{10}H_{12}N_4O_2$: C, H, N.

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Hydroxylaminolysis of Anilides. IV. α-Effect in the Hydroxylaminolysis of *p*-Nitroanilides. Unexpected Influence of *p*-Nitro Substituent

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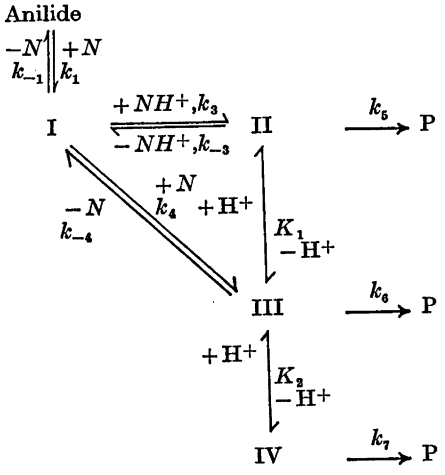
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The kinetics of the hydroxylaminolysis of *p*-nitroformanilide has been studied in the $[H^+]$ range $10^{-5.37} - 10^{-9.79}$, and of *p*-nitroacetanilide with 10 and 80 % of the hydroxylamine present as free base. The total hydroxylamine concentration varied from 0.2 to 3.0 M, the ionic strength being kept at 3.0 by addition of KCl. The reaction rates are in agreement with a mechanism previously postulated for anilides, where the primary addition intermediate (I, Schemes 1 and 2) is broken down to products *via* three cyclic intermediates (II, III and IV), which are in protolytic equilibrium. At low concentrations of the hydroxylamine system, the breakdown of I also seems to be catalyzed by H^+.

As a consequence of the *p*-nitro substitution, the rate of nucleophilic attack of hydroxylamine on the carbonyl is lowered. This unusual effect is explained by hydrogen bonding of the amide group with the atom in α-position to the nucleophile.

We have earlier postulated [1] that the hydroxylaminolysis of acetanilide follows a mechanism where the breakdown of the initial anilide-hydroxylamine intermediate (I) is assisted by the hydroxylammonium ion (NH^+) as well as by the hydroxylamine molecule (N), (see Scheme 1, where P denotes products). The investigation was later expanded [2] to see if the mechanism was applicable to other anilides as well. These anilides were originally chosen so that both electron donating and electron withdrawing ring substituents were represented. We found that for all the anilides studied, the hydroxylaminolysis reaction could be described by the mechanism postulated for acetanilide.

In the course of the investigation we also found, however, that the electron withdrawing substituent influenced the rate of the nucleophilic attack on the carbonyl carbon in an unusual direction, *i.e.* in the same direction as the electron donating *p*-methoxy group. In alkaline hydrolysis, the rate of the nucleophilic attack on the carbonyl carbon is usually enhanced a hundredfold or more by the introduction of a *p*-nitro group.[3] In the hydroxylaminolysis reaction, however, it is about ten times lower for *p*-nitroformanilide than for formanilide. This is even more surprising as electron donating substituents have been found to behave normally,[2] *i.e.* decrease the rate of the nucleophilic attack.



Scheme 1.

Table 1. Observed pseudo first order rate constants for the formation of *p*-nitroaniline from *p*-nitroformanilide at different concentrations of the hydroxylamine system. The percentages denote the fraction of free hydroxylamine base. The k_{obs} values at 99.9 and 99.97 % have been corrected for alkaline hydrolysis.[3]

	k_{obs}/min^{-1} 10^{-5}										
C	10.00 %	20.00 %	40.00 %	60.00 %	80.00 %	*C*	90.00 %	*C*	99.00 %	99.90 %	99.97 %
0.20	7.8	7.3	6.1	5.5	6.0	0.20	3.9	1.00	80.0	80	80
0.60	18.0	19.0	25.0	33.1	32.0	0.60	29.0	1.78	244	—	—
1.00	39.3	42.6	63.3	79.1	84.2	1.00	83.5	1.80	—	250	250
1.40	59.6	78.4	116	147	159	1.38	167	2.59	293	—	500
1.80	87.9	114	177	238	258	1.79	260	2.60	—	500	—
2.20	115	164	258	347	373	2.16	367				
2.60	144	212	339	454	499	2.57	512				
3.00	177	263	440	568	637	2.83	596				

We now present the rate constants for the hydroxylaminolysis of *p*-nitroformanilide and *p*-nitroacetanilide and offer an explanation for the unexpected behaviour of the *p*-nitrosubstituted anilides.

MATERIALS AND METHODS

p-*Nitroformanilide* was prepared by nitration of formanilide.[4] M.p. 194.5 – 195.5 °C, lit.[5] 194 – 195 °C.

p-*Nitroacetanilide* was prepared analogously. M.p. 214.5 – 215.5 °C, lit.[6] 214 – 216 °C.

All other chemicals used were Merck chemicals, reagent grade.

The kinetic experiments and the assay were performed as in our previous work,[2] with the following modification: To increase the solubility of the *p*-nitroacetanilide the reaction mixtures contained 20 % (v/v) dimethyl sulfoxide (DMSO). In the case of *p*-nitroformanilide no DMSO was needed.

The initial concentration of *p*-nitroacetanilide was 1.6×10^{-3} M and of *p*-nitroformanilide between 8×10^{-5} and 4.4×10^{-4} M. The absorption coefficient for the diazotized and coupled *p*-nitroaniline at 550 nm was 48 700 M^{-1} cm^{-1} in water and 50 000 M^{-1} cm^{-1} in the DMSO-water medium.

The k_{obs} values for *p*-nitroformanilide were determined from plots of log [remaining anilide] against time. Depending on the reaction rate, 20 – 50 % of the reaction was observed during the kinetic runs. In the case of *p*-nitroacetanilide the reaction was observed only initially (less than 4 % reacted) and the k_{obs} values were evaluated from plots of concentration of product against time. The determination was done graphically or by means of a Hewlett-Packard HP 9810A calculator (least squares regression).

The evaluation of the parameters in the dependence of p on $[H^+]$ was done on an IBM 370/155 computer, using a least squares regression program.

RESULTS

Observed pseudo first order rate constants, k_{obs}, for the formation of *p*-nitroaniline from *p*-nitroformanilide and *p*-nitroacetanilide at constant pH and constant total hydroxylamine concentration (C) are given in Tables 1 and 2. Eqn. (1), which has been derived from Scheme 1, and was introduced in a previous paper [2] from this laboratory, may be used to reproduce the k_{obs} values for *p*-nitroformanilide.

$$k_{obs} = k_1[N]pC/(1+pC) \quad (1)$$

In eqn. (1) $[N]$ is the concentration of free hydroxylamine base, and p is a pH-dependent variable.

Table 2. Observed pseudo first order rate constants for the formation of *p*-nitroaniline from *p*-nitroacetanilide at different concentrations of the hydroxylamine system. The percentages denote the fraction of free hydroxylamine base.

	k_{obs}/min^{-1} 10^{-6}	
C	10.00 %	80.00 %
0.60	1.55	2.48
1.00	3.11	7.45
1.40	5.18	13.3
1.80	7.61	24.0
2.20	10.2	36.0
2.60	12.9	51.2
3.00	15.7	68.2

Table 3. Experimental and theoretical p values. The experimental p values are the optimal ones for reproducing the experimental k_{obs} values by means of eqn. (1), using $k_1 = 9.0 \times 10^{-3}$. The theoretical p values have been calculated by means of eqn. (2), using the parameter values listed in Table 4.

% Base	p M^{-1}	p_{theor} M^{-1}	$-\log [H^+]$
10.00	0.61	0.57	5.37
20.00	0.32	0.34	5.72
40.00	0.22	0.22	6.14
60.00	0.18	0.17	6.50
80.00	0.14	0.14	6.92
90.00	0.125	0.126	7.27
99.00	0.103	0.107	8.27
99.90	0.103	0.102	9.27
99.97	0.103	0.101	9.79

The fit is good for all the points except at $C = 0.2$ and for the smaller C values at 10 % base, where the experimental k_{obs} values are higher than those calculated by means of eqn. (1). The optimal k_1 and p values have been determined as described previously,[2] and are listed in Table 3.

Since p-nitroacetanilide has been studied at only two base percentages, k_1 and p values have not been evaluated for this compound. However, it is evident from Table 2 that the dependency of k_{obs} on C is between first and second order at 10 % base and purely second order at 80 % base.

To illustrate the variation of the reaction rate with the pH, k_{obs} has been plotted *vs.* $-\log [H^+]$ for p-nitroformanilide in Fig. 1. The curves are drawn to connect the points and are not calculated. When $[H^+]$ is decreased, the rate increases to reach a maximum at about 90 % base, and then decreases slightly to reach a limiting value, which is very close to the maximum value.

The evaluation of the dependency of p in eqn. (1) on pH was done by means of eqn. (2) as described in Ref. 2. The resulting theoretical p values are listed in Table 3 together with the experimental ones obtained by means of eqn. (1). The optimal parameters of eqn. (2) are listed in Table 4.

The search for the optimal parameters was started from widely differing initial values but the final parameters differed only by less than 1 %. The only parameter that varied to any appreciable extent was K_{VI}, which varied between 1×10^5 and 2×10^5. The reliability of the constants was tested as in Ref. 2. A 3 % change in K_I, K_{III} and K_{IV}, a 5 % change in K_V and a 10 % change in K_{II} resulted in a 3 % change in the calculated p.

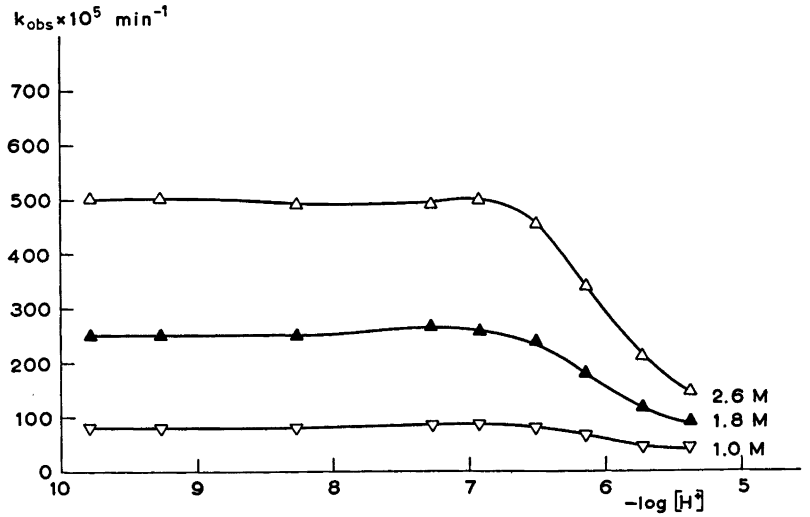


Fig. 1. p-Nitroformanilide. Plot of k_{obs} *vs.* $-\log [H^+]$ at three different C. The dots are experimental. The curves have not been calculated.

Table 4. Values of k_1 and of the parameters in eqn. (2) for four anilides.

	Acetanilide	*p*-Methoxy-acetanilide	Formanilide	*p*-Nitro-formanilide
k_1	7.0×10^{-4}	4.5×10^{-4}	8.0×10^{-2}	9.0×10^{-3}
$K_I = k_4/k_{-1}$ M^{-1}	1.30×10^{-2}	1.00×10^{-2}	1.89×10^{-2}	1.01×10^{-1}
$K_{II} = k_6/k_7K_2$ M^{-1}	2.14×10^{7}	6.30×10^{7}	1.24×10^{7}	3.79×10^{6}
$K_{III} = k_{-4}/k_7K_2$ M^{-1}	1.40×10^{8}	2.01×10^{10}	4.48×10^{7}	9.03×10^{7}
$K_{IV} = k_3/k_{-3}$ M^{-1}	2.30	9.51×10^{1}	9.52×10^{-1}	5.36
$K_V = k_5/k_7K_1K_2$ M^{-2}	8.85×10^{12}	3.81×10^{13}	2.34×10^{12}	1.89×10^{13}
$K_{VI} = k_{-3}/k_7K_1K_2$ M^{-2}	$< 10^{7}$	$< 10^{7}$	$< 10^{7}$	1.5×10^{7}

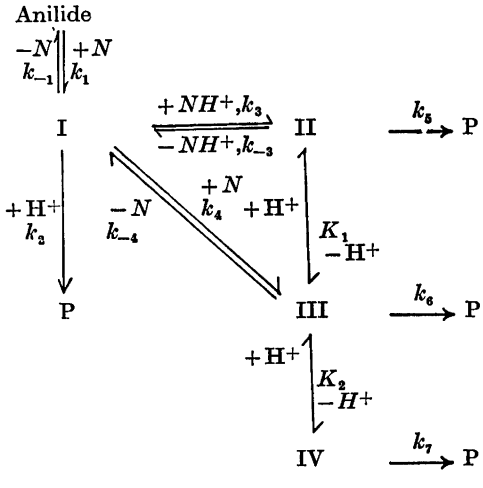
$$p = \frac{K_I + [K_{IV}/K_a + K_IK_{II}][H^+] + [K_{II}K_{IV}/K_a + K_IK_V][H^+]^2 + [K_{IV}K_V/K_a][H^+]^3}{1 + [1/K_a + K_{II} + K_{III}][H^+] + [(K_{II} + K_{III})/K_a + K_V + K_{VI}][H^+]^2 + [(K_V + K_{VI})/K_a][H^+]^3} \quad (2)$$

DISCUSSION

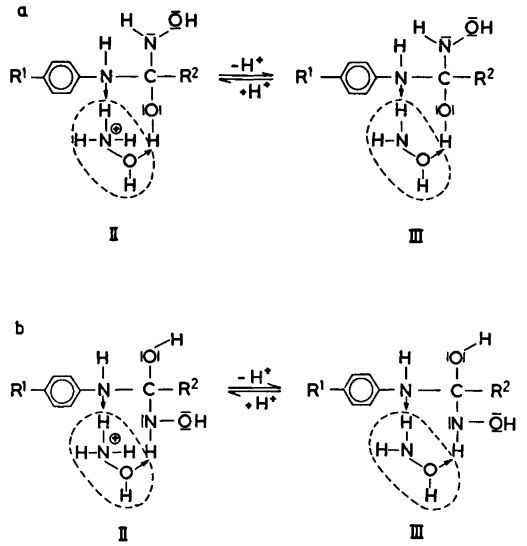
The applicability of eqns. (1) and (2) shows that the hydroxylaminolysis of *p*-nitroformanilide follows the mechanism postulated for the anilides studied previously. At $C = 0.2$ and at the lower part of the 10 % series, however, eqn. (1) fails to give a good fit to the experimental data. This disagreement between experimental and theoretical values cannot be explained by acid hydrolysis. At 10 % base the rate constant for the decomposition of the anilide due to acid hydrolysis can be estimated [7] to be about 9×10^{-7} min^{-1}, *i.e.* about 1 % of the rate of k_{obs} at $C = 0.2$. However, it may be explained by assuming an H^+-catalyzed path from the intermediate I to products in Scheme 1. Such a path has previously been suggested for formanilide [2] (see Scheme 2).

Breakdown of intermediate I. The influence of the *p*-nitro substituent on the steps in Scheme 1 is illustrated in Table 4. In the k_3 and k_4 steps a hydroxylammonium ion (k_3) or a hydroxylamine molecule (k_4) is bound to the intermediate I to form the intermediates II and III. These are shown with the catalyst bonded to the carbonyl hydrogen in Scheme 3a, and to the amine hydrogen in Scheme 3b.

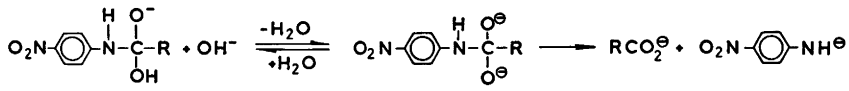
Substitution of an electron-withdrawing group at the *p*-position will lower the electron



Scheme 2.



Scheme 3.



Scheme 4.

density around the carbonyl carbon, which in turn will make the hydrogens at the oxygen or nitrogen more accessible for hydrogen bonding. This results in higher values of K_I and K_{IV}, that reflect the bonding of N and HN to I.

The ratio k_6/k_{-4} obtained by dividing K_{II} by K_{III}, is one seventh of that for formanilide. This may seem strange at first, since the amide bond should be weakened by the *p*-nitro group. However, at the same time the *p*-nitro substituent will decrease the ability of the free electron pair on the amide nitrogen to bind the extra hydrogen, which is necessary to make the aniline moiety a good leaving group.

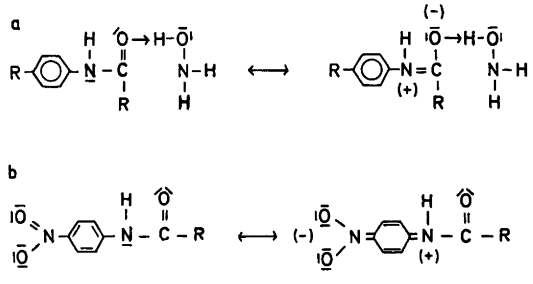
The ion R-ArNH$^-$, the stability of which is increased by the *p*-nitro group, is probably not dissociated from the intermediate III but rather from IV by the k_7 route. This is paralleled by the alkaline hydrolysis mechanism for *p*-nitroformanilide,[3] where this ion was suggested to be dissociated from an intermediate with two negative charges (see Scheme 4).

Formation of intermediate I. The really intriguing part of the hydroxylaminolysis of *p*-nitroformanilide is that the rate of the nucleophilic attack on the carbonyl carbon (k_1) is lowered almost ten times as a consequence of the introduction of the *p*-nitro group. The lowering of k_1 is reflected in the lowering of the total reaction rate by approximately the same factor. This effect has no parallel in the hydrolysis of anilides nor in any other reaction of nucleophiles with carbonyl compounds. In the alkaline hydrolysis,[3] the nucleophilic attack on *p*-nitroacetanilide is about 50 times faster than on acetanilide. Furthermore, in our previous work, the difference in k_1 for the hydroxylaminolysis reactions of different anilides has been well in line with that found in alkaline hydrolysis.

However, the present results are not completely unique. The rate data for *p*-nitroacetanilide in this work show the same decrease in k_{obs} when compared to acetanilide as those for *p*-nitroformanilide when compared to formanilide. Owing to the very low reaction rates and the low solubility of *p*-nitroacetanilide, the reaction was only studied at two pH values. Thus k_1 has not been evaluated, but the relative rates suggest that the decrease in k_1 for *p*-nitroformanilide is indeed a consequence of the *p*-nitro substitution and not due to special properties of the formyl group.

A *p*-nitro group in an anilide ring will decrease the electron density at the carbonyl carbon, thereby facilitating the nucleophilic attack. Since in this case the rate of nucleophilic attack is lowered, it seems that the rate of attack of the hydroxylamine on the anilide is determined not only by the attack of nitrogen on carbon, but also by some other factor. This conclusion is supported by the fact that hydroxylamine is about 1000 times more reactive as a nucleophile than is to be expected from its basicity.[8] A similar enhancement of the reaction rates has been found for other amine derivatives where there is a strongly electronegative element in the α-position to the nucleophile.[8] It has been suggested [9] that the abnormal reactivity is due to hydrogen bonding between the carbonyl oxygen and a hydrogen at the α atom.

There are two possible ways in which hydrogen bonding to the anilide may aid the nucleophilic attack, and by which the observed substituent effect can be accounted for: (1) Formation of a hydrogen bond to the carbonyl oxygen which increases by induction the electron density at the hydroxylamine nitrogen and helps to keep it in position for the nucleophilic attack. For most anilides, the hydrogen bond will be stabilized by the mesomeric structure shown in Scheme 5a (*cf.* Ref.



Scheme 5.

10), and k_1 will depend largely on the degree of electron density at the carbonyl carbon. For anilides with substituents capable of mesomeric interaction with the anilide nitrogen, such as a *p*-nitro group, the resonance structure shown in Scheme 5b will lessen the stabilization of the hydrogen bond. The hydroxylamine nitrogen will be deprived of its usual aid in the nucleophilic attack and k_1 will be smaller than expected. (2) A similar argument may be applied if the anilide nitrogen takes part in the hydrogen bonding. On introduction of a *p*-nitro group, the basicity of this nitrogen will be less reduced than that of the carbonyl oxygen. On the other hand, the geometry at the amide group will change owing to the mesomeric interaction of the *p*-nitro group with the anilide nitrogen. Thus in this case there are steric factors as well as a weaker hydrogen bond to account for the decrease in k_1.

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Short Communications

The Stereochemistry of Some Asymmetric Reformatsky Reactions

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We report here that the stereochemical outcome of five asymmetric Reformatsky reactions (1 – 5 in Table 1) is opposite to that predicted by the application of Prelog's generalization.[1] Thus, for the menthyl derivatives a preponderance of the L-hydroxy acids is expected (Fig. 1) but the D-isomers were found to be the main products.

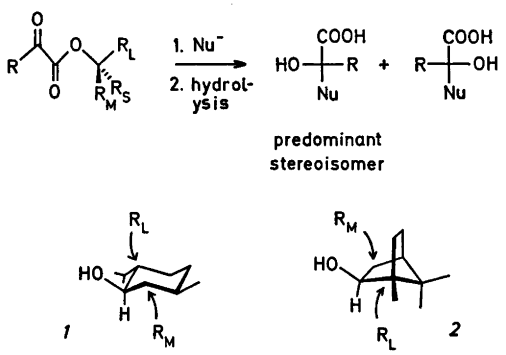


Fig. 1. Prelog's generalization applied to (−)-menthol (*1*) and (+)-borneol (*2*). L, large; M, medium; S, small.

(−)-Menthol (*1*) and (+)-borneol (*2*) have different chiral orders of the groups R_L, R_M, and R_S at the carbinol carbons and the different stereochemical results obtained from (−)-menthyl pyruvate and (+)-bornyl pyruvate are therefore expected.

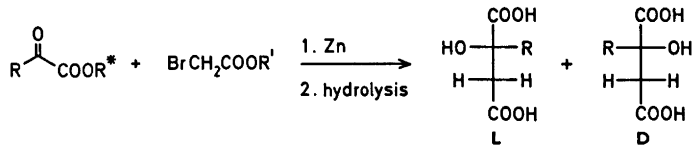
Unlike the reactions which originally formed the basis of Prelog's generalization, the addition of a Reformatsky reagent to a ketone has under certain conditions been shown to be reversible.[2] However, for reactions 1 and 2 (Table 1) we have been unable to demonstrate any such reversibility and hence the stereochemical results obtained should only be due to steric factors in the transition states leading to the Reformatsky adducts.

When THF was substituted by benzene the stereochemical result of reaction 1 was unaffected. In contrast, results according to Prelog's generalization were obtained when DMF was used as solvent and for reaction 2 when it was carried out in refluxing dimethoxymethane (two-step procedure)[3] or in DMSO, and also when it was run with the corresponding lithium[4] or chloromagnesium[5] reagents (8.0, 7.5, 3.7, 3.2 and 1.7 % enantiomeric excess of the *S* isomer respectively). The latter results thus show that the anomalous enantiomeric compositions obtained should be ascribed to the particular use of zinc rather than to the aldol-type condensation nature of the reactions. The influence of the metal ion on the steric courses of the reactions could possibly be discussed in terms of different tendencies of the ions (Zn > Mg > Li) to give chelate-like transition states involving both carbonyl groups of the α-keto esters. The results obtained in the more polar solvents (dimethoxymethane, DMF and DMSO) are consistent with this view since these solvents should compete with the carbonyl groups in bonding to zinc atoms, and the stereochemical outcome should then be similar to that of the corresponding lithium and magnesium reactions.

A few reactions that do not follow Prelog's generalization have been described.[13,14] The Reformatsky reactions described here are the first reported anomalous asymmetric syntheses with the frequently used substrates (−)-menthyl pyruvate and (+)-bornyl pyruvate.

Experimental. Granular zinc (Baker, 30 mesh) was purified before use.[6] Tetrahydrofuran was distilled over lithium aluminium hydride, and dimethoxymethane was repeatedly distilled over sodium. (−)-Menthyl pyruvate showed $[\alpha]_{578}^{24}$ −85.4° (*c* 2.0, ethanol); lit.[7] value: $[\alpha]_D$ −84.1° (*c* 1.9, ethanol). (−)-Menthyl phenylglyoxylate was prepared from phenylglyoxylic acid[8] and (−)-menthol by the same procedure as described for (−)-menthyl pyruvate.[7] It showed m.p. 73 – 74 °C and $[\alpha]_{578}^{25}$ −54° (*c* 3.4, chloroform); lit. values: m.p. 73 – 74 °C,[9] $[\alpha]_{578}$ −54° (*c* 3.0, chloroform).[10] (+)-Bornyl pyruvate was prepared in the same way as the other esters and showed $[\alpha]_{576}^{24}$ +50° (*c* 3.5, benzene); lit.[11] value: $[\alpha]_{576}^{25}$ +49° (*c* 3.0, benzene). In all the syntheses 1 – 5 the reagents were used in approximately

Table 1. Optical yields obtained in five asymmetric Reformatsky reactions.[a]



	R	R*	R′	Optical yields (%)	Configuration of hydroxy acid
1	CH_3	(−)-Menthyl	Et	2.2, 3.5, 1.5	*R* (D)
2	CH_3	(−)-Menthyl	*t*-Bu	3.7, 2.5	*R* (D)
3	C_6H_5	(−)-Menthyl	Et	~8	*S* (D)
4	CH_3	(+)-Bornyl	Et	2.8	*S* (L)
5	CH_3	(+)-Bornyl	*t*-Bu	2.0	*S* (L)

[a] All reactions were performed in refluxing THF.

20 % excess over the keto ester. The latter was used in 5 g quantities dissolved in 10 ml of THF. All reagents were mixed under nitrogen and the reactions were started by gentle heating; the spontaneous reflux was controlled be cooling with water but ceased after about 5 min. The mixtures were subsequently heated under reflux for 3 – 4 h (by which time all the zinc had been consumed) and then poured into dilute hydrochloric acid-ice and extracted three times with diethyl ether. The combined organic layers were dried (Na_2SO_4) and concentrated. Yields were estimated by NMR with piperonal as internal standard and found to be 50 – 85 %. Analytical and preparative GLC, measurements of optical rotations (in chloroform) and transformation of crude mixed esters to diethyl citramalate were performed as previously described.[4] The purity of the diethyl citramalate was checked by both NMR and analytical GLC and no menthol or borneol could be detected. Optically pure (*S*)-diethyl citramalate shows $[\alpha]_{578}$ +20.1° (*c* 2.2).[4] The optical yield in reaction 3 was determined as follows. The mixed ester was hydrolysed with 1 M NaOH and after acidification the diacid was extracted with diethyl ether and esterified with diazomethane. The resulting ester was hydrogenated in methanol, using 5 % rhodium on alumina as catalyst, to yield dimethyl 2-cyclohexylmalate. After preparative GLC and hydrolysis the CD curve of the molybdate complex of the 2-cyclohexylmalic acid was compared with that of the complex of (*S*)-2-isopropylmalic acid (erroneously denoted *R* in the previous paper [12]). The optical yield was estimated assuming that these acids in optically pure forms show the same molecular ellipticities.

Attempted epimerisations. The crude mixed ester from reaction 2 was purified by removal of volatile components under reduced pressure and was then dissolved in hexamethylphosphortriamide. An equimolar amount of lithium hydride was added and the mixture was stirred for 24 h (23 °C) and worked up as usual. A 100 % recovery of mixed ester was obtained (GLC, phenanthrene as internal standard). Routine hydrolysis and esterification gave (*R*)-diethyl citramalate in an optical yield of 2.3 %. When this experiment was performed at 60 °C (24 h) the recovery was again quantitative and (*R*)-diethyl citramalate was obtained in an optical yield of 1.9 %.

The same experiment was performed using the mixed ester from reaction 2 which had been carried out in two steps with dimethoxymethane as solvent. No change in sign or optical yield for diethyl citramalate was obtained either at 25 or 60 °C, but recoveries of mixed ester were only 96 % and 84 %, respectively. We have also been unable in other ways to demonstrate reversibility of reactions; for example separate experiments have been carried out, firstly in which the Reformatsky adduct was prepared in dimethoxymethane and the solvent then replaced by THF and secondly in which anhydrous zinc bromide was added to the Reformatsky adduct obtained from the above-mentioned chloromagnesium reagent.

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Improvements in the Preparation of Crystalline D-Mannose from the Doum Palm Kernel*

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D-Mannose is usually prepared[1–5] by acid hydrolysis of the D-mannan of the ivory nut, followed either by direct crystallisation of the sugar from the hydrolysate, or preparation and recrystallisation of methyl α-D-mannopyranoside,[6,7] from which it is recovered by acid-hydrolysis.[8,9] Like ivory nut, the kernel of the African doum palm (*Hyphaene thebaica*) has been used for the manufacture of buttons, and contains a D-mannan.[10–12]

In a recent publication,[13] the doum palm kernel was described as an alternative source of D-mannose, obtained by acid hydrolysis of the kernel shavings and direct crystallisation from glacial acetic acid, in 20 % overall yield.

In the present work the method is improved by first preparing crystalline methyl α-D-mannopyranoside from the doum palm kernel shavings by methanolysis, in a good yield (50 – 52 %). Methyl α-D-mannopyranoside is then hydrolysed with sulfuric acid to give crystalline D-mannose in 70 % yield from the mannoside. The overall yield is about 34 % of the doum palm kernel.

Experimental. Melting points are uncorrected. Concentrations were performed at reduced pressure below 50 °C. Thin-layer chromatography (TLC) was performed on silica gel (Kiesel gel G, Merck) using butanol – pyridine – water (10:3:3) as a solvent and ceric sulfate as a spray reagent.

Methyl α-D-mannopyranoside. The doum palm kernels (500 g) were defatted with light petroleum (b.p. 60 – 80 °C) for a week, then ground in a mill. The dry, finely-ground kernels (100 g) were added in small portions to 75 % sulfuric acid (100 ml) in a mortar, at such a rate that the temperature was not allowed to exceed 30 °C, and the meal was well kneaded after each addition. The meal was kept for 15 h at 25 – 30 °C with occasional kneading until the suspension was nearly a homogeneous paste. At the end of this period, a mixture of dry, acetone-free methyl alcohol (100 ml) and concentrated hydrochloric acid (25 ml) was added under vigorous mixing, and the soft paste was transferred to a 2 l flask and diluted with absolute methyl alcohol (600 ml). The mixture was boiled under reflux for 8 h, and after addition of charcoal (15 g), it was refluxed for a further 1 h, and filtered hot. The residue was washed with hot absolute methyl alcohol (50 ml). The filtrate and washings were combined and kept at room temperature for 24 h whereupon methyl α-D-mannopyranoside crystallised in colourless cubes. After 3 days the crystals were collected, washed with absolute methyl alcohol (25 ml), dry acetone, and dried; yield, 50 g. After concentration of the mother liquor in a current of air, another 2 g was obtained. The total yield was 52 % of the original meal. m.p.* 158 – 165 °C, $[\alpha]_D^{20}$ +72° (*c* 2, water). This product was recrystallised from 85 % ethanol with 87 % recovery. It was chromatographically pure (TLC), R_F 0.48, m.p. 193 °C, $[\alpha]_D^{20}$ +82° {lit. m.p. 195 °C, $[\alpha]_D^{20}$ +80° (*c* 1, water)}.

When the unextracted meal was used, the yield of methyl α-D-mannopyranoside was 36 % of the original meal, and 16 – 20 % of unreacted meal was recovered.

D-Mannose. A solution of methyl α-D-mannopyranoside (20 g) in 2 N sulfuric acid (320 ml)

* This work was carried out in part at the Organic Chemistry Laboratories, The Norwegian Institute of Technology, University of Trondheim, Trondheim-NTH, Norway.

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* M.p. of the crude methyl α-D-mannopyranoside isolated from the ivory nut; 170 °C.

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D-Mannose is usually prepared[1–5] by acid hydrolysis of the D-mannan of the ivory nut, followed either by direct crystallisation of the sugar from the hydrolysate, or preparation and recrystallisation of methyl α-D-mannopyranoside,[6,7] from which it is recovered by acid-hydrolysis.[8,9] Like ivory nut, the kernel of the African doum palm (*Hyphaene thebaica*) has been used for the manufacture of buttons, and contains a D-mannan.[10–12]

In a recent publication,[13] the doum palm kernel was described as an alternative source of D-mannose, obtained by acid hydrolysis of the kernel shavings and direct crystallisation from glacial acetic acid, in 20 % overall yield.

In the present work the method is improved by first preparing crystalline methyl α-D-mannopyranoside from the doum palm kernel shavings by methanolysis, in a good yield (50 – 52 %). Methyl α-D-mannopyranoside is then hydrolysed with sulfuric acid to give crystalline D-mannose in 70 % yield from the mannoside. The overall yield is about 34 % of the doum palm kernel.

Experimental. Melting points are uncorrected. Concentrations were performed at reduced pressure below 50 °C. Thin-layer chromatography (TLC) was performed on silica gel (Kiesel gel G, Merck) using butanol – pyridine – water (10:3:3) as a solvent and ceric sulfate as a spray reagent.

Methyl α-D-mannopyranoside. The doum palm kernels (500 g) were defatted with light petroleum (b.p. 60 – 80 °C) for a week, then ground in a mill. The dry, finely-ground kernels (100 g) were added in small portions to 75 % sulfuric acid (100 ml) in a mortar, at such a rate that the temperature was not allowed to exceed 30 °C, and the meal was well kneaded after each addition. The meal was kept for 15 h at 25 – 30 °C with occasional kneading until the suspension was nearly a homogeneous paste. At the end of this period, a mixture of dry, acetone-free methyl alcohol (100 ml) and concentrated hydrochloric acid (25 ml) was added under vigorous mixing, and the soft paste was transferred to a 2 l flask and diluted with absolute methyl alcohol (600 ml). The mixture was boiled under reflux for 8 h, and after addition of charcoal (15 g), it was refluxed for a further 1 h, and filtered hot. The residue was washed with hot absolute methyl alcohol (50 ml). The filtrate and washings were combined and kept at room temperature for 24 h whereupon methyl α-D-mannopyranoside crystallised in colourless cubes. After 3 days the crystals were collected, washed with absolute methyl alcohol (25 ml), dry acetone, and dried; yield, 50 g. After concentration of the mother liquor in a current of air, another 2 g was obtained. The total yield was 52 % of the original meal. m.p.* 158 – 165 °C, $[\alpha]_D^{20}$ +72° (*c* 2, water). This product was recrystallised from 85 % ethanol with 87 % recovery. It was chromatographically pure (TLC), R_F 0.48, m.p. 193 °C, $[\alpha]_D^{20}$ +82° {lit. m.p. 195 °C, $[\alpha]_D^{20}$ +80° (*c* 1, water)}.

When the unextracted meal was used, the yield of methyl α-D-mannopyranoside was 36 % of the original meal, and 16 – 20 % of unreacted meal was recovered.

D-Mannose. A solution of methyl α-D-mannopyranoside (20 g) in 2 N sulfuric acid (320 ml)

* This work was carried out in part at the Organic Chemistry Laboratories, The Norwegian Institute of Technology, University of Trondheim, Trondheim-NTH, Norway.

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* M.p. of the crude methyl α-D-mannopyranoside isolated from the ivory nut; 170 °C.

was hydrolysed by steam distillation.[9] After 2 h, TLC showed the hydrolysis to be complete. Charcoal (1 g) was added, and the solution neutralised with barium hydroxide and barium carbonate. The precipitated barium sulfate was filtered off and washed with hot water. The solution and washings were combined and evaporated to a syrup which was dissolved in hot glacial acetic acid (25 ml). The solution was cooled, nucleated with a few crystals of D-mannose, and kept for 2 days with occasional stirring, and then for one day in a refrigerator. The crystalline D-mannose was filtered off, washed with cold ethanol, then ether and dried, yield 13 g; m.p. 130 – 131 °C, $[\alpha]_D^{20}$ + 14° (equil., water) {lit.[3] $[\alpha]_D^{20}$ + 14.2° (equil., water)}.

Acknowledgements. The author thanks Professor H. S. El Khadem, Michigan Technological University, for his keen interest. Also thanks are due to Dr. T. Anthonsen, Organic Chemistry Laboratories, The Norwegian Institute of Technology, University of Trondheim, Trondheim, Norway, for making available the facilities for this work, and to NORAD for a fellowship.

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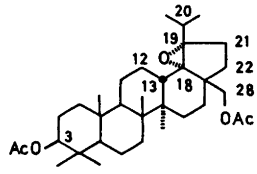
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Triterpenes. *p*-Toluenesulfonic Acid Catalysed Rearrangement of 18α,19α and 18β,19β Isomers of 3β,28-Diacetoxy-epoxylupane

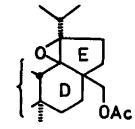
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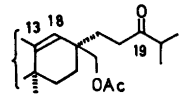
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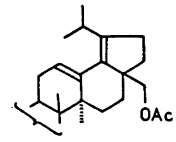
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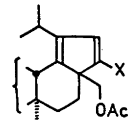
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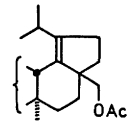
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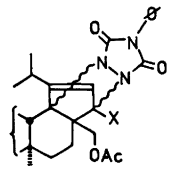
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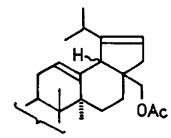
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8 X = H
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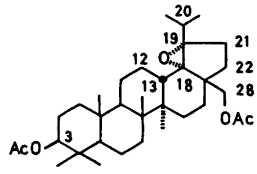
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Triterpenes. *p*-Toluenesulfonic Acid Catalysed Rearrangement of 18α,19α and 18β,19β Isomers of 3β,28-Diacetoxy-epoxylupane

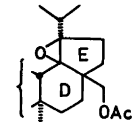
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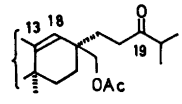
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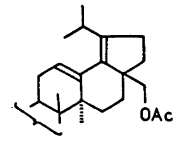
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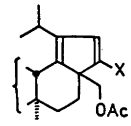
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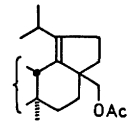
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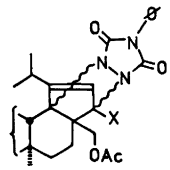
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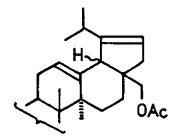
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9 X = Br



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The 18β,19β-epoxylupane derivative *2*, when treated with TosOH in benzene, gave in *ca.* 30 % yield the baccharane derivative *3* as compared to the *ca.* 90 % obtained with BF_3-etherate. The main products in the TosOH reaction were two conjugated dienes, together *ca.* 70 %. The less polar diene on $AgNO_3$ impregnated silica plate was identical with the compound *4* obtained in a 10 % yield from the BF_3-catalysed reaction of the β-epoxide *2*. The more polar diene had the structure *5*. Prolonged reaction time increased the proportional amount of diene *4*. Isomerisation of diene *5* to diene *4* was confirmed by a separate experiment under the same conditions. For comparison, the diene *5* was prepared from *6 via* a low-temperature bromination-dehydrobromination. This reaction at room temperature gave a mixture of products, among them the bromodiene *7*, which was also obtained from *6 via* a NBS/dehydrobromination sequence. Both the diene *5* and bromodiene *7* form a Diels-Alder adduct *8* and *9* with 4-phenyl-1,2,4-triazoline-3,5-dione. The ^1H NMR spectra of the adducts show the known [2] low field δ value (6.20 and 6.25) for the C-21 proton.

Reaction of the 18α,19α-epoxy derivative *1* with TosOH affords *ca.* 85 % of the baccharane derivative *3*, while BF_3-etherate yields this compound quantitatively. The TosOH catalysed reaction gives, in addition, *ca.* 15 % of the unconjugated diene *10*.

Experimental. For general experimental information see Ref. 1.

Reaction of 3β,28-diacetoxy-18β,19β-epoxylupane (2) with TosOH. 3β,28-Diacetoxy-18β,19β-epoxylupane [1] (*2*) (0.5 g) in dry benzene (20 ml), $TosOH.H_2O$ (0.1 g), and Ac_2O (0.15 g) was refluxed for 5 h. The mixture was washed with $NaHCO_3$ solution, dried, the solvent evaporated and the residue chromatographed on silica plates. The more polar of the two main fractions was 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (*3*) crystallised from EtOH (0.1 g) m.p. 113 °C, $[\alpha]_D$ −35° (*c* 1.0). (Ref. 1, m.p. 115 °C, $[\alpha]_D$ −34.8°.) The less polar fraction was chromatographed on a 10 % $AgNO_3$ impregnated silica plate to give two compounds. The less polar compound was identified as 3β,28-diacetoxy-lupa-12,18-diene [1] (*4*) crystallised from EtOH (0.15 g) m.p. 140 °C, $[\alpha]_D$ +241° (*c.* 1.1). (Ref. 3, m.p. 140−143 °C, $[\alpha]_D$ +219°). The more polar 3β,28-diacetoxy-lupa-18,21-diene (*5*) crystallised from EtOH (0.1 g) had m.p. 217 °C, $[\alpha]_D$ −118° (*c* 1.0); λ_{max} (abs. EtOH) 253 nm (ε 3500); *m/e* (% rel. int.) 524 (13, M^+), 464 (18), 451 (35), 361 (13), 274 (12), 261 (13), 249 (7), 214 (21), 201 (100), 200 (45), 199 (23), 189 (44), 187 (52), 173 (82); δ (CCl_4) 6.30 (2 H, s, C-21 and C-22 protons; Eu(DPM)$_3$ addition splits the singlet to AB quartet *J* 5.3 Hz), 4.40 (1 H, m, 3αH), 4.40 and 3.35 (à 1 H, d, *J* 11 Hz, C-28 protons), 3.3−2.2 (2 H, m, C-13 and C-20 protons), 1.95 (6 H, s, acetyl protons), 1.25−0.75 (group of 7 methyls). Anal. $C_{34}H_{52}O_4$: C, H.

Isomerisation of 3β,28-diacetoxy-lupa-18,21-diene (5). 3β,28-Diacetoxy-lupa-18,21-diene (*5*) (0.2 g) in dry benzene (10 ml), $TosOH.H_2O$ (0.1 g), and Ac_2O (0.1 g) was refluxed for 2 h. The mixture was worked up as above and chromatographed on a 10 % $AgNO_3$ impregnated silica plate. The least polar main fraction (20 mg) was identical with 3β,28-diacetoxy-lupa-12,18-diene (*4*) obtained above.

Bromination of 3β,28-diacetoxy-lup-18-ene (6). To the solution of 3β,28-diacetoxy-lup-18-ene (*6*) (0.52 g) in $CHCl_3$ (10 ml) was added bromine (0.16 g) in CCl_4 (1 ml) at −40 °C. After 2 h the reaction mixture was washed with water, $NaHCO_3$ solution, dried and chromatographed on 10 % $AgNO_3$ impregnated silica plates. The main product was identical with 3β,28-diacetoxy-lupa-18,21-diene (5) obtained above. The reaction mixture contained, in addition, small amounts of starting material *6* and bromodiene *7*, characterised below.

At room temperature a similar reaction gave a complex mixture with increased amount of bromodiene *7*.

Reaction of 3β,28-diacetoxy-lup-18-ene (6) with NBS. 3β,28-Diacetoxy-lup-18-ene (*6*) (0.5 g), *N*-bromosuccinimide (0.7 g), and dibenzoylperoxide (0.2 g) in CCl_4 (30 ml) were refluxed for 15 min. Succinimide was filtered off, the mixture washed with $NaHCO_3$ solution, dried and evaporated. Chromatography on 10 % $AgNO_3$ impregnated silica plates gave 22-bromo-3β,28-diacetoxy-lupa-18,21-diene (*7*), crystallised from EtOH (0.3 g) m.p. 179 °C, $[\alpha]_D$ −116° (*c* 0.95); λ_{max} (abs. EtOH) 269 nm (ε 6100): ν 1730, 1615, 1550, 1225, 1215, δ (CCl_4) 6.45 (1 H, s, C-21 proton), 4.50 (1 H, m, 3αH), 4.47 and 3.95 (à 1 H, 2 d, *J* 11 Hz, C-28 protons), 3.15 (1 H, sept. *J* 6.5 Hz, C-20 proton), 2.7 (1 H, m, C-13 proton), 2.05 and 1.92 (à 3 H, s, acetyl protons), 1.2−0.75 (group of 7 methyls); *m/e* 604, 602. Anal. $C_{34}H_{51}BrO_4$: C, H.

Diels-Alder adducts 8 *and* 9. 3β,28-Diacetoxy-lupa-18,21-diene (*4*) (0.1 g) and 4-phenyl-1,2,4-triazoline-3,5-dione (0.05 g) in dry benzene (5 ml) were allowed to stand overnight. The solvent was evaporated and the residue chromatographed on a silica plate. Crystallisation from EtOH gave adduct *8* (0.05 g) m.p. 213 °C (decomp.), $[\alpha]_D$ −11° (*c* 1.0): δ ($CDCl_3$) 7.38 (5 H, phenyl), 6.20 (1 H, d, *J* 3 Hz, C-21 proton), 4.83 (1 H, d, *J* 3 Hz, C-22 proton), 4.8−4.1 (3 H, m, 3αH and C-28 protons), 2.10 and 2.05 (à 3 H, s, acetyl protons), 1.2−0.85 (group of 7 methyls).

Similar treatment of 22-bromo-3β,28-diacetoxy-lupa-18,21-diene (*7*) gave adduct *9*, m.p. 172 °C (decomp.), $[\alpha]_D$ +35° (*c* 1.0); δ ($CDCl_3$) 7.37 (5 H, phenyl), 6.25 (1 H, s, C-21 proton), 3.5 (3 H, m, 3αH and C-28 protons), 2.12 and 2.02 (à 3 H, s, acetyl protons), 1.15−0.8 (group of 7 methyls).

Reaction of 3b,28-diacetoxy-18α,19α-epoxylupane (1) with TosOH. 3β,28-Diacetoxy-18α,19α-epoxylupane [1] (*1*) (1.0 g), TosOH.H_2O (0.15 g) and Ac_2O (0.2 g) in dry benzene (25 ml) were refluxed for 35 min and worked up. Chromatography on silica plates gave 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (*3*) (0.8 g) identical with the compound above, and 3β,28-diacetoxy-lupa-12,19(21)-diene (*10*), crystallised from EtOH (0.11 g), m.p. 124 °C, $[\alpha]_D$ −6° (*c* 1.0); *m/e* (% rel. int.) 524 (7, M^+), 464 (12), 451 (21), 404 (5), 391 (6), 274 (11), 261 (24), 225 (5), 215 (11), 214 (15), 213 (7), 203 (11), 202 (25), 201 (100), 200 (11), 199 (18), 190 (16), 189 (25), 187 (21), 185 (17), 171 (21); δ (CCl_4) 5.4 (1 H, m, C-21 proton), 5.2 (1 H, m, C-12 proton), 4.35 (1 H, m, 3αH), 3.92 (2 H, AB quart. *J* 11 Hz, C-28 protons), 2.9 (1 H, br. s, C-18 proton), 1.95 (6 H, s, acetyl protons), 1.1 − 0.8 (group of 7 methyls). Anal. $C_{34}H_{52}O_4$: C, H.

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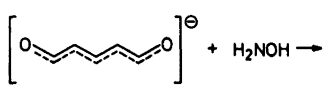
Derivatives and Reactions of Glutacondialdehyde. VI. Isoxazoline Formation from the Glutacondialdehyde Anion

JAN BECHER and JENS PETER JACOBSEN

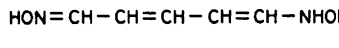
Department of Chemistry, Odense University, DK-5000 Odense, Denmark

The formation of pyridines [1] and pyrylium salts [2] as well as 3-formyl-2(1*H*)-pyridinethiones [3] from the glutacondialdehyde anion (*1*) is well-known, while the formation of other heterocyclic systems from *1* has not been reported.

Baumgarten reported [4] the reaction of *1* and hydroxylamine to give the dioxime of glutacondialdehyde to which he assigned the structures *2a* or *2b*:

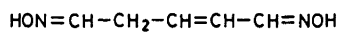


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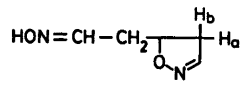
2a

or



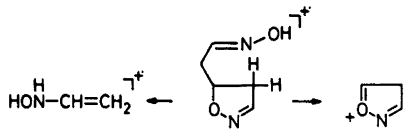
2b

As a part of our interest in the chemistry of *1* we have reinvestigated this reaction, and found that the anion *1* reacts with hydroxylamine in a more interesting manner than could be anticipated.[4] In this contribution we wish to report that the correct structure of the reaction products is *3*, thus giving a new synthesis of the Δ^2-isoxazoline system:



3

The UV spectrum of the product showed bands at $\lambda_{max} = 215$ nm (log $\varepsilon = 3.44$) indicating the absence of a highly conjugated system as in the simple glutacondialdehyde derivatives.[6] The mass spectrum of the compound is in accordance with structure *3*. The molecular ion peak is seen at *m/e* = 128, while dominant fragment ion peaks are seen at *m/e* = 59 and 70. The fragmentation of *3* can be rationalized in the following way:



m/e = 59(66%) *m/e* = 128(6.4%) *m/e* = 70(100%)

The ^1H NMR spectrum of *3* shows in the cases of H(4a) and H(4b) the usual pattern of non-equivalent ring protons. The chemical shift and coupling constants are given in Table 1. The values of the coupling constants and chemical shift are in close agreement with values in similar compounds as reported by several authors.[5] The ^{13}C NMR spectrum of *3* consists of five lines as stated in Table 1. The assignment of the lines has been done by recording the gated decoupled spectrum.

Reaction of 3b,28-diacetoxy-18α,19α-epoxylupane (1) with TosOH. 3β,28-Diacetoxy-18α,19α-epoxylupane [1] (*1*) (1.0 g), TosOH.H_2O (0.15 g) and Ac_2O (0.2 g) in dry benzene (25 ml) were refluxed for 35 min and worked up. Chromatography on silica plates gave 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (*3*) (0.8 g) identical with the compound above, and 3β,28-diacetoxy-lupa-12,19(21)-diene (*10*), crystallised from EtOH (0.11 g), m.p. 124 °C, $[\alpha]_D$ −6° (*c* 1.0); *m/e* (% rel. int.) 524 (7, M^+), 464 (12), 451 (21), 404 (5), 391 (6), 274 (11), 261 (24), 225 (5), 215 (11), 214 (15), 213 (7), 203 (11), 202 (25), 201 (100), 200 (11), 199 (18), 190 (16), 189 (25), 187 (21), 185 (17), 171 (21); δ (CCl_4) 5.4 (1 H, m, C-21 proton), 5.2 (1 H, m, C-12 proton), 4.35 (1 H, m, 3αH), 3.92 (2 H, AB quart. *J* 11 Hz, C-28 protons), 2.9 (1 H, br. s, C-18 proton), 1.95 (6 H, s, acetyl protons), 1.1 − 0.8 (group of 7 methyls). Anal. $C_{34}H_{52}O_4$: C, H.

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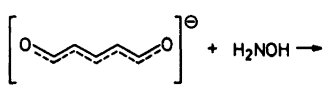
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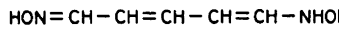
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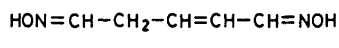


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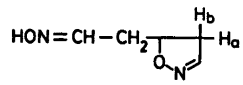
2a

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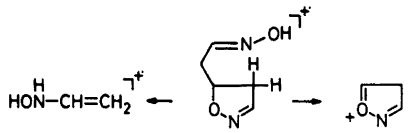
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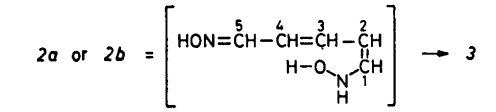
Table 1. Chemical shift δ(i) [a] and coupling constants J_{ij} in *3*.

Atom (i)	δ(i)	Atoms (i, j)	$\lvert J_{ij}\rvert$ (Hz)
H(3)	7.4 (7.4)	H(3), H(4a)	1.7 (1.7)
H(4a)	3.0 (3.0)	H(3), H(4b)	1.7 (1.7)
H(4b)	2.6 (2.6)	H(4a), H(4b)	17.8 (17.8)
H(5)	4.6 (4.6)	H(4a), H(5)	10.0 (10.0)
H(6)	2.5 (2.5)	H(4b), H(5)	7.4 (7.4)
H(7)	6.6 (7.2)	H(5), H(6)	5.7
H(8)	11.0 (10.5)	H(6), H(7)	5.4
C(3)	147.1 (146.9)		
C(4)	40.3 (40.0)		
C(5)	74.7 (75.2)		
C(6)	30.3 (34.6)		
C(7)	146.0 (146.0)		

[a] Relative to TMS.

The NMR spectrum of the reaction product before purification consisted of two sets of lines, which we assigned to the two isomers, *syn* and *anti*, in ratio 1:1. After distillation only one of the isomers was obtained with m.p. 87–89 °C. The values in brackets in Table 1 are the ones associated to the other isomer. The differences of the chemical shifts in the two compounds have been used in the assignment of the lines.

The mechanism for the formation of *3* are similar to well-known syntheses of isoxazolines and can be explained by assuming *2a* or *2b* as an intermediate. The C(3)–C(4) double bond in *2a* or *2b* may after isomerization add the hydroxylamine moiety to give *3*:



We have shown [6] that addition of for example an alcohol to the C(3)–C(4) double bond in the acetals of *1* readily takes place.

Experimental. Microanalyses were carried out in the Microanalytical Department of the University of Copenhagen by Mr. Preben Hansen.

Instrumentation: UV, Beckman ACTA III; MS, AEI-MS 902; ^{13}C NMR and ^{1}H NMR, Jeol. FX-60.

2-Isoxazolin-5-yl acetaldehyde oxime 3. Following the reported procedure,[4] the sodium salt of glutacondialdehyde (*1*) (15 g) and hydroxylamine was refluxed in absolute ethanol. Evaporation of the ethanol and continuous extraction of the residue with ether yielded nearly colourless crystals, 4.2 g (34 %). Distillation of the crude product yielded as the main fraction (b.p. 136–138 °C/0.5 mmHg) colourless crystals of *3*, m.p. 87–89 °C. The distillate as well as the crude product gave rise to only one spot by TLC on silica gel with different solvents as eluents. (Baumgarten reported a yield of 32 % and m.p. 88 °C). Anal. $C_5H_8O_2N_2$: C, H, N.

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Reduction of the 610 nm Absorption Band of Ceruloplasmin by Phenothiazine Derivatives

ROLF A. LØVSTAD

Institute of Medical Biochemistry, University of Oslo, Karl Johans gt. 47, Oslo 1, Norway

Ceruloplasmin (ferroxidase, coeruloplasmin, EC 1.16.3.1) is a blue serum protein, which catalyzes the oxidation of phenothiazine derivatives to free radicals.[1,2] The protein molecule contains paramagnetic Type-1 and Type-2 Cu(II) as well as diamagnetic copper.[3] The 610 nm absorption band of the enzyme is due to the Type-1 Cu(II), which is involved in the catalytic process. Experiments suggest that electrons are initially transferred from substrate to the blue Type-1 copper.[3-7] The reduction of the 610 nm chromophore by ascorbate and certain aryl diamines and diphenols exhibited a second order dependence on the substrate concentration, and differences in the rate of reduction correlated to differences in the ionization potential of the reducing substrates.[8]

In the present communication the transient reduction of the 610 nm chromophore by various phenothiazine derivatives has been studied in order to find out if a correlation exists between the rate of reduction and the electron donor ability of the phenothiazines. Fig. 1 shows some typical progress curves

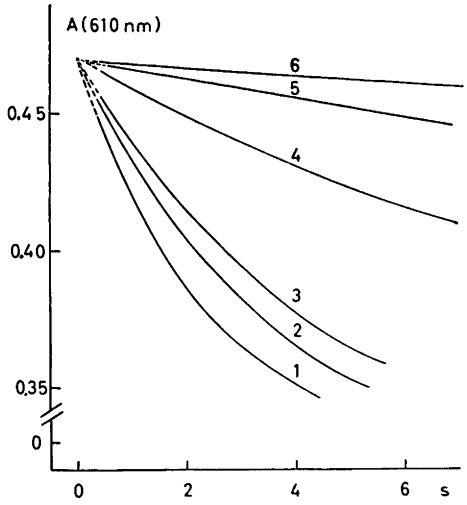


Fig. 1. Time course of the 610 nm absorbance change during the reduction of ceruloplasmin by phenothiazine derivative. 1, 1.25 mM promazine; 2, 1 mM promazine; 3, 0.63 mM promazine; 4, 1.25 mM alimemazine; 5, 2.5 mM diethazine; 6, 1.25 mM promethazine.

obtained on mixing ceruloplasmin with phenothiazine derivatives. The rate of reduction was found to increase with increasing substrate concentration, the reaction initially exhibiting a second order dependence on the substrate concentration. A rate constant, k, was calculated according to the equation, $k = \ln (A_0/A)/[S]t$, where A_0 represents the 610 nm absorption at zero time and [S] the substrate concentration. Three different concentrations of each substrate were used for the determination of the k-value (five in the case of chlorpromazine). The average k-values are listed in Table 1 together with the Hammett σ_{para}-values for the substituents in the 2-position of the phenothiazine ring.[9] It has been reported that the electron donor ability of the phenothiazines progressively falls as the σ_{para}-value increases,[10] and that the nature of the side chain in 10-position of the phenothiazine ring does not significantly influence the electron donor ability. In Fig. 2

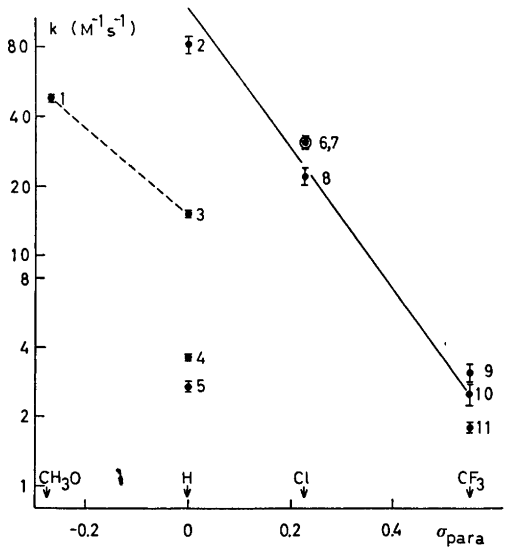
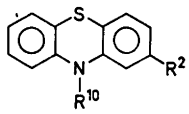
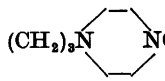
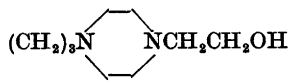
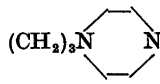
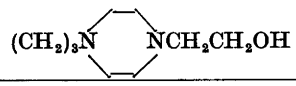


Fig. 2. The rate constant, k, plotted against the Hammett σ_{para}-value for the substituent in the 2-position of the phenothiazine ring. 1, Levomepromazine; 2, promazine; 3, alimemazine; 4, diethazine; 5, promethazine; 6, chlorpromazine; 7, perphenazine; 8, prochlorperazine; 9, trifluoperazine; 10, fluphenazine; 11, triflupromazine. Standard deviations are indicated on the figure.

the rate constant, k, is plotted semilogarithmically against the Hammett σ_{para}-value for the phenothiazines studied. A simple correlation between the rate of reduction of the 610 nm chromophore and the electron donor ability of the substrate could not be obtained. Promazine, alimemazine, promethazine, and di-

Table 1. Second order rate constants and Hammett σ_{para}-values for the phenothiazine derivatives.



Compound	R^2	R^{10}	k/ M^{-1} s^{-1}	σ_{para}-values
Levomepromazine	CH_3O	$CH_2CH(CH_3)CH_2N(CH_3)_2$	48	−0.27
Alimemazine	H	$CH_2CH(CH_3)CH_2N(CH_3)_2$	15	0.00
Promazine	H	$(CH_2)_3N(CH_3)_2$	82	0.00
Promethazine	H	$CH_2CH(CH_3)N(CH_3)_2$	2.7	0.00
Diethazine	H	$(CH_2)_2N(C_2H_5)_2$	3.6	0.00
Chlorpromazine	Cl	$(CH_2)_3N(CH_3)_2$	31	0.23
Prochlorpromazine	Cl		22	0.23
Perphenazine	Cl		31	0.23
Trifluprom azine	CF_3	$(CH_2)_3N(CH_3)_2$	1.8	0.55
Trifluoperazine	CF_3		3.1	0.55
Fluphenazine	CF_3		2.5	0.55

ethazine, having a proton in 2-position and hence equal electron donor ability,[10] gave different k-values. Phenothiazines with three carbon atoms between the ring nitrogen and the side chain nitrogen atom (promazine and alimemazine) reacted faster than those with only two (promethazine and diethazine). The former were also more rapidly oxidized by ceruloplasmin.[2] The present experiments demonstrate that the reaction between Type-1 Cu(II) and substrate is influenced by the nature of the side chain in 10-position.

Among the phenothiazines with a propyl side chain (promazine, chlorpromazine, perphenazine, prochlorperazine, trifluprom azine, fluphenazine, and trifluoperazine) the rate of decolourization was found to decrease with decreasing electron donor ability of the substrate as shown in Fig. 2 ($\log k \simeq -3.3\ \sigma_{para} + 2.2$). Similarly, levomepromazine, characterized by a methylated three carbon side chain (Table 1), reacted faster than its analog, alimemazine, displaying a lower electron donor ability. Alimemazine had a lower k-value than promazine, indicating that the carbon bound methyl group lowers the rate of reaction between the enzyme and phenothiazine.

Previous results, based on steady state kinetics, suggested that ceruloplasmin had greater affinity for chlorphenothiazines than for trifluphenothiazines.[2] The present observation that chlorphenothiazines react faster with Type-1 copper than the trifluphenothiazines (Fig. 2) is in accordance with this suggestion.

Materials. Human ceruloplasmin was purchased from AB Kabi and crystallized according to the method of Deutsch.[11] The purified enzyme had an absorbance ratio, A_{610}/A_{280}, of 0.038. Enzyme concentrations were calculated from the 610 nm absorption ($\varepsilon = 10.9\ mM^{-1}\ cm^{-1}$).[11] Promazine was obtained from AB Ferrosan, chlorpromazine from A/S Dumex, levomepromazine from AB Mekos, trifluprom azine and fluphenazine from E. R. Squibb & Sons, perphenazine from Schering Corp., alimemazine,

promethazine, diethazine, prochlorperazine and trifluoperazine from Pharma Rhodia, NADH from Sigma Chem. Co. and 2,2′-dipyridyl from E. Merck AG. Aqueous solutions were prepared in deionized, glass-distilled water.

Methods. Kinetic experiments were performed in a Beckman DK-1 recording spectrophotometer. A solution of phenothiazine derivative, NADH and 2,2′-dipyridyl was rapidly mixed with ceruloplasmin, using a stopped flow apparatus. The reaction mixture contained 43 μM ceruloplasmin, 0.25 mM NADH, phenothiazine derivative (0.63 – 5 mM) and 62 μM 2,2′-dipyridyl in 0.4 M sodium acetate buffer, pH 5.5, at 20 °C. Phenothiazine derivatives are oxidized to coloured radicals by ceruloplasmin.[1] Addition of NADH, which spontaneously reduces the radicals generated,[1] effectively prevents accumulation of these compounds. The rate of 610 nm chromophore reduction by phenothiazines was independent of the NADH concentration. NADH alone did not reduce the blue colour of the enzyme. 2,2′-Dipyridyl was added in order to prevent trace iron from activating the reaction.[8,12] Preliminary experiments established that the variation of the 2,2′-dipyridyl concentration between 62 μM and 0.38 mM had no effect on reaction velocities observed.

Acknowledgements. The author thanks Prof. O. Walaas, Dr. med. E. Walaas and Dr. H. Osmundsen for their help and interest and the pharmaceutical companies for the gift of phenolthiazine derivatives. This work was supported by grants from A. Jahres fond and Prof. E. Langfeldts fond.

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Synthesis of Optically Active Alcohols from 3-Cyclohexene-1-carboxylic Acid

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(*S*)-2-Propyl-1-dodecanol has been prepared from (*R*)-3-cyclohexene-1-carboxylic acid by use of the Kolbe electrolytic synthesis and the Wittig reaction. After changes of the number of carbon atoms in the reagents, the same route also allows the preparation of (*R*)-2-propyl-1-dodecanol from (*R*)-3-cyclohexene-1-carboxylic acid. The CD spectra of (*S*)-3-carboxyethyl-γ-butyrolactone, (*R*)-3-carboxyethyl-γ-butyrolactone methyl ester, and (*S*)-3-propyl-γ-butyrolactone are reported.

3-Cyclohexene-1-carboxylic acid, *1*, which is commercially available, has been resolved and the configurations of the enantiomers have been determined.[1–3] This acid is an attractive starting material for a variety of compounds [4,5] and in the present communication we describe the synthesis of optically active branched alcohols from *1*.

Reduction of (*R*)-3-cyclohexene-1-carboxylic acid, *1*, to the alcohol *2* with lithium aluminum hydride proceeded without racemization [2] (Chart 1). Ozonization of *2* followed by oxidative work-up gave the γ-lactone acid *3*.[2] Its structure follows from mass and NMR spectra of the methyl ester and the ring size from a carbonyl absorption at 1778 cm^{-1} in the infrared spectrum. This lactone acid, which contains a free carboxyl function and masked carboxyl and hydroxyl functions, is the key compound in the synthetic sequence, which can be elaborated in several directions. Kolbe electrolysis with a salt of a monocarboxylic acid or with the salt of a half-ester of a dicarboxylic acid converts *3* into a lactone containing an alkyl or an esterified carboxyalkyl side chain. The lactone ring can then be opened and the carboxyl and hydroxyl functions can be modified separately. It should also be observed that both enantiomers of the desired product can be obtained from one enantiomer of *3* by varying the reagents.

We have examplified the described approach with the synthesis of the two forms of 2-propyl-1-dodecanol, *7a* and *7b*. One enantiomer was prepared from *3* in the following way. Kolbe electrolysis of *3* in the presence of sodium acetate replaced the carboxyl group by a methyl group. 3-Propyl-γ-butyrolactone, *4a*, was formed in good yield and dimeric products were easily eliminated by distillation. The lactone was then converted into the hemiacetal

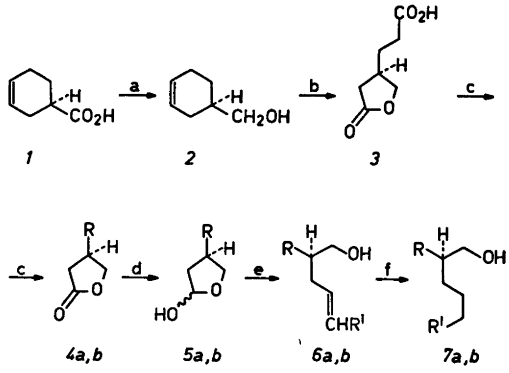


Chart 1. Scheme for the synthesis of optically active 2-alkylated alcohols: a. $LiAlH_4$, b. O_3 − 30 % H_2O_2 + HCOOH, c. Kolbe electrolysis, CH_3COONa or $C_8H_{17}COONa$, d. $[(CH_3)_2CHCH_2]_2AlH$, e. $C_6H_{16}=P(C_6H_5)_3$ or $CH_2=P(C_6H_5)_3$, f. H_2 + Pd/C.

5a by reduction with diisobutylaluminum hydride and the hemiacetal was allowed to react with the Wittig reagent octylidenetriphenylphosphorane.[6] Finally, the olefinic alcohol *6a* was catalytically reduced to the desired saturated alcohol *7a*, which was dextrorotatory.

The other enantiomer was obtained using the same type of reactions. Kolbe electrolysis of *3* in the presence of sodium nonanoate gave the 3-decyl-γ-butyrolactone, *4b*, which was reduced with diisobutylaluminum hydride to the hemiacetal *5b*. With methylenetriphenylphosphorane this compound yielded the unsaturated alcohol *6b*, which finally was reduced to the levorotatory enantiomer of 2-propyl-1-dodecanol, *7b*. The main problem we have encountered in the preparations described is the separation of desired and dimeric products formed in the Kolbe electrolysis.

Intermediates *3* and *4* (Chart 1) are chiral 3-substituted γ-lactones whose chiroptical properties have attracted considerable interest for some time.[7–11] We have therefore recorded the CD spectra for *3*, its methyl ester, and *4a* (*cf.* Exp.). Compounds (*S*)-*3* and (*S*)-*4a* give rise to CD curves displaying positive Cotton effects, while the (*R*)-*3* methyl ester gives a negative one for the n-π^* transition [12] at 218 nm. If we suppose that the preferred conformations of the γ-lactones have the 3-substituents in pseudoequatorial positions, as indicated in Fig. 1, our results are in agreement with the rule proposed by Legrand and Bucourt [9] and the findings by Beecham.[10]

In conclusion, we want to point out that, by variation of the reagents, the types of optically active compounds shown in Fig. 2 can be prepared from 3-cyclohexene-1-carboxylic acid.

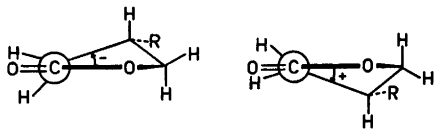


Fig. 1. Preferred conformations of 3-substituted γ-butyrolactones showing the sign of the dihedral angle $O-C(=O)-C_3-C_3$.

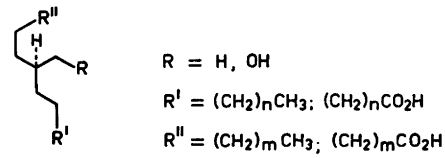


Fig. 2. Optically active compounds from 3-cyclohexene-1-carboxylic acid.

EXPERIMENTAL

General methods. GLC analyses were carried out on a Perkin-Elmer 900 instrument fitted with flame ionization detectors and 3 mm × 180 cm stainless steel columns packed with 3 % SE-30 on Gas Chrom Q. Column chromatographic separations were performed on Merck Kiselgel 60, particle diameter 0.063 – 0.200 mm. The eluents were continuously analysed with a Pye Unicam LCM2 detector. The CD spectra were determined with a Cary 60 instrument. The IR spectra were recorded on a Beckman IR 9 spectrophotometer. MS were determined on an AEI 902 mass spectrometer or on an LKB 9000 mass spectrometer connected to a gas chromatograph. The 270 MHz NMR spectra were determined in $CDCl_3$ with a Bruker WH270 instrument.

(R)-*3-Propyl-γ-butyrolactone*, 4a. (*R*)-3-Carboxyethyl-γ-butyrolactone, *3*, (0.5 g, 3.2 mmol) of 97 % optical purity [2] and acetic acid (3.6 g, 60 mmol) were separately dissolved in 5 and 10 ml of anhydrous methanol, respectively. The solutions were neutralized by dropwise addition of 1 M sodium methoxide solution. The combined methanolic solutions were electrolysed in a Kolbe electrolysis apparatus with mercury cathode and rotating platinum anode.[13] The initial current was 4 A and after 2 h it had decreased to 0.4 A. The reaction mixture was evaporated under reduced pressure, 5 ml of water was added, and the resulting mixture was extracted with 4 × 10 ml of ethyl ether. The combined organic solutions were washed twice with a saturated solution of sodium bicarbonate, twice with water, dried over anhydrous magnesium sulfate, and evaporated giving 500 mg of crude *4a*, $[\alpha]_D + 3.2°$ ($CHCl_3$, *c* 26). IR: 1778 cm^{-1} (lactone carbonyl). ^1H NMR (270 MHz): δ 0.94 (3 H, t), 1.20 – 1.55 (2 H, m), 1.37 (1 H, t), 1.45 (1 H, t), 2.09 – 2.28 (1 H, m), 2.44 – 2.71 (2 H, m), 3.92 (1 H, t), and 4.42 (1 H, t). MS: mol. wt. obs. 128.082 ± 0.003, calc. for $C_7H_{12}O_2$ 128.084.

(R)-*3-Decyl-γ-butyrolactone*, 4b. (*R*)-3-Carboxyethyl-γ-butyrolactone, *3*, (1.0 g, 6.3 mmol) of 97 % optical purity and nonanoic acid (4.0 g, 25.2 mmol) were reacted as described for the preparation of *4a*. The product (2.04 g) was purified by LC on silica gel (gradient elution with benzene – ethyl acetate) giving 300 mg of *4b*, $[\alpha]_D + 5.1°$ ($CHCl_3$, *c* 5), of 95 %

purity (GC). IR: 1778 cm^{-1} (lactone carbonyl). ^1H NMR (270 MHz): δ 0.88 (3 H, t), 1.19–1.39 (16 H, m), 1.39–1.55 (2 H, m), 2.09–2.28 (1 H, m), 2.44–2.71 (2 H, m), 3.92 (1 H, t), and 4.42 (1 H, t). MS: mol. wt. obs. 226.194 ± 0.003, calc. for $C_{14}H_{26}O_2$ 226.193.

(2RS,4R)-2-Hydroxy-4-propyltetrahydrofuran, 5a. In a dry nitrogen atmosphere *4a* (244 mg, 1.9 mmol) was dissolved in 10 ml of anhydrous toluene. A solution of diisobutylaluminium hydride [6,14] (8 mmol) in 10 ml of anhydrous toluene was added dropwise to the cooled, −70 °C, lactone solution over a period of 10 min. The solution was stirred at −70 °C and after 45 min, 10 ml of 4 M 2-propanol in toluene was added dropwise, followed by 2 ml of water. The reaction mixture was allowed to assume room temperature and was filtered. Toluene, as well as 2-propanol and water, was removed by evaporation under reduced pressure giving 100 mg of crude *5a*, $[\alpha]_D + 21°$ ($CHCl_3$, *c* 3). The IR spectrum showed no lactone carbonyl absorption. ^1H NMR (60 MHz): δ 0.92 (3 H, t), 1.1–2.6 (7 H, m), 3.2–4.4 (2 H, m), 4.6 (1 H, broad s), and 5.5 (1 H, broad d).

(2RS,4R)-2-Hydroxy-4-decyltetrahydrofuran, 5b. The lactone *4b* (240 mg, 1.33 mmol) was dissolved in 10 ml of anhydrous toluene and was reacted with diisobutylaluminium hydride (16 mmol) in 10 ml of anhydrous toluene as described above for the preparation of *5a* giving 240 mg of *5b*, $[\alpha]_D + 20°$ ($CHCl_3$, *c* 7). The IR spectrum showed no lactone carbonyl absorption. ^1H NMR (60 MHz): δ 0.7–2.5 (24 H), 3.2–3.7 (2 H, m), 3.7–4.4 (1 H, m), and 5.5 (1 H, broad d).

(R)-2-Propyl-4-dodecen-1-ol, 6a. A solution of octylidentriphenylphosphorane, from octyltriphenylphosphonium bromide [15,16] (2.73 g, 6.0 mmol) in 16 ml of dimethyl sulfoxide, was added dropwise to a solution of the lactol *5a* * (700 mg, 5.4 mmol) dissolved in 4 ml of dimethyl sulfoxide under dry nitrogen atmosphere.[14] The reaction mixture was stirred at 70 °C. After 24 h, 30 ml of water was added. The aqueous phase was extracted with 5 × 15 ml of pentane. The combined organic phases were washed three times with water, dried over anhydrous magnesium sulfate, and evaporated. The crude product was purified by LC on silica gel (benzene) giving 150 mg of *6a*, $[\alpha]_{546} - 2.0°$ (MeOH, *c* 6). ^1H NMR (60 MHz): δ 0.88 (3 H, t), 0.90 (3 H,t), 1.1–1.6 (15 H), 1.80 (1 H, s), 1.9–2.3 (4 H, m), 3.55 (2 H, d), and 5.3–5.6 (2 H, m). MS: mol. wt. obs. 226.226 ± 0.003, calc. for $C_{15}H_{30}O$ 226.230.

(R)-2-Decyl-4-penten-1-ol, 6b. Methylenetriphenylphosphorane [15] (20 mmol) dissolved in 20 ml of dimethyl sulfoxide was added dropwise to a solution of *5b* (180 mg, 0.79 mmol) dissolved in 2 ml of dimethyl sulfoxide [14] under dry nitrogen atmosphere. The reaction mixture was treated as described for the preparation of *6a*. The crude product was purified by preparative TLC on silica gel (benzene) giving 25 mg of *6b*, $[\alpha]_D + 8°$ ($CHCl_3$, *c* 2). MS: $M^+ = 226$. ^1H NMR (60 MHz): δ 0.88 (3 H, t), 1.1–2.3 (22 H), 3.55 (2 H, d), and 4.7–5.3 (3 H, m).

(R)-2-Propyl-1-dodecanol, 7a. Compound *6a* * (140 mg, 0.44 mmol) was dissolved in 15 ml of ethanol and hydrogenated at atmospheric pressure with 8 mg of 10 % Pd/C as catalyst. The suspension was filtered and the filtrate was evaporated giving 110 mg of a product, which was further purified by preparative TLC on silica gel with benzene as eluent. Yield: 53 mg of *7a*,* $[\alpha]_D - 0.7°$ (MeOH, *c* 1). ^1H NMR (270 MHz): δ 0.88 (3 H, t), 0.91 (3 H, t), 1.0–1.6 (24 H), and 3.5–3.6 (2 H, broad d). MS: mol. wt. obs. 210.233 ± 0.003, calc. for $C_{15}H_{30}$ ($M^+ - H_2O$) 210.235.

(S)-2-Propyl-1-dodecanol, 7b. Compound *6b*, (20 mg, 0.08 mmol) was dissolved in 10 ml of methanol and catalytically hydrogenated at 2.7 atm with 6 mg of 10 % Pd/C for 1 h in a Parr hydrogenation apparatus. The suspension was filtered and the filtrate was evaporated. The product was further purified by TLC giving 13 mg of *7b*, $[\alpha]_D + 7°$ ($CHCl_3$, *c* 1.5). ^1H NMR (270 MHz): δ 0.86 (3 H, t), 0.89 (3 H, t), 1.0–1.6 (24 H), and 3.5–3.6 (2 H, broad d). MS: mol. wt. obs. 210.240 ± 0.004, calc. for $C_{15}H_{30}$ ($M^+ - H_2O$) 210.235.

(S)-3-Carboxyethyl-γ-butyrolactone, 3. Crude *(S)-3*, $[\alpha]_D + 0.16°$ (MeOH, *c* 6), was prepared from *(S)-1* of 53 % optical purity as described in Ref. 2. CD: $[\theta]_{218} + 420$ (EtOH).

(R)-3-Carboxyethyl-γ-butyrolactone methyl ester. *(R)-3* methyl ester, $[\alpha]_D - 2.4°$ (ethyl ether, *c* 4), was prepared from optically pure *(R)*-3, esterified with CH_2N_2, and purified by chromatography over silica gel (ethyl ether) twice. CD: $[\theta]_{218} - 790$ (EtOH).

(S)-3-Propyl-γ-butyrolactone, 4a. *(S)-4a*, $[\alpha]_D - 5.3°$ (EtOH, *c* 0.6), was prepared from *(S)*−1 of 53 % optical purity as described for *(R)*−*4a* and was purified by distillation and by chromatography over silica gel (CH_2Cl_2–MeOH, 10:1). CD: $[\theta]_{218} + 540$ (EtOH).

* Prepared in an experimental series starting with *(R)*-3-cyclohexene-1-carboxylic acid of 52 % optical purity. Compound *7a* has, compared to *7b*, a specific rotation which is too low. We can offer no adequate explanation for this finding.

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The Crystal and Molecular Structure of 2,6-*trans*-Diphenylhexamethylcyclotetrasiloxane

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The crystal structure of 2,6-*trans*-diphenylhexamethylcyclotetrasiloxane, $C_{18}H_{28}O_4Si_4$, has been determined and refined using three-dimensional X-ray diffractometer data. The unit cell is monoclinic, space group $C2/c$ with the constants $a=23.147$ Å, $b=8.759$ Å, $c=14.877$ Å, $\beta=128.08°$. There is half a formula unit in the asymmetric unit. The structure was solved by the "heavy atom" technique and refined by least-squares procedures. The final R-value was 0.050 for 1635 symmetry independent reflexions. The eight-membered siloxane ring has a "chair form" configuration. The intramolecular bond distances and bond angles are discussed. The present structure is compared with that of the *cis* form.

The structure of the *trans* form of 2,6-diphenylhexamethylcyclotetrasiloxane was determined in order to compare it with the structure of the *cis* form [1] which has a very marked estrogen-ike activity.[2] Unlike the *cis* form the *trans* form has no pronounced biological activity. The material used for the X-ray crystallographic analysis was synthesized by Dow-Corning, USA, and was in the form of large, colourless crystals placed at our disposal by Kabi AB, Stockholm. The material was recrystallized by slow evaporation of a methanolic solution.

EXPERIMENTAL

A number of crystals were investigated by oscillation and Weissenberg diagrams which indicated a monoclinic system with systematic absences characteristic for either of the space groups Cc or $C2/c$. Unit cell parameters: $a=23.147$, $b=8.759$, $c=14.877$ Å, $\beta=128.08°$. The density of the crystals showed that the unit cell contained 4 molecules. For the recording of the intensities a crystal with the dimensions $0.2\times0.4\times0.25$ mm was mounted about its b-axis and sealed within a capillary of Lindemann glass since the crystals slowly evaporated in open air. A Philips linear automatic diffractometer (PAILRED) with graphite monochromatized Mo$K\alpha$-radiation ($\lambda=0.7107$ Å) was used for collecting the intensity data. Excluding systematic absences, 2788 symmetry independent reflexions of the layers $h0l$ through $h11l$ were recorded within $\sin\theta/\lambda\leq0.66$ Å^{-1}, using the ω-scanning technique. Out of these 1137 did not differ significantly from the background intensity which was measured for 40 s at the beginning and the end of each scan. The standard reflexions measured two times in each layer as a check of the stability of the crystal and the instrument showed no significant changes. The integrated intensities were corrected for Lorentz and polarization factors but not for extinction or absoption. The latter could be neglected because of the low linear absorption coefficient of the material ($\mu=2.67$ cm^{-1} for Mo$K\alpha$). The structure factors were placed on an approximately absolute scale by Wilson statistics and the Wilson plot gave a correlation coefficient for the K-kurve of -0.961 and an overall temperature factor $B=2.6$ Å^2. The normalized structure factors, $|E|$'s, were computed, but the $|E|$ value distribution did not clearly indicate whether the structure was centrosymmetric or not. For that reason the non-centrosymmetric space group Cc was assumed for the structure determination.

An IBM 360/75 computer was employed for all calculations in this study, using our local crystallographic program system.[3]

DETERMINATION AND REFINEMENT OF THE STRUCTURE

The structure was solved by the "heavy atom" technique. In the three-dimensional Patterson maps several of the strongest peaks

Table 1. Fractional atomic coordinates for non-hydrogen atoms ($\times 10^5$) and for hydrogen atoms ($\times 10^4$). Anisotropic temperature parameters ($\times 10^5$) for non-hydrogen atoms and isotropic temperature factors for hydrogen atoms. Standard deviations in parentheses. β_{ij} are the coefficients in the expression: $\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)]$.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Si(1)	31833 (5)	32707(10)	44922 (7)	278 (3)	1307(13)	657 (7)	−146 (9)	599 (8)	−18(14)
Si(2)	15294 (5)	27098(11)	32670 (7)	232 (3)	1307(13)	559 (6)	−85 (9)	401 (7)	71(14)
O(1)	34679(12)	23226(26)	56370(18)	367 (8)	1363(33)	795(18)	111(26)	760(21)	251(38)
O(2)	22981(12)	34308(32)	36635(19)	283 (8)	1980(45)	835(20)	−373(28)	532(21)	221(46)
C(1)	35582(16)	52377(38)	48863(25)	258(10)	1362(45)	633(22)	−49(32)	479(25)	170(49)
C(2)	43015(20)	55170(48)	55373(36)	278(11)	1748(65)	1218(38)	−140(40)	590(35)	−6(76)
C(3)	45891(24)	69703(60)	58626(43)	344(15)	2312(91)	1449(49)	−832(56)	639(44)	−394(98)
C(4)	41391(31)	81882(55)	55462(46)	563(22)	1621(74)	1512(52)	−659(63)	975(56)	−421(94)
C(5)	34143(28)	79652(55)	49184(50)	514(20)	1456(70)	1970(64)	51(54)	1014(60)	183(99)
C(6)	31197(20)	65093(48)	45812(37)	307(13)	1561(61)	1389(42)	−31(42)	613(39)	154(80)
C(7)	34822(26)	22168(50)	37835(38)	628(19)	1738(64)	1313(40)	−76(54)	1471(50)	−194(79)
C(8)	14348(25)	7642(51)	27370(33)	576(18)	1884(67)	934(33)	−502(55)	943(41)	−856(75)
C(9)	7827(22)	39607(58)	21762(35)	384(14)	2541(86)	1015(35)	547(55)	724(38)	1098(87)

Atom	x	y	z	B	Atom	x	y	z	B
H(2)	4621(21)	4646(47)	5748(31)	5.5	H(7C)	3322(21)	1198(51)	3600(33)	5.5
H(3)	5116(20)	7055(47)	6266(31)	5.5	H(8A)	1833(22)	102(47)	3352(33)	5.5
H(4)	4307(20)	9195(47)	5746(31)	5.5	H(8B)	1397(21)	690(48)	2066(32)	5.5
H(5)	3091(20)	8818(47)	4728(31)	5.5	H(8C)	1092(22)	146(46)	2605(32)	5.5
H(6)	2609(20)	6401(49)	4119(31)	5.5	H(9A)	846(21)	4897(46)	2515(31)	5.5
H(7A)	3946(22)	2011(50)	4179(33)	5.5	H(9B)	803(20)	3969(48)	1522(31)	5.5
H(7B)	3336(21)	2846(47)	3096(32)	5.5	H(9C)	296(21)	3820(50)	1812(32)	5.5

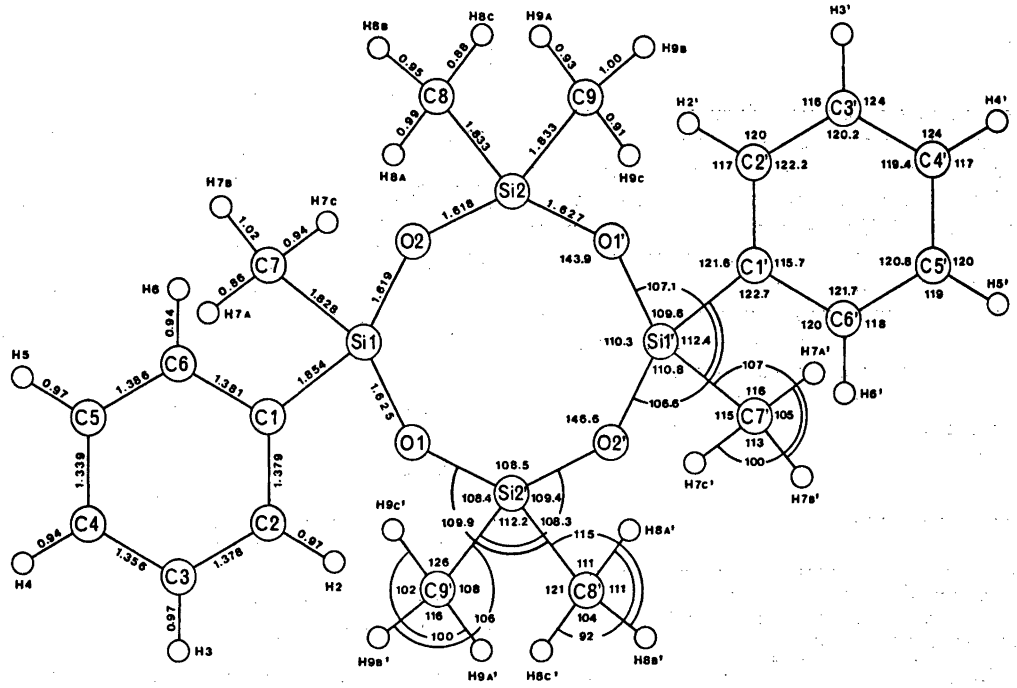


Fig. 1. The numbering of the atoms and bond distances and bond angles in the 2,6-*trans*-diphenylhexamethylcyclotetrasiloxane molecule.

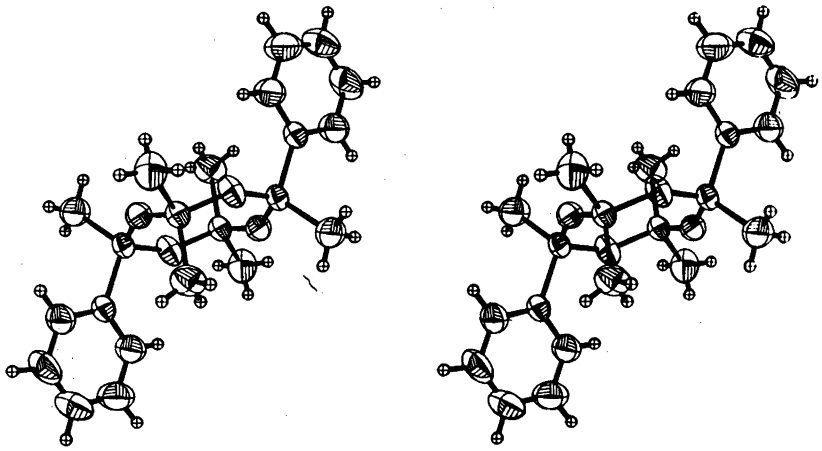


Fig. 2. The configuration of the 2,6-*trans*-diphenylhexamethylcyclotetrasiloxane molecule. The thermal ellipsoids for the non-hydrogen atoms are scaled to 50 % probability. Hydrogen atoms are shown as equally large spheres. The drawing is made with the plotting program ORTEP by Johnson.[12]

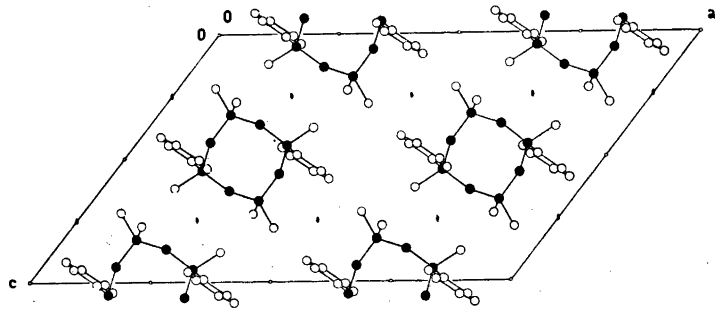


Fig. 3. Projection along *b* of the crystal structure of 2,6-*trans*-diphenylhexamethylcyclotetrasiloxane. The filled, dotted and open circles represent the atoms O, Si, and C, respectively.

could be interpreted as caused by four silicon atoms arranged in a slightly distorted square parallel to the $a-c$ plane at a height of $y \approx 0.30$. Phased by the four atomic positions of the silicon atoms thus determined, three-dimensional electron density maps were calculated. After a few cycles of Fourier syntheses it was easy to locate all the 26 non-hydrogen atoms of the molecule. The positional coordinates were subsequently refined by the full-matrix least-squares method. Atomic scattering factors were taken from International Tables for X-Ray Crystallography [4] except for those of the hydrogen atoms which were taken from the data of Stewart *et al.*[5] After one cycle of refinement using individual isotropic temperature factors the discrepancy index R ($R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$) dropped from an initial value of 0.25 to 0.165. One cycle of refinement with anisotropic temperature factors lowered the R-value to 0.086. At this stage it became clear that the structure indeed was centrosymmetric. After one further cycle of refinement with anisotropic temperature factors for the 13 non-hydrogen atoms of the asymmetric unit the R-value dropped to 0.080 when the centrosymmetric space group $C2/c$ was adopted. A difference Fourier synthesis prepared at this stage revealed the positions of the 14 hydrogen atoms. Three additional cycles of refinement of the positional and thermal parameters of the non-hydrogen atoms and one cycle of refine-

ment of the positional parameters of the hydrogen atoms gave a final R-value of 0.050 for 1635 observed structure amplitudes. Eight reflexions which obviously were erroneous were excluded from the last refinements. The shifts were all below the e.s.d.'s in the last cycle. The hydrogen atoms were assigned isotropic temperature factors of 5.5 Å^2. Weights were applied to $|F_o|$ according to $w=1/(a+|F_o|+b|F_o|^2)$ with $a=25.0$ and $b=0.01$. The positional and thermal parameters for the final structure are given in Table 1. On request, a list of the final observed and calculated structure factors may be obtained from the authors.

DESCRIPTION AND DISCUSSION OF THE STRUCTURE

Intramolecular bond distances and bond angles uncorrected for thermal motion are given in Fig. 1 which also shows the numbering system of the atoms. The estimated standard deviations for bond lengths are: Si – O = 0.002 Å, Si – C = 0.004 Å, C – C = 0.005 – 0.006 Å and C – H = 0.03 – 0.05 Å. For bond angles involving silicon atoms the e.s.d.'s are 0.2 – 0.3° and for C – C – C angles 0.4 – 0.6°. The average Si – O and Si – C_{meth} distances are 1.622 Å and 1.831 Å, respectively, and the Si – C_{phen} distance is 1.828 Å. The angles around the silicon atoms are all close to the expected tetrahedral value and the mean Si – O – Si angle is 145.2°. These values are in good agreement with corresponding parameters reported for 2,6-*cis*-diphenylhexamethylcyclotetrasiloxane,[1] for 1,1,2,2-tetramethyl-3,3,4,4-tetraphenylcyclotetrasiloxane,[6] and for octamethylcyclotetrasiloxane.[7] The benzene ring attached to Si(1) is planar (maximal deviation 0.003 Å) but slightly distorted. The angle C(6) – C(1) – C(2) of the benzene ring is thus quite small (115.7°). This type of distortion is also found in the 2,6-*cis*-diphenylhexamethylcyclotetrasiloxane [1] as well as in several other compounds where benzene rings are attached to a silicon compound.[8–10] The deviation from the regular hexagonal geometry of the benzene ring upon substitution for a hydrogen atom with an electron-withdrawing functional group also effects the bond lengths.[11] In the present structure the bonds C(3) – C(4) and C(4) – C(5) are considerably shorter than the aromatic standard value but this shortening can mainly be attributed to the effects of thermal motion.

The eight-membered siloxane ring possesses a centre of symmetry. Since the Si – O – Si angles are large (around 145°) the ring is relatively planar with a largest deviation from the plane for Si and O atoms of 0.3 Å. Owing to the symmetry the planes of the benzene rings are parallel and the angle between the least-squares plane through the siloxane ring and the plane through the benzene ring is 105°. The shape of the molecule can be seen in Fig. 2 which also depicts the ellipsoids of thermal motion. The cyclotetrasiloxane ring has a "chair form" configuration. The atoms Si(1) and O(2) lie 0.75 Å below and Si(1′) and O(2′) 0.75 Å above a plane through the atoms Si(2), O(1′), Si(2′) and O(1). This configuration ($\bar{1}$) differs from that of the *cis* form [1] ($\bar{4}2$ m) but is the same as found in the structure of octamethylcyclotetrasiloxane.[7] The packing of the molecules in the present structure is shown in Fig. 3 which is a projection along the b-axis. It was most interesting to find that in this projection the packings of the *cis* [1] and *trans* forms are virtually identical. This is also reflected in the cell parameters. When the cell edges and angles of the *cis* form are transferred to fit the shape of the unit cell of the present compound one obtains: $a=24.232$ Å, $b=8.829$ Å, $c=15.155$ Å and $\beta=132.07°$.

There are no short intermolecular distances. The closest carbon-hydrogen and hydrogen-hydrogen approaches are 3.00 Å and 2.51 Å, respectively. The rather loose packing of the structure is in accordance with the low density (1.17 g cm^{-3}) of the compound.

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Preparation of Some Thiophene-, Selenophene- and Furanthiols. Ionisation Potentials in Tautomer Analysis

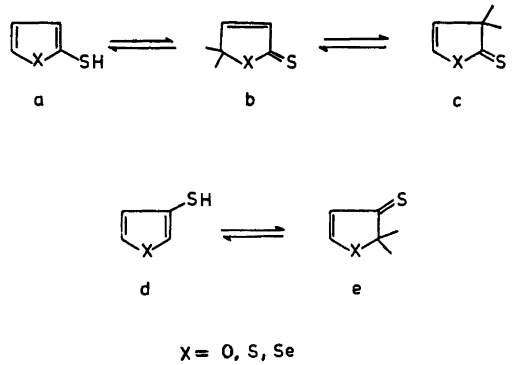
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5-Methyl-2-mercapto and 2,5-dimethyl-3-mercapto derivatives of furan, thiophene and selenophene have been prepared by treatment of the corresponding aryllithiums with sulfur. Upon alkylation by an ion pair extraction method, only the *S*-alkylated compounds were obtained. The potentially tautomeric thiol systems exist mainly in the thiol form in liquid solution and gas phase, as found by IR and NMR spectroscopy and by a study of ionization potentials.

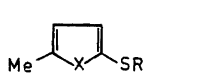
Analysis of some tautomeric 2- and 3-hydroxythiophenes, -selenophenes, and -furans by ionisation potential measurements in the gas phase in the mass spectrometer have shown that the 2-isomers are largely present in the lactone forms, whereas the 3-isomers are present in both the ketone and hydroxy forms.[1,2]

We report herein comparative studies of tautomeric thiol analogues (Scheme 1) in the mass spectrometer as well as studies of tautomeric equilibria in liquid state and in solutions.



Scheme 1.

The thiol compounds studied are the 5-methyl-2-mercapto and 2,5-dimethyl-3-mercapto derivatives of furan, thiophene and selenophene. As unsubstituted 3-hydroxythiophene decomposes the methyl substituents were introduced in order to obtain stable compounds.[3,4] Thiophenethiols can be prepared in various ways,[5–12] but the method used in this work is treatment of an aryllithium with sulfur followed by hydrolysis. The aryllithium was obtained by direct metalation in the 2-series,



	R	X		R	X
1	H	O	*7*	H	O
2	H	S	*8*	H	S
3	H	Se	*9*	H	Se
4	Me	O	*10*	Me	O
5	Me	S	*11*	Me	S
6	Me	Se	*12*	Me	Se

Scheme 2.

and by halogen-metal exchange in the 3-series. The halogen-metal exchange was performed at −70 °C in the furan and thiophene case, and at −110 °C in the selenophene case to avoid ring-opening.[13] Three of the six resulting thiol systems were not previously known (*cf.* Experimental part). Upon alkylation of these potentially tautomeric systems by an ion pair extraction method, only *S*-alkylated products were obtained with either dimethyl sulfate or methyl iodide as the alkylating agents.

Table 1. ^{1}H NMR parameters for 5-methyl-2-mercapto and 2,5-dimethyl-3-mercapto derivatives of furan, thiophene and selenophene at 60 MHz.

		δ-Values					J-Values							
		$2CH_3$	$5CH_3$	SH	3H	4H	3,4	3,SH	4,SH	$2CH_3$,SH	$5CH_3$,SH	4,$5CH_3$	3,$5CH_3$	$2CH_3$,$5CH_3$
1	[a]	–	2.23	3.40	6.31	5.88	3.0	1.9	–	0.5 [c]	1.0 [c]	1.0	0.4 [c]	–
2	[a]	–	2.37	3.40	6.81	6.50	3.4	1.6	–	–	1.1	1.1	0.4	–
3	[a]	–	2.46	3.63	6.96	6.63	3.6	1.4	–	–	1.1	1.2	–	–
7	[b]	2.20	2.14	2.49	–	5.74	–	–	–	1.2	–	0.9	–	0.5
8	[b]	2.28	2.28	2.83	–	6.37	–	–	0.4	0.8	–	1.0	–	–
9	[b]	2.37	2.36	2.88	–	6.53	–	–	0.4	0.8	–	1.1	–	0.7

[a] Deuteriochloroform solution. [b] Carbon disulfide solution. [c] Obtained by decoupling experiments at 100 MHz.

The ^{1}H NMR data of the thiols and their sulfide analogues are given in Tables 1 and 2, respectively. It can be seen that the chemical shifts as well as the coupling constants increase in the series furan < thiophene < selenophene. The only exceptions are the thiol *ortho* couplings in the two furan thiol systems, $J_{3S,H}$ and $J_{2\ CH_3,\ SH}$, which are larger than those of the thiophene and selenophene derivatives.

The ^{1}H NMR spectra of the potentially tautomeric thiols showed only the presence of the thiol tautomer. The same conclusion was reached from the IR spectra, which had absorptions in the region 2525 – 2545 cm^{-1} characteristic for the thiol form. The spectral data support earlier conclusions reached from dipole moment measurements that the thiol form of the five-membered heterocyclic thiols predominates to more than 95 %.[14,15] In potentially tautomeric hydroxy derivatives of five-membered heterocyclic compounds, however, the relative concentration of the hydroxy form may be small, and in some cases the hydroxy form is not seen by the usual spectroscopic techniques.[3–5,16] These findings agree with the general observation that in thione-enethiol tautometic equilibria, a higher percentage of the enethiol tautomer will be present than of the enol in an analogous keto-enol system.[17] Furthermore, the physical data for the monothiated carboxylic acids favour the thiolcarboxylic acid rather than the thionocarboxylic acid structure.[18] As expected from the above results the thiol form of the heterocyclic thiols was found to predominate in the gas phase in the mass spectrometer by measurements of ionisation potentials (IP). From Table 3 it is seen that in both the 2- and 3-series the methylthio derivatives of furan and thiophene (*4* and *5*) and (*10* and *11*), respectively, have closely similar IP values whereas the values for the corresponding selenophenes

Table 2. ^{1}H NMR parameters for 5-methyl-2-methylmercapto and 2,5-dimethyl-3-methylmercapto derivatives of furan, thiophene and selenophene at 60 MHz in deuteriochloroform solution.

	δ-Values					J-Values				
	$2CH_3$	$5CH_3$	SCH_3	3H	4H	34	4,$5CH_3$	3,$5CH_3$	4,$2CH_3$	$2CH_3$,$5CH_3$
4	–	2.25	2.32	6.28	5.90	3.0	1.0	0.4	–	–
5	–	2.37	2.35	6.81	6.50	3.4	1.1	0.4	–	–
6	–	2.46	2.40	6.93	6.65	3.6	1.2	–	–	–
10	2.26	2.20	2.21	–	6.24	–	0.8	–	0.4	0.6
11	2.36	2.35	2.28	–	6.58	–	1.0	–	–	0.5
12	2.48	2.44	2.29	–	6.79	–	1.2	–	–	–

Table 3. Ionisation potentials of 2-methyl-2-thiol, 2,5-dimethyl-3-thiol and the corresponding methylthio derivatives of furan, thiophene and selenophene.

Compound	IP ± 0.05 eV
1	8.45
2	8.48
3	8.17
4	8.15
5	8.13
6	7.84
7	8.23
8	8.22
9	7.90
10	7.91
11	7.96
12	7.73

(*6* and *12*) are 0.2 – 0.3 eV lower. These findings are in accordance with previous results obtained for the parent heterocycles and 3-methoxy derivatives.[2,17] The IP values for the thiols vary in the same manner as the IP values for the respective methylthio analogues and are *ca.* 0.3 eV higher. This difference is similar to that between thiophenol (8.93 eV) and thioanisol (8.60 eV), and between pyridine-3-thiol and 3-methylthio pyridine.[20] The IP values for the thione tautomers in the 2-series (*b* and *c*) and in the 3-series (*e*), or of model compounds thereof, are expected to vary significantly with the nature of the heteroatom.[1,2] Unfortunately, suitable compounds were not available for study, but the available data show that the thiol tautomers *a* and *d* are the predominant species in the gas phase. Their hydroxy analogues, however, are mainly present as lactones or ketones.[1,2] The difference in tautomeric preferences is another reflection of the relative tendencies of oxygen and sulfur for double bond formation.[18]

EXPERIMENTAL

General. ^{1}H NMR spectra were obtained with a Varian A-60 high resolution spectrometer. The IR spectra were recorded on a Perkin-Elmer model 257 instrument. The gas chromatograph used was a Perkin-Elmer 900 analytical instrument. Mass spectra were obtained with an LKB 9000 mass spectrometer. The ionisation efficiency curves were recorded with an AEI MS-902 mass spectrometer and the IPs obtained by the semilog-plot method as previously described,[28] with xenon as the reference gas. The reservoir and the connecting tubes were kept at 130 °C and the ion source at 230 °C. The IPs are the average of three determinations, the deviation being 0.05 eV. Elemental analyses were carried out at the Analytical Department at the Chemical Center, Lund, and by Dornis und Kolbe, Mikroanalytisches Laboratorium, Mülheim/Ruhr.

5-Methyl-2-mercaptofuran, 1 (Procedure A). To a solution of 24.6 g (0.300 mol) of 2-methylfuran (Aldrich) in 250 ml of dry diethyl ether, 235 ml of 1.40 N butyllithium in hexane was added at such a rate that the reaction mixture was gently refluxing. When the addition was complete, the reaction mixture was refluxed for another 2.5 h, whereupon it was cooled to −70 °C and 9.6 g (0.30 mol) of sulfur was added in one portion. The reaction mixture was stirred for half an hour at −70 °C and then slowly warmed to −30 °C, whereupon it was hydrolyzed with 750 ml of water and ice. The phases were separated and the water phase was extracted twice with ether. The combined water phases were covered with ice and ether and acidified with one equivalent of cold 2 N hydrochloric acid. The ether phase was separated and the water phase extracted twice with ether. The combined ether phases were washed with water until neutral reaction and dried over magnesium sulfate. VPC analysis of the ether solution showed only one component. Upon distillation under nitrogen, 13.7 g (40 %) of 5-methyl-2-mercaptofuran was obtained, b.p. 43 – 44 °C/1.6 kPa, n_D^{20} = 1.5165, IR (film), SH = 2525 cm^{-1}, for NMR data see Table 1. Anal. C_5H_6OS: C, H. M. Wt. 114.

5-Methyl-2-mercapto thiophene, 2, was prepared according to procedure A from 18.5 g (0.190 mol) of 2-methylthiophene, 135 ml of 1.40 N butyllithium and 6.1 g (0.190 mol) of sulfur, yielding 11.9 g (48 %) of the product, b.p. 72 – 73 °C/1.7 kPa, n_D^{20} = 1.5850 (lit.[5] 64 °C/1.3 kPa, n_D^{20} = 1.5904) IR (film): SH = 2525 cm^{-1}, for NMR data see Table 1.

5-Methyl-2-mercapto selenophene, 3, was prepared according to procedure A from 11.8 g (0.040 mol) of 2-methylselenophene,[21] 65 ml of 1.40 N butyllithium and 2.9 g (0.090 mol) of sulfur, yielding 5.6 g (35 %) of the product, b.p. 93 – 94 °C/1.6 kPa, n_D^{20} = 1.6320. IR (film): SH = 2525 cm^{-1}. For NMR data see Table 1. Anal. C_5H_6SSe: C, H, S. M.Wt. 178 (^{80}Se).

2,5-Dimethyl-3-mercaptofuran,[22] *7*, (Procedure B). To a solution of 11.1 g (0.068 mol) of 2,5-dimethyl-3-iodofuran[23] in 75 ml of dry diethyl ether at −70 °C, 55 ml of 1.37 N butyllithium in hexane was added dropwise with stirring under nitrogen. 15 min after the addition was complete, 2.4 g (0.075 mol) of sulfur was added in one portion. The stirring was

continued at −70 °C for another 90 min, whereupon the temperature was raised to −15 °C, and the reaction mixture poured into 200 ml of cold water. The phases were separated and the ether phase extracted with small portions of cold 10 % potassium hydroxide solution. The combined aqueous phases were acidified with cold dilute hydrochloric acid and immediately extracted with ether. The combined ether phases were washed with cold water and dried over magnesium sulfate. Distillation under nitrogen gave 4.5 g (78 %) of the product, b.p. 54–56 °C/1.6 kPa, n_D^{20} = 1.5084. For NMR data see Table 1.

2,5-Dimethyl-3-mercaptothiophene, *8*, was prepared according to procedure B from 17.0 g (0.071 mol) of 2,5-dimethyl-3-iodothiophene,[24] 55 ml of 1.34 N butyllithium and 2.4 g (0.075 mol) of sulfur, yielding 7.6 g (74 %) of the product, b.p. 80–82 °C/1.36 kPa, n_D^{20} = 1.5776 (lit.[25] b.p. 95–96 °C/2.6 kPa, n_D^{17} = 1.566). For NMR data see Table 1.

2,5-Dimethyl-3-mercaptoselenophene, *9*, was prepared according to procedure B from 20.0 g (0.700 mol) of 2,5-dimethyl-3-iodoselenophene,[26] 55 ml of 1.34 N butyllithium and 2.4 g (0.075 mol) of sulfur at −110 °C, yielding 4.0 g (30 %) of the product, b.p. 94–97 °C/1.3 kPa, n_D^{20} = 1.6122. For NMR data see Table 1. Anal. C_6H_8SSe: C, H, Se. M.Wt. 192 (^{80}Se).

Alkylation of the 5-methyl-2-mercaptofuran system with methyl iodide (Procedure C). A freshly prepared solution of 13.5 g (0.04 mol) of tetrabutylammonium hydrogen sulfate and 3.20 g (0.08 mol) of sodium hydroxide in 40 ml of water was added dropwise and with stirring to 4.55 (0.04 mol) of 5-methyl-2-mercaptofuran and 11.40 g (0.08 mol) of methyl iodide in 40 ml of chloroform at 30 °C. The reaction mixture was stirred vigorously for another 10 min, whereupon the phases were separated and the water phase extracted with chloroform. When the combined organic phases were concentrated and the residue treated with diethyl ether, tetrabutylammonium iodide precipitated. After filtration, the filtrate was washed with water and dried over magnesium sulfate. Distillation under nitrogen gave 3.5 g (68 %) of 5-methyl-2-methylthiofuran, *4*, b.p. 56–57 °C/1.6 kPa, n_D^{20} = 1.5190. For NMR data see Table 2. Anal. C_6H_8OS: C, H. M.Wt 128.

Alkylation of the 5-methyl-2-mercapto thiophene system with methyl iodide was carried out according to procedure C, using 3.25 g (0.025 mol) of 5-methyl-2-thiophenethiol, 7.1 g (0.050 mol) of methyl iodide, 8.5 g (0.025 mol) of tetrabutylammonium hydrogen sulfate and 2.0 g (0.05 mol) of sodium hydroxide, to yield 2.6 g (72 %) of 5-methyl-2-methyl-thiophene *5*, b.p. 83–84 °C/1.6 kPa, n_D^{20} = 1.5785 (lit.[27] b.p. 76–77 °C/1.3 kPa, n_D^{20} = 1.5785). For NMR data see Table 2.

Alkylation of the 5-methyl-2-mercaptoselenophene system with methyl iodide was carried out according to procedure C, from 2.00 g (0.01 mol) of 5-methyl-2-mercaptoselenophene, 3.25 g (0.022 mol) of methyl iodide, 3.82 g (0.01 mol) of tetrabutylammonium hydrogen sulfate and 0.90 g (0.023 mol) of sodium hydroxide, yielding 1.3 g (60 %) of 5-methyl-2-methylthioselenophene, *6*, b.p. 100–102 °C/1.6 kPa, n_D^{20} = 1.6140. For NMR data see Table 2. Anal. C_6H_8SSe: C, H, S. M.Wt 192 (^{80}Se).

Alkylation of the 2,5-dimethyl-3-mercaptofuran system with methyl iodide was carried out according to procedure C, using 1.3 g (0.010 mol) of 2,5-dimethyl-3-mercaptofuran, 3.3 g (0.023 mol) of methyl iodide, 4.0 g (0.012 mol) of tetrabutylammonium hydrogen sulfate and 0.9 g (0.023 mol) of sodium hydroxide, to yield 0.9 g (63 %) of 2,5-dimethyl-3-methylthiofuran, *10*, b.p. 62–64 °C/1.3 kPa, n_D^{20} = 1.5057. For NMR data see Table 2. Anal. $C_7H_{10}OS$: C, H, S. M.Wt 142.

Alkylation of the 2,5-dimethyl-3-mercaptofuran system with dimethyl sulfate was carried out according to procedure C with a small modification in the work-up, using 1.3 g (0.010 mol) of 2,5-dimethyl-3-mercaptofuran, 3.1 g (2.3 ml, 0.025 mol) of dimethyl sulfate, 4.0 g (0.012 mol) of tetrabutylammonium hydrogen sulfate and 0.9 g (0.023 mol) of sodium hydroxide. Upon treatment with diethyl ether the ammonium salt forms an oily slurry, which was separated and the ether phase was worked up as described for procedure C, yielding 1.2 g (85 %) of 2,5-dimethyl-3-methylthiofuran, *10*, b.p. 60–63 °C/1.5 kPa, identical with the compound described above.

Alkylation of the 2,5-dimethyl-3-mercaptothiophene system with methyl iodide was carried out according to procedure C, from 1.4 g (0.0097 mol) of 2,5-dimethyl-3-mercaptothiophene, 3.3 g (0.023 mol) of methyl iodide, 4.0 g (0.012 mol) of tetrabutylammonium hydrogen sulfate and 0.9 g (0.023 mol) of sodium hydroxide, yielding 0.7 g (45 %) of 2,5-dimethyl-3-methylthiothiophene, *11*, b.p. 100–101 °C/1.7 kPa, n_D^{23} = 1.1507. For NMR data see Table 2. Anal. $C_7H_{10}S_2$: C, H, S. M.Wt 158.

Alkylation of the 2,5-dimethyl-3-mercaptoselenophene system with methyl iodide was carried out according to procedure C, using 1.0 g (0.010 mol) of 2,5-dimethyl-3-mercaptoselenophene, 3.3 g (0.023 mol) of methyl iodide, 4.0 g (0.012 mol) of tetrabutylammonium hydrogen sulfate and 0.9 g (0.023 mol) of sodium hydroxide, to yield 1.1 g (55 %) of 2,5-dimethyl-3-methylthioselenophene, *11*, b.p. 104–106 °C/1.3 kPa, n_D^{20} = 1.6005. For NMR data see Table 2. Anal. $C_7H_{10}SSe$: C, H, Se. M.Wt 206 (^{80}Se).

Alkylation of the 2,5-dimethyl-3-mercaptoselenophene system with dimethyl sulfate was carried out according to the modified procedure C described above for the furan system, using 1.2 g (0.0063 mol) of 2,5-dimethyl-3-mercaptoselenophene, 2.0 g (1.5 ml, 0.016 mol) of dimethyl sulfate, 2.5 g (0.0074 mol) of tetrabutylammonium hydrogen sulfate and 0.6 g (0.015 mol) of sodium hydroxide, to yield

0.7 g (54 %) of 2,5-dimethyl-3-methylthioselenophene, *12*, b.p. 104–106 °C/1.3 kPA, identical with the compound described above.

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Studies on Organophosphorus Compounds. XV.* Synthesis of 2-Arylbenzothiazoles and 2-(α-Pyridyl)naphthothiazoles by Sulfur Oxidation in Hexamethylphosphoric Triamide (HMPA). ^{13}C NMR Studies

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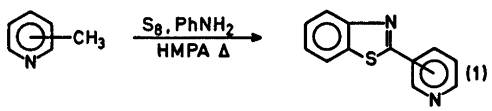
Heating primary aromatic amines in HMPA with elemental sulfur and α- or γ-picolines, benzylic esters, benzyl alcohol, acetophenone, and thiobenzanilide, respectively, produced 2-substituted thiazoles fused with aromatic rings. The following products have been synthesized: 2-(α-Pyridyl)benzothiazole, 2-(γ-pyridyl)benzothiazole, 2-(α-pyridyl)naphtho[1,2-*d*]thiazole, 2-(α-pyridyl)naphtho[2,1-*d*]thiazole, 2-benzylbenzothiazole, 6-methyl-2-phenylbenzothiazole, 2-phenylbenzothiazole, 4-methyl-2-phenylbenzothiazole, 2-(*p*-methoxyphenyl)benzothiazole. 2-Phenylbenzothiazole was obtained by heating thiobenzanilide with *o*-mercaptoaniline in HMPA. ^{13}C NMR spectra of benzothiazole, 2-phenylbenzothiazole, 2-(α-pyridyl)-benzothiazole, 2-(α-pyridyl)naphtho[1,2-*d*]thiazole, and 2-(α-pyridyl)naphtho[2,1-*d*]thiazole are reported. Proton-coupled ^{13}C NMR spectra and selective proton decoupling were used for signal assignments.

Recently much work has been done to find new ways of synthesizing 2-arylbenzothiazoles, as some of these compounds are found to be very effective antiinflammatory, analgesic, and antipyretic agents.[1–3] In an earlier report [4] we have shown that 2-phenylbenzothiazole was smoothly produced when benzyl alcohol, benzaldehyde, benzyl benzoate, *N*-benzylideneaniline, or thiobenzanilide were oxidized with elemental sulfur in the presence of aniline and hexamethylphosphoric triamide (HMPA) as co-solvent. The role of HMPA in these reactions has not been clarified but is still under investigation. Also in other reactions [4–6] elemental sulfur in HMPA is a very convenient thiation agent at elevated temperatures. A few reports have been given that 2-arylbenzothiazoles were produced from anilines and suitable aromatic compounds [7–9] but the yields reported were poor and the reaction conditions needed were rather severe. Compared to these results our synthesis of 2-phenylbenzothiazole [4] seemed so advantageous that a further investigation was started.

RESULTS AND DISCUSSION

As shown earlier α- and γ-picolines are smoothly oxidized by elemental sulfur in HMPA to the corresponding *N*,*N*-dimethylthiopicolinamides.[5] This prompted us to investigate reactions of picolines with elemental sulfur and primary aromatic amines in HMPA. Emmert [7,8] has shown that reaction of α- and γ-picolines with aniline and elemental sulfur as the main products produce the corresponding thioanilides. These reactions were performed under reflux for about 48 h. As by-products (< 25 %) the corresponding 2-pyridylbenzothiazoles were formed. In the presence of HMPA the reactions proceeded much more rapidly at lower temperatures to give high yields of the benzothiazoles, *1*, (82 and 62 % of the α- and γ-isomer, respectively).

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In the reaction of γ-picoline no *N*-phenylthioisonicotinamide could be isolated while the reaction of α-picoline produced 5 % yield of *N*-phenyl-α-thiopicolinamide.

α-Picoline was also reacted with α- and β-naphthylamine in the presence of sulfur and with HMPA as co-solvent. In these reactions naphthothiazoles were the only products isolated and they were formed in very high yields under rather mild reaction conditions compared to similar reactions performed by Emmert *et al.*[7,8] Thus reaction of α-naphthylamine produced 2-(α-pyridyl)naphtho[1,2-*d*]thiazole, *2*, in 86 % yield.

In the ^{1}H NMR spectrum of *2* a significant steric interaction is observed in the *peri*-hydrogen H-8 which appears as a multiplet at $\delta_8 \sim 9.0$. Further the assignment of structure *2* has been confirmed by ^{13}C NMR spectroscopy.

β-Naphthyl amine similarly produced 2-(α-pyridyl)naphtho[2,1-*d*]thiazole, *3*, in 83 % yield. Structure *3* was assigned by ^{13}C NMR spectroscopy.

Structural assignments by ^{13}C NMR. ^{13}C NMR studies of benzothiazoles have only been reported for 2-methylbenzothiazole.[15] However, only the signals of the quaternary carbon atoms and of the CH_3-group were assigned. It was our hope to obtain a complete assignment of the ^{13}C NMR spectra of the naphthothiazoles *2* and *3* to confirm the proposed structures conclusively.

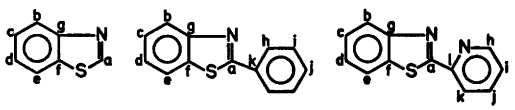
Benzothiazole, 4. ^{1}H NMR parameters for benzothiazole have been reported by Tobiason and Goldstein,[16] so an assignment of the signals from the proton bearing carbon atoms were straight-forward by selective decoupling of the protons. As the chemical shifts in the ^{1}H NMR spectrum are highly concentration dependent.[16] selective proton decoupling was performed at different concentrations. The chemical shifts are given in Table 1. The shift values for the quaternary carbon atoms δ 133.73(f) and δ 153.31(g) were in good agreement with the values given for 2-methylbenzothiazole [δ 135.5(f) and δ 153.3(g)].

2-Phenylbenzothiazole, 5. The signals of low intensity from the quaternary carbon atoms in the decoupled spectrum, δ(a), δ(f), δ(g), and δ(k) were easily assigned and δ(a), δ(f), and δ(g) (167.99, 135.12, 154.22) correspond nicely to the corresponding shifts in 2-methylbenzothiazole (166.4, 135.5, 153.3)[15] and 2-(α-pyridyl)benzothiazole, *6* (169.32, 136.15, 154.30). For δ(b), δ(c), δ(d), and δ(e) we found almost the same values as the corresponding signals in benzothiazole, *4*, and in 2-(α-pyridyl)-benzothiazole, *6* ($\Delta\delta < 0.45$). Further the corresponding selective proton decoupling frequencies appeared in the same order in the three compounds *4*, *5*, and *6*. The high intensity in the proton decoupled spectrum assigned two of the signals as δ(h) and δ(i) because of the equivalency of the two *ortho*-positions and the two *meta*-positions in the phenyl ring. It is known from other 2-phenyl substituted heterocycles that $\delta_{ortho} < \delta_{meta}$, and from ^{1}H NMR it has been shown that $\delta_{Hortho} < \delta_{Hmeta}$.[17] This gave the individual assignment of δ(h) and δ(i).

2-(α-Pyridyl)benzothiazole, 6. The signals from the benzothiazole part of the molecule were assigned according to the principles given above for 2-phenylbenzothiazole and the δ-values were in good agreement with the two compounds as seen in Table 1. Long-range ^{13}CH coupling constants for the pyridine and 2-bromopyridine have been determined by Hansen and Jakobsen.[18] From the undecoupled ^{13}C NMR spectrum of *6*, first order long-range ^{13}CH coupling constants were found which fitted very well with the reported constants. The chemical shifts of the α-carbon and of the γ-carbon in pyridine [18] have typical down-field values. The corresponding shifts for *6* were δ 149.57 [h; δ (pyridine) = 150.10] and δ 136.88 [j; δ (pyridine) = 135.85]. From the ^{1}H NMR spectrum of *6* δ_H(h), δ_H(i), and δ_H(k) were assigned. So selective proton decoupling easily afforded the corresponding carbon-13 shift values. The complete assignment is summarized in Table 1.

*2-(α-Pyridyl)naphtho[1,2-*d*]thiazole, 2.* ^{13}C NMR signals from the pyridine part of the molecule were easily assigned by first order long-range ^{13}CH coupling constants, selective proton decoupling, and typical down-field values for δ 149.51 (l) and 136.82 (h). The chemical shifts thus assigned differed very

Table 1. ^{13}C NMR chemical shifts of benzothiazoles.



4 *5* *6*

δ_X	*4*	*5*	*6*
a	153.75	167.99	169.32
b	123.63	123.27	123.58
c	126.10	126.28	126.21
d	125.47	125.16	125.59
e	121.81	121.60	121.96
f	133.73	135.12	136.15
g	153.31	154.22	154.30
h		128.99	149.57
i		127.57	125.15
j		130.91	136.88
k		133.68	120.71
l			151.40

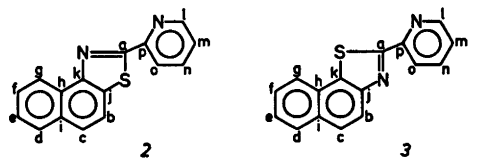
little from the corresponding shifts in the pyridine ring of *6*. The maximum difference was $\Delta\delta = 0.37$. Of the five remaining quaternary signals only assignment of δ(i) and δ(j) could cause some doubt. However, δ(i) was expected to be of higher intensity than δ(j) as C(i) is surrounded by two geminal protons while C(j) has only one geminal proton. Using additivity rules the assignment thus obtained fits very well with the calculated values for δ(i) and δ(j) as indicated in Table 3. Because of steric interaction the peri-proton Hg absorbs at very low field [δ_H 9.0(g)], so C(g) was easily assigned by selective proton decoupling. As C(b) is the only proton bearing carbon which has no $^3J_{CH}$ couplings it appears as the only sharp doublet in the undecoupled spectrum. As pointed out by Ernst [19] small $^3J_{CH}$– values are expected when the coupling nuclei are locked in a *cis*-conformation compared to *trans*-conformations. The assignment of C(c) is thus obtained. ($^3J_{C(c)H(d)}^{cis} \sim 4.5$ Hz). C(d) has both a $^3J_{CH}^{cis}$- and a $^3J_{CH}^{trans}$-coupling and from the long-range splittings of the remaining 3 signals C(d) is assigned leaving C(e) and C(f) unassigned. As expected δ(d), δ(e), and δ(f) deviate very little from the corresponding shifts in naphthalene. δ (naphthalene) 128.10(α) and δ 128.10(d); δ (naphthalene) 126.00(β) and $\{\delta(e), \delta(f)\} = \{126.11, 126.95\}$.

*2-(α-Pyridyl)naphtho[2, 1-*d*]thiazole, 3.* As this compound resembles compound *2*, the same principles of assignment could be used, only that the *peri*-proton Hg in *3* was not shifted as far down-field as in compound *2*. The assigned δ-values are summarized in Table 2.

With 2-(α-pyridyl)benzothiazole, *6*, as model compound the δ-values of the substituted ring of the naphthalene part of compounds *2* and *3* were calculated as follows: δ_x^{calc} (*2* or *3*) = $\delta_x(6) + \delta_x$ (naphthalene $- \delta_c$ (benzene). Deviations from the observed δ-values are defined as $\Delta\delta_x = \delta_x^{obs} - \delta_x^{calc}$ and summarized in Table 3. From these values it can be seen that additivity rules are fulfilled quite well except for δ(k). As underlined by Ernst [19] this discrepancy is probably of steric origin due to interaction with the *peri*-hydrogen H(g).

As presented in an earlier paper [4] reaction of benzyl benzoate with aniline and sulfur in HMPA produced 2-phenylbenzothiazole and benzanilide. As benzanilide was formed in rather high yields we suggested that the benzothiazole was formed mainly from the alcoholic part of the ester. To verify this suggestion *p*-methoxybenzyl benzoate, *7*, was reacted under

Table 2. ^{13}C NMR chemical shifts of naphthothiazoles.



2 *3*

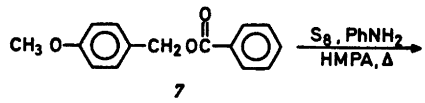
δ_X	*2*	*3*
a	168.10	168.20
b	119.29	121.84
c	126.37	127.41
d	128.10	128.88
e, f	126.11, 126.95	126.20, 127.01
g	123.78	125.29
h	128.96	128.26
i	132.02	131.20
j	133.18	152.40
k	150.61	133.73
l	149.51	149.57
m	124.78	124.89
n	136.82	136.91
o	120.52	120.38
p	151.75	151.44

Table 3. ^{13}C NMR chemical shift deviations from calculated values in naphthothiazoles.

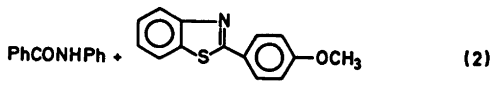
Compound	$\delta\Delta_X$[a] b	c	h	i	j	k
2	−0.18	1.17	0.15	0.58	−0.48	−3.30
3	0.75	1.59	1.07	0.38	0.59	−2.03

[a] $\Delta\delta_X = \delta_X$ (*2* or *3*) $- \delta_X$ (naphthalene) $+ \delta_X$ (benzene) $- \delta_X$ (*6*); δ_X (naphthalene): $\delta_\alpha = 128.10$; $\delta_\beta = 126.00$, $\delta_\gamma = 133.72$; δ_C (benzene) $= 128.49$.

similar conditions. Two main products were formed: benzanilide, *8*, in 64 % yield and 2-(*p*-methoxyphenyl)benzothiazole, *9*, in 78 % yield. Only traces of 2-phenylbenzothiazole were formed.



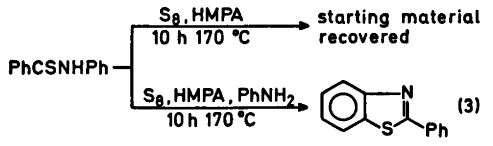
7



(2)

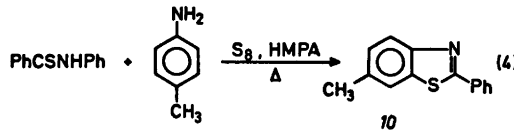
8 *9*

Often 2-arylbenzothiazoles are produced by oxidation of the corresponding thioanilides by potassium hexacyanoferrate(III) (Jacobsen oxidation).[10] As thioanilides could be isolated in many of our reactions and by short reaction times in good yields they were believed to be intermediates. However, a direct oxidation of these thioanilides by elemental sulfur cannot account for the formation of the thiazoles. No reaction took place when thiobenzanilide was heated with sulfur in HMPA. But by addition of aniline to this reaction mixture 2-phenylbenzothiazole was formed (82 %).[4]



(3)

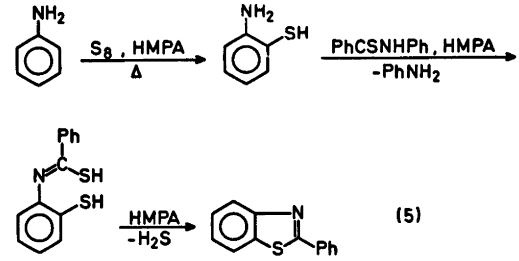
In fact we were able to react benzanilide with sulfur in HMPA above 200 °C to give thiobenzanilide (69 %) without producing any 2-phenylbenzothiazole.[11] Another indication that a simple oxidation of the thioanilides cannot account for the formation of the benzothiazoles was that reaction of thiobenzanilide with elemental sulfur in the presence of *p*-toluidine and HMPA did not produce any 2-phenylbenzothiazole, but 6-methyl-2-phenylbenzothiazole, *10*, was formed in 76 % yield.



(4)

10

We thus suggest that thioanilides are produced as intermediates, and *trans*-amidation of these with *ortho*-thiolated anilines, followed by H_2S elimination, produces the corresponding thiazoles. It is well-known that reaction of anilines with sulfur at elevated temperatures produces 2-mercaptoanilines or the corresponding polysulfides.[12,13]

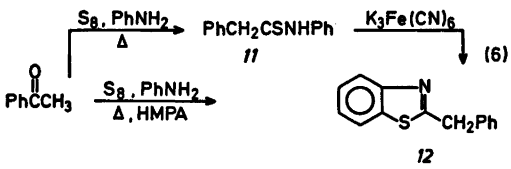


(5)

When *o*-mercaptoaniline was heated with thiobenzanilide at 170 °C in HMPA 2-phenylbenzothiazole was formed (60 %).

Reaction of benzyl alcohol with *o*-toluidine and sulfur in HMPA produced the expected 4-methyl-2-phenylbenzothiazole (68 %). In a similar experiment *o*-chloroaniline was reacted with benzyl alcohol. This reaction had to be run at rather high temperatures and the chlorine atom was substituted by sulfur to give 2-phenylbenzothiazole (43 %) and from reaction of benzyl alcohol with sulfur and HMPA was isolated *N*,*N*-dimethylthiobenzamide (46 %).

2-Phenylthioacetanilide, *11*, has been prepared in a Kindler reaction from acetophenone, aniline, and sulfur.[14] The thioanilide was then further oxidized by potassium hexacyanoferrate(III) to give 2-benzylbenzothiazole, *12*. In the presence of HMPA the benzothiazole, *12*,



was directly formed from acetophenone. It has earlier been reported that acetophenones are oxidized and degraded to *N,N*-dimethylthiobenzamides by heating with sulfur in HMPA.[5] As a by-product the degraded compound 2-phenylbenzothiazole was also formed in the reaction above.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 60 MHz on a Varian A-60 spectrometer. TMS was used as internal reference standard. ^{13}C NMR spectra were recorded at 20 MHz on a Varian CFT-20 instrument. $CDCl_3$ was used as solvent and the concentration was 15 % W/V. TMS was added as internal reference. Silica gel 60 (Merck) was used for column chromatography. 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.

2-(α-Pyridyl)benzothiazole, *6*. α-Picoline (9.3 g), sulfur (19.2 g), aniline (30 ml), and HMPA (10 ml) were heated together at 160–175 °C for 7.5 h. Non-reacted aniline and HMPA were removed by ether extraction (3 × 100 ml) from diluted hydrochloric acid (250 ml). The combined ether phases were washed with water (2 × 50 ml), dried ($MgSO_4$), and the ether evaporated. Column chromatography (ether/light petroleum) gave three pure products. *α-Thiopicolinanilide* (1.0 g, 5 %), m.p. 53 °C (lit.[8] m.p. 52 °C). NMR ($CDCl_3$): δ 7.2–8.9 (9 H, m), 12.6 (1 H, broad m). *N-Methyl-α-thiopicolinamide* (0.6 g, 4 %), m.p. 77 °C (lit.[20] m.p. 79 °C). NMR ($CDCl_3$): δ 3.4 (3 H, d), 7.3–8.8 (4 H, m), ~10.4 (1 H, broad). *2-(α-Pyridyl)-benzothiazole* (17.4 g, 82 %), m.p. 133 °C (lit.[8] m.p. 133–133.5 °C). NMR ($CDCl_3$): δ 7.1–8.7 (m). The ^{13}C NMR spectrum is summarized in Table 1.

2-(γ-Pyridyl)benzothiazole. γ-Picoline (9.3 g), sulfur (19.2 g), aniline (30 ml), and HMPA (10 ml) were heated together at 165–185 °C for 7 h. The reaction mixture was worked up as above. Column chromatography (ether/light petroleum) gave two pure products. *2-(N-Phenylamino)benzothiazole* (1.2 g), m.p. 159 °C (lit.[21] m.p. 161 °C). NMR ($CDCl_3$): δ 7.4 (9 H, m), 11.2 (1 H, broad). *2-(γ-Pyridyl)benzothiazole* (13.2 g, 62 %), m.p. 132–133 °C (lit.[7] m.p. 134–135 °C). NMR ($CDCl_3$): δ 7.2–8.2 (6 H, m), 8.8 (2 H, m).

2-(α-Pyridyl)naphtho[1,2-d]thiazole, *2*. α-Picoline (4.7 g), sulfur (9.6 g), α-naphthylamine (14.3 g), and HMPA (5 ml) were heated together at 165–170 °C for 7 h. The reaction mixture was very stiff, and was therefore dissolved in 25 ml of boiling acetone. Non-reacted aniline and HMPA were removed by ether extraction (3 × 100 ml) from diluted hydrochloric acid (250 ml). The combined ether phases were washed with water (2 × 50 ml) and dried ($MgSO_4$) and the ether evaporated. Column chromatography (ether/light petroleum) yielded *2-(α-pyridyl)naphtho[1,2-d]thiazole* (11.3 g, 86 %), m.p. 137 °C (lit.[8] m.p. 137–138 °C). NMR ($CDCl_3$): δ 7.1–8.7 (9 H, m), 9.0 (1 H, m). The ^{13}C NMR spectrum is summarized in Table 2.

2-(α-Pyridyl)naphtho[2,1-d]thiazole, *3*. α-Picoline (4.7 g), sulfur (9.6 g), β-naphthylamine (14.3 g), and HMPA (5 ml) were heated together at 170 °C for 7 h. The reaction mixture was worked up as for compound *2*, yielding *2-(α-pyridyl)naphtho[2,1-d]thiazole* (10.9 g, 83 %), m.p. 146–147 °C. NMR ($CDCl_3$): δ 7.1–8.7 (m). The ^{13}C NMR spectrum is summarized in Table 2. (Found: C 72.86; H 3.98; N 10.56; S 12.89. $C_{16}H_{10}N_2S$ requires: C 73.28; H 3.82; N 10.69; S 12.21).

2-(p-Methoxyphenyl)benzothiazole. *p*-Methoxybenzyl benzoate (12.1 g), sulfur (9.6 g), aniline (30 ml) and HMPA (10 ml) were heated together for 5 h at 170 °C. The same working-up procedure as for compound *6* gave two pure products. *2-(p-Methoxyphenyl)benzothiazole*, m.p. 122 °C (lit.[22] m.p. 121.5–122 °C). NMR ($CDCl_3$): δ 3.8 (3 H, s); 6.8–8.1 (8 H, m). (Found: C 69.75; H 4.55; N 5.80; S 13.30; $C_{14}H_{11}NOS$ requires: C 69.71; H 4.52; N 5.81; S 13.28 %). *Benzanilide* (6.3 g, 64 %), m.p. 161–162 °C (lit.[23] m.p. 160–161 °C). Spectral data were in accordance with an earlier prepared sample. On TLC small amounts of 2-phenylbenzothiazole (< 200 mg) were recognized, but we were unable to purify this compound adequately for further identification.

6-Methyl-2-phenylbenzothiazole. Thiobenzanilide (2.1 g), sulfur (1.6 g), *p*-toluidine (10 g), and HMPA (5 ml) were heated together at 170 °C for 10 h. The same working-up procedure as for compound *6* afforded *6-methyl-2-phenylbenzothiazole* (1.7 g, 76 %), m.p. 121–122 °C (lit.[24] m.p. 122–123 °C). NMR ($CDCl_3$): 2.4 (3 H, s); 7.1–8.1 (8 H, m).

2-Phenylbenzothiazole, *A*. Thiobenzanilide (1.1 g), *o*-mercaptoaniline (1 g) and HMPA (5 ml) were heated together at 170 °C for 10 h. Column chromatography (ether/light petroleum) of the reaction mixture afforded *2-phenylbenzothiazole* (0.67 g, 61 %), m.p. 113 °C (lit.[4] m.p. 113 °C). Spectral data were in accordance with an earlier prepared sample.[4]

2-Phenylbenzothiazole, *B*. Benzyl alcohol (5.4 g), sulfur (9.6 g), *o*-chloroaniline (25 ml), and HMPA (10 ml) were heated together at 175 °C for 12 h. The same working-up procedure as

for compound *6* afforded two pure products. *2-Phenylbenzothiazole* (4.5 g, 43 %). N,N-*Dimethylthiobenzamide* (3.8 g, 46 %), m.p. 67 °C (lit.[5] m.p. 67 °C). Spectral data were in accordance with an earlier prepared sample.[5]

4-Methyl-2-phenylbenzothiazole. Benzyl alcohol (5.4 g), sulfur (9.6 g), *o*-toluidine (25 ml), and HMPA (10 ml) were heated together at 170 °C for 10 h. The same working-up procedure as for compound *6* afforded *4-methyl-2-phenylbenzothiazole* (7.6 g, 67 %), m.p. 53 °C. NMR ($CDCl_3$): δ 2.8 (3 H, s), 7.2–8.2 (8 H, m). (Found: C 74.69; H 5.00; N 6.19; S 14.22; $C_{14}H_{11}NS$ requires: C 74.67; H 4.89; N 6.22; S 14.22).

2-Benzylbenzothiazole. Acetophenone (6.0 g), sulfur (12.8 g), aniline (20 ml), and HMPA (7 ml) were heated together at 185 °C for 6 h. The same working-up procedure as for compound *6* afforded two pure products from the rather complex reaction mixture, *2-phenylbenzothiazole* (1.6 g, 16 %). *2-Benzylbenzothiazole* (3.9 g, 35 %), b.p. 138 °C/0.25 mmHg (lit.[25] b.p. 157–163 °C/0.5 mmHg. Picrate m.p. 140–141 °C (lit.[14] m.p. 139–139.5). NMR ($CDCl_3$): 4.4 (2 H, s), 7.2–8.1 (9 H, m).

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Oxidation of 4-*O*-Methylglucuronic Acid and 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose with Chlorine in Aqueous Solution

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Treatment of 4-*O*-methylglucuronic acid with chlorine leads to 4-*O*-methylglucaric acid which is subjected to endwise oxidation resulting in the formation of methylpentaric and methyltetraric acids plus carbon dioxide. Demethylated aldaric acids are produced in minor amounts.

For 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose an oxidation of the xylose moiety to a xylonic acid moiety is a rapid reaction. Hydrolytic cleavage of the glycosidic bond results in the expected products.

A complex mixture of oligomeric acids and monomeric carboxylic acids, formed by fragmentation, is produced during the treatment of 4-*O*-methylglucuronoxylan with chlorine in aqueous solution.[1] These acids are also present in the effluent from the chlorination of wood pulp. To elucidate the reactions which occur during these treatments model experiments were made with 4-*O*-methylglucuronic acid and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

EXPERIMENTAL

4-*O*-Methylglucuronic acid (100 mg) and 2-*O*-(4-*O*-Me-α-D-GA*p*)-D-xylose (100 mg) were in separate experiments treated in darkness for 7 days at 28 °C with 200 cm³ of an aqueous solution containing 5.1 g of Cl_2 per litre. The excess chlorine was destroyed with sulfurous acid. After neutralization the reaction mixtures were kept at pH 8.5–9.0 for 5 h at room temperature to split lactones. The solutions were then applied to anion exchange columns (Dowex 1–X8, acetate).

No appreciable amounts of non-electrolytes were present in the effluents collected after washing with water, and no traces of sugar were detected. Monocarboxylic acids were eluted with 15 column volumes of 0.08 M sodium acetate and dicarboxylic acids with 15 column volumes of 0.3 M sodium acetate in 2 M acetic acid. Finally, oxalic acid was eluted with 4 column volumes of 0.5 M magnesium acetate.

After removal of the metal cations by cation exchange, the acid fractions were evaporated to dryness and weighed. The monocarboxylic acids were identified by GC of the trimethylsilyl (Me_3Si) derivatives on two stationary phases (OV-1 and QF-1) and by GC-MS.[2] In addition to this technique anion exchange chromatography in 0.3 M sodium acetate in 2 M acetic acid on preparative and analytical columns was employed for identification of the acids present in the dicarboxylic acid fraction.[3] An analytical column was used to determine the volume distribution coefficients, D_v.

The quantitative determinations of the organic acids by GC were based on the assumption that the peak areas were proportional to the weight of the derivatives.

Carbon dioxide was determined gravimetrically as barium carbonate.

Most of the methylated aldaric acids obtained in this work were identified in a study of the alkaline degradation of 4-*O*-methylglucuronic acid.[4] The structure determinations were based on analogies with the previously studied mass-spectrometric fragmentation of the derivatives of unsubstituted aldaric acids.[5] The same approach was used for the identification of 2-*O*-methylxylaric acid in this study. A peak at the *m/e*-value 467 (M–15) in the spectrum of the Me_3Si derivative indicated *m/e* 482 as the mass (M) of the molecular ion and suggested an *O*-methylpentaric acid. Moreover, an ion (*m/e* 349) of mass (M–15–118) was recorded

as anticipated for a pentaric acid. Peaks which could be explained by the predictable chain cleavages of a 2-*O*-methylpentaric acid were observed at *m/e* 321 and *m/e* 263. Prominent peaks at *m/e* 277 and *m/e* 219 were accounted for by secondary loss of carbon dioxide. Among the peaks in the upper part of the spectrum those at *m/e* 292 and *m/e* 234 were very prominent. They are characteristic of a structure-specific McLafferty-type rearrangement of a Me_3Si group and confirmed that the acid was a 2-*O*-methylpentaric acid. Since the acid was produced from 4-*O*-methylglucuronic acid, the 2-*O*-methylpentaric acid must be 2-*O*-methylxylaric acid.

RESULTS AND DISCUSSION

Table 1 shows that 82 % of the added 4-*O*-methylglucuronic acid was consumed after treatment with chlorine in aqueous solution for 7 days at 28 °C. Carbonic acid and dicarboxylic acids were the major reaction products. A carbon balance showed that the recovery was 97 %. Except for a slight amount of glucuronic acid formed by demethylation, no monocarboxylic acids were detected.

4-*O*-Methylglucaric acid formed by oxidation of the aldehyde group was the most abundant dicarboxylic acid. It has previously been shown [6] that aldonic acids are subjected to an endwise oxidation which results in a loss of C-1 as carbon dioxide and the formation of a new carboxyl group. A similar fragmentation of 4-*O*-methylglucaric acid explains the formation of 3-*O*-methylarabinaric (loss of C-1) and 2-*O*-methylxylaric (loss of C-6) acids. The presence of appreciable amounts of methylated tetraric acids indicates an endwise oxidation of the pentaric acids. The amount of *O*-methylerythraric acid derived from 3-*O*-methylarabinaric acid was somewhat less than that of *O*-methylthrearic acid which can be formed from both *O*-methylpentaric acids. Oxidation of the *O*-methyltetraric acids will give rise to *O*-methyltartronic acid. Gas chromatography indicated that only a trace amount was present.

Table 1. Organic acids formed on treatment of 4-*O*-methylglucuronic acid (MeGA; 100 mg) and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (MeGA*p*-Xyl; 100 mg) with chlorine for 7 days at 28 °C.

Product	Amount of acid produced from MeGA		MeGA*p*-Xyl		Distribution coefficient [b]
	mg	mol [a]	mg	mol [a]	D_v
Xylonic			3.9	8.0	
Threonic			0.6	1.5	
Glyceric			1.2	3.8	
Glycolic			4.3	19.2	
4-*O*-Methylglucuronic	18.3	18.3	12.0	19.6	
Glucuronic	0.6	0.6	0.2	0.4	
2-*O*-(4-*O*-Me-α-D-GA*p*)-D-xylose			0.3	0.3	
2-*O*-(4-*O*-Me-α-D-GA*p*)-D-xylonic			65.6	62.7	4.1
4-*O*-Methylglucaric (3-*O*-Methylgularic)	23.5	21.8	1.7	2.6	8.5
Glucaric	3.8	3.8	0.2	0.3	
3-*O*-Methylarabinaric	12.2	13.1	0.8	1.4	11.3
2-*O*-Methylxylaric	13.0	13.9	1.2	2.1	10.1
Arabinaric	1.2	1.4	0.1	0.2	
Xylaric	1.1	1.3	0.1	0.2	
O-Methylerythraric	6.1	7.7	0.6	1.2	9.3
O-Methylthrearic	7.8	9.9	0.7	1.5	14.7
Erythraric	0.8	1.1	<0.1	–	
Threaric	3.0	4.2	0.3	0.7	
Tartronic	0.2	0.3	0.3	0.9	
Oxalic	2.8	6.5	0.8	3.0	
Carbon dioxide	15.0	70.9	12.1	93.5	

[a] Mol per 100 mol starting material. [b] $D_v = \bar{V}/X - \varepsilon$, where $\bar{V}$ is peak elution volume, X is the column volume and ε the interstitial fraction ($\varepsilon = 0.39$). Marker: galactaric acid ($D_v = 12.5$ in 0.3 M sodium acetate – 2 M acetic acid, 30 °C.

A demethylation of the *O*-methylaldaric acids occurred to an appreciable extent, and, as expected, all demethylated species were present. Only a small amount of glucuronic acid was formed, indicating that the oxidation of 4-*O*-methylglucuronic acid was strongly favoured compared to demethylation.

Under the same reaction conditions the biouronic acid was consumed almost completely. The yield of products (Table 1) was 96 % calculated on a carbon basis. Fig. 1 shows that a primary reaction is an oxidation of the xylose moiety to a xylonic acid moiety,[6] and that this reaction is more rapid than the consecutive reactions which lead to fragmentation. Except for 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid, no other compounds containing two glycosidically linked moieties were formed. Evidently the 4-*O*-methylglucuronic acid substituent at C-2 in the xylonic acid moiety and the pyranosyl structure of the uronic acid moiety prevent an endwise oxidation of this compound.

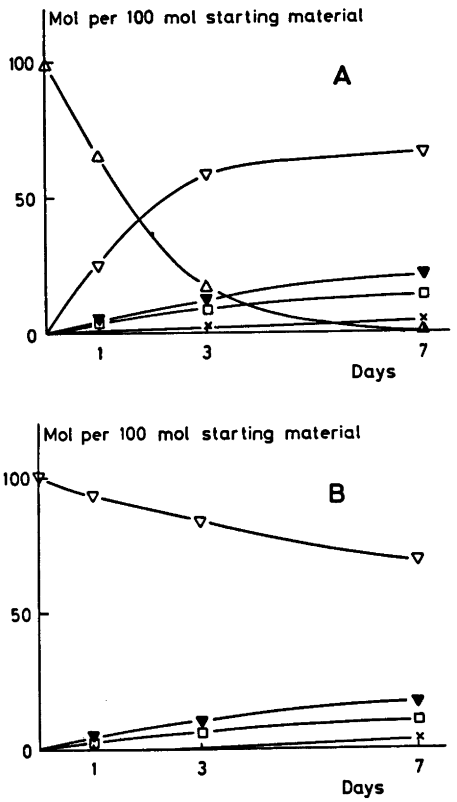


Fig. 1. Treatment of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (A) and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid (B) with chlorine (4.8 g/l) in aqueous solution at 27.3 °C. △, 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose; ▽, 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid; ▼, 4-*O*-Methylglucuronic acid; □, Xylonic acid; ×, 4-*O*-Methylglucaric and methylpentaric acids.

A hydrolytic cleavage of the glycosidic bond resulted in appreciable amounts of 4-*O*-methylglucuronic and xylonic acids already during an early period of the treatment. During the whole reaction period the concentration of 4-*O*-methylglucuronic acid was higher than that of xylonic acid. In consecutive reactions the liberated 4-*O*-methylglucuronic acid gave rise to the same reaction products as observed in the experiments with this acid (Table 1). The amount of carbon dioxide shows that the main part must be derived from the xylose moiety (~70 %). Xylonic acid was further degraded to threonic, glyceric, and glycolic acids.[6] The higher stability towards endwise oxidation of the uronic acid compared to xylonic acid is explained by the fact that in aqueous solution hexuronic acids are predominantly present in the pyranose form.

The experiments with 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid included in Fig. 1 confirm that this acid is decomposed to 4-*O*-methylglucuronic and xylonic acids. The higher concentration of the uronic acid confirms that its oxidative degradation is slower than that of the aldonic acid.

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Bacterial Carotenoids. LII. C_{50}-Carotenoids. 18.* Synthesis of the Model Compounds (2*R*,6*S*,2′*R*,6′*S*)-2,2′-Dimethyl-γ,γ-carotene and (2*R*,2′*R*,6′*S*)-2,2′-Dimethyl-β,γ-carotene

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The title compounds were synthesized from (+)−(2*S*,6*R*)-*cis*-γ-irone(*1*). The physical properties of the C_{42}-carotenes *5* and *6* and intermediates are reported for future stereochemical correlation of naturally occurring carotenoids with γ-end-groups.

We have reported earlier on the syntheses and chiroptical properties of the C_{42}-carotenes (2*R*,2′*R*)-2,2′-dimethyl-β,β-carotene,[1] (2*R*,6*S*,2′*R*,6′*S*)-2,2′-dimethyl-ε,ε-carotene and (2*R*,6*R*,2′*R*,6′*R*)-2,2′-dimethyl-ε,ε-carotene.[2] The chiroptical and ^{1}H NMR properties of these synthetic, optically active carotenes were decisive in assigning the absolute configuration in relation to the naturally occurring C_{50}-carotenoids *C.p.* 450 [(2*R*,2′*R*)-2-(4-hydroxy-3-hydroxymethyl-2-butenyl)-2′-(3-methyl-2-butenyl)-β,β-carotene] [1] and decaprenoxanthin [(2*R*,6*R*,2′*R*,6′*R*)-bis(4-hydroxy-3-methyl-2-butenyl)-ε,ε-carotene].[2]

Carotenes in the C_{40}-series and carotenoids in the C_{50}-series featuring the γ-end-group with an exocyclic double bond have been reported to occur naturally, namely β,γ-carotene [4,5] (optically active,[6] stereochemistry unknown); γ,γ-carotene [5] (*7*, assumed optically active) and sarcinaxanthin (optically active).[7,8]

The availability of (+)-(2S,6*R*)-*cis*-γ-irone (*1*)[1,9] suggested the synthesis of optically active C_{42}-carotenes in order to determine the absolute configuration of naturally occurring carotenoids of the same chromophore, containing the γ-end-group. We report now on the synthesis of (2*R*,6*S*,2′*R*,6′*S*)-2,2′-dimethyl-γ,γ-carotene (*5*) and (2*R*,2′*R*,6′*S*)-2,2′-dimethyl-β,γ-carotene (*6*).

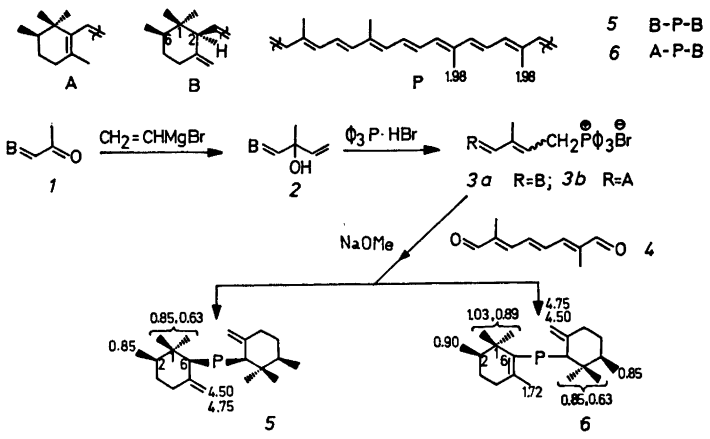
RESULTS AND DISCUSSION

Naturally occurring (+)-(2*S*,6*R*)-*cis*-γ-irone (*1*) was the key building block for the synthesis of *5* and *6*. The numbering system for starting irone [9] and early intermediates differ from that of the carotenes; see Scheme 1.

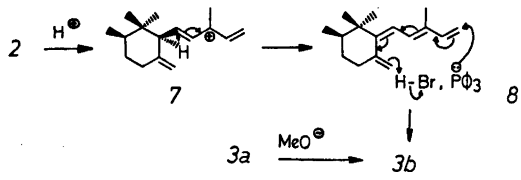
Reaction of *1* with vinylmagnesium bromide gave (2*S*,6*R*)-vinyl-γ-ironol (*2*) in nearly quantitative yield. Treatment of *2* with triphenylphosphonium bromide gave the phosphonium salt *3* (possibly a mixture of *3a* and *3b*). Condensation of the phosphoran derived therefrom with the dial *4* gave two products (2*R*, 6*S*, 2′*R*, 6′*S*)-2,2′-dimethyl-γ,γ-carotene (*5*) and (2*R*,2′*R*, 6′*S*)-2,2′-dimethyl-β,γ-carotene (*6*). The latter product resulted from isomerization of the labile γ end-group to the thermodynamically more stable β end-group. A similar isomerization of a γ to a β end-group was previously noted during a Horner reaction with γ-ionone.[10,11] However, ^{1}H NMR analysis of the vinyl-γ-ironol (*2*) demonstrated that no isomerization occurred during the Grignard reaction from *1*. Isomerization may have been effected during formation of the phosphonium salt through intermediates *7* and *8* (Scheme 2), since compounds similar to *8* are

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reported to provide phosphonium salts with triphenylphosphine hydrobromide.[12] Alternatively base catalyzed isomerization of *3a* may have occurred during generation of the phosphoran (Scheme 2). Either of these alternatives would lead to the *β*-type phosphonium salt *3b* and account for the presence of the dimethyl-*β,γ*-carotene *6* in the final reaction mixture.



The optically active C_{42}-carotenes *5* and *6* had electronic absorption spectra and IR spectra identical with their C_{40}-counterparts.[10,11] The mass spectra of *5* and *6* showed the predicted molecular ions and the typical loss of toluene (M − 92), xylene (M − 106) and also M − 158.[13] Other fragment ions originated from fragmentation patterns similar to those of the corresponding C_{40}-carotenes[10,11] with the exception that the *m/e* values were increased due to the methyl substitutents at C-2,2′. Qualitative CD spectra of *5* and *6* are given in Fig. 1.

The ^{1}H NMR and CD data reported here have been used for stereochemical correlation of naturally occurring C_{50}- and C_{40}-carotenoids containing the *γ* end-group.[8,14]

EXPERIMENTAL

Materials and methods. General methods, including instrumentation, chromatography and the GLC separation of irones from Iris oil have been described elsewhere.[1,2]

(+)-(2S,6R)-cis-γ-Irone (1). 1 isolated by preparative GLC was better than 98 % pure as measured by analytical GLC. *1* had the following properties: CD see Fig. 1; $[\alpha]_D^{20}$ (EPA) 589 nm = 4°, 578 nm = 5°, 546 nm = 6°, 436 nm = 21°,

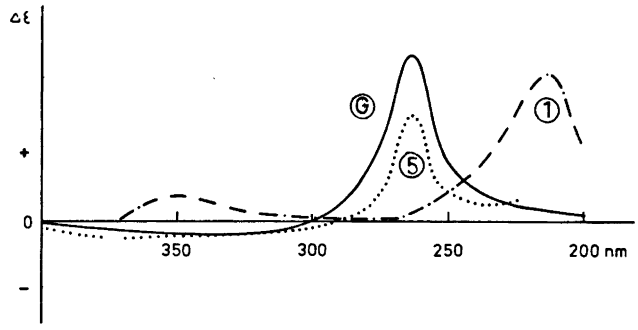


Fig. 1. Qualitative CD spectra in EPA solution of (2*S*,6*R*)-*cis*-*γ*-irone (*1*), (2*R*,6*S*,2′*R*,6′*S*)-2,2′-dimethyl-*γ,γ*-carotene (*5*), and (2*R*,2′*R*,6′*S*)-2,2′-dimethyl-*β,γ*-carotene (*6*).

365 nm = 112°; λ_{max} 222 nm (EPA), 227 nm (MeOH); IR(liq.) 3082 (m), 2965, 2937, 2860, 1650, 1630, 1455, 1370, 1258 (s), 1220, 1195, 1175 (m), 1145, 1110, 1049, 1025 (w), 1000 (s), 980, 960 (w), 895 (s) and 740 (m) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.63, 0.88 (6 H, *gem.* dimethyl), 0.88 (d, 3 H, J 5 Hz, $>CH-CH_3$), 1.0–1.7 (5 H, complex, $>CH_2$, $>CH-CH_3$), 2.27 (3 H, $O=C-CH_3$), 2.56 (d, 1 H, J 10 Hz, $>CH-C=C$), 4.45, 4.80 (2 H, $>C=CH_2$), 6.03 (d, 1 H, J 16 Hz, $>CH-C=O$) and 6.95 (q, 1 H, J_1 16 Hz, J_2 10 Hz, $>CH=CH-C=O$; *m/e* 206 (M), 191 (M−15), 149 (M−57) and 121 (M−85).

(+)-(2S,6R)-cis-γ-Vinyl-ironol (2). *1* (570 mg) gave *10* (590 mg, 90 %) when reacted with vinylmagnesium bromide using earlier described methods.[3] *2* had $[\alpha]_D^{20}$ (EPA) 589 nm = 7°, 578 nm = 8°, 546 nm = 9°, 436 nm = 17°, 365 nm = 33°; IR (liq.) 3390 (s) 3082 (m), 2970, 2930, 2860, 1645 (s), 1455, 1410, 1390, 1370 (m), 1265, 1195, 1140, 1100 (w), 990, 920 (s), 895 (m), 740 (s) and 650 (m) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.62, 0.88 (6 H, *gem.* dimethyl), 0.83 (d, 3 H, J *ca.* 5 Hz, $>CH-CH_3$), 1.0–1.7 (5 H, complex, $>CH_2$, $>CH-CH_3$), 1.38 (3 H, $CH_3-CH-OH$), 2.27 (d, 1 H, broad, $>CH-C=C<$), 3.65 (1 H, broad, OH), 4.73, 4.48 (2 H, $>C=CH_2$), 5.03 (q, 1 H, J_1 10 Hz, J_2 2 Hz, *cis* $-CH=CH_2$), 5.22 (q, 1 H, J_1 17.5 Hz, J_2 2 Hz, *trans* $-CH=CH_2$), 5.73 (1 H, $=CH-COH$), 5.70 (d, 1 H, J 9 Hz, $-CH=CH_2$), and 6.02 (q, 1 H, J_1 17.5 Hz, J_2 10.5 Hz, $-CH=CH-COH$); *m/e* 234 (M), 216 (M−18) and 263 (M−71).

(2S,6R)-cis-γ-Ironylidene-ethyltriphenylphosphonium bromide (3a). *3a* was prepared by reacting *2* with triphenylphosphonium bromide following earlier described procedure;[3] yield 70 % from *2* (570 mg).

(2R,6S,2'R,6'S)-2,2'-Dimethyl-γ,γ-carotene (5). The phosphoran generated from *3a* with sodium methoxide was reacted with 2,7-dimethylocta-2,4,6-trienedial (*4*, 15 mg) and the products isolated and purified by chromatography using procedures described earlier;[3] yield 17.5 mg, 31 % based on dial *4*. Both *5* and the isomerization product *6* were obtained. Crystallized from MeOH–$CHCl_3$ solution, *5* had m.p. 165–168 °C. The electronic absorption and IR spectra could not be distinguished from those of γ,γ-carotene;[10,11] CD spectrum is shown in Fig. 1; ^1H NMR signals are indicated on structure *5* in Scheme 1; *m/e* 564 (M), 485 (M−79), 472 (M−92), 458 (M−106), 427 (M−137), 406 (M−158), 460, (M−204), 347 (M−217), and 282. R_F = 0.33 on Schleicher & Schüll (S&S) 288 alumina paper developed with light petroleum.

(2R,2'R,6'S)-2,2'-Dimethyl-β,γ-carotene (6) was an isomerization product formed during the synthesis of *13;* yield *ca.* 5 mg. *6* had m.p. 156–158 °C after crystallization from MeOH–$CHCl_3$ solution. The electronic absorption and IR spectra could not be distinguished from those of β,γ-carotene.[10,11] The CD spectrum of *6* is reproduced in Fig. 1 and ^1H NMR signal assignments included in Scheme 1; *m/e* 564 (M), 485 (M−79), 472 (M−92), 458 (M−106), 427 (M−137), 413 (M−141), 406 (M−158), 460 (M−204), 347 (M−217) and 282. R_F = 0.40 on S&S 288 alumina paper developed with light petroleum.

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Bacterial Carotenoids. LIII. C_{50}-Carotenoids. 19.* Absolute Configuration of Sarcinaxanthin and Sarcinaxanthin mono-β-D-glucoside. Isolation of Sarcinaxanthin Diglycoside

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By improved ^{1}H NMR data sarcinaxanthin is shown to be centrosymmetric with terminal methylene groups in substituted γ-rings, consistent with IR and MS data. (2*R*,6*R*,2′*R*,6′*R*)-chirality for sarcinaxanthin (*4*) follows from CD and ^{1}H NMR data in comparison with appropriate models.

Evidence is presented that sarcinaxanthin occurs in *Sarcina lutea* at least partly esterified with a $C_9H_{11}COOH$ acid.

A previously characterized C_{50}-carotenoid β-D-glucoside is by ^{1}H NMR and CD data shown to be sarcinaxanthin mono-β-D-glucoside (*9*).

A dihexoside, presumably sarcinaxanthin diglucoside (*10*), was isolated for the first time.

Previously characterized less polar carotenoids were not present in three batches.

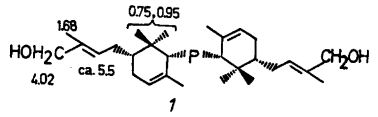
CD data for sarcinaxanthin (*4*) and decaprenoxanthin (*1*) are discussed in relation to preferred conformations.

Previous work [1,2] on the configuration of sarcinaxanthin from *Sarcina lutea* established its isomeric relationship to the C_{50}-diol decaprenoxanthin (*1*).[3–5] The structures *2* and *3* (Scheme 1) with substituted ε end groups were compatible with available ^{1}H NMR evidence and lacking Retro-Diels-Alder fragmentation on electron impact.[2] For differentiation between these alternatives this project was undertaken.

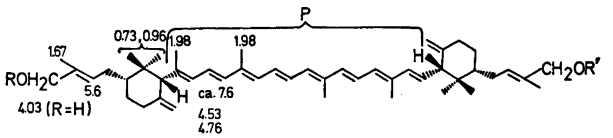
RESULTS AND DISCUSSION

The previous time-averaged ^{1}H NMR spectrum of sarcinaxanthin could not be reproduced

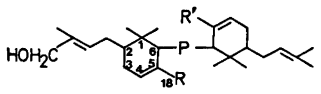
* Part LII, No. 18. *Acta Chem. Scand. B 31* (1977) 212.



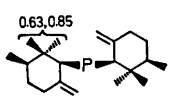
1



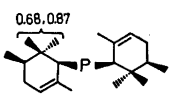
4 R=R′=H; *4a* R=$C_9H_{11}COO$, R′=H; *4b* R=$C_9H_{11}COO$, R′=Ac
9 R=β-D-gluc., R′=H; *9a* R=$(Ac)_4$-β-D-gluc., R′=Ac
10 R=R′=gluc., *10a* R=R′=$(Ac)_4$-β-D-gluc.



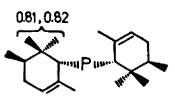
2 R=CH_3, R′=CH_2OH
3 R=CH_2OH, R′=CH_3



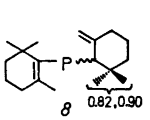
5



6



7



8

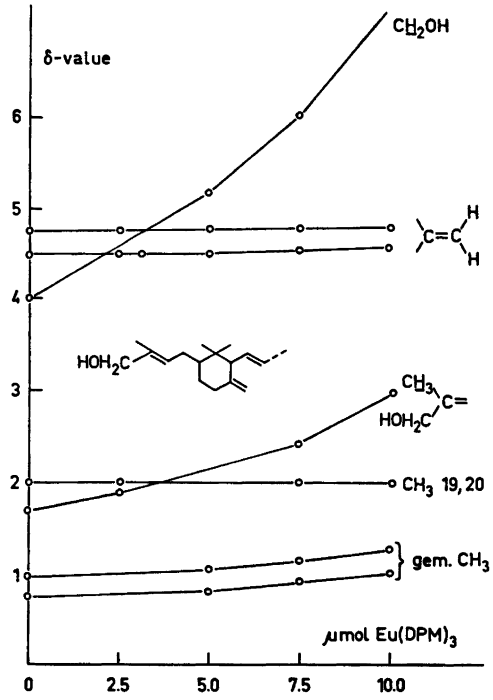


Fig. 1. Induced chemical shifts of ^{1}H NMR signals of sarcinaxanthin (*4*, 10 μmol) upon addition of Eu(dpm)$_3$ at constant volume (0.2 ml).

in the δ 1.5 – 1.8 region. Due to two false singlets in this region signals at δ 4.53, 4.76 had been overlooked. The latter signals, present in the previous and present spectrum, can now be assigned to two terminal methylene groups in substituted γ end-groups,[6,7] consistent with medium intensity IR-absorption at 890 cm^{-1}.[6,7] The centrosymmetrical structure *4* (still disregarding stereochemistry) followed from the ^{1}H NMR spectrum and shifts induced upon addition of Eu(dpm)$_3$ reagent, Fig. 1. ^{1}H NMR assignments are included in Scheme 1.

The hydroxylated side-chain was assigned *E*-configuration on the basis of the chemical shifts of the $-CH_3$(δ 1.67), $-CH_2OH$(δ 4.03) and olefinic proton (δ 5.6) as compared with those of decaprenoxanthin (*1*)[3,4] and an aliphatic model.[8]

The Cotton effect (see Fig. 2) of sarcinaxanthin is opposite to that of the synthetic C_{42} model *5*,[9] favouring opposite absolute configuration at C-6,6′ in sarcinaxanthin (*4*)

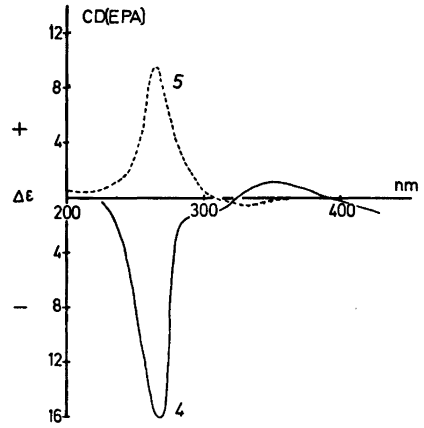


Fig. 2. CD spectrum of sarcinaxanthin (*4*) and of (2*R*, 6*S*, 2′*R*, 6′*S*) – 2,2′-dimethyl-γ,γ-carotene (*5*)[9] in EPA (diethyl ether, isopentane, ethanol 5:5:2).

and *5*; *cf.* previous arguments for the stereochemistry of decaprenoxanthin (*1*) from CD-comparison with the C_{42} model *6*.[5,10] The configuration at C-2,2′ in sarcinaxanthin may, like in the ε-series,[11,5] have little influence on the CD spectrum. In the ε-series the difference in chemical shift of the *gem*-dimethyl signals (Δ) within the 2,6-*cis* series (*4* and *6*, Δ = *ca.* 0.20) *vs.* the 2,6-*trans* (*7*, Δ = 0.02) were used for assignment of absolute configuration of C-2,6; one of the two *gem*-dimethyl groups being more shielded in the 2,6-*cis* series. In the γ-series only the 2,6-*cis* C_{42} model (*5*) is available. However, the chemical shift difference of the *gem*. dimethyl signals for *5* (Δ = 0.22) and sarcinaxanthin (Δ = 0.23) coincide so well that a 2,6-*cis* relationship in sarcinaxanthin (*4*) is likely. For comparison Δ = 0.08 for the unsubstituted γ end-group in optically active β,γ-carotene (*8*).[6]

The close agreement in chemical shifts of the *gem*. dimethyl groups in the C_{42} models *5* and *6*[9] (Scheme 1) with ε and γ end-groups, as well as for decaprenoxanthin (*1*) and sarcinaxanthin (*4*), together with nearly identical Cotton effects for decaprenoxanthin (*1*)[5] and sarcinaxanthin (*4*, Fig. 2), suggest a similar geometry of these ε and γ end-groups, in spite of the different location of the double bond. This is confirmed by molecular models: The ε-ring of *1* and *6* is planar in the C-3,4,5,6 region with the polyene chain quasiequatorial

and the C-2 substituent equatorial, whereas the γ-ring of *4* and *5* is planar in the C-4,5,6,18 region with the polyene chain and the C-2 substituent equatorial.

$\Delta\varepsilon$-Values for decaprenoxanthin (*1*, $\Delta\varepsilon = -5$,[12] -5[13] value from Ref. 12 used, -15.1[11]) and sarcinaxanthin (*4*, $\Delta\varepsilon = -16$) are comparable. The high $\Delta\varepsilon$-value (15.5) for α-ionone has been explained [15] by predominance of the conformation with quasiequatorial side chain in order to allow interaction between the enone and the isolated olefin chromophore. In (+)-*cis*-α-irone ($\Delta\varepsilon = 12.2$) a certain destabilization of this conformation has been ascribed to the axial C-2 methyl group. For (+)-γ-ionone and (+)-*cis*-γ-irone very low $\Delta\varepsilon$-values (*ca.* 0.002–0.2) are reported. It was concluded [15] that no significant coupling between the electric transition moments of the enone/olefin chromophores occurs. Since $\Delta\varepsilon$-values of *ca.* 16 are not exceptionally high in the carotenoid series (*cf.* zeaxanthin [16]) the similar $\Delta\varepsilon$-values for decaprenoxanthin (*1*) [14] and sarcinaxanthin (*4*) give no reason to assume any predominance of conformations with quasiaxial or axial polyene chain. Such conformations would be destabilized in the C_{42}- and C_{50}-series.

In connection with the present assignment of absolute configuration to sarcinaxanthin (*4*) it should be pointed out that the chirality at C-2 is the same in C_{50}-carotenoids with β[17], ε,[5] ψ,[18] and γ end-groups, and that the configuration at C-6 is the same for C_{50}-carotenoids with ε [5] and γ end groups in cases hitherto studied. Biosynthetic considerations have been made.[5,18,19]

Although saponification was included in the isolation procedure the presence of small amounts of a sarcinaxanthin monoester (*4a*) esterified with a $C_9H_{11}COOH$ acid could be demonstrated (Batch 1) by acetylation, saponification and $LiAlH_4$-reduction experiments and high precision MS.

A C_{50}-carotenoid β-D-monoglucoside, tentatively identified as sarcinaxanthin β-D-monoglucoside,[20] comprised 20–40 % of Batches 1, 2, and 3. The ^{1}H NMR and CD spectra of the pentaacetate *9a* here prepared are consistent with structure *9* for the mono-β-D-glucoside. Structure *9* is compatible with previously reported in-chain fragmentations of the deuterioacetate *9b* on electron impact.[20]

Sarcinaxanthin dihexoside (*10*) was isolated for the first time and comprised around 30 % of Batches 1 and 3. It was characterized as the octaacetate *10a* by chromatographic behaviour, electronic and mass spectrum. By analogy with *9a*. a di-β-D-glucoside structure appears likely.

Minor, less polar C_{40}- and C_{50}-carotenoids previously isolated [2] were not present in Batches 1, 2, and 3.

The variation in carotenoid content of *Sarcina lutea*,[1,2,21–23] particularly in mass culture, reflects sensitivity of the carotenoid synthesis to cultivation conditions.

So far sarcinaxanthin and its derivatives represent the only C_{50}-carotenoids with substituted γ end-groups.

EXPERIMENTAL PART

Materials and general methods were the same as before.[2] ^{1}H NMR 100 MHz FT spectra were recorded on a Varian XL–100 instrument and CD spectra on a Roussel Jouan Dicrographe.

Biological material. Sarcina lutea from the Department of Biochemistry at this University [2] was grown in mass cultures (150 l) by the previously described procedure;[2] Batch 1 provided *ca.* 300 g of wet cells (51 g dry cell residue after acetone extraction) and Batch 2 370 g of wet cells. Batch 3 constituted lyophilized cells, 600 g, of *S. lutea*, provided by Dr. A. G. Andrewes.

Pigment extraction. Cells were lysed by lysozyme treatment and extracted with acetone;[2] total yield of crude carotenoids: Batch 1, 53 mg, Batch 2, *ca.* 5 mg and Batch 3, 24 mg.

Separation of individual carotenoids were best effected on acetylated polyamide columns [2] or TLC (SiO_2) after standard saponification with 5 % KOH in methanol [24] to remove contaminants.

Sarcinaxanthin (*4*). Batch 1 yielded 9 mg of *4*, crystallized from acetone. *4* had adsorptive properties, electronic, IR and MS spectra as previously described.[2] ^{1}H NMR ($CDCl_3$) at 60 MHz and 100 MHz differed from the published spectrum:[2] δ 0.73 s and 0.96 (s, 6 H + 6 H, *gem.* dimethyl), 1.67 (s, 6 H, side-chain CH_3), 1.98 (s, 12 H, in-chain CH_3), 2.3 (m, *ca.* 10 H, allylic CH_2 and CH), 4.03 (s, 4 H, CH_2OH), 4.53 and 4.76 2 H + 2 H, $=CH_2$), *ca.* 5.6 (t, 2 H, isopropylidene H), and 6–7 (m, *ca.* 10 H, olefinic H). Induced shifts upon addition of Eu(dpm)$_3$ are illustrated in Fig. 1. The CD spectrum is reproduced in Fig. 2.

Sarcinaxanthin monoester (*4a*). In spite of prior saponification a monoester *4a*, 0.5 mg,

less polar than *4* and with $R_F = 0.63$ on Schleicher & Schüll (S&S) No. 287 paper with 10 % acetone in light petroleum (a.pe.) was isolated from Batch 1. After acetylation a monoacetate *4b* ($R_F = 0.85$ on S&S 287 10 % a.pe.) was obtained; *m/e* 894 (M), 802 (M − 92), 788.5725 ($C_{54}H_{76}O_4 = M - C_8H_{10}$): $M = C_{62}H_{86}O_4$), 746.5621 ($C_{53}H_{74}O_3 = M - C_8H_{10} = C_3H_2O$). Treatment of *4b* with $LiAlH_4$ in dry ether gave sarcinaxanthin (*4*) judged by MS and adsorptive properties. *4a* upon standard alkali treatment provided sarcinaxanthin (*4*) judged by co-chromatography.

Sarcinaxanthin mono-β-D-glucoside (*9*). *9*, eluted from acetylated polyamide with 10 % methanol in benzene, rechromatographed on TLC (SiO_2, eluted with 50 % a.pe.), was acetylated by a standard procedure.[24] The pentaacetate *9a*, purified by TLC (SiO_2, eluted with 30 % a.pe.; $R_F = 0.58$ on S&S No. 287 paper 10 % a.pe., *cf.* Ref. 20) had electronic spectrum like *9*; MS data as previously reported;[20] ^1H NMR ($CDCl_3$): δ 0.73 (s), 0.98 (s, 6 H + 6 H, *gem.* dimethyl), 1.60 (s, 3 H, side-chain CH_3), 1.67 (3 H, side-chain CH_3), 1.98 (*ca.* 12 H, in-chain CH_3); 2.00, 2.02 and 2.09 (*ca.* 15 H, acetate CH_3), 4.20 [*ca.* 2 H, $-CH_2O$ gluc (Ac)$_4$], 4.5 and 4.75 (*ca.* 4 H, $=CH_2$), methine H and olefinic H; CD (EPA) $A\varepsilon$ 260 nm (−10.4 pk.), 240 nm (−5 tr.), 215 nm (−7.8 pk.).

Sarcinaxanthin diglycoside (*10*). *10*, eluted with benzene-methanol from acetylated polyamide had $R_F = 0.10$ on S&S No. 287 paper (30 % a.pe.). After standard acetylation the octaacetate (*10a*, 0.5 mg from Batch 1) was obtained; $R_F = 0.23$ on S&S No. 287 (10 % a.pe.), electronic spectrum as for *4*; *m/e* 1364 (M), M − 42, M − 92, M − 106, M − 158, M − 92 − 106, M − 289, M − 346, 331, 169, 145, 109, 43.

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The Crystal and Molecular Structure of 4-Amino-3-(2,6-dichlorobenzylidenehydrazono)-2-methyl-1,2,4-triazoline Hydrochloride Dihydrate

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The crystal structure of the title compound has been determined by three-dimensional single crystal X-ray diffraction methods.

The crystals are triclinic, space group $P\bar{1}$, with unit cell dimensions $a = 11.722(4)$ Å, $b = 10.453(4)$ Å, $c = 7.167(2)$ Å, $\alpha = 89.55(3)°$, $\beta = 110.60(2)°$ and $\gamma = 107.66(4)°$. The structure was solved by direct methods and refined to an R-value of 0.066 for 1574 observed independent reflexions.

The cation is essentially planar. The dihedral angle between the five- and six-membered rings is 1.5°. The C—N bonds in the triazoline ring and the methylene-hydrazino chain indicate a conjugated system.

The analysis of the crystal structure of 4-amino-3-(2,6-dichlorobenzylidenehydrazono)-2-methyl-1,2,4-triazoline (A) hydrochloride dihydrate is part of a program to study the structure-activity relationships in a number of 4-amino-3-benzylidenehydrazino-1,2,4-triazole hydrochlorides.[1] Among them, 4-amino-3-(2,6-dichlorobenzylidenehydrazino)-1,2,4-triazole (B) hydrochloride was found to possess a potent antihypertensive effect.[2] A complementary series of compounds has been prepared by Eriksson and Florvall [2] in order to study the effect of structural variations in the phenyl and triazole part, the amino group and the intermediate methylene-hydrazino chain of the active compound, B-hydrochloride. The monomethylation of compound B was effected with dimethyl sulfate. Since there is a potential hydrazino-hydrazono tautomerism of B, three possible monomethylation products had to be considered.

Chemical evidence of the existence of an amino group in the methylation product was

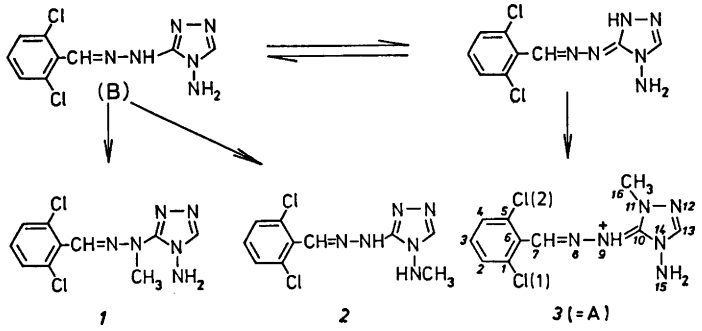


Fig. 1. Potential hydrazino-hydrazono tautomerism of 4-amino-3-(2,6-dichlorobenzylidenehydrazino)-1,2,4-triazole with possible monomethylation products.

given by Florvall and Eriksson;[2] thereby structure 2 was excluded. It did not prove possible, however, to distinguish unambiguously between the two structural possibilities 1 and 3 by spectroscopic techniques. The present crystallographic analysis has shown that the monomethylation product of B is 3 (=A) (*cf.* Fig. 1); *i.e.* the triazole ring is methylated – not the intermediate chain.

EXPERIMENTAL

A sample of A-hydrochloride was kindly provided by Dr. L. Florvall, Astra Läkemedel AB, Södertälje. The substance was recrystallized from an ethanol–water solution of hydrogen chloride to colourless, prismatic crystals of A-hydrochloride dihydrate, $C_{10}H_{10}Cl_2N_6$.$HCl.2H_2O$. A crystal with the approximate dimensions $0.04 \times 0.07 \times 0.54$ mm^3 was mounted on an automatic diffractometer, Philips PW 1100, equipped with a graphite monochromator to reflect Cu$K\alpha$ radiation. The least-squares refined unit cell parameters given in Table 1 were derived from an X-ray powder diffraction photograph taken with strictly monochromatized Cu$K\alpha_1$ radiation in a Guinier-Hägg focussing camera using potassium chloride (a= 6.2930 Å) as an internal standard. The photograph was measured by an automatic film scanner system described by Malmros and Werner.[3]

Integrated intensities were obtained by the diffractometer using the $\theta - 2\theta$ scan technique. The scan speed was 1.2° min^{-1}. Backgrounds were estimated by stationary counting at $\pm 1°$ from the position of the peak maximum. Three standard reflexions were measured at regular intervals to monitor crystal stability and crystal orientation. Their intensities remained essentially constant throughout the runs. Significant counts, larger than $3\sigma_{count}$, were recorded for 1574 independent reflexions out of the 2512 accessible for $2\theta \leq 60°$. σ_{count} is the Poisson statistical counting error. Corrections were made for Lorentz and polarisation factors, but not for absorption.

Table 1. Crystal data. 4-Amino-3-(2,6-dichlorobenzylidenehydrazono)-2-methyl-1,2,4-triazoline hydrochloride dihydrate, $C_{10}H_{10}Cl_2N_6.HCl.2H_2O$, triclinic.

a= 11.722(4) Å,	b= 10.453(4) Å,	c= 7.167(2) Å,
α= 89.55(3)°	β= 110.60(2)°	γ= 107.66(4)°
V= 778.4 Å^3,	FW= 357.6,	Z= 2.

D_{calc}= 1.526 g cm^{-3}. Space group $P\bar{1}$.

STRUCTURE DETERMINATION AND REFINEMENT

The structure was determined by use of the program system MULTAN,[4] starting with 300 reflexions with $|E| \geq 1.24$. An E map calculated from the set of phases with the highest combined figure of merit revealed the positions of all non-hydrogen atoms. Full-matrix least-squares refinements were performed using a modified version of the program LALS.[5] Weights were assigned as $1/\sigma(F)^2$, where $\sigma(F)$ was determined from the value σ_{cs} (F) yielded by counting statistics with a 2.5 % addition for instrumental instability according to the formula:

$$\frac{\sigma(F)}{F} = \left[\left(\frac{\sigma_{cs}(F)}{F}\right)^2 + \left(\frac{2.5}{100}\right)^2\right]^{1/2}$$

The atomic scattering factors for oxygen, nitrogen and carbon were taken from Freeman,[6] for chlorine from International Tables for X-Ray Crystallography,[7] and for hydrogen from Stewart, Davidson and Simpson.[8]

The positions of all hydrogen atoms except those from the water molecules were derived partly from chemical and geometrical considerations and partly from difference synthesis maps. The hydrogen atom parameters were not refined but included in the structure factor calculations using an isotropic temperature factor of 4 Å^2. The four crystal water hydrogen atoms were not included in the calculations. For all non-hydrogen atoms anisotropic temperature factors were used. The positional and thermal parameters of these atoms were refined to an R value of 0.066. The final atomic parameters are listed in Table 2. A list of the observed and calculated structure factors may be obtained from the author on request.

RESULTS AND DISCUSSION

The molecular structure revealed by this analysis is the triazoline 3 shown in Fig. 1. The atomic numbering is given in the figure, and a perspective view of the cation and the chloride ion is shown in Fig. 2. The bond lengths and bond angles, uncorrected for thermal motion are listed in Table 3.

The average C–Cl distance, 1.736Å, is in agreement with the distance 1.737 Å reported

Table 2. Atomic coordinates and thermal parameters with their standard deviations. The anisotropic temperature factor is of the form exp $-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+\beta_{12}hk+\beta_{13}hl+\beta_{23}kl)$.

Atom	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
(a) Non-hydrogen atoms ($\times 10^4$)									
Cl(1)	8630(2)	6981(2)	8263(4)	64(2)	52(2)	332(9)	10(4)	159(7)	−6(7)
Cl(2)	4163(2)	2833(2)	7049(5)	64(2)	43(2)	367(9)	11(2)	125(7)	26(7)
Cl(3)	2971(3)	512(2)	1090(5)	106(3)	65(13)	429(11)	49(4)	221(9)	25(8)
C(1)	7706(7)	5281(7)	7875(12)	59(8)	37(8)	138(24)	−1(12)	40(23)	27(21)
C(2)	8363(8)	4377(8)	7886(15)	79(9)	72(9)	248(30)	78(15)	148(28)	34(27)
C(3)	7684(9)	2999(8)	7650(15)	95(10)	77(11)	260(31)	102(16)	140(29)	72(27)
C(4)	6424(8)	2561(7)	7428(14)	93(10)	39(8)	224(28)	61(14)	89(27)	43(23)
C(5)	5776(8)	3494(7)	7404(13)	61(8)	54(8)	151(25)	16(13)	52(24)	17(22)
C(6)	6418(8)	4910(7)	7674(13)	64(8)	48(7)	137(22)	42(12)	82(21)	21(19)
C(7)	5850(7)	5963(7)	7705(13)	52(8)	52(8)	174(25)	35(12)	51(23)	28(22)
N(8)	4694(6)	5782(6)	7540(11)	54(7)	47(6)	195(22)	46(11)	83(20)	33(18)
N(9)	4395(6)	6961(6)	7616(11)	54(7)	43(7)	231(23)	38(11)	75(20)	15(19)
C(10)	3213(7)	6901(7)	7433(12)	53(8)	58(8)	132(23)	63(13)	72(22)	23(21)
N(11)	2157(6)	5885(6)	7181(11)	55(7)	44(6)	186(21)	32(11)	69(19)	32(18)
N(12)	1139(6)	6312(7)	7022(12)	60(7)	84(9)	248(25)	52(12)	116(22)	32(22)
C(13)	1609(8)	7618(8)	7201(15)	76(10)	74(9)	258(31)	59(15)	106(28)	35(27)
N(14)	2858(6)	8027(6)	7429(11)	48(7)	60(7)	236(23)	37(11)	97(20)	13(20)
N(15)	3736(7)	9349(6)	7632(13)	77(7)	38(7)	364(28)	34(11)	152(24)	41(21)
C(16)	1934(9)	4411(8)	6100(15)	84(9)	46(8)	233(29)	15(14)	120(27)	0(24)
O(1)	8500(6)	171(6)	6038(11)	118(8)	100(7)	289(22)	86(12)	156(21)	81(20)
O(2)	1202(8)	1259(7)	7084(13)	159(10)	130(9)	436(29)	44(15)	240(29)	−29(26)

(b) Hydrogen atoms ($\times 10^4$)

Atom	x/a	y/b	z/c
H(2)	9319	4719	8080
H(3)	8153	2329	7626
H(4)	5954	1560	7294
H(7)	6427	6932	7877
H(9)	5077	7865	7811
H(13)	1091	8245	7203
H(151)	3750	9860	9000
H(152)	3500	9860	6600
H(161)	2705	4104	8037
H(162)	1826	4090	5608
H(163)	1128	3915	7248

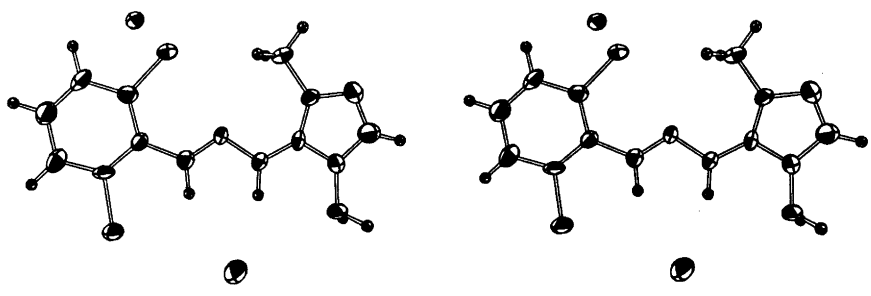


Fig. 2. Stereoscopic view of the cation and the chloride ions in the crystal structure. The non-hydrogen atoms are represented by thermal ellipsoids scaled to include 50 % probability.

Table 3. Bond lengths and bond angles with their standard deviations.

(a) Bond distances (Å)

Cl(1) – C(1)	1.742(7)
Cl(2) – C(5)	1.729(8)
C(1) – C(2)	1.387(10)
C(1) – C(6)	1.394(10)
C(2) – C(3)	1.397(11)
C(3) – C(4)	1.358(12)
C(4) – C(5)	1.403(10)
C(5) – C(6)	1.422(10)
C(6) – C(7)	1.453(10)
C(7) – N(8)	1.272(9)
N(8) – N(9)	1.386(8)
N(9) – C(10)	1.327(9)
C(10) – N(11)	1.319(9)
C(10) – N(14)	1.361(9)
N(11) – N(12)	1.365(8)
N(11) – C(16)	1.481(9)
N(12) – C(13)	1.296(10)
C(13) – N(14)	1.344(10)
N(14) – N(15)	1.423(8)

(b) Bond angles (deg)

Cl(1) – C(1) – C(2)	115.2(6)
Cl(1) – C(1) – C(6)	120.2(6)
C(2) – C(1) – C(6)	124.5(7)
C(1) – C(2) – C(3)	117.8(8)
C(2) – C(3) – C(4)	121.0(8)
C(3) – C(4) – C(5)	120.2(7)
Cl(2) – C(5) – C(4)	116.6(6)
Cl(2) – C(5) – C(6)	121.8(6)
C(4) – C(5) – C(6)	121.6(7)
C(1) – C(6) – C(5)	114.9(7)
C(1) – C(6) – C(7)	118.9(7)
C(5) – C(6) – C(7)	126.2(7)
C(6) – C(7) – N(8)	126.0(7)
C(7) – N(8) – N(9)	114.5(6)
N(8) – N(9) – C(10)	120.1(6)
N(9) – C(10) – N(11)	132.9(7)
N(9) – C(10) – N(14)	122.4(6)
N(11) – C(10) – N(14)	105.0(7)
C(10) – N(11) – N(12)	112.2(6)
C(10) – N(11) – C(16)	129.8(6)
N(12) – N(11) – C(16)	118.0(6)
N(11) – N(12) – C(13)	104.2(6)
N(12) – C(13) – N(14)	111.4(7)
C(10) – N(14) – C(13)	107.5(6)
C(10) – N(14) – N(15)	122.0(6)
C(13) – N(14) – N(15)	130.5(6)

by Rudman [9] for aromatic C – Cl bonds, which are not adjacent to other C – Cl bonds. The bond angle C(1) – C(6) – C(7) (118.9°) is 7.3° less than the angle C(5) – C(6) – C(7), 126.2°. This fact may be attributed to a repulsion between Cl(2) and N(8). The three N – N bonds are all in resonance between a double bond, 1.26 Å, and a single bond 1.46 Å[10]. C(7) – N(8), 1.272 Å, is a pure double bond. Also N(12) – C(13), 1.296 Å, is within the limits of error a double bond. The remaining C – N bonds in the five-membered ring and in the intermediate chain all show some degree of double bond character, thus indicating a conjugated system.

The cation is essentially planar. The dihedral angle between the benzylidene and the tri-

Table 4. Least-squares planes. The planes are described in terms of an orthogonal vector basis. **p** || **c** and **n** in the **bc**-plane.

Plane A: $0.2600\mathbf{m} - 0.0743\mathbf{n} + 0.9627\mathbf{p} = 4.2950$
Plane B: $0.2623\mathbf{m} - 0.0777\mathbf{n} + 0.9619\mathbf{p} = 4.3111$
Plane C: $0.2506\mathbf{m} - 0.0497\mathbf{n} + 0.9668\mathbf{p} = 4.4553$

Perpendicular distances of atoms (in Å) from the least-squares planes

Plane A		Plane B		Plane C	
C(1)	– 0.005	C(6)	– 0.002	C(10)	0.003
C(2)	– 0.001	C(7)	– 0.003	N(11)	0.001
C(3)	0.001	N(8)	0.008	N(12)	– 0.005
C(4)	0.005	N(9)	0.003	C(13)	0.007
C(5)	– 0.011	C(10)	– 0.004	N(14)	– 0.006
C(6)	0.011	N(11)	– 0.001	N(15)[a]	– 0.030
C(7)[a]	0.016			C(16)[a]	– 0.033
Cl(1)[a]	0.051				
Cl(2)[a]	– 0.048				

[a] These atoms are not included in the least-squares planes.

Table 5. Hydrogen-bond distances and code for superscripts.

Hydrogen-bond distances (Å)

O(1) .. O(2[i])	2.835(10)
O(1) .. O(2[ii])	2.877(10)
Cl(3) .. O(1[ii])	3.074(7)
Cl(3) .. O(2[iii])	3.148(8)
Cl(3) .. N(9[iv])	3.232(6)
Cl(3) .. N(15[v])	3.270(8)

Code for superscripts:

i	$1+x,y,z$
ii	$1-x,-y,1-z$
iii	$x,y,z-1$
iv	$1-x,1-y,1-z$
v	$x,y-1,z-1$

azoline ring is 1.5°. Details of least-squares planes calculated for various portions of the molecular frameworks are given in Table 4.

The protonated N(9) and N(15) are hydrogen bonded to the chloride ions (*cf.* Table 5). Chains of crystal water, also hydrogen bonded to the chloride ions, are present in the structure. The hydrogen bonds are summarized in Table 5.

A discussion of the structure-activity relations and a comparison with three other closely related compounds is given by Werner, Florvall and Stjernström.[1]

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Synthesis of Cyclopropyl-substituted Heterocyclic Compounds

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When vinyl-substituted heterocyclic aromatic compounds with electron deficient vinyl double bond were treated with sulfur ylides formation of the corresponding cyclopropyl derivatives occurred in fair to good yields. The reaction of dichlorocarbene with 2-vinylpyridine afforded 3-chloroindolizine.

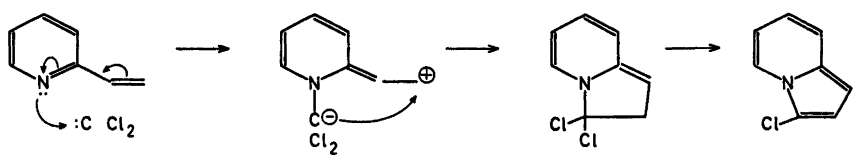
Simple syntheses of cyclopropylpyridines from vinylpyridines have recently been reported.[1,2] The present study describes some attempts to prepare cyclopropyl-substituted *N*-heterocyclic aromatic compounds starting from vinyl-substituted 5- and 6-membered heterocyclic rings.

The vinyl compounds were prepared according to known methods with two exceptions. Eisch *et al.*[3] recently reported a convenient preparation of the unstable 2-vinyl-1,2-dihydroquinoline, and it was easily oxidized with chloranil to give 2-vinylquinoline (*1*) in 35 % yield. 2-Vinylbenzoxazole (*2*) was chosen as an example of a vinyl-substituted 5-membered ring with an sp^2-hybridized nitrogen. 3-Chloro-2′-hydroxypropananilide (*3*), prepared from 3-chloropropionyl chloride and *o*-aminophenol, was treated with catalytic amounts of *p*-toluenesulfonyl chloride to give ring closure with loss of water and hydrogen chloride producing compound *2* in 54 % yield.

The addition of carbenes to double bonds is an easy approach to cyclopropanes; however, the vinyl double bond attached α or γ to an sp^2-hybridized ring nitrogen is electron deficient, and it was not surprising that the reaction of these compounds with dichlorocarbene[4] gave no isolable yield of the corresponding cyclopropyl derivatives. In all cases a black tarry product resulted from which no pure substance could be obtained with one exception. From the reaction of 2-vinylpyridine a small amount of 3-chloroindolizine (*4*) was isolated. The compound was unstable and structural proof was based on spectroscopic data. The UV spectrum was quite similar to that reported for indolizine itself[5] and the NMR spectrum showed only the presence of aromatic protons. The fragmentation pattern of the mass spectrum was as expected for a 3-substituted indolizine.[6] A reasonable explanation for its formation is depicted in Scheme 1.

The nitrogen lone pair attacks dichlorocarbene to give a zwitterion which cyclizes; under the strong alkaline condition hydrogen chloride is eliminated to give the product *4*.

Conjugation of a vinyl double bond with an sp^2-hybridized nitrogen atom as in 2-vinylpyrrole (*5*) and *N*-methyl-2-vinylpyrrole (*6*) or a sulfur atom as in 2-vinylthiophene (*7*) makes the former more electron rich and should therefore be more inclined to carbene additions. Indeed the thiophene *7* reacted readily with dichlorocarbene to give 2-(2,2-dichlorocyclopropyl)thiophene (*8*)[7] in 42 % yield. The



4

Scheme 1.

Table 1. Yields of cyclopropanes in the reaction of dichlorocarbene and of the ylides *9* and *10* with vinyl-substituted heterocyclic compounds.

Vinyl compound	% Yield of the cyclopropyl product with :CCl_2	Ylide *9*	Ylide *10*
2-Vinylpyridine (*11*)	[a]	63[b]	7[b]
4-Vinylpyridine (*12*)	0	60[b]	19[b]
2-Vinylpyridazine (*13*)	0	42	20
4-Vinylpyrimidine (*14*)		8	
2-Vinylquinoline (*1*)	0	46	18
2-Vinylbenzoxazole (*2*)		37	7
2-Vinylpyrrole (*5*)		[c]	
N-Methyl-2-vinylpyrrole (*6*)	0	0	0
2-Vinylthiophene (*7*)	42	0	0

[a] Low yield of 3-chloroindolizine. [b] From Ref. 2. [c] 60 % yield of *N*-methyl-2-vinylpyrrole.

pyrrole *6*, however, gave only a black tarry product.

In addition to the carbene route to cyclopropanes sulfur ylides have long proved their significance.[8] Dimethylsulfonium (*9*) and dimethylsulfoxonium methylide (*10*) afford cyclopropyl derivatives from vinyl- and styrylpyridines.[1,2] The reaction can be envisioned as a Michael-type condensation where the intermediate carbanion undergoes ring closure with loss of dimethyl sulfide or dimethyl sulfoxide.

The results from reactions of dichlorocarbene and of the ylides *9* and *10* with the vinyl-substituted heterocyclic compounds are summarized in Table 1. Polymerization to varying degrees accompanied most of the reactions.

The ylide *9* gave under identical conditions more than twice the yields of cyclopropanes than the ylide *10*. When the vinyl group was α or γ to an sp^2-hybridized ring nitrogen in a 5- or 6-membered ring cyclopropanes were formed in moderate to good yields, except in the reaction with 4-vinylpyrimidine where polymerization was the predominant reaction. The conjugation of the vinyl group with the electronegative nitrogen and the aromatic ring enables these compounds to be efficient in Michael-type additions.

With compound *5*, however, the ylide *9* acted as a methylating agent, and compounds *6* and *7* were, as expected, both recovered unchanged after prolonged treatment with the ylides.

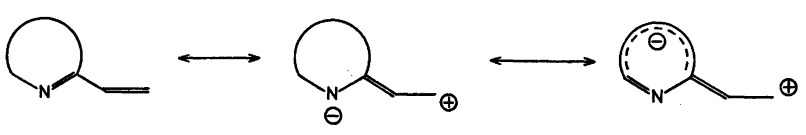
EXPERIMENTAL

Melting points were determined on a micro hot stage. UV spectra were recorded on a Cary 14 Spectrophotometer, IR spectra on a Perkin-Elmer 457 Grating Infrared Spectrophotometer, 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, 70 eV. Elemental analyses were performed by I. Beetz, West Germany.

2-Vinylquinoline (1). To the solution of dried, crude 2-vinyl-1,2-dihydroquinoline in tetrahydrofuran, prepared from 9 g (70 mmol) quinoline,[3] chloranil (17.4 g, 70 mmol) and xylene (40 ml) were added and the mixture was refluxed for 30 min. After removal of the solvents the crude product was dissolved in light petroleum (100 ml), washed with 10 % aqueous sodium hydroxide (2 × 20 ml) and water (2 × 15 ml) and then dried. The black liquid residue was distilled to give 2.3 g of unreacted quinoline and 1.9 g (35 % based on the quinoline used) of compound *1*, b.p. 63–65 °C/0.01 mmHg, (lit:[13] 100–101 °C/2 mmHg). The NMR spectrum was identical with that previously reported.[3]

3-Chloro-2'-hydroxypropananilide (3). 3-Chloropropionyl chloride (15.2 g, 120 mmol) was added dropwise with stirring to a dispersion of *o*-aminophenol (25 g, 240 mmol) in benzene (100 ml) and left at room temperature for 4 h. The mixture was filtered and the white solid washed thoroughly with ether and recrystallized from water to give 13 g (54 %) of the anilide *3*, m.p. 124–125 °C. Anal. $C_9H_{10}ClNO_2$: C, H. MS (*m/e*): 199 (M). ^{1}H NMR (DMSO-d_6): δ 2.94 (2 H, t, J 6.5 Hz), 3.88 (2 H, t, J 6.5 Hz), 6.57–7.05 (4 H, complex), 7.58–7.82 (1 H, complex), 9.37 (1 H, broad s).

2-Vinylbenzoxazole (2). The anilide *3* (11 g, 55 mmol) and *p*-toluenesulfonyl chloride (0.2 g) in xylene (200 ml) were refluxed in a flask equipped with a water separator for 6 h until



Scheme 2.

1 ml (55 mmol) of water was collected. The solvent was removed and the residue distilled to give 4.3 g (54 %) of compound *2*, b.p. 69 °C/0.2 mmHg. Anal. C_9H_7ON: C, H. MS [*m/e* (% rel. int.)]: 145 (100, M), 144 (43.5), 64 (36.0), 63 (38.5), 39 (11.3), 38 (14.5). ^1H NMR ($CDCl_3$): δ 5.77 (1 H, dd, J 2.6 and 9.5 Hz), 6.37 (1 H, dd, J 2.6 and 17.0 Hz), 6.78 (1 H, dd, J 9.5 and 17.0 Hz), 7.15–7.80 (4 H, complex). IR (film): 1530 (s), 1450 (s), 1240 (s), 935 (s), 830 (s) cm^{-1}.

2-Vinylpyrrole (5),[9] *N-methyl-2-vinylpyrrole (6)*,[9] *2-vinylthiophene (7)*,[10] *2-vinylpyrazine (13)*,[11] and *4-vinylpyrimidine (14)*[12] were prepared according to known methods.

Reactions with dichlorocarbene

3-Chloroindolizine (4). To a solution of 2-vinylpyridine (*11*) (5.3 g, 50 mmol) and triethylbenzylammonium chloride (TEBA) (150 mg) in chloroform (18 g, 155 mmol) was added 50 % aqueous sodium hydroxide (10 ml, 125 mmol). The mixture was vigorously stirred for 14 h. The two layers of the black mixture were separated and the organic phase chromatographed on Silica gel 60 with chloroform to give colourless liquid *4* which turned black within 1 h. MS [*m/e* (% rel. int.)]: 153 (35), 151 (100, M), 116 (39.9), 115 (23.6), 89 (35.2), 63 (19.6). ^1H NMR ($CDCl_3$): δ 6.40–8.10 (complex). UV (abs. ethanol): 233.5, 294.0, 282.0, 348.5 nm; for indolizine itself[5] (log ε): 237.5 (4.51), 295 (3.56), 285 (3.45), 275.5 (3.34), 346.5 (3.29) nm.

2-(2,2-Dichlorocyclopropyl)thiophene (8). To a solution of 2-vinylthiophene (*7*) (0.55 g, 5 mmol), 1-nitroso-2-naphthol (20 mg), and TEBA (20 mg) in chloroform (3.6 g, 32 mmol) was added 50 % aqueous sodium hydroxide (2 ml, 25 mmol). The mixture was vigorously stirred for 5 h. The two layers were separated and the organic phase was dried. Distillation gave 0.4 g (42 %) of compound *8*, b.p. 97 °C/9 mmHg, (lit.:[7] b.p. 64–65 °C/49 mmHg). Anal. $C_7H_6Cl_2S$: C, H. MS [*m/e* (% rel. int.)]: 196 (2.4), 194 (14.6), 192 (21.5, M), 159 (35.0), 157 (100), 122 (40.7), 121 (46.1), 69 (10.1), 60 (10.5), 45 (30.4), 39 (13.0). ^1H NMR (CCl_4): δ 1.73 (1 H, dd, J 7.0 and 8.5 Hz), 1.98 (1 H, dd, J 7.0 and 10.0 Hz), 2.90 (1 H, dd, J 8.5 and 10.0 Hz), 6.75–6.98 (2 H, complex), 7.12 (1 H, dd, J 2.0 and 4.7 Hz). IR (film): 1430 (s), 1118 (s), 1055 (s), 850 (s) cm^{-1}.

Reactions with ylides

Reactions with dimethylsulfonium (*9*) and sulfoxonium methylide (*10*) are performed as described previously.[2]

2-Cyclopropylpyridazine (15). B.p. 68 °C/11 mmHg. Anal. $C_7H_8N_2$: C, H. MS [*m/e* (% rel. int.)]: 120 (40.0, M), 119 (100), 118 (4.2), 94 (5.3), 78 (5.4), 65 (6.3), 52 (5.4), 39 (11.1). ^1H NMR (CCl_4): δ ~1.05 (4 H, m), ~2.00 (1 H, m), ~8.33 (3 H, m). IR (film): 1418 (s), 1140 (s), 1042 (s), 1010 (s) cm^{-1}.

4-Cyclopropylpyrimidine (16). M.p. 43–45 °C. Anal. $C_7H_8N_2$: C, H. MS [*m/e* (% rel. int.)]: 120 (16.3, M), 119 (100). ^1H NMR (CCl_4): δ ~1.08 (4 H, m), ~1.90 (1 H, m), 7.20 (1 H, dd, J 1.5 and 5.0 Hz), 8.61 (1 H, d, J 5.0 Hz), 9.10 (1 H, broad s). IR (film): 1585 (s), 1030 (m), 991 (s) cm^{-1}.

2-Cyclopropylquinoline (17). B.p. 65–70 °C/0.01 mmHg (lit.:[14] b.p. 145–148 °C/17 mmHg). MS [*m/e* (% rel. int.)]: 169 (49.1, M), 168 (100), 167 (30.0), 83.5 (13.5). Mol.wt., obs. 169.0868, calc. for $C_{12}H_{11}N$ 169.0891. IR (film): 1601 (s), 1502 (s), 1425 (s), 1030(sh), 1020 (m), 819 (s) cm^{-1}.

2-Cyclopropylbenzoxazole (18). B.p. 73 °C/0.07 mmHg. Anal. $C_{10}H_9NO$: C, H. MS [*m/e* (% rel. int.)]: 159 (100, M), 158 (72.5), 133 (52.3), 130 (13.8). ^1H NMR (CCl_4): δ 1.18 (4 H, m), 2.18 (1 H, m), 7.12–7.68 (4 H, m). IR (film): 1618 (s), 1575 (s), 1452 (s), 1240 (s), 1155 (s), 1078 (s), 1030 (m), 940 (s) cm^{-1}.

Acknowledgement. The author is indebted to Professor Lars Skattebøl for valuable discussions.

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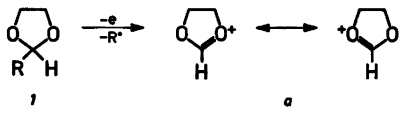
Reactions Between Formaldehyde and Polyhydric Alcohols III.* An Unexpected Isotope Effect in the Mass Spectra of Deuterated 1,3-Dioxolanes

J. U. R. NIELSEN, S. E. JØRGENSEN, N. FREDERIKSEN, R. B. JENSEN, GUSTAV SCHROLL and D. H. WILLIAMS**

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4-Methyl-1,3-dioxolane-2,2-d_2 (*2a*) displays in its mass spectrum an M − H peak which is more abundant than the M − D peak. From the study of the mass spectra of related compounds it was found that the hydrogen lost from *2a* originates from C-5. The kinetics are discussed and a mechanism for this unexpected reaction is proposed.

The mass spectra of 1,3-dioxolanes (*1*) have been extensively studied [2–5] and discussed [6] in previous work. The most outstanding feature of their spectra is the relatively low activation energy for loss of a group R (R = H or alkyl) from C-2, due to the relatively high stability of the delocalized oxonium ion *a* which is formed.



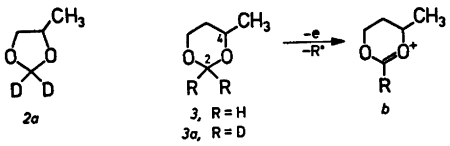
Scheme 1.

It was therefore extremely surprising to find that 4-methyl-1,3-dioxolane-2,2-d_2 (*2a*), displays in its mass spectrum an M − H peak which is more abundant than the M − D peak (Fig. 1). The present paper reports a study made to try to understand this surprising result.

* Part II, see Ref. 1.

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RESULTS AND DISCUSSION



Scheme 2.

Early evidence cited as supporting a low activation energy for the formation of a delocalized oxonium ion such as *a* was found in the mass spectrum of 4-methyl-1,3-dioxane (*3*), in which the M − H peak is over three times the abundance of the M − CH_3 peak.[6] It was argued [6] that since methyl radical loss is normally preferred over hydrogen radical loss in mass spectra (where this competition can occur for −H and $-CH_3$ substituents attached to the same carbon atom), then the dominant M − H ion must be due to the delocalized ion *b* (R = H). This conclusion has now been verified by an examination of the mass spectrum of 4-methyl-1,3-dioxane-2,2-d_2 (*3a*): the M − H ion from *3* is replaced by an M − D ion from *3a*.

The unusual phenomenon in the mass spectrum of *2a* was examined further through a comparison of the spectra of the parent compound *2* and the additional deuterated derivatives *2b* and *2c* (Fig. 1). Only deuterium is lost from the molecular ion of *2c*, establishing loss of D from positions C-2 and/or C-5 in forming the M − D ion in this case. The 5,5-d_2-derivative *2b*

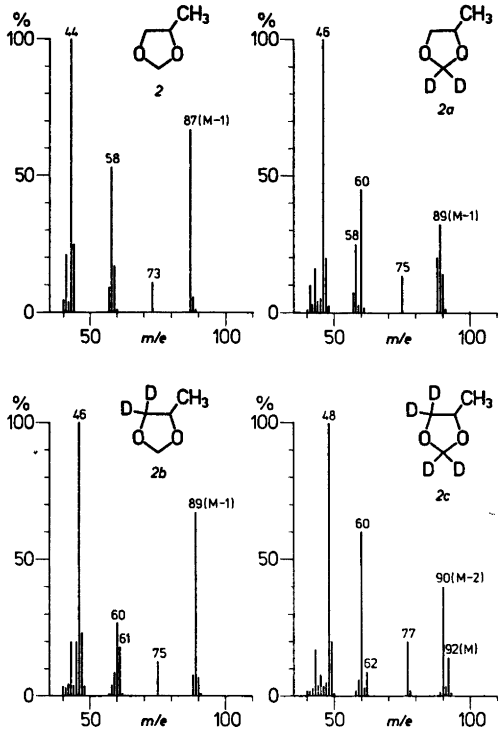
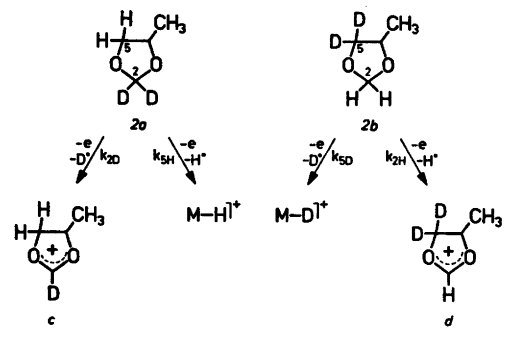


Fig. 1. Mass spectra of 4-methyl-1,3-dioxolane (*2*) and deuterated analogues (*2a* – *2c*).

forms almost exclusively M – H, establishing the preferential formation of ion *d* from this compound. Thus, the preferential loss of H from *2a* must occur from C-5, and the dominance of this process over D loss from C-2, to give *c*, must be due to an isotope effect.



Scheme 3.

Table 1. Abundances of M, M – H and M – D peaks in the mass spectra of *2*, *2a*, *2b* and *2c*.

Compound	% Σ_{40}[a] M	M – H	M – D	Metastable ion observed for
2 (d_0)	0.8	20.7	–	$M^+ \rightarrow [M-H]^+$
2a (2,2-d_2)	4.0	10.2	6.5	$M^+ \rightarrow [M-D]^+$
2b (5,5-d_2)	1.1	19.1	2.3	$M^+ \rightarrow [M-H]^+$
2c (2,2,5,5-d_4)	4.4	0.4	12.8	$M^+ \rightarrow [M-D]^+$

[a] Corrected for ^{13}C.

Relevant data for the abundances of M, M – H and M – D peaks in the spectra of *2*, *2a*, *2b*, and *2c* are given in Table 1.

The metastable ion data suggest that loss of H (or D) from C-2 is always the lowest activation energy process. Thus, $E_0(2D) < E_0(5H)$ and $E_0(2H) < E_0(5D)$. From the abundance of the M – H and M – D ions it can be concluded that on average, for reactions occurring in the ion source, $k_{5H} > k_{2D}$ and $k_{2H} > k_{5D}$. In total, the data indicated the following sequences of activation energies and frequency factors:

$$E_0(2H) < E_0(2D) < E_0(5H) < E_0(5D);$$

$$\nu_{2D} < \nu_{5H};$$

corresponding to crossing $\log_{10} k$ *vs.* E curves for the competing loss of H and D from ionized *2a*. It was not possible to observe any significant change in the (M – H)/(M – D) ratio for *2a* by lowering the electron energy from 70 eV to *ca.* 12 eV, indicating a rapid rise of k_{2D} and k_{5H} with E.

The dramatic effect of deuterium substitution on the activation energy for the most facile dissociation of these ionized dioxolanes may be illustrated by the molecular ion abundances which are evident in Fig. 1 and Table 1. The fraction of the total ion current (% Σ_{40}) carried by the molecular ion is increased by a factor of 5 in the presence of deuterium substitution at C-2, whereas substitution at C-5 only leads to an increase of *ca.* 40 %. This clearly indicates that the most facile decomposition mode of ionized *2* is the loss of H from C-2. Our observations show that increasing the strength of the C(2) – H bond towards homolysis by only *ca.* 4 kJ mol^{-1} (by deuterium substitution), the fraction of molecular ions

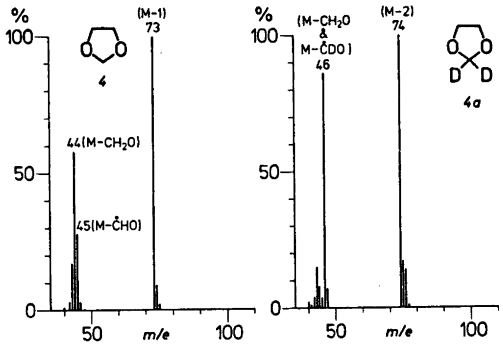
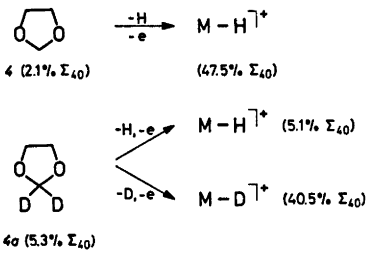


Fig. 2. Mass spectra of 1,3-dioxolane (*4*) and 1,3-dioxolane-2,2-d_2 (*4a*).

which do not decompose before reaching the collector is increased by a factor of 5.

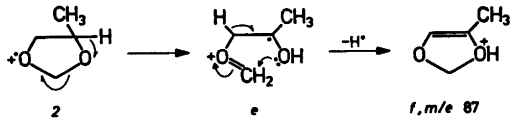
It remains to explain why 4-methyl-1,3-dioxolane (*2*) loses H from C-5 when substituted with deuterium at C-2. The phenomenon does not occur in the spectrum (Fig. 2) of 1,3-dioxolane-2,2-d_2 (*4a*) since this compound shows a great preference for D over H loss from M^+ in source reactions. The isotope labelling at C-2 in this case leads to an approximated doubling of the % Σ_{40} for the molecular ion.



Scheme 4.

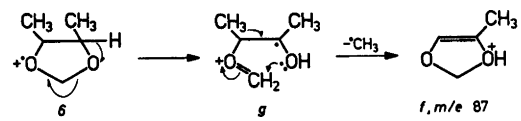
Thus, it is the presence of the C-4 methyl group in *2* and its deuterated analogues which gives rise to the phenomenon. This is further supported by the mass spectrum of 4-ethyl-1,3-dioxolane-2,2-d_2 (*5a*), which displays M−H and M−D peaks in the ratio 2.3:1. The presence of metastable peaks corresponding to the loss of H as well as D from ionized *5a* indicates, that the activation energy for loss of H from C-5 is only slightly greater than that for loss of D from C-2.

One possibility is that C−O bond cleavage in the molecular ion of *2* is favoured by participation of the C-4 hydrogen atom when the radical *e* so produced is secondary rather than primary; hydrogen radical loss from C-5 can then result in the formation of a cyclic oxonium ion *f*.



Scheme 5.

The striking anomaly in the spectrum of *2a* (Fig. 1) is emphasized by the greater abundance of the peak due to loss of H from C-5 compared to that due to loss of CH_3 from C-4, notwithstanding the greater stability of the latter radical by 79 kJ mol^{-1}. The sequence *2*→*e*→*f* not only accomodates this anomaly by using the methyl group to stabilize the radical centre while labilizing the hydrogen radical to be lost, but also leads to the prediction that, since a methyl radical is much more stable than a hydrogen radical, a 4,5-dimethyl-1,3-dioxolane (*6*) should undergo methyl radical loss (*6*→*g*→*f*) in preference to H expulsion.



Scheme 6.

This prediction is realized in the mass spectra of either diastereoisomer of *6* (Fig. 3; the spectra of *Z*-isomer being reproduced). Also as anticipated, the poor competition of H loss relative to CH_3 loss from *6* (1:6) is worsened in the 2,2-d_2-analogue *6a* [(M−H):(M−D): (M−CH_3) = 1:2:30], (see also Fig. 3).

EXPERIMENTAL

The mass spectra were recorded on an AEI MS902 mass spectrometer at 70 eV. The samples were introduced through the glass inlet system. The gas chromatographic separations were made on a Perkin-Elmer F21 pre-

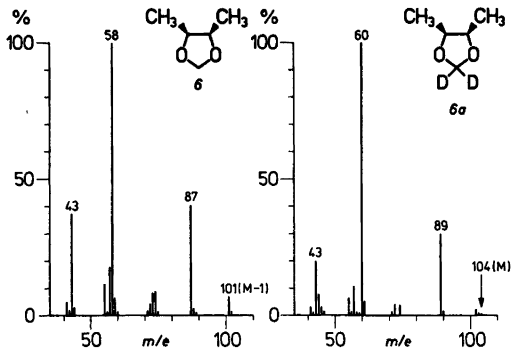


Fig. 3. Mass spectra of *Z*-4,5-dimethyl-1,3-dioxolane (*6*) and *Z*-4,5-dimethyl-1,3-dioxolane-2,2-d_2 (*6a*).

parative gas chromatograph using a 10 % Carbowax 1500 column at 70 °C. The 1,3-dioxolanes were prepared according to the general procedure given below. The diols were commercial samples unless otherwise stated. The perdeuterioparaformaldehyde and the lithium aluminium deuteride used were more than 98 % isotopically pure reagents. The purity of the products was checked by NMR spectrometry and GLC.

General procedure for the preparation of 1,3-dioxolanes. Equimolar amounts (*ca.* 0.1 mol) of the diol and paraformaldehyde (or perdeuterioparaformaldehyde) and a catalytic amount of concentrated sulfuric acid were stirred and heated (oil bath, *ca.* 130 °C) in a flask equipped with a distillation condenser. The dioxolane and water formed during the reaction were distilled off as an azeotrope. With the exceptions of the cases of 1,3-dioxolane (*4*) and 1,3-dioxolane-2,2-d_2 (*4a*), the distillates separated into two phases. The dioxolane was isolated and dried over anhydrous potassium carbonate. The samples of *4* and *4a* contained an equimolar amount of water.

1,2-Propandiol-1,1-d_2 was prepared by the reduction of 5-methyl-1,3-dioxolan-4-one (prepared according to Salomaa and Laiho [7]) with lithium aluminium deuteride.

DL-E- *and Z-4,5-dimethyl-1,3-dioxolane (6) and* DL-E- *and Z-4,5-dimethyl-1,3-dioxolane-2,2-*d_2 *(6a).* From 2,3-butanediol (containing equimolar amounts of *meso-* and DL-2,3-butanediol) and paraformaldehyde a mixture of DL-E- and *Z*-4,5-dimethyl-1,3-dioxolane (*6*) was formed. The isomers were separated by preparative GLC. The d_2-analogues were obtained in a similar manner.

4-Methyl-1,3-dioxane (3). MS [*m/e* (% rel. int.)]: 102 (6, M), 101 (98, [M−H]), 87 (20, [M−CH_3]), 72 (59), 71 (5), 58 (20), 57 (25), 55 (73), 45 (43), 44 (18), 43 (100), 42 (39), 41 (25).

4-Methyl-1,3-dioxane-2,2-d_2 *(3a).* MS [*m/e* (% rel. int.)]: 104 (8, M), 103 (5, [M−H]), 102 (58, [M−D]), 89 (47, [M−CH_3]), 72 (77), 71 (6), 60 (22), 59 (5), 58 (7), 57 (14), 55 (47), 47 (16), 46 (20), 45 (25), 44 (21), 43 (100), 42 (42), 41 (23).

4-Ethyl-1,3-dioxolane (5). MS [*m/e* (% rel. int.)]: 102 (9, M), 101 (65, [M−H]), 73 (65), 72 (40), 57 (9), 55 (34), 46 (7), 45 (48), 44 (100), 43 (40), 42 (12), 41 (17); *m** 102→101, obs. 100.1, calc. 100.0.

4-Ethyl-1,3-dioxolane-2,2-d_2 *(5a).* MS [*m/e* (% rel. int.)]: 104 (15, M), 103 (35, [M−H]), 102 (15, [M−D]), 75 (67), 74 (27), 72 (21), 57 (7), 55 (20), 47 (38), 46 (100), 45 (15), 43 (19), 42 (13), 41 (15); *m** 104→103, obs. 102.1, calc. 102.1; 104→102, obs. 100.1, calc. 100.0.

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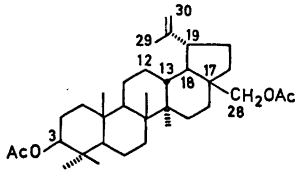
Acta Chemica Scandinavica B 31 (1977) 231—234

Triterpenes. BF_3 Catalysed Rearrangement of 3β,28-Diacetoxy-18(α or β),19(α or β)-epoxylupane. A Novel Route to Baccharane Derivatives

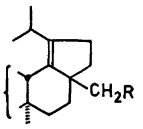
ELIAS SUOKAS and TAPIO HASE

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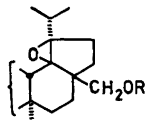
Both the 3β,28-diacetoxy-18α,19α-epoxylupane (*4a*) and the corresponding 18β,19β-isomer (*3a*) gave 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (*5a*) on treatment with BF_3-etherate. 18β,19β-Epoxy isomer (*3a*) gave, in addition, 3β,28-diacetoxy-lupa-12,18-diene (*6*). The baccharane derivative *5a* was also synthesized *via* another route. The stereochemistry of the isomeric 18,19-epoxy-lupane derivatives *3* and 4 is discussed.



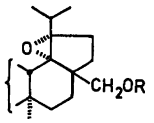
1



2a R=OAc
b R=H



3a R=Ac
b R=H
c R=$COCF_3$



4a R=Ac
b R=H
c R=$COCF_3$

The rather rare tetracyclic triterpenes with a six-membered D-ring include the baccharane series, the parent hydrocarbon of which has been shown to be 18,19-secolupane.[1,2] In this communication we report a novel route to a polyfunctional baccharane derivative starting from lupane derivatives. Nomenclature based on the lupane skeleton is applied throughout.

Betulin diacetate (*1*) can be transformed to its Δ^{18}-isomer (*2a*), either through successive dehydrogenation and hydrogenation steps,[3,4] or *via* acid catalysed double-bond migration.[5] On epoxidation of *2a*, both *m*-chloroperbenzoic acid and performic acid give as the main product the same epoxide isomer; only a few percent of the other epoxide isomer was formed. Treatment of *2a* with ozone yields only the epoxide obtained as a minor product from the peracid epoxidation. Epoxidation of triterpenes with peracids usually occurs by attack from the α-side, on account of steric hindrance due to methyl groups on the β-side. When considering oxidative attack of the double bond in compound *2a*, it is difficult to conclude from Dreiding models, however, which side is the less hindered. The ^1H NMR spectra of the epoxides show different chemical shifts for the protons at the acetoxy carbon C-28. In the epoxide obtained with peracids the C-28 protons have higher δ-values (in *3a* doublets at δ 3.80 and 4.50) and are magnetically more different than those of the epoxide produced by ozone (in *4a* doublets at δ 3.74 and 4.20). This difference can be explained by hindered rotation about the C-17 – C-28 axis and the influence of the magnetic anisotropy of a β-epoxy oxygen, thus indicating the β-epoxy structure *3* for the main epoxide produced by peracid and the α-epoxy structure *4* for the epoxide obtained by treatment with ozone.

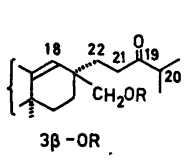
Further information was sought from the NMR shift reagent spectra of epoxides *3c* and *4c*, where hydroxy groups are protected as

trifluoroacetates. The $Eu(DPM)_3$ or $Eu(fod)_3$ induced shifts in the spectrum of the presumed β-epoxide agree with the β-structure *3c* but they do not strictly exclude the possibility of the α-structure *4c*. As to the presumed α-epoxide *4c*, the induced shifts are so small that it is evident that Eu-reagent does not form a dipolar complex with the epoxy oxygen thus preventing clear deductions.

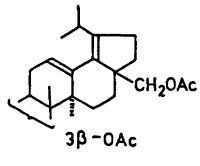
Both epoxides are highly resistant toward anionic species (HO^-, H^-, several R^-, *etc.*) and attempted determination of the stereostructure of the epoxides with chemical methods based on these reagents failed.

Results from X-ray crystallographic measurements,[6] however, confirmed the assumption that the epoxide obtained with peracids has β-epoxy structure *3* and thus the epoxide produced by ozone the α-epoxy structure *4*.

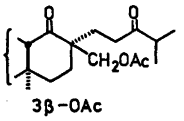
The reason for different stereochemical outcome of ozone and peracid epoxidation is not clear. Any directive effect of C-28 oxygen function is excluded as the corresponding Δ^{18}-lupane derivative *2b*, lacking C-28 oxygen, similarly gives different epoxides as main products when treated with ozone [8] and peracid.[7] The different stereochemistry may be due to the fact that ozone is diradical [8] and peracid ionic in character.



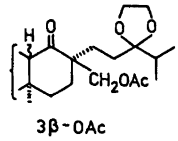
5a R = Ac
b R = $COCF_3$



6

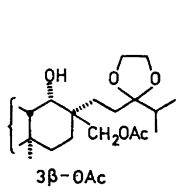


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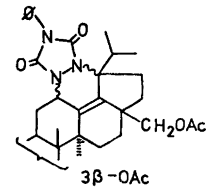


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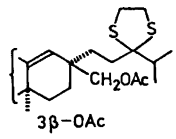
Both epoxides *3a* and *4a* rearrange to the baccharane derivative *5a* on treatment with BF_3-etherate in benzene, and the β-epoxide *3a* gives, in addition, as a minor product, the conjugated diene *6*. The double-bond structure of the latter *6* is confirmed by the formation of Diels-Alder adduct *10*. *5a* is reduced to the methylene compound *12 via* formation of the thioacetal *11* followed by Raney-Ni desulfurisation. The $Eu(DPM)_3$ induced shifts to lower field in ^1H NMR spectrum of the trifluoroacetate derivative *5b* are in the expected order: protons at C-22 (2 H, tr, $J = 8$ Hz) $\simeq$ at C-20 (1 H, m) > at C-21 (2 H, tr, $J = 8$ Hz) > at C-29 and C-30 (6 H, d, $J = 7$ Hz).



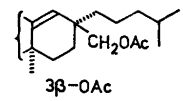
9



10



11



12

The baccharane derivative *5a* was also synthetised *via* an independent route. Oxidation of the double bond in *2a* with $RuO_2 - NaIO_4$ results in the diketone *7*, the side chain carbonyl of which is selectively protected as an ethylene acetal to give the ketoacetal *8*. $NaBH_4$ reduction of *8* gave a mixture of compounds, among them the hydroxy acetal *9*. The 13βH,-18αOH-structure follows from the 4 Hz half-height width of the broad singlet of 18βH at δ 3.33. Dehydration of hydroxy acetal *9* in HMPT and subsequent regeneration of the side chain carbonyl at C-19 produces the baccharane derivative *5a*, identical (TLC, m.p., $[\alpha]_D$, IR, ^1H NMR, and MS) with the compound *5a* obtained from the BF_3-catalysed rearrangement of epoxides *3a* and *4a*.

EXPERIMENTAL

Melting points are uncorrected. ^1H NMR spectra were recorded on a Jeol JNM – PMX 60 spectrometer, the spectra with shift reagent in CS_2 and others in $CDCl_3$ (unless stated otherwise) and related to internal TMS, the IR

spectra on a Perkin-Elmer 125 spectrometer using KBr pellets, mass spectra on a Perkin-Elmer 270 B mass spectrometer, specific rotations in $CHCl_3$ solution on a Perkin-Elmer 141 polarimeter, and elemental analyses with a F & M 185 CHN analyser.

Derivatives of 18β,19β-epoxylupane 3a, 3b *and* 3c. (a) 3β,28-Diacetoxy-lup-18-ene [3,5] (*2a*) was epoxidised with *m*-chloroperbenzoic acid, as described earlier,[5] to 3β,28-diacetoxy-18β,19β-epoxylupane (*3a*) m.p. 222–223 °C (EtOH), $[\alpha]_D$ +25° (*c* 1.0). (Ref. 5, m.p. 222 °C, $[\alpha]_D$ +25.5°). δ 4.50 (1 H, m, 3αH), 4.50 and 3.80 (à 1 H, d, 11 Hz, AB system of C-28 protons). X-Ray crystallographic data will be published [6] later. A small amount (about 5 % from *3a*) of 3β,28-diacetoxy-18α,19α-epoxylupane (*4a*), characterised below, was also obtained.

(b) 3β,28-Diacetoxy-lup-18-ene (*2a*) (0.2 g), CH_2Cl_2 (3 ml), EtOAc (5 ml), HCO_2H (2 ml), and 30 % H_2O_2 (1 ml) were stirred for 1 h at room temperature. The mixture was washed with water, $NaHCO_3$-solution, dried, and the solvent evaporated. Crystallisation from Et_2O–MeOH gave 3β,28-diacetoxy-18β,19β-epoxylupane (*3a*) (0.17 g) with physical constants as above.

Hydrolysis of *3a* (1.0 g) with KOH (1.0 g) in boiling EtOH (30 ml) for 1 h, work up, and chromatography on silica plates ($CHCl_3$-EtOAc 9:1 eluent), and crystallisation from EtOH gave 3β,28-dihydroxy-18β,19β-epoxylupane (*3b*) (0.75 g), m.p. 245 °C, $[\alpha]_D$ +29° (*c* 1.04). Reacetylation in boiling Ac_2O gave the starting epoxide *3a*.

3β,28-Dihydroxy-18β,19β-epoxylupane (*3b*) (1.0 g) in CH_2Cl_2 (20 ml) and $(CF_3CO)_2O$ (1.0 ml) was allowed to stand for 15 min at room temperature. Most of the solvent was evaporated under reduced pressure. The residue crystallised from EtOH resulting in 3β,28-di(trifluoroacetoxy)-18β,19β-epoxylupane (*3c*) (0.5 g), m.p. 124 °C, $[\alpha]_D$ +23° (*c* 1.28); M+ 650; ν 1775, 1225, 1160, 950, 870, 775, 730, 655; δ 4.58 (1 H, m, 3αH), 4.46 and 4.15 (à 1 H, d, 11 Hz, AB system of C-28 protons), 1.05 and 1.00 (à 3 H, d, 7 Hz, methyls of isopropyl group), 1.12, 1.06, 0.94 (à 3 H, s), 0.86 (6 H, s).

Derivatives of 18α,19α-epoxylupane 4a, 4b *and* 4c. 3β,28-Diacetoxy-lup-18-ene (*2a*) (1.0 g) in EtOAc–CH_2Cl_2 (1:1, 50 ml) was ozonised at −75 °C until the solution remained faintly blue. Excess ozone was driven off with a stream of nitrogen and the solution allowed to reach room temperature. Evaporation of the solvent, chromatography on silica plates ($CHCl_3$ eluent), and crystallisation from EtOH gave 3β,28-, diacetoxy-18α,19α-epoxylupane (*4a*) (0.7 g), m.p. 210 °C, $[\alpha]_D$ +36.6° (*c* 1.02); M+ 542; δ 4.44 (1 H, m, 3αH), 4.20 and 3.74 (à 1 H, d, 11 Hz, AB system of C-28 protons), 2.02 and 1.98 (à 3 H, s, acetyl groups), 1.10–0.85 (group of 7 Me). Anal. $C_{34}H_{54}O_5$: C, H.

Hydrolysis of *4a* (1.0 g) as the corresponding β-epoxide *3a* above gave 3β,28-dihydroxy-18α,19α-epoxylupane (*4b*) (0.5 g) crystallised from Et_2O–MeOH m.p. 214 °C, $[\alpha]_D$ +22.6° (*c* 1.0). Reacetylation in boiling Ac_2O gave the starting diacetate *4a*.

3β,28-Di(trifluoroacetyl)-18α,19α-epoxylupane (*4c*) was prepared as the corresponding β-epoxy derivative *3c* above. Crystallisation from MeOH–Et_2O gave m.p. 225 °C, $[\alpha]_D$ +30° (*c* 1.0); M+ 650; ν 1770, 1210, 1155, 920, 875, 775, 730; δ 4.74 (1 H, m, 3αH), 4.59 and 4.05 (à 1 H, d 11 Hz, AB system of C-28 protons), 1.15–0.89 (group of 7 Me).

BF_3-catalysed rearrangement of 3β,28-diacetoxy-18β,19β-epoxylupane (3a) *and trifluoroacetate* 3c. 3β,28-Diacetoxy-18β,19β-epoxylupane (*3a*) (0.5 g) in dry benzene (30 ml) and BF_3-etherate (1 ml) was allowed to stand for 20 min at room temperature. The reaction was interrupted by addition of methanol (5 ml) and the mixture washed with water and $NaHCO_3$-solution, dried and the solvent evaporated. Chromatography on silica plates ($CHCl_3$–light petroleum, b.p. 60–80 °C, 3:1 eluent) gave two compounds. The less polar 3β,28-diacetoxy-lupa-12,18-diene (*6*) crystallised from EtOH (30 mg), m.p. 140 °C, $[\alpha]_D$ +220° (*c* 1.03). (Ref. 4, m.p. 140–143 °C, $[\alpha]_D$ +219 °.) λ_{max} (EtOH) 234 nm (ε 7600); M+ 524; ν 3020, 1730, 1240; δ (CCl_4) 5.35 (1 H, br. tr, 3 Hz, C-12 vinyl proton), 4.45 (1 H, m, 3αH), 3.28 (2 H, AB quart, 11 Hz, C-28 protons), 1.05–0.89 (group of 7 Me).

The more polar 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (*5a*) crystallised from EtOH (0.35 g), m.p. 115 °C, $[\alpha]_D$ −34.8° (*c* 1.15); M+ 542; ν 1730, 1710, 1240; δ (CCl_4) 4.93 (1 H, br. s, C-18 vinyl proton), 4.40 (1 H, m, 3αH), 3.79 (2 H, AB quart, 11 Hz, C-28 protons), 2.00 and 1.96 (à 3 H, s, acetyl methyls), 1.12 (6 H, s), 1.00–0.80 (group of 5 Me). Anal. $C_{34}H_{54}O_5$: C, H.

Similar treatment of 3β,28-di(trifluoroacetoxy)-18β,19β-epoxylupane (*3c*) (1.0 g) with BF_3-etherate gave 3β,28-di(trifluoroacetoxy)-18,19-secolup-13(18)-en-19-one (*5b*) (0.7 g), m.p. 125 °C (from EtOH), $[\alpha]_D$ −24.8° (*c* 1.25); ν 1775, 1710, 1220, 1160.

BF_3-catalysed rearrangement of 3β,28-diacetoxy-18α,19α-epoxylupane (4a). The treatment with BF_3-etherate of 3β,28-diacetoxy-18α19α-epoxylupane (*4a*) as the β-epoxide *3a* above resulted in quantitatively 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (*5a*) identical with the compound *5a* obtained from the β-epoxide *3a*.

Diels-Alder adduct (10). 3β,28-Diacetoxy-lupa-12,18-diene (*6*) (0.1 g) and 4-phenyl-1,2,4-triazoline-3,5-dione (50 mg) in dry benzene (10 ml) were allowed to stand overnight. The solvent was evaporated and the residue chromatographed on silica plates ($CHCl_3$ eluent). Crystallisation from EtOH gave adduct *10* (60 mg), m.p. 160 °C decomp., $[\alpha]_D$ +162° (*c* 0.56); ν 1730, 1690; δ (CCl_4) 7.40 (5 H, m, phenyl), 4.65 (2 H, m, 3αH and C-12 proton), 3.95 (2 H, br. s, C-28 protons), 2.04 and 2.00

(à 3 H, s, acetyl methyls), 1.12–0.85 (group of 7 Me). Anal. $C_{40}H_{57}N_3O_6$: C, H, N.

Reduction of 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (5a). 3β,28-Diacetoxy-18-19-secolup-13(18)-en-19-one (*5a*) (0.5 g), ethane-1,2-dithiol (2.0 g), and BF_3-etherate (0.3 ml) was allowed to stand for 15 min at room temperature, methanol (15 ml) was added and the precipitated thioacetal *11* (0.3 g) m.p. 160 °C, $[\alpha]_D$ −29° (*c* 1.0), refluxed with Raney-Ni in EtOH for 1 h. The Raney-Ni was filtered off, the solvent evaporated and the residue chromatographed on silica plates ($CHCl_3$-light petroleum, b.p. 60–80 °C, 3:1 eluent). Crystallisation from Et_2O-MeOH gave 3β,28-diacetoxy-18,19-secolup-13(18)-ene (*12*) (0.16 g), m.p. 90 °C, $[\alpha]_D$ −40° (*c* 1.47); *ν* 3020, 1735, 1265, 1245, 756; *δ* 5.00 (1 H, br. s, C-18 vinyl proton), 4.50 (1 H, m, 3αH), 3.88 (2 H, AB quart, 11 Hz, C-28 protons), 2.05 (6 H, s, acetyl methyls), 0.95–0.80 (group of 7 Me). Anal. $C_{34}H_{56}O_4$: C, H.

3β,28-Diacetoxy-18,19-secolupan-18,19-dione (7). 3β,28-Diacetoxy-lup-18-ene (*2a*) (1.0 g) in CCl_4 (30 ml), $NaIO_4$ (1.0 g) in water (20 ml), and a catalytic amount of RuO_2 was shaken vigorously for 20 h. The water layer was removed and the CCl_4 phase washed with water, added MeOH (1 ml), dried, and chromatographed on a short silica column ($CHCl_3$-light petroleum, b.p. 60–80 °C, 2:1 eluent). Crystallisation from EtOH gave 3β,28-diacetoxy-18,19-secolupan-18,19-dione (*7*) (0.8 g), m.p. 166 °C, $[\alpha]_D$ +38.7° (*c* 1.2); M+ 588; *ν* 1740, 1720, 1705, 1250; *δ* 4.45 (1 H, m, 3αH), 4.44 and 3,95 (à 1 H, d, 11 Hz, AB system of C-28 protons), 3.9–2.2 (4 H, m), 2.03 (6 H, s, acetyl methyls), 1.15–0.8 (group of 7 Me). Anal. $C_{34}H_{54}O_6$: C, H.

3β,28-Diacetoxy-18,19-secolupan-18,19-dione 19-ethylene acetal (8). 3β,28-Diacetoxy-18,19-secolupan-18,19-dione (*7*) (1.0 g), ethylene glycol (5 ml), *p*-toluenesulfonic acid (0.1 g), and benzene (50 ml) were refluxed for 4 h and water removed from the condensed azeotrope. The mixture was washed with $NaHCO_3$-solution, dried, and the solvent evaporated. Crystallisation from Et_2O-MeOH gave 3β,28-diacetoxy-18,19-secolupan-18,19-dione 19-ethylene acetal (*8*) (0.6 g), m.p. 192 °C, $[\alpha]_D$ +43° (*c* 1.0); M+ 602; *δ* 4.43 (1 H, m, 3αH), 4.42 and 3.90 (à 1 H, d, 11 Hz, AB system of C-28 protons), 3.87 (4 H, s, ethylene acetal protons). Anal. $C_{36}H_{58}O_7$: C, H.

3β,28-Diacetoxy-18α-hydroxy-18,19-secolupan-19-one ethylene acetal (9). 3β,28-Diacetoxy-18,19-secolupan-18,19-dione 19-ethylene acetal (*8*) (1.0 g) in EtOH (50 ml) and $NaBH_4$ (0.5 g) was stirred for 1 h. Benzene (50 ml) was added and the solution washed with water, dried, and the solvent evaporated. The reaction mixture consisting of several compounds was chromatographed on silica plates ($CHCl_3$ + 2 % EtOAc eluent). The least polar main component 3β,28-diacetoxy-18α-hydroxy-18, 19-secolupan-19-one ethylene acetal (*9*) crystallised from EtOH (0.2 g), m.p. 163 °C, $[\alpha]_D$ +10.8° (*c* 1.02); M+ absent, M+ − 43 561; *ν* 3500, 1730, 1240; *δ* 4.40 (1 H, m, 3αH), 4.1–3.7 (6 H, m, C-28 and 19-ethylene acetal protons), 3.33 (1 H, br. s, half-height width 4 Hz, C-18βH). Anal. $C_{36}H_{60}O_7$: C, H.

3β,28-Diacetoxy-18,19-secolup-13(18)-en-19-one (5a) via dehydration. 3β,28-Diacetoxy-18α-hydroxy-18,19-secolupan-19-one ethylene acetal (*9*) (0.2 g) in hexamethylphosphoric triamide (3 ml) was heated for 10 min at 240 °C. To the cooled mixture was added benzene (30 ml), washed with water and 1 M H_2SO_4. Benzene was evaporated and the partially hydrolysed acetal was dissolved in acetone and 1 M H_2SO_4 added. The mixture was stirred for 2 h, worked up, and chromatographed on silica plates ($CHCl_3$ eluent). Crystallisation from Et_2O-MeOH gave 3β,28-diacetoxy-18,19-secolup-13(18)-en-19-one (*5a*) (0.1 g) identical with the product from the BF_3-rearrangement above.

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Synthesis of the 1,3,4-Triaza- and 1,4-Diazacycl[3.3.3]azine Systems

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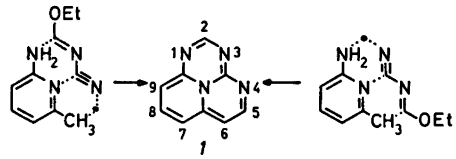
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1,3,4-Triazacycl[3.3.3]azine, *1*, and 3-cyano-1,4-diazacycl[3.3.3]-azine, *2a*, have been prepared in low yields from 2-amino-6-methylpyridine by condensation with *N*-cyanoformimidate and ethoxymethylenemalononitrile, respectively. Electrophilic bromination of *1* yielded the three predicted monosubstituted derivatives, but due to *peri* interactions only two di- and no trisubstituted derivatives.

This communication reports synthesis and electrophilic bromination studies of the new unsubstituted 1,3,4-triazacycl[3.3.3]azine, *1*, and the synthesis of 3-cyano-1,4-diazacycl-[3.3.3]azine, *2a*.* The latter system carrying four ring substituents has been prepared earlier [3,4] by different methods. The present work is a continuation of our studies on bridged annulenes,[10,13] which are of considerable theoretical interest.[13]

Condensation of 1 mol of 2-amino-6-methylpyridine, *3*, with 2 mol of ethyl *N*-cyanoformimidate, *4*, gave *1* directly in low yield. It is most reasonable to assume that the first step in the reaction is an attack of the 2-amino group in *3* on *4*. If, instead, the 6-methyl group should react first, the same azacyclazine results. Therefore, no isomers can be formed in the reaction between *4* and a 2,6-disubstituted pyrimidine.[5]

The triazacyclazine *1* is a blue crystalline compound, rather stable both in the solid state and in solution. Its mass spectrum is typical for heteroaromatics with a large molecular ion and abundant doubly charged ions.

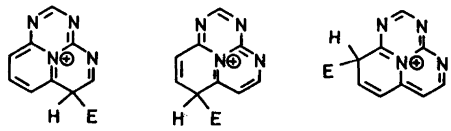


In the mass spectrum of *1* two consecutive losses of HCN are observed. The ^{1}H NMR chemical shift values are in the same region (δ 5.3 – 6.9, *cf.* Experimental) as those of the isomeric 1,3,6-triazacycl[3.3.3]azine (δ 4.9 – 6.5) [6] and 2,5,8-trimethyl-1,4,7-triazacycl[3.3.3]-azine (δ 5.5) [4,7] which indicate that they possess a similar degree of aromaticity. In general, physical and chemical properties are very similar for the four known triaza systems.[4–7]

Electrophilic substitution in *1* occurred in positions 6, 7, and 9 as expected (*cf.* structures on Chart 1 and resonance structures in Ref. 6), with approximately the same rate as in the isomeric systems.[4,6] With *N*-bromosuccinimide (NBS), *1* yielded a mixture of three monobromo, two dibromo, but no tribromo derivatives. The most abundant monobromo compound (*ca.* 40 % of total yield of brominated product) is 6-bromo-*1*, *5a*, since its NMR spectrum displays a one-proton singlet signal (H-5) and an ABX-type absorption. The two other monobromo compounds (*ca.* 10 %) could not be separated from each other by thin-layer chromatography. An NMR spectrum of the mixture showed two pairs of AX-type signals from each compound in a 2:1 ratio and they are therefore the 7- and 9-bromo derivatives *5b* and *5c*. Treatment of *5a* with NBS gave only one dibromo compound which

* For definitions and nomenclature of cyclazines, *cf.* Refs. 1 and 2.

Chart 1. Some resonance structures for electrophilic substitution intermediates of *1*.

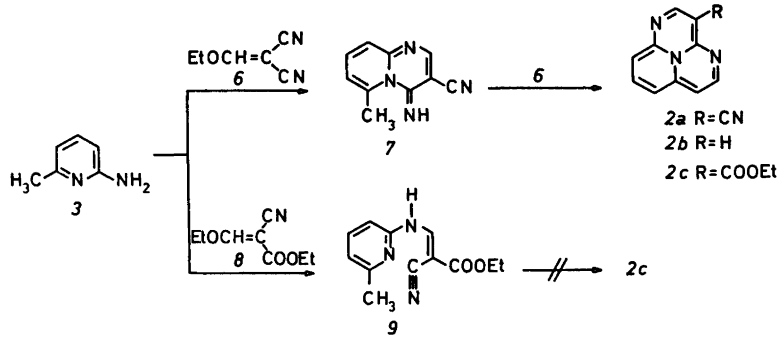


thus is 6,7-dibromo-*1*, *5d*, or 6,9-dibromo-*1*, *5e*. We prefer *5e* since the *peri* relation of bromine atoms in the 6 and 7 positions would cause bond and angle deformations of the ring system.[8] The second dibromo compound isolated (*ca.* 20 %) should the be 7,9-dibromo-*1*, *5f*. Attempts to brominate *5e* or *5f* with NBS resulted in decomposition of the cyclazine system. In other cyclazine systems [4-6] complete bromination has occurred in the positions predicted from resonance structures of the electrophilic substitution intermediates similar to those in Chart 1. The failure to produce a tribromo derivative of *1* is therefore most likely due to the *peri* interaction which would result from two bromine atoms at C-6 and C-7.[8]

The second part will describe experiments directed toward the preparation of 1,4-diazacycl[3.3.3]azine, *2b*, which is one of seven isomers, with non-adjacent, peripheral nitrogen atoms.[5] A tetrasubstituted derivative, dimethyl 3-cyano-2-methylthio-1,4-diazacycl[3.3.3]azine-5,6-dicarboxylate, was reported recently.[3] The possibility of preparing *2b* from this compound by removing as many as four substituents from the not too stable ring system without destroying it seemed rather small. A monosubstituted derivative would therefore be a better precursor for the parent system. Condensation of equimolar amounts of 2-amino-6-methylpyridine, *3*, and ethoxymethylenemalononitril, *6*, at 100 °C for 1 h yielded the bicyclic system *7*. Its structure is in agreement with NMR and mass spectral data (*cf.* Experimental). Ring closure of *7* to *2a* was unusually difficult and it did not succeed with 1 additional mol of *6* under the above-mentioned conditions or in other solvents (*cf.* Experimental). Attempts with acetic formic anhydride or acetic anhydride [9] with or without pyridine as a base were also unsuccessful. Only after careful purification of *7*, followed by the heating of it with 1 mol of *6* for 7 h at 150 °C without solvent, did we obtain *2a*, unfortunately in a yield too low for subsequent preparative decyanation with polyphosphoric acid (PPA) [14] to *2b*. Condensation of *3* with ethoxymethylene cyanoacetate, *8*, which would give *2c* was also tried, but from this reaction only the open intermediate (C≡N, IR) *9* resulted. Ring closure of *9* under the different conditions used to obtain *2a* was totally unsuccessful in the present case.

The structure of *2a* is supported by ^{1}H NMR and mass spectral data (*cf.* Experimental). It is a green crystalline compound, fairly stable both in the solid state and in solution. The ^{1}H NMR chemical shift values for *2a* (δ 5.2 – 6.9) are in the same region as those for the isomeric 3-cyano-1,9-diaza system (δ 5.1 – 6.8).[10] Electron attracting groups seem to stabilize the cycl[3.3.3]azine systems considerably and bring the δ-values for the

Chart 2. Schemes for the synthesis of the 1,4-diazacycl[3.3.3]azine system.



ring protons downfield. Therefore it is difficult to evaluate the effect of the relative positions of the peripheral N-atoms in isomeric azacycl-[3.3.3]azine systems on their "aromaticity" [11] from substituted cyclazine systems like *2a*.

EXPERIMENTAL

General. NMR spectra were recorded with a Jeol MH-60 and a Bruker WH 270 spectrometer, using tetramethylsilane (TMS) as internal reference. UV and visible spectra were measured in ethanol solution with a Cary Model 15 spectrophotometer. IR spectra were determined in KBr with a Perkin-Elmer 337 spectrophotometer. Mass spectra were obtained with a GEC-AEI 902 mass spectrometer at the Department of Medical Biochemistry, University of Göteborg. Thin-layer chromatography was performed on silica gel 60F 254 (Merck) and the colorless spots were visualized with short-wave ultraviolet light.

Preparation of 1,3,4-triazacycl[3.3.3]azine, 1. A mixture of 500 mg (4.6 mmol) of 2-amino-6-methylpyridine, *3*, and 900 mg (9.2 mmol) of ethyl *N*-cyanoformimidate,[12] *4*, was kept at 140 °C for 4 h and then cooled to room temperature. The product was dissolved in EtOAc. The insoluble material was filtered off and the solution evaporated to dryness. The residue was purified first by column chromatography on silica gel (EtOAc-MeOH; 9:1), then by preparative TLC (EtOAc-MeOH; 3:1). Yield: < 5 % of a blue solid *1*, m.p. 191–194 °C. UV: λ_{max} (ε) 217 (9470), 247 (18 720); 332 sh. (14 450), 337 (15 150), 356 sh. (8870), 368 sh. (5470), 375 sh. (5280), 525 sh. (220), 569 (420), 617 (570), 672 (380) nm, NMR ($CDCl_3$): δ 5.31 (1H, d, H-6), 5.53 (1 H, d, H-7 or H-9), 5.60 (1 H, d, H-7 or H-9), 6.69 (1 H, d, H-5), 6.75 (1 H, t, H-8), 6.85 (1 H, s, H-2), $J_{5,6}=6.0$, $J_{7,8}=J_{8,9}=8.0$ Hz, MS: M+ = 170.0585. Calc. for $C_9H_6N_4$: 170.0592.

Bromination of 1. (a) To a solution of 32 mg (0.18 mmol) of *1* in 3 ml of CH_2Cl_2, 33 mg (0.18 mmol) of NBS was added. The mixture was applied directly to preparative TLC plates (EtOAc-MeOH; 5:1). Three bands, two blue ($R_F=0.10$, $R_F=0.52$) and one blue-green ($R_F=0.30$) were scraped off and eluted. The latter and the blue with the largest R_F value contained the dibrominated compounds *5f* and *5e*, respectively. Yield: 4 mg of *5f*, m.p. 260 °C and 5 mg of *5e*, m.p. 265–267 °C. The blue zone ($R_F=0.10$), on the other hand, consisted of three components, all monobrominated *1*. One of them, *5a*, could be separated from the others on preparative TLC plates developed three times in EtOAc. Yield: 7 mg, m.p. 235–237 °C, of a green solid, *5a*, and 2 mg of a green solid, *5b*+*5c*. *5a:* NMR ($CDCl_3$) δ 5.63 (1 H, d, H-7 or H-9), 5.83 (1 H, d, H-7 or H-9), 6.75 (1 H, t, H-8), 6.76 (1 H, s, H-2 or H-5), 6.81 (1 H, s, H-2 or H-5), $J_{7,8}=J_{8,9}=8.0$ Hz, MS: M+ = 247.9690 ± 0.003. Calc. for $C_9H_5N_4{}^{79}Br$: 247.9698. *5b*+*5c*: NMR ($CDCl_3$) δ 5.36 (1 H, d, H-7 or H-9), 5.52 (1 H, d, H-6), 6.70 (1 H, d, H-5), 6.73 (1 H, s, H-2), 6.78 (1 H, d, H-8), $J_{5,6}=6.0$, $J_{7,8}$ or $J_{8,9}=9.2$ Hz and 5.20 (1 H, d, H-6), 5.30 (1 H, d, H-7 or H-9), 6.61 (1 H, d, H-5), 6.85 (1 H, s, H-2), 6.86 (1 H, d, H-8), $J_{5,6}=6.3$, $J_{7,8}$ or $J_{8,9}=8.7$ Hz. The integrals indicate a 2:1 ratio of *5b* and *5c*. MS:M+ = 247.9680 ± 0.003. Calc. for $C_9H_5N_4{}^{79}Br$: 247.9698. *5e:* NMR (acetone-d_6) δ 6.01 (1 H, d, H-7), 7.05 (1 H, s, H-2 or H-5), 7.07 (1 H, s, H-2 or H-5), 7.45 (1 H, d, H-8), $J_{7,8}=8.7$ Hz, MS: M+ = 327.876 ± 0.003. Calc. for $C_9H_4N_4{}^{79}Br^{81}Br$: 327.8784. *5f:* NMR ($CDCl_3$) δ 5.77 (1 H, d, H-6), 6.95 (1 H, d, H-5), 7.07 (1 H, s, H-2 or H-8), 7.29 (1 H, s, H-2 or H-8), $J_{5,6}=6.6$ Hz, MS: M+ = 327.878 ± 0.003. Calc. for $C_9H_4N_4{}^{79}Br^{81}Br$: 327.8784.

(b) To a solution of 8 mg (0.04 mmol) of *1* in 2 ml of CH_2Cl_2 25 mg (0.14 mmol) of NBS was added. As performed above, the mixture was applied to preparative TLC plates. This time dibrominated *5e* resulted and only traces of *5f*.

Preparation of 7. A mixture of 5.0 g (46.3 mmol) of 2-amino-6-methylpyridine, *3*, and 5.7 g (46.7 mmol) of ethoxymethylenemalononitrile, *6*, was kept at 100 °C for 15 min. The reaction product was air-dried overnight; 9 g of crude material was obtained. Chromatographic purification on Al_2O_3 (CH_2Cl_2) yielded 7 g (82 %) of a white solid *7*, m.p. 180 °C. IR: 3310, 3250 (NH), 2220 cm^{-1}(C≡N), UV: λ_{max} (ε) 266 sh. (10 190), 273 (11 600), 326 (28 780) nm, NMR (DMSO-d_6): δ 2.43 (3 H, s, CH_3), 7.01 (2 H, d, H-7 and H-9), 7.67 (1 H, t, H-8), 8.62 (1 H, s. H-2), $J_{7,8}=J_{8,9}=7.6$ Hz, MS: M+ = 184.

Ring closure of 7 *to* 2a. (a) An attempt to synthesize *2a* from *7* by using one additional mol of *6* at 100 °C was not successful. The result was also negative when a small amount of base (pyridine or NaOEt) was present. The use of hexamethylphosphoric triamide (HMPA) or *o*-dichlorobenzene as solvents and varying the temperature from 100 °C to 170 °C also failed to give *2a*. The reactions were followed by analytical TLC.

(b) Formylation ($HCOOH/Ac_2O$) or acetylation (Ac_2O) of *7* with or without pyridine present as a base, at different temperatures 20 to 140 °C) and different reaction times (up to 2 days) gave no ring closure.

(c) A mixture of 1.0 g (5.4 mmol) of *7* and 0.7 g (5.7 mmol) of *6* was heated at 150 °C for 7 h. The work-up procedure was the same as for *1*, yielding < 1 % of a green solid *2a*. IR: 2220 cm^{-1} (C≡N), NMR ($CDCl_3$): δ 5.18 (1 H, d, H-6), 5.31 (1 H, d, H-7 or H-9), 5.66 (1 H, d, H-7 or H-9), 6.45 (1 H, d, H-5), 6.62 (1 H, t, H-8), 6.83 (1 H, s, H-2), $J_{5,6}=6.3$, $J_{7,8}=J_{8,9}=8.2$ Hz, MS: M+ = 194.058 ± 0.003. Calc. for $C_{11}H_6N_4$: 194.0592.

Preparation of 9. A mixture of 5.0 g (46.3 mmol) of 2-amino-6-methylpyridine, *3*, and 7.8 g (46.2 mmol) of ethoxymethylenecyanoacetate, *8*, was kept at 100 °C for 15 min. The sample was air-dried overnight and purified on Al_2O_3 (CH_2Cl_2). Yield: 9 g (84 %) of a white solid *9*, m.p. 117–118 °C. IR: 3290, 3240 (NH), 2210 (C≡N), 1670 cm^{-1} (C=O), UV: λ_{max} (ε) 268 sh. (6410), 274 (8980), 326 (34 000) nm, NMR ($CDCl_3$): δ 1.37 (3H, t, ester protons), 2.50 (3 H, s, CH_3), 4.30 (2 H, q, ester protons), 6.64 (1 H, d, H-3 or H-5), 6.92 (1 H, d, H-3 or H-5), 7.56 (1 H, t, H-4), 8.75 (1 H, d, vinyl proton), $J_{7,8} = J_{8,9} = 7.7$ Hz, MS: $M^+ = 231$.

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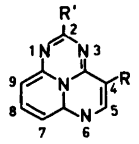
Some Reactions of the 2-Methyl-1,3,6-triazacycl[3.3.3]azine System

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The following properties of the 2-methyl-1,3,6-triazacycl[3.3.3]azine system have been studied: the reactivity of the methyl group, catalytic hydrogenation, nitration, alkylation, and nucleophilic substitution. Hydrogenation leads to a dihydro derivative, alkylation to a mixture of *N*-alkylated products, and attack by piperidine proceeds *via* an aryne intermediate.

The present communication describes some further properties and reactions of the earlier prepared 2-methyl-1,3,6-triazacycl[3.3.3]azine,* *1*,[1–3] and its 4-cyano derivative *2*.[1,4,5]



1 R=H, R'=Me
2 R=CN, R'=Me
3 R=CN, R'= CH=CHPh
8 R=NH_2, R'=Me
9 R=Br, R'=Me

Methyl groups on carbon atoms adjacent to nitrogen in aromatic heterocycles often display considerable reactivity, *e.g.* in 2-methylpyrimidine.[8] The NMR spectrum of *2* in CF_3COOD showed that the methyl protons had exchanged to 50 % after 10 min, while the intensity of all other proton signals remained unchanged for several hours. In a CF_3COOD solution containing a trace of concentrated DCl, exchange of H-5 was also observed from a small decrease in its NMR signal after *ca.* 2 h. Reaction of *2* with benzaldehyde in the presence of $ZnCl_2$ gave the styryl compound *3*.[8] Attempts to convert *3* to the corresponding aldehyde by ozonolysis were unsuccessful since the cyclazine system was decomposed by ozone.

Catalytic hydrogenation of *2* yielded two products, *4* and *5*, of the same molecular weight. The structure of *4*, 5,6-dihydro-*2*, follows from the presence of a CH_2 signal (not coupled with the NH in DMSO-d_6) and an A_2X-type absorption in the aromatic region of the NMR spectrum. In air, on TLC plates, and on sublimation, *4* is smoothly oxidized back to *2*. A mass spectrum of *4* shows a small molecular ion and a large M − 2 ion indicating that aromatization takes place in the mass spectrometer also. Compound *5*, isomeric with *4* and showing the same behavior, is probably the 1,2- or 2,3-dihydro compound, but lack of material did not allow a structural assignment. The parent compound *1* is more resistant to catalytic hydrogenation than *2* and most of the starting material is recovered unchanged when the conditions are the same as those used above.

The central nitrogen atom in cyclazines is completely non-basic [6] and in azacyclazines protonation occurs on the peripheral *N*-atoms.[2,9] When *1* is treated with methyl iodide, a mixture of three quaternary salts, *6a* − *c* is formed in the ratio 85:10:5 (NMR, *cf.* Experimental). The major component is probably the 6-methylated compound, *6a*, since alkylation of N-6 is sterically less hindered than that of N-1 and N-3; the NMR chemical shift values do not allow a distinction between *6b* and *6c*, respectively. Attempts to alkylate *2* with methyl iodide were unsuccessful also under forcing conditions. On the other hand, *2* reacted smoothly with methyl fluorosulfonate to form a mixture of two quaternary salts in a 95:5 ratio (NMR, *cf.* Experimental). Again, the major component is probably the 6-methyl derivative, *7a*.

* For definitions and nomenclature of cyclazines, *cf.* Refs. 6 and 7.

None of the methods available to date for the synthesis of azacycl[3.3.3]azines has led to amino compounds which could be converted into diazonium salts. When *2* was heated in polyphosphoric acid (PPA) containing $NH_2OH.H_2SO_4$,[10] a small yield of the 4-amino compound *8* was obtained. This conversion is not synthetically useful, however.

Treatment of *1* with $Cu(NO_3)_2-Ac_2O$ produced the three isomeric 4-, 7-, and 9-nitro compounds. All three possessed the same R_F-value in the solvent systems tried and they could not be separated. Nitration, like bromination, of *1*[3] proceeded very smoothly and rapidly and the mixture of derivatives was produced in 40 % yield.

In order to investigate the behavior of the 1,3,6-triazacycl[3.3.3]azine system toward nucleophiles, 4-bromo-*1*, *9*, was refluxed for *ca.* 2 days with piperidine. Instead of the expected substitution product, the 5-piperidino derivative resulted. Its structure was supported by NMR-shift reagent studies (*cf.* Experimental). This reaction therefore seems to require an elimination-addition process *via* the 4,5-dihydro derivative.[11] The conditions used in the present case are notably mild for the generation of such an intermediate.

EXPERIMENTAL

General spectrometric conditions and chromatographic procedures were the same as those described in Ref. 13.

The ozonization experiment was performed according to the following procedure: The reaction solution was kept at −60 °C by the use of a dry ice−ethanol bath. A mixture of 3 % of ozone in oxygen, produced by a Welsbach Model T-23 ozonator, was passed through the solution at a rate of 70 ml/min. The reaction was continued until elemental iodine was liberated from a 1 % aqueous solution of potassium iodide through which the effluent gases were passed. Excess of ozone was then removed by passing a stream of nitrogen through the solution for 20 min.

Preparation of 4-cyano-2-styryl-1,3,6-triazacycl[3.3.3]azine, 3. To a mixture of 170 mg (0.81 mmol) of *2* in 3 ml (29.6 mmol) of benzaldehyde a small amount of $ZnCl_2$ was added. The solution was kept at 110 °C for 20 min, during which time it turned dark-green. Some of the benzaldehyde was evaporated under reduced pressure. Chromatography of the residue on 15 g of silica gel (EtOAc−$CHCl_3$); 1:9) gave 110 mg of product from which benzoic acid was eliminated by sublimation at 120°/2 Torr. Purification of the sublimation residue by preparative TLC (EtOAc) yielded 60 mg (25 %) of a green solid *3*, m.p. 235−238 °C. IR: 2230 (C≡N), 1630 cm^{-1} (C=C), UV: λ_{max} (ε) 215 (17 450), 238 (13 960), 295 sh. (7280), 352 (51 200), 378 sh. (27 850), 388 sh. (18 940), 397 (21 200), 407 sh. (5050) in ethanol and 555 sh. (200), 600 (326), 643 (410), 707 (315 nm in acetone). NMR ($CDCl_3$): δ 5.94 (1 H, d, H-7 or H-9), 6.11 (1 H, d, H-7 or H-9), 6.41 (1 H, d, vinyl proton), 7.12 (1 H, t, H-8), 7.6−7.3 (6 H, m, phenyl protons and H-5), 7.78 (1 H, d, vinyl proton), $J_{7,8}=J_{8,9}=8.2$, J(vinyl protons) = 15.8 Hz (indicating *trans* configuration). MS: $M^+=297.096\pm0.004$. Calc. for $C_{18}H_{11}N_5$: 297.1014.

Ozonolysis of 3. A solution of 15 mg of *3* in 30 ml of CH_2Cl_2 was ozonized for 7 min. The blue solution had then turned yellow. To destroy the ozonides, a small amount of Pd/C was added and the solution was hydrogenated for 16 h. After filtration and evaporation, a yellow sirup was obtained in which no blue or green compounds could be detected by TLC.

Catalytic hydrogenation of 2 *to* 4 *and* 5. A mixture of 1 g of *2* and 100 mg (10 %) Pd/C in 250 ml of abs. ethanol was shaken under H_2 (3.1 atm.) for three days in a Parr hydrogenation apparatus. Unreacted starting material and catalyst were removed from the solution by filtration. After the solution was evaporated to dryness, 700 mg of a green solid remained which was purified by column chromatography on silica gel with EtOAc-$CHCl_3$ (1:3) as the eluent. Yield: 200 mg (20 %) of a yellow product *4*, m.p. 243 °C. Preparative TLC (EtOAc-MeOH; 3:1) separated *5* ($R_F=0.45$) from *4* ($R_F=0.37$) giving < 1 % yield of *5*. *4* IR: 3480 (NH), 2200 cm^{-1} (C≡N), UV:λ_{max} (ε) 262 (1055), 346 (2250), 377 sh. (1290), 410 sh. (690) nm, NMR (DMSO-d_6): δ 2.15 (3 H, s, CH_3), 3.88 (2 H, s, CH_2), 6.30 (2 H, d, H-7 and H-9), 7.67 (1 H, t, H-8), $J_{7,8}=J_{8,9}=8.0$ Hz, MS: $M^+=211$, *5* MS: $M^+=211$.

Catalytic hydrogenation of 1. A mixture of 42 mg of *1* and 7 mg of (10 %) Pd/C in 20 ml of abs. ethanol was hydrogenated as described above. During three days the solution did not change color and no hydrogenation product could be detected by TLC.

Methylation of 1 *to* 6a−c. A solution of 73 mg (0.39 mmol) of *1* in 3 ml (48.1 mmol) of CH_3I in a sealed tube was kept at 60 °C for 4 h. Unreacted starting material was dissolved in $CHCl_3$ and the remaining red product was isolated by filtration, washed with $CHCl_3$, and dried. Yield: 75 mg (95 %) of *6*. The three N-CH_3 signals indicate the presence of the three isomers *6a−c*. NMR (D_2O) of the major isomer: δ 2.14 (3 H, s, 2-CH_3), 3.27 (3 H, s, N-CH_3), 5.55 (1 H, d, H-4), 6.61 (2 H, d, H-7 and H-9), 7.36 (1 H, d, H-5), 7.88 (1 H, t, H-8), $J_{4,5}=7.7$, $J_{7,8}=J_{8,9}=8.6$ Hz. The two minor

isomers have their methyl signals at 2.20, 3.06 and 2.23, 3.13, respectively. The ring-proton signals overlap and are therefore difficult to assign. The integral ratio for the three methyl groups in *6a–c* is 17:2:1, respectively. Electrophoresis (pyridine-AcOH-H_2O; 20:5:220) of *6* was performed on cellulose plates (400 V) and showed the presence of only one spot, hereby proving the existence of only monomethylated *1* in the mixture. The electrophoresis was carried out on a Camag Thin Layer Electrophoresis apparatus.

Methylation of 2 (a) with methyl iodide. One hundred mg (0.47 mmol) of *2* and 0.7 ml (11.3 mmol) of CH_3I in a sealed tube were reacted for 17–24 h at different temperatures between 20 and 100 °C. At lower temperatures, the starting material was recovered unchanged, but at higher temperatures it was destroyed. No methylated product could be isolated.

(b) with methyl fluorosulfonate. To a suspension of 100 mg (0.47 mmol) of *2* in 4 ml of CH_3CN, 1 g (8.8 mmol) of CH_3OSO_2F was added dropwise. The blue solution turned red and after 1 min, 5 ml of CH_2Cl_2 was added. The red precipitate which formed was collected by filtration, washed with CH_2Cl_2 and air-dried. Yield: 40 mg (37 %). The red amorphous product, *7*, was very unstable in the solid state and from an MeOH or H_2O solution the starting material was reformed almost immediately. The NMR signals of the methylated product indicated the presence of two isomers in a 95:5 ratio. NMR (CF_3COOD) of the major isomer: δ 2.38 (3 H, s, 2-CH_3), 3.99 (3 H, s, *N*-CH_3), 6.95 (1 H, d, H-7 or H-9), 7.27 (1 H, d, H-7 or H-9), 8.04 (1 H, t, H-8), 8.25 (1 H, s, H-5), $J_{7,8}=J_{8,9}=8.5$ Hz.

Preparation of 4-amino-1,3,6-triazacycl[3.3.3]azine, 8. A mixture of 257 mg (1.2 mmol) of *2* and 612 mg (4.7 mmol) of $NH_2OH.H_2SO_4$ in 5 ml of PPA was heated to 190 °C. Ice water was added and the solution was neutralized with 10 % aqueous $NaHCO_3$ and then extracted with chloroform. The extract was dried ($MgSO_4$) and evaporated to dryness. Preparative TLC (EtOAc-MeOH; 3:1) yielded 9 mg (4 %) of a green solid, *8*, m.p. 220 °C. IR: 3450, 3310 cm^{-1} (NH), UV: λ_{max} (ε) 213 (9900), 237 (11 770), 263 (13 460), 307 (9100), 353 (10 840), 394 (6240), 531 sh. (40), 577 (130), 629 (190), 691 (150) nm, NMR ($CDCl_3$): δ 1.65 (3 H, s, CH_3), 5.10 (1 H, d, H-7 or H-9), 5.76 (1 H, d, H-7 or H-9), 6.46 (1 H, q, H-8), 6.99 (1 H, s, H-5), $J_{7,8}=8.7$, $J_{8,9}=7.4$ Hz, MS: $M^+=199.084\pm0.003$. Calc. for $C_{10}H_9N_5$: 199.0858.

Acetylation of 8. To a solution of 2 mg of *8* in 0.5 ml of *p*-xylene, 1 drop of acetic anhydride was added. The mixture was kept at 30 °C for 5 min, then evaporated to dryness yielding a green solid. TLC (EtOAc-MeOH; 1:1) showed the presence of a blue compound with almost the same R_F value as *8*, m.p. 207 °C. MS: $M^+=241$.

Nitration of 1. To a solution of 80 mg of $Cu(NO_3)_2.3H_2O$ in 2 ml of acetic anhydride, 40 mg of *1* was added. The mixture was left at room temperature for 25 min and it was then neutralized with aqueous $NaHCO_3$ (10 %), yielding a green solution which was extracted with chloroform. The combined extracts were dried ($MgSO_4$) and then evaporated to dryness. On preparative TLC (EtOAc-MeOH; 3:1) of the dark-green product, only one band ($R_F=0.42$) was observed. Extraction of this band yielded 20 mg of a green solid, m.p. 180–182 °C. The NMR spectrum ($CDCl_3$) showed it to be a mixture of three isomers. The methyl groups have the chemical shift values 1.91, 2.01 and 2.08 ppm and the integral ratio of these signals is 1:1.5:1, MS: $M^+=229$.

Reaction of 4-bromo-2-methyl-1,3,6-triazacycl[3.3.3]azine 9, *with piperidine*. A mixture of 90 mg of 4-bromo-*1* in 8 ml of piperidine was kept at 80–90 °C for two days. The solution was evaporated to dryness. Column chromatography on 4 g of silica gel with EtOAc as the eluent, gave a red product which was further purified by preparative TLC (EtOAc-MeOH; 3:1). Yield: 20 mg (22 %), m.p. 125–127 °C. UV: λ_{max} (ε) 234 (15 820), 260 (17 940), 285 sh. (17 270), 309 (25 800), 325 sh. (19 820), 367 sh. (4760), 428 sh. (160), 450 sh. (260), 480 sh. (470), 513 (650), 552 (540) nm. NMR ($CDCl_3$): δ 1.62 (6 H, s, piperidine protons), 1.84 (3 H, s, CH_3), 3.51 (4 H, s, piperidine protons), 5.02 (1 H, s, H-4), 5.55 (1 H, d, H-7 or H-9), 5.90 (1 H, d, H-7 or H-9), 6.95 (1 H, t, H-8), $J_{7,8}=J_{8,9}=8.3$ Hz, MS: $M^+=267.146\pm0.002$. Calc. for $C_{15}H_{17}N_5$: 267.1484.

Lanthanide-shift measurements. The different Δ_{Eu}-values [12] were determined as follows: a weighed amount of the actual substance was dissolved in dry $CDCl_3$ and known amounts of Eu(fod)$_3$-d_{27} dissolved in the same solvent were added. After each addition, the NMR tube was left spinning in the probe for about 15 min before the NMR spectrum was determined. The Δ_{Eu}-values obtained in *ca.* 0.15 M solution and at shift reagent/substrate ratios from 0 to 2 are summarized in Table 1.

Table 1. Δ_{Eu}-values for the protons in 5-piperidino-*1*.

Proton	Δ_{Eu}
H-4	3.8
H-7	0.8
H-8	0.8
H-9	5.4
2-CH_3	5.0
Piperidine	0.3 and 0.8

The high value for the 2-methyl protons indicates that the coordination of Eu takes place preferably at N-1 and N-3. The chemical shift value of the one-proton singlet (δ 5.02) and the high Δ_{Eu}-value for the same proton indicate that H-4 is present. Therefore we conclude that the piperidine moiety is located in the 5-position. The low degree of coordination at the 6-position is therefore a result of the steric hindrance of the piperidine moiety.

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Animal Carotenoids. 12.* Chirality of Asterinic Acid

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The chirality of monoacetylenic asterinic acid [(3*S*,3′*S*)-7,8-didehydroastaxanthin, *1a*] has been established by $NaBH_4$-reduction and hydrolysis of the corresponding diesters *1b* and *1c* providing the tetrol *9* and CD-correlation with diatoxanthin [(3*R*,3′*R*)-7,8-didehydro-β,β-carotene-3,3′-diol, *10*].

Diacetylenic asterinic acid [(3*S*,3′*S*)-7,8,7′,8′-tetradehydro-β,β-carotene-3,3′-diol, *2a*] was assigned the some absolute configuration by similar conversion to the diacetylenic tetrol *11* and CD-correlation with alloxanthin [(3*R*,3′*R*)-7,8,7′,8′-tetradehydro-β,β-carotene-3,3′-diol, *12*].

IR and CD properties of the diacetates *1C* and *2C* of the naturally occurring α-ketols are reported.

Asterinic acid (asterinsäure), first isolated by von Euler and Hellström [1] from the starfish *Asterias rubens* (Linné), has also been encountered in the soft coral *Alcyonium digitatum* (Linné) [2] and recently in lobster roe.[3]

Asterinic acid occurs as a protein complex in *Asterias rubens*,[1,3] presumably also in the lobster eggs,[1,3] and is known to be a mixture of 7,8-didehydro- and 7,8,7′,8′-tetradehydroastaxanthin (*1* and *2*, Scheme 1) from chromatographic and spectroscopical (electronic, IR, ^1H NMR and mass spectra) evidence.[5,6]

We now report on the absolute configuration of these acetylenic derivatives of astaxanthin (*3*).

RESULTS AND DISCUSSION

The chirality of astaxanthin (*3*) *ex* lobster was assigned in our laboratory by conversion to a diastereomeric mixture of tetrols (*4*) by $LiAlH_4$-reduction. Conformational analysis of the tetrols (*4*) revealed that the chirality at C-3(3′) was decisive for the preferred half-chair conformation of the cyclohexene end groups, dictating the sign of the Cotton effect.[7] Since the CD of the tetrol mixture (*4*) was identical with that of zeaxanthin (*5*) of known 3*R*,3′*R* configuration,[8] the same chirality at C-3,3′ of the tetrols *4* and zeaxanthin (*5*) and consequently also in astaxanthin (*3*) was concluded. The chirality of astaxanthin (*3*) has since been confirmed by Kienzle [9] by total synthesis of (3*S*,3′*S*)-astaxanthin (*3*), thus verifying the validity of the arguments used in our configurational assignment.

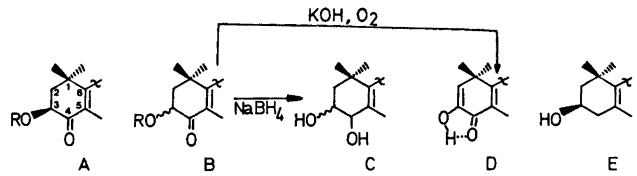
It is therefore evident that the chirality of the mono- and diacetylenic derivatives (*1* and *2*) of astaxanthin (*3*) could be solved by the same approach.

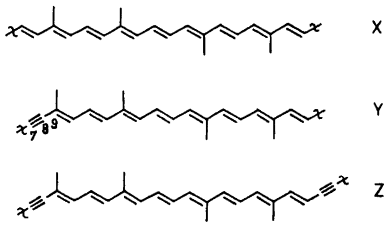
A mixture of astaxanthin (*3*) and the monoacetylenic (*1*) and diacetylenic derivative *2* were reisolated from *Asterias rubens via* the crude protein complex. Also present were fatty acid diesters *1b*, *2b* and *3b* of *1*, *2* and *3*, respectively.

The mixed α-ketols *1*, *2* and *3* were converted to the diacetates *1c*, *2c* and *3c* to effect better chromatographic separation and less facile conversion to the corresponding diosphenols *6*, *7* and *8*. The monoacetylenic diacetate *1c* and the diacetylenic diacetate *2c* were characterized by electronic, IR and mass spectra.

To simplify the isolation the monoacetylenic diesters *1b* and *1c* were treated with $NaBH_4$, rather than $LiAlH_4$, resulting in reduction of the keto groups, followed by alkaline hydrolysis of the ester functions to provide the monoacetylenic tetrol *9* with retention of configuration at C-3,3′ and mixed configuration at C-4,4′. After TLC purification the tetrol *9* was characterized

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1	B-Y-B	R=H
1a	A-Y-A	R=H
1b	B-Y-B	R=Acyl
1c	B-Y-B	R=Acetyl
2	B-Z-B	R=H
2a	A-Z-A	R=H
2b	B-Z-B	R=Acyl
2c	B-Z-B	R=Acetyl

3	A-X-A	R=H
3b	A-X-A	R=Acyl
3c	A-X-A	R=Acetyl
4	C-X-C	
5	E-X-E	
6	D-X-D	
7	D-Y-D	
8	D-Z-D	
9	C-Y-C	
10	E-Y-E	
11	C-Z-C	
12	E-Z-E	

by electronic, mass and CD spectra. The CD spectrum (Fig. 1) showed agreement with that of authentic all-*trans* diatoxanthin (*10*)[10] of known 3*R*,3′*R*-configuration.[11] By the same argumentation as used in the configurational assignment of astaxanthin (*3*), the diastereomeric mixture of monoacetylenic tetrols *9* must have the same chirality at C-3,3′ as diatoxanthin (*10*). Consequently the monoacetylenic astaxanthin from which the tetrol *9* was prepared has the same absolute configuration and is (3*S*,3′*S*)-7,8-didehydroastaxanthin (*1a*).

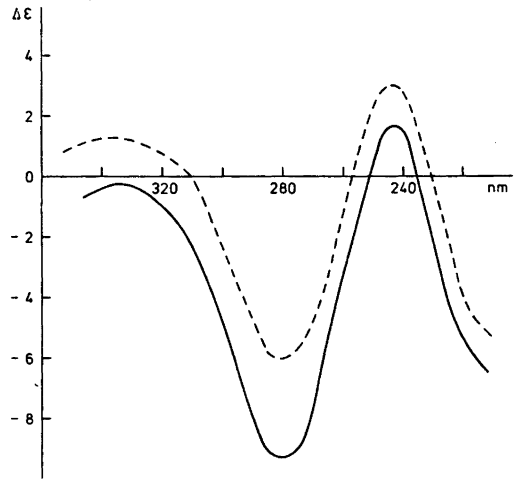


Fig. 1. CD spectra in EPA solution of ——— the monoacetylenic tetrol *9* and of – – – diatoxanthin (*10*).

One point must be commented on. It is known that triple bonds in 7,8-position of carotenoids favour *cis* configuration of the adjacent double bond.[12] Moreover, a *cis* bond in the polyene chain reverses the sign of the Cotton effect.[10,11] However, the spectral characteristics (λ_{max} and insignificant *cis* peak) in the electronic spectrum of the tetrol *9* for which the CD was recorded, are incompatible with dominance of a 9-*cis* tetrol.

The natural diacetylenic diesters *2b*, were converted by $NaBH_4$ followed by alkaline hydrolysis to the diacetylenic tetrol *11*, the CD of which was correlated with that of the corresponding diacetylenic 3,3′*R*-diol alloxanthin (*12*) of known 3*R*,3′*R*-configuration,[11] Fig. 2. As references are used the CD spectrum of all-*trans* alloxanthin (*12*) calculated by a Koenig-Kramers transformation[13–15] of the published[11] ORD spectrum and the measured CD spectra

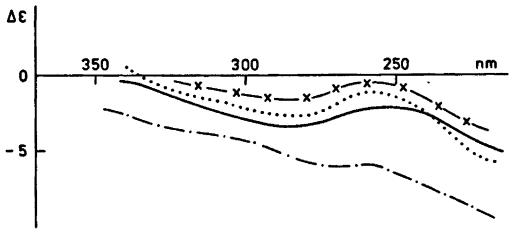


Fig. 2. CD spectra in EPA solution of ——— the diacetylenic tetrol *11*, — — alloxanthin (*12*) *ex Microciona prolifera*,[16] — × — alloxanthin (*12*) *ex Asterias rubens* and of · · · alloxanthin (*12*) in solution calculated from the published [11] ORD spectrum. *Δε*-values only valid for the three former curves.

of alloxanthin *ex Asterias rubens* and *ex* the sponge *Microciona prolifera*.[14] In all cases a negative Cotton effect is observed below 310 nm. It is concluded that the diacetylenic tetrol *11* has the same chirality at C-3,3′ as alloxanthin (*12*) and thus that the diacetylenic asterinic acid is (3*S*,3′*S*)-7,8,7′,8′-tetradehydroastaxanthin (*2a*).

In conclusion it is here shown that the naturally occurring 7,8-didehydro- and 7,8,7′,8′-tetradehydro derivatives of astaxanthin from starfish have the same chirality as astaxanthin (*3*), *ex* lobster, zeaxanthin (*5*), diatoxanthin (*10*), and alloxanthin (*12*). The stereochemical result is consequently compatible with a biosynthetic

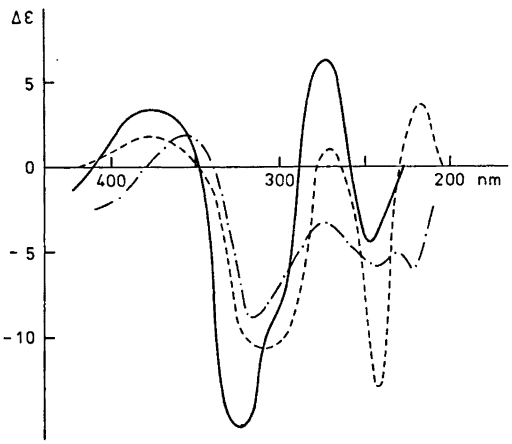


Fig. 3. CD spectra in EPA solution of — — — astaxanthin (*3*),[7] ——— 7,8-didehydroastaxanthin diacetate (*1c*) and —·— 7,8,7′,8′-tetradehydroastaxanthin diacetate (*2c*); *Δε*-values not valid for *3*.

precursor relationship to any of these 3,3′-diols. It should be mentioned that astaxanthin occurs with 3*S*,3′*S* chirality (*3*) in all sources studied hitherto,[11,17] except a red yeast [18] which produces the enantiomeric (3*R*,3′*R*)-astaxanthin.

ORD spectra of zeaxanthin (*5*), diatoxanthin (*10*) and alloxanthin (*12*) are published by Bartlett *et al.*[11] CD spectra of the same series are reported elsewhere [7] and in the present work. In Fig. 3 are compiled the CD spectra of (3*S*,3′*S*)-astaxanthin (*3*), (3*S*,3′*S*)-7,8-didehydroastaxanthin diacetate (*1c*) and (3*S*,3′*S*)7,8,7′,8′-tetradehydroastaxanthin diacetate (*2c*). It is obvious that triple bonds in 7,8(7′,8′)-positions change drastically the chiroptical properties of carotenoids. Introduction of such triple bonds cause a gradual "flattering" of the CD spectra ascribed to chromophoric changes.

EXPERIMENTAL

Materials and methods were those commonly employed in the Trondheim laboratory.[19–21] Chromatography was effected by TLC System A (kieselgel G; 0.75 or 1 mm layers) or System B [22] (kieselgel 30 g, MgO 9 g, $Ca(OH)_2$ 12 g, $CaSO_4$ 3 g, and water 93 ml; 0.75 or 1 mm layers) and circular kieselguhr paper Schleicher & Schüll No. 287 (System C), using mixtures of acetone in hexane (A-hex) for development.

CD spectra were recorded on a Roussel-Jouan Dicrographe. *Δε*-Values are based on calculated concentrations. The following $E(1\ \%,\ 1\ cm)$ values at λ_{max} in acetone were used for *1c* and *2c* 2100, for *9* and *11* 2250, and for *10* and *12* 2350.

Biological material. Bluish-violet starfishes *Asterias rubens* (190 specimens, 7.3 kg live weight) were collected near Røskje, Nord-Trøndelag, August 1974.

Pigment isolation. The coloured parts of the back skin were cut out (rubber gloves) and rinsed quickly in cold water. The residue (1.2 kg wet weight) was minced in a Waring blendor with water, and the suspension extracted with water (5 l + 3.5 l) at room temperature for 2 days,[1,5,6] followed by decantation and filtration.

Addition of aqueous, saturated NH_4Cl-solution to aliquots of the aqueous extract in various proportions failed to precipitate the protein complex. The crude protein complex in the aqueous extract was therefore split by solvent extraction using at optimum conditions aqueous extract – acetone – diethyl ether 1:1.2:1, providing 22.7 mg carotenoids $E(1\ \%,\ 1\ cm) = 2500$) after transfer to ether. Lipids were removed from the crude extract by repeated precipitation from acetone at low temperature.

Pigment separation was effected by preparative TLC. The results are given in Table 1.

Table 1. Pigment separation by preparative TLC (System A, 20 % A-hex). u, unidentified.

Zone	R_F	Yield mg	% of total	Compound
1	0.96	0.12	0.5	β,β-carotene
2	0.86	0.57	2.5	alloxanthin (*12*) diester
3	0.70	0.23	1.0	u
4	0.57	10.0	46.0	diesters *1b*, *2b* and *3b*
5	0.50	0.12	0.5	u
6	0.42	0.91	4.0	u
7	0.25	0.10	0.5	u
8	0.16	6.6	29.0	α-ketols *1a*, *2a* and *3*
9	0.08	3.6	16.0	alloxanthin (*12*) and diosphenols *6*, *7* and *8*

β,β-Carotene was identified from electronic and mass spectra and co-chromatography (System C, hexane) with an authentic sample.

Alloxanthin (*12*) diester had upon rechromatography (System A, 15 % A-hex) $R_F = 0.71$. Saponification followed by chromatography (System A, 50 % A-hex) gave alloxanthin (*12*) by the criteria given below.

Free alloxanthin (*12*) isolated by rechromatography (System B, 60 % A-hex) had $R_F = 0.26$, slightly more strongly adsorbed than authentic zeaxanthin (*5*); λ_{max} (acetone) (430), 454 and 483 nm; *m/e* 564 (100 %), M−15, M−92, M−106.

The mixed diesters (Zone 4) *1b*, *2b* and *3b* were identified from R_F-value, λ_{max} (acetone) 475 and (502) nm and by alkali treatment providing the mixed diosphenols *6*, *7* and *8*; *m/e* (%): 592 (M_1; 27), 590 (M_2; 32), 588 (M_3; 18), 575 (M_1-17; 3), 573 (M_2-17; 2), 500 (M_1-92; 3), 498 (M_3-92; 2), 496 (M_3-92; 1), 486 (M_1-106; 3), 484 (M_2-106; 2) and 482 (M_3-106; 1), *cf.* Ref. 6.

The mixed diesters *1b*, *2b* and *3b* were separated in System B (35 % A-hex): R_F *3b* 0.67, *1b* 0.43 and *2b* 0.18.

The mixed α-ketols *1a*, *2a* and *3* (Zone 8) were crystallized from diethyl ether, yield 4 mg, and identified from R_F-value, λ_{max} (acetone 476 and 503 nm) and *m/e* 596 (M_1), 594 (M_2), 592 (M_3), M_n-92 and M_n-106 peaks.

Standard acetylation [18] of the mixed α-ketols gave the diacetates *1c*, *2c* and *3c* with unchanged λ_{max} and *m/e* (%): 680 (M_1; 9), 678 (M_2; 29), 676 (M_3; 36), 620 (M_1-60; 2), 618 (M_2-60; 4), 616 (M_3-60; 3), 588 (M_1-92; 2), 586 (M_2-92; 1), 584 (M_3-92; 1) and 574 (M_1-106; 1), *cf.* Ref. 6. The diacetates were separated in System B (60 % A-hex), R_F *3c* 0.87, *1c* 0.51 and *2c* 0.21.

7,8-Didehydroastaxanthin natural diester (*1b*) had λ_{max} (acetone) 478 and (CS_2) 503 (535) nm; ν_{max} (KBr) 2920, 2850, 2161 (−C≡C−), 1740 (ester), 1680 (conj. C=O), 1568, 1520, 1468, 1380, 1365, 1290, 1270, 1243, 1160, 1120, 1090, 1072, 1040, 964 (*trans*-CH=CH−), 929, 830 (>C=CH−), 742 and 720 cm^{-1}.

1b (0.2 mg) in chloroform (0.5 ml) and ethanol (5 ml) was treated with $NaBH_4$ (0.1 g). The reaction was monitored by TLC and after 1 h 10 % methanolic KOH (2 ml) was added. Extractive isolation, followed by TLC (System A, 60 % A-hex) gave tetrol *9* as a major product; $R_F = 0.51$, 0.08 mg (39 % yield); λ_{max}(tetrahydrofurane) (430), 456 and 484 nm; *m/e* 598 (M); CD (EPA) Fig. 1.

7,8-Didehydroastaxanthin diacetate (*1c*) had λ_{max} (CS_2) 500 nm; ν_{max} (KBr) 2958, 2922, 2855, 2161 (−C≡C−, medium), 1740 (ester) 1672 (conj. C=O), 1600, 1571, 1551, 1460, 1375, 1345, 1304, 1288, 1270, 1240, 1232, 1180, 1150, 1122, 1070, 1045, 975, 952, 905, 834 and 760 cm^{-1}; CD (EPA) Fig. 3.

The diacetate *1c* was reduced with $NaBH_4$ and hydrolyzed with KOH in the same manner as *1b* above, providing the tetrol *9* with properties as reported above.

7,8,7',8'-Tetradehydroastaxanthin natural diester (*2b*) had λ_{max} (acetone) 482 (510), (CS_2) 503, 535 nm.

2b (0.2 mg) was reduced with $NaBH_4$ and hydrolyzed with KOH in the same manner as *1b*. Extractive isolation and chromatography in System A (60 % A-hex) gave the diacetylenic tetrol *11*, yield 0.1 mg (48 %); $R_F = 0.50$; λ_{max} (acetone) (428) 454 and 483 nm, % III/II [23] = 26, (tetrahydrofurane) (432), 457 and 485 nm; *m/e* 596 (M); CD (EPA) Fig. 2.

7,8,7',8'-Tetradehydroastaxanthin diacetate (*2c*) had λ_{max} (CS_2) 503, (535) nm; ν_{max} (KBr) 2958, 2922, 2161 (−C≡C−, relatively stronger than for *1c*), 1740 (ester), 1672 (conj. C=O), 1600, 1571, 1551, 1460, 1378, 1348, 1304, 1288, 1270, 1240, 1232, 1180, 1150, 1122, 1070, 1045, 975, 952, 905, 834 and 740 cm^{-1}; CD (EPA) Fig. 3.

The diacetate *2c* was converted to the diacetylenic tetrol *11* by the same procedure as for *2b*.

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Reaction of Carbohydrates with Hydrogen Bromide. Preparation of Some 6-Deoxy-D-mannofuranoses

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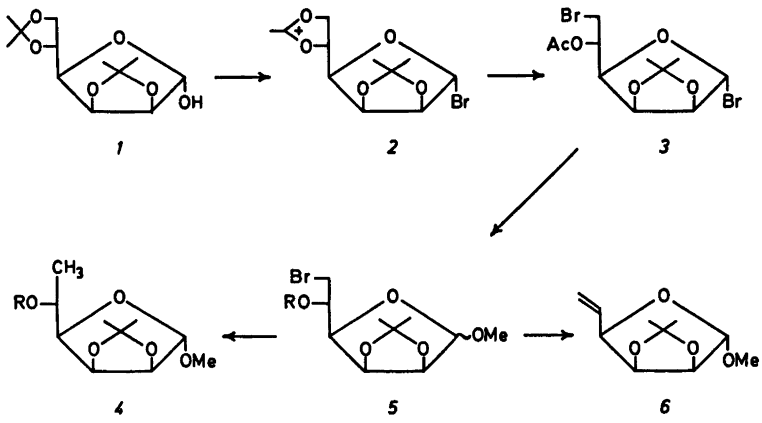
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Reaction of 2,3-5,6-di-*O*-isopropylidene-α-D-mannofuranose (*1*) with hydrogen bromide in acetic acid gave 5-*O*-acetyl-6-bromo-6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranosyl bromide (*3*). With methanol *3* gave the methyl furanoside (*5*) which was converted into methyl 5-*O*-benzoyl-6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranoside (*4c*) and into methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (*6*). Hydrolysis of *4* gave D-rhamnose.

In a previous paper [1] the conversion of methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside into tri-*O*-benzoyl-6-bromo-6-deoxy-D-galactofuranosyl bromide by treatment with hydrogen bromide in acetic acid (HBA) was described. The reaction was assumed to proceed *via* a ring-contraction to a furanose derivative, formation of a 5,6-benzoxonium ion, and subsequent reaction of the latter with bromide ions.

This reaction indicated that hexofuranoses might also yield 6-bromo-6-deoxy-derivatives by treatment with HBA and a number of compounds has now been studied in order to investigate this reaction. In the present paper the reaction of 2,3-5,6-di-*O*-isopropylidene-α-D-mannofuranose (*1*) with HBA is described.

When *1* was treated with HBA for 2 h at room temperature it was converted into a syrup which, as seen from NMR spectra, consisted almost exclusively of 5-*O*-acetyl-6-bromo-6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranosyl bromide (*3*). This product was not purified, but was only characterized through its ^{1}H and ^{13}C NMR spectra. Treatment of *3* with methanol in the presence of silver carbonate gave methyl 5-*O*-acetyl-6-bromo-6-deoxy-2,3-*O*-isopropylidene-β-D-mannofuranoside (*β-5b*). When *3* was treated with methanol alone the corresponding



1 *2* *3*

4 *5* *6*

a, R=H; b, R=Ac; c, R=Bz

α-anomer was formed, presumably *via β-5a* and subsequent anomerization catalyzed by the liberated hydrogen bromide. The acidic conditions, arising in the latter reaction, also caused deacetylation of *α-5b* and the crude *α-5a* thus obtained was benzoylated. This gave crystalline methyl 5-*O*-benzoyl-6-bromo-6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranoside (*α-5c*). Reduction of *α-5a* with lithium aluminium hydride yielded the corresponding 6-deoxy-compound (*4a*), which was converted into the known [2] crystalline 5-*O*-benzoate (*4c*).

Hydrolysis of *4a* with aqueous acid gave a 65 % yield of D-rhamnose, thus confirming its structure. Alternatively, *1* could be converted into crystalline D-rhamnose in 30 % overall yield *via 3*, *5*, and *4* without purification of any of the intermediates.

Treatment of *5c* with zinc in boiling acetic acid yielded the previously described [3] methyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hex-5-enofuranoside (*6*).

Thus the simple treatment of the readily available diisopropylidene derivative with hydrogen bromide offers a convenient route to a number of D-rhamnofuranose derivatives and to 5,6-unsaturated compounds.

The first step in the conversion of *1* into *3* must be loss of the 5,6-isopropylidene group and formation of 2,3-*O*-isopropylidene-D-mannofuranose. This was confirmed by the fact that methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside also gave *3* in high yield when treated with HBA. The next step is probably acetylation at C5 or C6 and subsequent formation of the acetoxonium ion (*2*). The latter finally reacts with bromide ions to give *3*.[1,4]

EXPERIMENTAL

Melting points are uncorrected. Preparative thin layer chromatography (TLC) was performed on 20 × 40 cm plates using 1 mm layers of Merck silica gel PF_{254}. ^1H NMR spectra were measured at 270 MHz and ^{13}C NMR spectra at 22.63 MHz on Bruker instruments using deuteriochloroform as solvent and TMS as internal reference.

5-O-Acetyl-6-bromo-6-deoxy-2,3-O-isopropylidene-α-D-mannofuranosyl bromide (3). To a 33 % solution of hydrogen bromide in acetic acid (25 ml) was added 2,3-5,6-di-*O*-isopropylidene-α-D-mannofuranose (*1*) [5] (5.0 g) and the mixture was stirred at room temperature for 2 h. It was then diluted with dichloromethane (100 ml) and washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated. The syrupy residue (7.5 g), (~100 %) consisted of almost pure *3* as seen from ^1H and ^{13}C NMR spectra. It was not purified further. ^1H NMR: δ 6.39 (H1), 5.15 (H2), 4.85 (H3), 4.49 (H4), 5.27 (H5), 3.86 (H6), 3.59 (H6′); J_{12} 0 Hz, J_{23} 5.8, J_{34} 3.8, J_{45} 8.5, J_{56} 2.8, $J_{56'}$ 4.1, $J_{66'}$ 11.6. ^{13}C NMR: 91.5 ppm (C1), 89.5 (C2), 81.1 (C3), 77.7 (C4), 67.6 (C5), 32.5 (C6).

Methyl 5-O-acetyl-6-bromo-6-deoxy-2,3-O-isopropylidene-β-D-mannofuranoside (*β-5b*). A solution of crude *3* (1.8 g) in methanol (20 ml) was stirred with silver carbonate (2.5 g) overnight at room temperature. Filtration and evaporation gave 1.45 g of crude product. Purification by TLC using ether-pentane (3:1) as eluent gave 968 mg (62 %) of *β-5b* as a syrup, $[\alpha]_D^{20}$ +10.7° (*c* 0.4, $CHCl_3$). Anal. $C_{12}H_{19}BrO_6$: C, H, Br. ^1H NMR: δ 4.70 (H1), 4.66 (H2), 4.72 (H3), 3.98 (H4), 5.22 (H5), 3.88 (H6), 3.72 (H6′); J_{12} 3.6 Hz, J_{23} 6.3, J_{34} 4.4, J_{45} 8.4, J_{56} 3.0, $J_{56'}$ 3.6, $J_{66'}$ 11.4. ^{13}C NMR: 103.5 ppm (C1), 79.3 (C2), 78.5 (C3), 75.2 (C4), 69.0 (C5), 33.3 (C6).

Methyl 5-O-benzoyl-6-bromo-6-deoxy-2,3-O-isopropylidene-α-D-mannofuranoside (α-5c). A solution of *3* (7.5 g) in methanol (100 ml) was kept at room temperature for 24 h. Pyridine (5 ml) was then added and the solution was evaporated. The residue was dissolved in chloroform and washed with 4 N hydrochloric acid and with aqueous $NaHCO_3$, dried and evaporated. The residue (5.9 g ≃ 100 %) consisted of methyl 6-bromo-6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranoside (*α-5a*) as seen from ^1H and ^{13}C NMR spectra.

Benzoylation with benzoyl chloride in pyridine in the usual manner and crystallization from pentane gave 4.0 g (52 %) of *α-5c*, m.p. 50 – 55 °C. Two recrystallizations from pentane gave the pure product, m.p. 56 – 57 °C, $[\alpha]_D^{20}$ +27.8° (*c* 2.7, $CHCl_3$). Anal. $C_{17}H_{21}BrO_6$: C, H, Br. ^1H NMR: δ 4.93 (H1), 4.59 (H2), 4.77 (H3), 4.39 (H4), 5.44 (H5), 4.00 (H6), 3.87 (H6′); J_{12} 0 Hz, J_{23} 5.9, J_{34} 3.6, J_{45} 8.5, J_{56} 2.9, $J_{56'}$ 3.3, $J_{66'}$ 11.3 ^{13}C NMR: 106.9 ppm (C1), 84.9 (C2), 79.1 (C3), 78.1 (C4), 69.5 (C5), 33.7 (C6).

Methyl 5-O-benzoyl-6-deoxy-2,3-O-isopropylidene-α-D-mannofuranoside (4c). A solution of *α-5c* (3.8 g) in ether (30 ml) was treated with $LiAlH_4$ (400 mg) in ether (30 ml) for 24 h at room temperature. The mixture was worked up by careful addition of ethyl acetate followed by 1 N sulfuric acid, separation of the ether phase and extraction of the aqueous phase with ether. The combined ether solutions were washed with aqueous $NaHCO_3$, dried and evaporated to give 2.28 g (82 %) of crude methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-mannofuranoside (*4a*) as a syrup. Benzoylation with benzoyl chloride in pyridine gave the benzoate *4c* which was crystallized from pentane, yield 2.11 g (51 %), m.p. 72 – 74 °C, $[\alpha]_D^{20}$ +19.7° (*c* 2.3, $CHCl_3$) (reported [2] m.p. 75 – 76 °C, $[\alpha]_D^{20}$ +19.9°). ^{13}C

NMR: 107.2 ppm (C1), 84.9 (C2), 79.5 (C3), 81.7 (C4), 69.0 (C5), 17.5 (C6).

D-*Rhamnose*. Crude *4a* (1.55 g) was stirred at 100 °C for 1 h with 15 ml 0.5 N hydrochloric acid. The solution was then neutralized with "Amberlite" IR-4B and evaporated to dryness. The residue (834 mg, 65 %) was recrystallized from ethanol-ether to give 554 mg (41 %) of D-rhamnose monohydrate, m.p. 87 – 89 °C, undepressed in admixture with an authentic sample.

Alternatively *1* (25 g) was treated with HBA as described above. The crude dibromo-compound (*3*) was treated with methanol to give α-*5a*, which was reduced with $LiAlH_4$ to give crude α-*4a*. Hydrolysis as described above gave 7 g crude product and crystallization from ethanol-ether gave 5.2 g (28 %) of D-rhamnose, m.p. 87 °C.

Methyl 5,6-dideoxy-2,3-O-isopropylidene-α-D-lyxo-*hex-5-enofuranoside* (6). A solution of *5c* (550 mg) in acetic acid (5 ml) was boiled for 1 h with zinc dust (500 mg). The mixture was then filtered and the filtrate was poured into water. The product was extracted with chloroform and the extract was washed with water, aqueous $NaHCO_3$, dried and evaporated. The residue was purified by TLC (ether-pentane 1:2) to give pure *6* (130 mg, 57 %) as a syrup, $[\alpha]_D^{20}$ +28.0° (*c* 0.7, $CHCl_3$) (reported [3] $[\alpha]_D$ +27.8°). Anal. $C_{10}H_{16}O_4$: C, H. ^1H NMR: δ 4.90 (H1), 4.57 (H2), 4.67 (H3), 4.37 (H4), 6.00 (H5), 5.40 (H6), 5.32 (H6′); J_{12} 0 Hz, J_{23} 6.0, J_{34} 3.8, J_{45} 7.3, J_{56} 17.5, $J_{56'}$ 10.5, $J_{66'}$ 1.8, J_{46} 1.0, $J_{46'}$ 0.9. ^{13}C NMR 107.2 ppm (C1), 81.6, 81.2 (C2) (C3), 85.4 (C4), 132.4 (C5), 119.1 (C6).

Microanalyses were performed by Novo Microanalytical Laboratory. The pulsed NMR spectrometers were provided by the Danish National Science Research Council.

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Crystal and Molecular Structure of 1-Hydroxybiuret

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1-Hydroxybiuret, $H_2NCONHCONHOH$, has been prepared and isolated in the pure state. The melting point of the compound is 146 – 147 °C (decomp.), and the pK_A value was determined to 9.58(1). The structure of 1-hydroxybiuret has been determined using three-dimensional diffractometer-collected X-ray data. The compound crystallizes in the space group $P\bar{1}$ with four formula units ($C_2H_5N_3O_3$) in the unit cell with the dimensions $a = 7.054(2)$, $b = 6.958(2)$, $c = 10.793(2)$ Å, $\alpha = 106.65(2)°$, $\beta = 96.34(2)°$, $\gamma = 71.03(3)°$. The structure was solved by direct phasing techniques, and was refined by full-matrix least-squares calculations to an R-value of 0.035.

Both of the two crystallographically unrelated molecules form an *intra*-molecular NH···O bond. Significant differences were found in the dimensions of the two molecules. The crystals are built up of layers of hydrogen bonded molecules.

1-Hydroxybiuret, $H_2NCONHCONHOH$, has been shown to inhibit the DNA synthesis in an *in vitro* Ehrlich ascites tumor test system.[1] The substance used in the experiments was prepared by reaction of methyl allophanate with hydroxylamine [eqn. (1)], but no physical data of the compound, obtained by evaporation of the reaction mixture to dryness, were given.

$$H_2NCONHCOOCH_3 + H_2NOH,HCl + NaOH \rightarrow H_2NCONHCONHOH + CH_3OH + NaCl \quad (1)$$

This work presents the preparation and the crystal structure determination of the compound. The structure determination is part of an X-ray study of hydroxylamine derivatives, which inhibit the DNA synthesis in different cell systems, probably by preventing the bioreduction of ribonucleotides. Hydroxyurea, the parent compound in this series, has been shown to inhibit the enzyme system ribonucleotide reductase of *E. coli* by destroying the organic free radical of protein B2, a subunit of the enzyme system.[2] A series of hydroxyurea analogs, including 1-hydroxybiuret in the pure state, has recently been prepared in our laboratory and tested for inhibitory action on the same enzyme system. 1-Hydroxybiuret was found to be a less potent inhibitor than is hydroxyurea.[3]

Preliminary investigations in our laboratory seem to indicate a correlation between the ability of the hydroxyurea analogs to inactivate protein B2 and the ability of the compounds to destroy (reduce) the nitroxide radical in Fremy's salt [$(KO_3S)_2NO\cdot$]. Hydroxylamine and easily oxidizable substituted hydroxylamines have been reported to be oxidized by Fremy's salt.[4] Further investigations in this field are in progress.

EXPERIMENTAL

Synthesis. 1-Hydroxybiuret was prepared from methyl allophanate and hydroxylamine as earlier proposed.[1] To a solution of 10.0 g (0.085 mol) of methyl allophanate[5] in anhydrous methanol (800 ml) was added 5.9 g (0.085 mol) of hydroxylammonium chloride and 5.2 g (0.130 mol) of sodium hydroxide. After standing at room temperature for about 14 days the solution was adjusted to pH 7 with a methanolic solution of hydrogen chloride and evaporated to dryness *in vacuo*. The residue was extracted with six 25 ml portions of ethanol. The combined ethanol extracts were evaporated to dryness *in vacuo* and the residue (5.3 g), which by TLC was shown to consist of several compounds, was subjected to column chromatography [silica gel: 200 g; eluent: benzene – methanol – ethyl acetate (4:2:1) containing 1 %

Table 1. Crystal data for 1-hydroxybiuret.

Mol. formula	$C_2H_5N_3O_3$
Mol. weight	119.1
Melting point, °C	146 – 147 (decomp.)
Space group	$P\bar{1}$
a, Å	7.054(2)
b, Å	6.958(2)
c, Å	10.793(2)
α,°	106.65(2)
β,°	96.34(2)
γ,°	71.03(3)
V, Å^3	479.9
Z	4
D_x, g cm^{-3}	1.65
D_m, g cm^{-3}	1.64
μ Mo$K\alpha$ cm^{-1}	1.64

of formic acid]. The fractions giving a blue colour reaction with iron(III) chloride on TLC were collected and evaporated to dryness *in vacuo* to give 1.0 g of crude 1-hydroxybiuret. Recrystallization of the product from methanol gave 0.5 g (5 %) of slightly coloured crystals, m.p. 146 – 147 °C (decomp.). Anal. $C_2H_5N_3O_3$: C, H, N. The pK_A value of the compound was determined to 9.58(1) by electrometrical titration in aqueous solution at 21 °C, according to the method described by Albert and Serjeant.[6] The pH values were measured on a Radiometer pH meter 26.

X-Ray crystallography. Single crystals suitable for the X-ray work were obtained by slow evaporation from a solution of the compound in methanol. The size of the crystal chosen for data collection was $0.14 \times 0.20 \times 0.30$ mm^3. Some crystal data of 1-hydroxybiuret are given in Table 1. The density was measured by flotation in a mixture of carbon tetrachloride and methyl iodide. The melting point was determined with a hot stage microscope (Mikroskop Heiztisch Ernst Leitz G.m.b.H., Wetzlar).

The lattice parameters of the compound were calculated from 32 diffractometer-measured θ-values ($\lambda = 0.71069$ Å). The crystal was mounted in a glass capillary and oriented with the b-axis parallel to the ϕ-axis of the goniostat. The intensity data were collected on a NONIUS three-circle automatic diffractometer, using the same technique as described earlier.[7] Intensities of reflexions were measured in the range $2.5° \leq \theta \leq 25.0°$; of the 1578 independent reflexions 1170 had $I(\text{net}) \geq 2.5\sigma(I)$, and were considered observed. The data were corrected for Lorentz and polarization effects, but no corrections for absorption or extinction were made.

STRUCTURE DETERMINATION

The structure was solved by direct methods using the automatic phasing program MULTAN.[8] Refinement of the positional and thermal parameters of the non-hydrogen atoms, using the full-matrix least-squares method and anisotropic temperature factors, led to an R-value of 0.062. The quantity minimized was $\sum w(|F_o| - |F_c|)^2$, where the weights were initially taken as unity.

All hydrogen atoms were located in the difference electron density map. The final cycles of refinement included all positional parameters, as well as anisotropic temperature factors for the non-hydrogen atoms. The isotropic thermal parameters of the hydrogen atoms were fixed at the values of the parameters of the atoms to which they are bonded. The weighting scheme used in the last cycles of refinement was of the form $w = 1/\{1 + [(|F_o| - 7.0)/8.0]^2\}$.

The final R-value is 0.035 for all observed reflexions. The scattering factors used for hydrogen were those of Stewart, Davidson and Simpson,[9] and for oxygen, nitrogen and carbon

Table 2. Fractional coordinates and thermal parameters for the non-hydrogen atoms. The thermal parameters ($\times 10^2$) are of the form: $\exp[-2\pi^2 (U_{11}h^2a^{*2} + \cdots 2U_{12}hka^*b^* \cdots)]$

ATOM	X/A	Y/B	Z/C	U11	U22	U33	U12	U13	U23
N11	0.5443(3)	0.2186(4)	0.4504(2)	2.7(1)	6.1(1)	2.5(1)	-1.9(1)	0.0(1)	1.0(1)
C12	0.3721(3)	0.2608(4)	0.5040(2)	2.8(1)	3.5(1)	2.5(1)	-1.1(1)	-0.1(1)	1.3(1)
O12	0.3482(2)	0.3269(3)	0.6237(1)	3.0(1)	5.7(1)	2.0(1)	-1.6(1)	-0.7(1)	1.1(1)
N13	0.2094(3)	0.2295(3)	0.4261(2)	2.1(1)	4.5(1)	2.2(1)	-1.2(1)	0.1(1)	1.2(1)
C14	0.1952(3)	0.1991(4)	0.2942(2)	2.9(1)	3.0(1)	2.3(1)	-0.9(1)	0.2(1)	0.9(1)
O14	0.3423(2)	0.1573(3)	0.2259(1)	2.9(1)	5.8(1)	2.5(1)	-1.3(1)	0.4(1)	1.2(1)
N15	0.0153(3)	0.2145(4)	0.2396(2)	2.7(1)	5.9(1)	1.8(1)	-1.4(1)	0.1(1)	1.0(1)
O16	-.1499(2)	0.2554(3)	0.3120(2)	3.0(1)	5.7(1)	3.1(1)	-1.2(1)	0.6(1)	1.2(1)
N21	0.7200(3)	0.3313(4)	0.7834(2)	2.7(1)	7.1(2)	3.1(1)	-1.8(1)	-0.4(1)	2.3(1)
C22	0.8827(3)	0.3048(4)	0.7226(2)	2.9(1)	3.7(1)	2.4(1)	-1.5(1)	-0.4(1)	1.3(1)
O22	0.8839(2)	0.3095(3)	0.6092(2)	3.2(1)	7.1(1)	2.5(1)	-2.2(1)	-0.4(1)	2.1(1)
N23	1.0636(3)	0.2717(3)	0.7924(2)	2.6(1)	4.7(1)	2.4(1)	-1.6(1)	0.0(1)	1.4(1)
C24	1.0952(3)	0.2416(4)	0.9138(2)	3.2(1)	3.5(1)	2.2(1)	-1.0(1)	0.0(1)	0.9(1)
O24	0.9618(2)	0.2482(3)	0.9809(2)	3.3(9)	7.4(1)	2.8(1)	-1.9(1)	0.0(1)	2.3(1)
N25	1.2873(3)	0.1952(4)	0.9567(2)	2.9(1)	6.3(2)	2.4(1)	-1.3(1)	-0.1(1)	2.0(1)
O26	1.4256(3)	0.2392(3)	0.8955(2)	3.3(1)	6.0(1)	3.9(1)	-1.3(1)	0.6(1)	1.7(1)

Table 3. Fractional coordinates and thermal parameters for the hydrogen atoms.

ATOM	X/A	Y/B	Z/C	B
H111	0.548(4)	0.186(4)	0.367(3)	2.7
H112	0.643(4)	0.245(4)	0.496(3)	2.7
H13	0.113(4)	0.254(4)	0.464(2)	2.2
H15	0.000(4)	0.200(4)	0.157(3)	2.4
H16	-.216(4)	0.417(5)	0.335(3)	3.0
H211	0.728(4)	0.321(4)	0.869(3)	3.0
H212	0.611(4)	0.352(4)	0.742(3)	3.0
H23	1.158(4)	0.263(4)	0.754(2)	2.4
H25	1.306(4)	0.206(4)	1.035(3)	2.8
H26	1.507(4)	0.108(5)	0.855(3)	3.3

those of Cromer and Mann.[10] Final parameters for the non-hydrogen atoms are given in Table 2, and the hydrogen atom parameters are given in Table 3. Notation of the atoms is given in Fig. 1. The observed and calculated structure factor data are available from the author on request. The programs used in the crystallographic analysis were the same as mentioned earlier.[7]

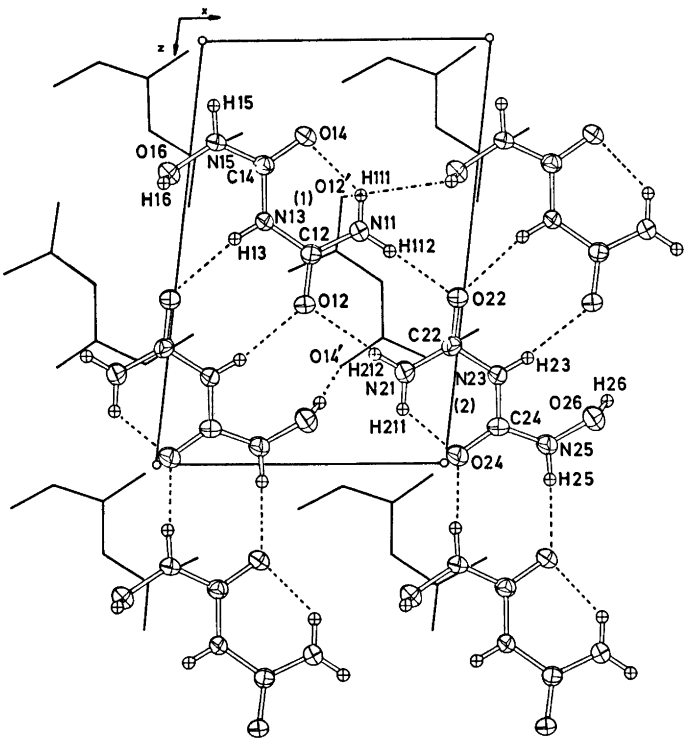


Fig. 1. The structure of 1-hydroxybiuret viewed along the b^*-axis. Dashed lines indicate hydrogen bonds within a layer, ····· indicate hydrogen bonds to a molecule above or below this layer.

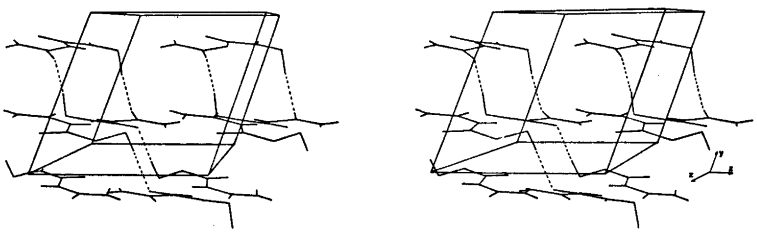


Fig. 2. Stereoscopic diagram of the structure. Only hydrogen bonds between the layers are indicated.

Table 4. Distances and angles concerning the hydrogen bonding system.

X–H···Y	Distances (Å) X···Y	H···Y	Angle (°) X–H···Y
N11–H111···O14	2.669(3)	2.00(3)	134(2)
N11–H112···O22	2.921(3)	2.08(3)	179(3)
N13–H13···$O22_{x-1,y,z}$	3.005(3)	2.24(3)	167(3)
N15–H15···$O24_{x-1,y,z-1}$	2.842(3)	2.00(3)	165(3)
O16–H16···$O12_{-x,1-y,1-z}$	2.702(3)	1.67(3)	173(3)
N21–H211···O24	2.655(3)	1.95(3)	130(2)
N21–H212···O12	2.979(3)	2.16(3)	167(3)
N23–H23···$O12_{x+1,y,z}$	3.033(3)	2.26(3)	161(2)
N25–H25···$O14_{x+1,y,z+1}$	2.964(3)	2.16(3)	165(3)
O26–H26···$O14_{-x+2,-y,-z+1}$	2.735(3)	1.82(3)	177(3)
Non-bonded short distances			
O16···$N11_{x-1,y,z}$	2.871(3)		
O26···$N21_{x+1,y,z}$	2.838(4)		

Table 5. Deviations of atoms (Å) from the least-squares planes through the indicated atoms. The equations of the planes are in direct (unit cell) space. Atoms defining the planes are asterisked.

Molecule (1)

Urea plane I: $0.4818x + 6.7034y - 3.6449z - 0.0872 = 0$
Urea plane II: $1.1184x + 6.8041y - 1.7094z - 1.0678 = 0$

Molecule (2)

Urea plane I: $1.1294x + 6.5463y + 0.2415z - 3.1701 = 0$
Urea plane II: $1.1306x + 6.2935y + 1.3904z - 4.0177 = 0$

Molecule (1) Atom	I	II	Molecule (2) Atom	I	II
N11	−.001*		N21	.001*	
C12	.004*		C22	−.003*	
O12	−.001*		O22	.001*	
N13	−.001*	−.001*	N23	.001*	−.004*
C14		.002*	C24		.012*
O14		−.001*	O24		−.004*
N15		−.001*	N25		−.004*
O16		−.031	O26		.345
H111	.09		H211	−.03	
H112	.06		H212	.00	
H13	−.02	−.01	H23	.05	.00
H15		.03	H25		.20
H16		.96	H26		−.44
Angle I:II	12.3°		Angle I:II	6.2°	

RESULTS AND DISCUSSION

The crystal structure of 1-hydroxybiuret consists of roughly planar layers of molecules that are extensively hydrogen bonded to one another, *cf.* Figs. 1–2. The dimensions of the hydrogen bonds are listed in Table 4. Each of the two crystallographically unrelated molecules is connected to three neighbouring molecules by pairs of NH···O bonds. The terminal hydroxyl groups connect adjacent layers by forming OH···O bonds. The hydroxyl groups

Table 6. Bond lengths (Å) and angles (°) for the two crystallographically non-equivalent molecules of 1-hydroxybiuret.

N11-C12	1.312(3)	N21-C22	1.320(4)
C12-O12	1.252(3)	C22-O22	1.234(3)
C12-N13	1.384(3)	C22-N23	1.395(3)
N13-C14	1.375(3)	N23-C24	1.369(3)
C14-O14	1.247(3)	C24-O24	1.231(3)
N15-O16	1.381(3)	N25-O26	1.391(4)
N11-H111	0.86(3)	N21-H211	0.94(3)
N11-H112	0.84(3)	N21-H212	0.84(3)
N13-H13	0.78(3)	N23-H23	0.80(3)
N15-H15	0.86(3)	N25-H25	0.82(3)
O16-H16	1.04(3)	O26-H26	0.91(3)
N11-C12-O12	122.7(2)	N21-C22-O22	124.2(2)
N11-C12-N13	119.3(2)	N21-C22-N23	117.3(2)
O12-C12-N13	118.0(2)	O22-C22-N23	118.5(2)
C12-N13-C14	125.8(2)	C22-N23-C24	126.9(2)
N13-C14-O14	123.1(2)	N23-C24-O24	124.0(2)
N13-C14-N15	117.1(2)	N23-C24-N25	115.8(2)
O14-C14-N15	119.8(2)	O24-C24-N25	120.2(2)
C14-N15-O16	121.3(2)	C24-N25-O26	118.5(2)
H111-N11-C12	118(2)	H211-N21-C22	121(2)
H111-N11-H112	120(3)	H211-N21-H212	121(3)
H112-N11-C12	121(2)	H212-N21-C22	118(2)
H13-N13-C12	114(2)	H23-N23-C22	115(2)
H13-N13-C14	119(2)	H23-N23-C24	118(2)
H15-N15-C14	120(2)	H25-N25-C24	117(2)
H15-N15-O16	119(2)	H25-N25-O26	116(2)
H16-O16-N15	106(2)	H26-O26-N25	103(2)

of both molecules are hydrogen bonded to oxygen atoms (O14′ and O12′) of a molecule symmetry related to molecule (1). The environments of the two unrelated molecules are thus most different in the *y* direction. The short distances between the hydroxyl oxygen atoms and nitrogen atoms of neighbouring NH_2-groups within the same layer are not considered as hydrogen bonds. The lengths of these non-bonded distances are also given in Table 4.

As 3-hydroxybiuret [7] the molecule of 1-hydroxybiuret can be considered to be composed of two overlapping urea moieties. Each of these individual moieties is planar within experimental error, *cf.* Table 5, while each of the two molecules as a whole is rather non-planar. The angles between the two planes are 12.3 and 6.2° for molecule (1) and (2), respectively. The terminal N – O bond is twisted out of the plane of the urea moiety in both molecules. The torsion angle N13 – C14 – N15 – O16 is – 1.2(4)°, and N23 – C24 – N25 – O26 is 18.0(3)°. These small conformational variations are probably due to the different environments of the two molecules in the *y* direction.

The overall conformation of the 1-hydroxybiuret molecules is the same, *i.e.* the conformation that is stabilized by an *intra*-molecular NH···O hydrogen bond. A similar *intra*-molecular contact was also found in the three crystal structures of 3-hydroxybiuret,[7] in perdeuterated biuret,[11] triuret,[12] and in the oxalurate ion, $H_2NCONHCOCOO^-$.[13] None of these molecules were found to be planar. The angles between the two urea moieties (or urea moiety and peptide unit) [13] are of the same magnitude as found in the 1-hydroxybiuret molecules, *i.e.* 5 – 15°.

Bond lengths and angles of the 1-hydroxybiuret molecules are given in Table 6. Some differences between corresponding bonds and angles are found. In fact greater variations in the dimensions of the two crystallographically unrelated molecules in the crystal structure of 1-hydroxybiuret are observed than in the dimensions of the 3-hydroxybiuret molecules in the three different crystal structures of this compound.[7]

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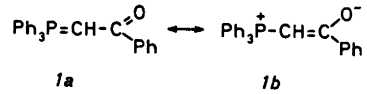
^{13}C Chemical Shifts of Phosphonium Ylides Stabilised by *p*-Substituted Phenacyl Groups; Substituent Effects and Comparison with Arsonium Analogues

PAUL FRØYEN [a] and DAVID G. MORRIS [b]

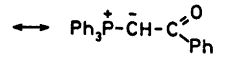
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^{13}C Chemical shift assignments are presented for a series of triphenylphosphonium ylides in which a *p*-substituted phenacyl group is linked to the carbanion. The ylide carbon is deshielded by electron-withdrawing substituents such that their chemical shifts correlate linearly with the dual substituent parameters of Swain and Lupton with the resonance term predominant. Chemical shifts of the carbonyl carbon do not show an analogous correlation and enhanced conjugation is indicated for the *p*-methoxy derivative from, *inter alia*, the carbonyl carbon chemical shift. A comparison of the shieldings of the carbons of the phenyl rings bound to phosphorus with those of the corresponding carbons of the analogous arsonium ylides indicates minor but subtle differences which are discussed in terms of the transmission modes of substituent effects. For these carbons the probable relevance of π polarisation as a mechanism for redistribution of electron density is indicated from a comparison of chemical shifts of the ylide, triphenylphosphine and a triphenylphosphonium salt.

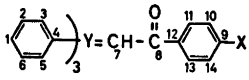
Recently a number of studies have been made of the ^{13}C and ^{31}P NMR spectra of phosphorus ylides;[1–3] in most of these cases greater stability has been conferred on the ylide by delocalisation of the formal negative charge on the ylide carbon over a stabilising group. Accordingly the ylide may, in the case of a stabilising phenacyl group be represented by a number of resonance structures *1a-c*, where additionally there exists the possibility of delocalisation of the negative charge on the ylide carbon onto phosphorus by means of $d\pi-p\pi$ bonding; this latter feature is denied to ylides derived from first row elements. The extent of this $d\pi-p\pi$ bonding is considered to be relatively small,[3,4] although P–C interatomic distances in several ylides have been found [5] to be intermediate between estimated values of the corresponding single and double bond lengths.[4]



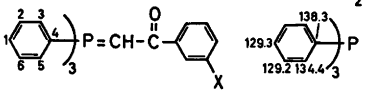
1a *1b*



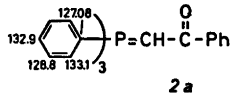
1c



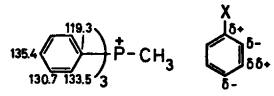
Series *2* Y=P *a* X=H *e* X=Cl
Series *3* Y=As *b* X=Me *f* X=Br
c X=OMe *g* X=CN
d X=F *h* X=NO_2



4 *5*



2a



6 *7*

Recent calculations [5] which include 3*d* orbitals on phosphorus indicated, with respect to analogous calculations not involving 3*d* orbitals, a significantly reduced charge separation in an unstabilised prototype phosphonium ylide with concomitant increase in P–C overlap population which arises from bonding between P_{3dxz} and C_{2px} orbitals together with a smaller, although significant, involvement of the P_{3dyz} orbital in bonding which results in the ability of phosphorus to conjugate in two perpendicular planes. Further, calculations showed that the electron density in the phosphorus carbon σ bond was small.

In order to extend our previous studies on ^{13}C chemical shifts of the corresponding *p*-substituted arsonium [6] and *m*-substituted phosphonium [7] ylides and to effect comparison between these series we have examined the ^{13}C chemical shifts of a series of nine ylides *2a–h* where a *p*-substituted phenacyl group is bonding to the ylide carbon C(7).

RESULTS AND DISCUSSION

^{13}C Chemical shifts for the series *2a–h* were determined in $CDCl_3$ at a common concentration and are presented in Table 1, with the coupling constant between the particular carbon and phosphorus given in parentheses. The assignment of carbon shifts was carried out as in our earlier paper [6] with additional assistance from the couplings to phosphorus in the case of carbons (1)–(8), where ample precedent exists. Additionally, we note, as with the corresponding *m*-substituted series,[7] a coupling $^3J_{P-C-C-C(12)} \approx 15$ Hz.

The ylide carbon C(7) in the series *2a–h* is deshielded by electron-withdrawing substituents and the chemical shift variation of this carbon correlates with the dual substituent field (*F*) and resonance (*R*) parameters of Swain and Lupton [8] as indicated in eqn. (1), with a correlation coefficient of 0.925; in this equation the

$$\delta[C(7)] = 1.57F + 3.11R \quad (1)$$

weighting of *R* is 55.8 %. This latter value is rather lower than that obtained for the corresponding *p*-substituted acetophenones [9] and arsonium ylides.[6] With respect to arsonium ylides the lower weighting of *R* arises from both an enhancement of *F* and an attenuation of *R*, although in both these ylide series the *R* weighting is appreciably higher than that obtained for the corresponding carbon, β to the aromatic ring, in both *p*-substituted styrenes [10] and phenylacetylenes.[11]

By way of contrast no correlation of carbonyl, C(8) chemical shifts with *F* and *R* is obtained; for this carbon electron-withdrawing

Table 1. ^{13}C Chemical shifts (ppm) in *p*-substituted phosphonium ylides *2a-h*.

Compound	Substituent	C(4)	C(3), C(5)	C(2), C(6)	C(1)	C(7)	C(8)
2a	H	127.08(91.5)	133.07(10.3)	128.82(12.2)	132.01(2.0)	50.54(112.1)	184.80(3.4)
2b	Me	127.51(91.1)	133.23(10.1)	128.87(12.4)	132.00(1.9)	50.03(112.6)	185.06(3.2)
2c	MeO	127.06(91.1)	133.15(10.2)	128.91(12.1)	132.12(1.8)	50.12(113.0)	182.71(3.2)
2d	F	126.96(90.6)	133.10(10.1)	128.91(12.2)	132.11(1.8)	50.47(112.5)	183.56(3.8)
2e	Cl	126.92(90.0)	133.17(10.1)	128.99(12.2)	132.15(–)	50.98(112.3)	183.56(3.4)
2f	Br	126.86(91.7)	133.14(10.1)	128.93(12.2)	132.15(2.1)	50.98(111.8)	183.57(4.3)
2g	CN	126.27(91.5)	133.06(10.3)	128.99(12.3)	132.32(–)	52.93(111.2)	182.14(3.6)
2h	NO_2	126.26(91.4)	133.12(10.4)	129.06(12.3)	132.41(2.4)	53.55(110.7)	181.16(3.6)
		C(12)	C(11), C(13)	C(10), C(14)	C(9)	C(15)	
2a	H	129.26	127.67	126.89	141.30(15.0)	–	
2b	Me	139.32	128.47	127.03	138.80(15.1)	21.34	
2c	MeO	156.76	110.90	128.32	135.48(15.1)	56.29	
2d	F	163.67	114.35	–	137.64(15.1)	–	
2e	Cl	135.18	128.42	127.82	139.98(15.4)	–	
2f	Br	123.65	130.78	128.68	140.21(15.0)	–	
2g	CN	112.33	131.94	127.47	145.42(15.0)	119.09	
2h	NO_2	148.25	123.02	127.76	147.40(15.1)	–	

substituents cause deshielding. As in the corresponding arsonium ylide series [6] greater shielding than expected is shown for C(8) of the *p*-methoxy derivative *2c*. Together with a smaller than usual substituent chemical shift for C(12) and greater than normal deshielding of the methoxy carbon in *2c* this suggests enhanced conjugation by the electrons of the methoxy oxygen lone-pair as has been found for carboxyl carbons in *p*-substituted benzoic acids,[12] where the substituents Me_2N, MeO, F which cause enhanced shielding can be brought into line by means of the Yukawa-Tsuno equation which uses a common extra-conjugative coefficient. In *2d* we do not find excessive shielding of C(8) and we were unable to extend the series *2a–h* to include the substituent Me_2N.

In the phosphonium ylides *2a–h* the ylide carbon C(7) is shielded by *ca.* 7 ppm from C(7) in the corresponding arsonium series *3a–h*, whereas the carbonyl carbon C(8) in *2a–h* is deshielded by 3 ppm from the series *3a–h* for a given substituent. This behaviour may be rationalised by a greater electron density on C(7) and smaller electron density on C(8) in the phosphonium ylide series. The more ketone like behaviour of C(8) in the phosphonium ylides is in accord with greater polarity of the formal carbonyl bond in arsonium ylides.[15]

Although originally chosen as ballast the triphenylphosphonium and arsonium moieties of the *p*-substituted ylides show interesting trends in their ^{13}C chemical shifts. Thus the heteroatom bonded C(4) and C(2),C(6) are more shielded in the phosphonium series *2a–h* for a given substituent, whereas C(3), C(5) and C(1) are more shielded in the arsonium series *3a-h*. This pattern of alternation is accompanied by attenuation with increasing separation from the heteroatom, P or As (Table 2). Interpretation of these effects, which are novel to monosubstituted aromatic rings, is something of a problem, particularly since ^{13}C chemical shifts of triphenyl phosphine [1] and arsine oxides * do not conform to the above pattern. Here the heteroatom bonded carbon is more shielded in Ph_3AsO, in contrast to the corresponding carbon in triphenylphosphine and arsine.[15] We also note that carbons bonded to chlorine generally absorb at lower field than those bonded to bromine.[16]

The ^{13}C shift variations in Table 2 do not conform to the effects of substituent induced orbital repulsion [17–19] as formulated for first row elements; presently we expect greater orbital repulsion for the less diffuse phosphorus orbitals with the effect that C(4) in the series *2a-h* would be deshielded relative to the series *3a-h*. Recently Reynolds [18] has invoked π-polarisation effects to explain the large high field ipso shift in the cation form of phenylalanine with respect to the zwitterionic form; however, this change is accompanied by much smaller low field shifts for the remaining carbons. Other postulated substituent effects include the ill-defined inductoelectromeric ef-

* Chemical shifts of Ph_3AsO (numbering as in series *2* and *3*) are C(4) 132.83, C(3) 131.54, C(2) 129.39, C(1) 132.19 ppm.

Table 2. ^{13}C Chemical shifts (ppm) of carbons of unsubstituted aromatic rings in *m*-substituted phosphonium ylides *4a–h*. Values in parentheses denote the relevant coupling constant to phosphorus.

Compound	Substituent	C(4)	C(3)	C(2)	C(1)
4a	H	127.08(91.5)	133.07(10.3)	128.82(12.2)	132.01(2.0)
4b	Me[a]	–	133.24(10.2)	128.93(12.0)	132.07(2.2)
4c	MeO[b]	126.97(91.2)	133.07(10.1)	128.83(12.5)	132.02(2.0)
4d	F	126.71(91.4)	133.07(10.5)	128.89(11.7)	132.15(2.5)
4e	Cl	126.55(91.5)	132.98(10.3)	128.85(12.1)	132.13(1.8)
4f	Br	–	133.13(10.2)	128.93(12.0)	132.17(2.9)
4g	CN[c]	126.46(91.4)	133.15(10.1)	129.05(12.3)	132.38(3.2)
4h	NO_2	126.34(91.5)	133.13(10.4)	129.04(12.6)	132.41(1.8)

[a] δC(15) = 21.50 ppm. [b] δC(15) = 55.24 ppm. [c] δC(15) = 111.80 ppm.

fect [17] which lacks experimental support, the short range σ inductive effect and the π inductive effect which effects π electron changes as in *7* for a $+I_\pi$ substituent.

The data of Table 2 are accommodated most economically in terms of differential inductive effects of the partially positively charged phosphorus and arsenic atoms as these are considered to be transmitted through both saturated and unsaturated frameworks. The pattern obtained in Table 2 does not correspond to differential π polarisation effects between phosphorus and arsenic except for C(4) and C(1); however the point has been made that when the substituents which form the basis for comparison are directly bonded to the aromatic ring explicit manifestation of the π polarisation pattern may not be observed on account of other factors, in particular conjugative effects.[18] Although conjugative effects are considered to be absent between phosphorus and the three associated phenyl rings in certain phosphonium ylides on the basis of earlier basicity measurements,[23] ^{13}C chemical shifts may provide a more sensitive measure for such effects.

A possible incursion of π polarisation can be seen from comparison of the triphenylphosphorus carbon shifts in the sequence *5*, *2a* and *6* where the progressive increase of positive charge on the phosphorus brings about corresponding greater shielding at C(4), the phosphorus bound carbon, accompanied by deshielding at C(1), with rather small variations at C(2) and C(3). The shifts at C(4) and C(1) are in accord with enhancement and reduction, respectively, of electron density at these carbons.

Additionally in this series we note that the ^{31}P chemical shifts of $+6$,[24] -16.5,[25] and -21.1 [3b] ppm (a negative value indicates deshielding with respect to external 85 % H_3PO_4) for *5*, *2a* and *6* agree with expectation based on progressively greater positive charge on phosphorus. However, for the series *2a–h* the ^{31}P chemical shifts are, within experimental error, invariant to the nature of the substituent.

Currently we are investigating the chemical shift variations in the series of phosphorus salt precursors of the ylide series *2a-h* in order to investigate further and clarify the substituent dependence of chemical shifts.

EXPERIMENTAL

^{13}C NMR spectra were recorded at 25.2 MHz with a Varian XL 100 spectrometer operating in the Fourier transform mode with proton noise decoupling. The radio-frequency pulse width was 10 μs. The accuracy of individual line positions was 0.05 ppm except for carbonyl carbons where the accuracy was 0.02 ppm. The probe temperature was 35 °C. The spectral data refer to solutions of ylide (0.1 M) in $CDCl_3$ (lock signal) and are quoted relative to internal tetramethylsilane as standard.

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Short Communications

Phosphoramides. IV.* A New Synthesis of Biologically Important Formamidines

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N-Aryl-*N'*,*N'*-dimethylformamidines represent a new class of pesticides.[1,2] Especially interesting[3] is the acaricide-insecticide *N*-(4-chloro-2-methylphenyl)-*N'*,*N'*-dimethylformamidine (chlordimeform). The active compounds are usually prepared by a Vilsmeyer condensation of DMF and an aniline [4] or by treatment of aryl isocyanates with DMF.[5]

In a previous investigation it was observed that *N*,*N*-dimethyl-*N'*-phenylformamidine was formed by refluxing formanilide in hexamethylphosphoric triamide (HMPT) (1).[6] As formanilide may be obtained by heating aniline and formic acid this opens up a new route.

$$C_6H_5NHCHO \xrightarrow{HMPT} C_6H_5-N=CH-NMe_2 \quad (1)$$

Formamidines were now obtained by heating an aniline with formic acid to reflux temperature in HMPT. The yields of formamidines in this new synthesis are given in Table 1. Especially it should be noted that chlordimeform was obtained in a yield as high as 91 %.

Experimental. In all experiments commercial HMPT (Pierrefitte-Auby) was used.

General procedure. An aniline (0.1 mol), formic acid (0.13 – 0.17 mol, see Table 1), and HMPT (50 ml) were heated at reflux temperature in an oil bath (250 °C) for 3 – 8 h. The reaction mixture was allowed to cool to 100 °C and was then taken up in 400 ml of N NaOH and extracted four times with ether. The combined ether phases were washed with water and distilled. Correct spectroscopic data and satisfactory microanalyses were obtained for all new compounds.

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* Part III, Pedersen, E. B. *Synthesis. In press.*

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Table 1. Yields obtained in the reaction $RC_6H_4NH_2 \xrightarrow[HMPT]{HCOOH} RC_6H_4N=CH-NMe_2$.

R	HCOOH/mol	Reaction time/h	Yield/%	B.p./(°C/mmHg)	n_D^{25}
H	0.13	3	35	68 – 70/0.08	1.5924
2-CH_3	0.13	3	67	66 – 68/0.08	1.5762
4-CH_3	0.13	3	54	77 – 79/0.08	1.5828
4-Bu	0.17	8	52	108 – 110/0.09	1.5973
4-OCH_3	0.15	5	64	103 – 104/0.09	1.5883
4-Cl	0.13	3	76	90 – 92/0.09	1.6020
2-CH_3-4-Cl	0.17	6.5	91	96 – 99/0.08	1.5908

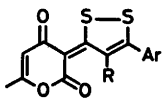
Electron Impact Induced Fragmentation of 3-(5′-Aryl-1′,2′-dithiol-3′-ylidene)-6-methyl-2*H*-pyran-2,4-diones and 3-(5′-Aryl-1′,2′-dithiol-3′-ylidene)-3*H*-benzo[*b*]pyran-2,4-diones

ERIK G. FRANDSEN

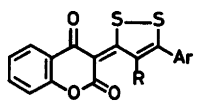
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α-(1′,2′-Dithiol-3′-ylidene)ketones and -aldehydes cannot be considered normal carbonyl compounds as it has been shown that a rather strong oxygen-sulfur interaction is present.[1,2] Therefore, it is necessary to consider 3-(5′-aryl-1′,2′-dithiol-3′-ylidene)-6-methyl-2*H*-pyran-2,4-diones (*1*) and 3-(5′-aryl-1′,2′-dithiol-3′-ylidene)-2*H*-benzo[*b*]pyran-2,4-diones (*2*) as resonance hybrids of structures such as *x*, *y* and *z*.* ^{19}F NMR and ^{13}C NMR studies [3] indicate that *1* and *2* are relatively polar substances, whose structures are best represented by *z*. In contrast, the structure of simple alkyl and aryl substituted α-(1′,2′-dithiol-3′-ylidene)-ketones and -aldehydes is best described by the non-polar form corresponding to *x*.

The mass spectra of these compounds were studied previously [4] in connection with the systematic studies of the physico-chemical properties of 1,2-dithioles performed in this laboratory.[3] We found it of interest to examine whether the greater polarity of *1* and *2* is reflected in their fragmentation on electron impact. Representative mass spectra are shown in Fig. 1. The molecular ions generally give rise to the base peaks.



1



2

Compounds	Ar	R
1a,2a	C_6H_5	H
1b,2b	*p*-$CH_3OC_6H_4$	H
1c,2c	*p*-FC_6H_4	H
1d,2d	C_6H_5	CH_3
1e,2e	C_6H_5	C_6H_5
1f,2f	$-CH=CH-CH=CH-$	

3-(1′,2′-Dithiol-3′-ylidene)-6-methyl-2H-pyran-2,4-diones (1). The predominant fragmentation mode is loss of ketene from the molecular ions giving rise to ion *a*, which decomposes by extrusion of carbon monoxide or ketene forming ions *b* and *c*, respectively. Using metastable defocusing technique, it was shown that *c* is likewise formed directly from the molecular ions.

The decomposition of *c* depends on the substitution pattern of the dithiole ring. If the ring bears no substituent in position 4′ (*1a – 1c*), *c* eliminates CO or HS_2· giving rise to ions *d* and *e*, respectively. A mechanism for the extrusion of HS_2· observed in the fragmentation of related compounds has been proposed.[4] Accordingly, 4′-substituted compounds should be incapable of losing HS_2· and ion *e* was not observed in the decomposition of these compounds (*1d – 1f*). The structure of *e* is probably *3*.*

$$Ar-C\equiv C-C\equiv C-C\equiv O^+$$

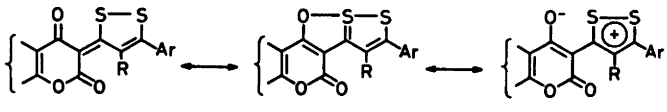
3

Loss of a methyl group and carbon monoxide have been found in the fragmentation of some 2-pyrones.[5] Extrusion of these fragments from the molecular ions of *1* is found in most cases, but the intensities of the corresponding peaks are generally low (< 10 %). The structures of the resulting ions *f* and *g*, respectively, may be formulated in analogy to those proposed for the fragment ions derived from the 2-pyrones.[5] Ion *f* decomposes by extrusion of CO yielding ion *h*.

Elimination of a hydrogen atom is a possible process, but only for compounds *1d* (63 %) and *1f* (16 %) the abundance exceeds 5 %. In these cases the structural requirements for formation of a six-membered ring are present.

* The elemental composition was shown by accurate mass measurements to be in accordance with the proposed structure.

* We shall for the sake of consistency and brevity refer to all compounds by the dithiole name corresponding to form *x*.



x *y* *z*

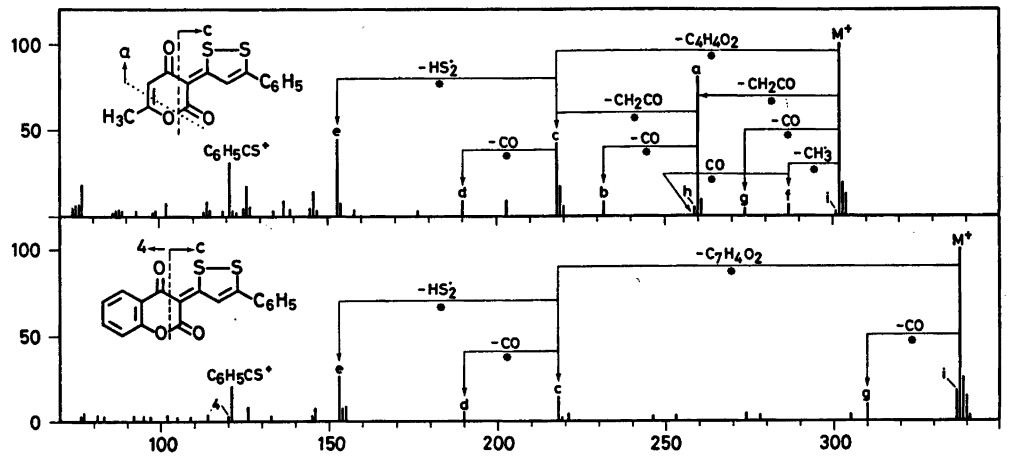
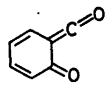


Fig. 1. Mass spectra of 3-(5′-phenyl-1′,2′-dithiol-3′-ylidene)-6-methyl-2*H*-pyran-2,4-dione (*1a*) and 3-(5′phenyl-1′,2′-dithiol-3′-ylidene)-2*H*-benzo[*b*]pyran-2,4-dione (*1b*).

*3-(1′,2′-Dithiol-3′-ylidene)-2*H*-benzo[b]pyran-2,4-diones (2).* The important decomposition modes are principally the same as described for *1* (*cf.* Fig. 1). However, the intensities of the resulting peaks are generally lower.

Ions c are formed from the molecular ions by loss of the fragment *4*. The reversed charge distribution gives rise to a peak at *m/e* 120 ($C_7H_4O_2$), but only in the spectrum of *2f* the intensity (54 %) exceeds 6 %. The further fragmentation of *c* is in accordance with the decomposition modes described for *1*.

Loss of carbon monoxide and hydrogen from the molecular ions are possible processes. The abundance of the $[M-H]^+$ ions are greatly increased [10 % (*2b*) to 94 % (*2d*)], whereas elimination of CO also in this series gives rise to peaks of low intensities.



4

In both series of compounds, the primary decomposition modes are related to the pyrone part of the molecules. Analogously, the fragmentations of simple α-(1,2-dithiol-3-ylidene) ketones are reported to be connected with the carbonyl group. Thus, the incorporation of the carbonyl group in the quasiaromatic ring and the resulting change in charge distribution do not in principle change the decomposition modes, reflecting the high stability of the 1,2-dithiole ring upon electron impact.

Experimental. The mass spectra were recorded on an AEI MS-902 mass spectrometer by Mrs. E. Wolff-Jensen, The H. C. Ørsted Institute, University of Copenhagen, or on a Varian MAT 311A by Mr. C. Jepsen, Department of Chemistry, Odense University. The ion source temperature was typically 130 °C (70 eV, direct sample insertion). The preparation of the compounds is reported elsewhere.[3]

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The Reaction of 4-Substituted Thiosemicarbazides with Phenyl Isocyanide. 1,3,4-Thiadiazoles and 1,2,4-Triazoles

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In connection with our investigations on the reactivity of isocyanides towards hydrazines [1] we were interested in the reaction between thiosemicarbazides and phenyl isocyanide. No α-addition products or products from cleavage of the N^4-C bond as observed for reaction with thioureas [2] were isolated, instead we found cyclization by insertion of $=CH-$ between N and S or between N and N. Similar insertion reactions of $=CH-$ with isocyanides have been reported for reaction with bifunctional compounds such as diamines, aminoalcohols, and aminothiols.[3]

The reactions were carried out in refluxing benzene with copper(I) chloride as catalyst. Three thiosemicarbazides were examined, namely 4-methyl-, 4-phenyl- and unsubstituted thiosemicarbazide.

4-Methylthiosemicarbazide (*1a*) was the most slowly reacting thiosemicarbazide (1.75 h) giving almost exclusively 2,4-dihydro-4-methyl-3*H*-1,2,4-triazole-3-thione (*3a*). Thiosemicarbazide (*1b*) and 4-phenylthiosemicarbazide (*1c*) required both 0.75 h for consuming the isocyanide. Thiosemicarbazide (*1b*) gave only 2-amino-1,3,4-thiadiazole (*2b*), and 4-phenylthiosemicarbazide (*1c*) gave a mixture of 2-phenylamino-1,3,4-thiadiazole (*2c*) and 2,4-dihydro-4-phenyl-3*H*-1,2,4-triazole-3-thione (*3c*). In all three reaction mixtures we found an almost quantitative yield of aniline determined by GLC analysis.

By TLC analysis we determined that only 4-phenylthiosemicarbazide formed both the thiadiazole and the triazole in high yield. 2-Methylamino-1,3,4-thiadiazole (*2a*) and 2,4-dihydro-3*H*-1,2,4-triazole-3-thione (*3b*) could only be detected as traces (less than 1 %) in the other two reaction mixtures.

It is known that thiosemicarbazides can be cyclized to either thiadiazoles (*2*) or triazoles (*3*) or a mixture,[4,5] depending on the reaction conditions [6] and the cyclizing agent.[7] The substituent (alkyl or aryl) in the 4-position of the thiosemicarbazide has been reported important for the product distribution with triethyl orthoformate as cyclizing agent [8] as reported in this communication when phenyl isocyanide is used as cyclizing agent.

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 JEOL JNM MH 60/II instrument. IR spectra were recorded on a Perkin-Elmer model 225 grating spectrograph or model 157 NaCl spectrophotometer. Mass spectra were taken on an AEI-902 instrument operating at 70 eV. GLC-analyses were carried out on a Perkin-Elmer F11 gas chromatograph equipped with a Chromosorb 103 column. Melting points are uncorrected.

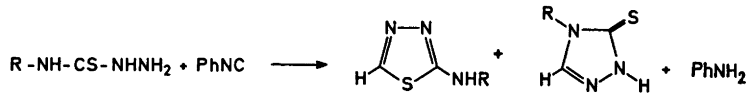
All the compounds mentioned have previously been described in the literature.[9–13] Their identities were established by IR, NMR and mass spectrometry. The IR spectra were compared with spectra of authentic samples.

*2,4-Dihydro-4-methyl-3*H*-1,2,4-triazole-3-thione (*3a*).*[8] A solution of phenyl isocyanide (0.05 mol) and copper(I) chloride (0.00075 mol) in 50 ml of benzene was refluxed with 4-methylthiosemicarbazide (*1a*) (0.05 mol) for 1.75 h. The reaction mixture was cooled in ice and the precipitate filtered off and washed with ether. Yield 81 % of yellow crystals, m.p. 163–165 °C.

*2-Amino-1,3,4-thiadiazole (*2b*)* [9] was prepared from thiosemicarbazide (*1b*) as described above. Reflux for 0.75 h consumed the phenyl isocyanide and left a syrupy mass after decantation. Recrystallization from ethanol gave 30 % of light yellow crystals, m.p. 188–190 °C.

When the reaction was carried out without copper(I) chloride the phenyl isocyanide was consumed after 2 h yielding 65 % of 2-amino-1,3,4-thiadiazole (*2b*).

*2-Phenylamino-1,3,4-thiadiazole (*2c*)* [11] and *2,4-dihydro-4-phenyl-3*H*-1,2,4-triazole-3-thione (*3c*).*[12] A solution of phenyl isocyanide (0.10 mol) and copper(I) chloride (0.0015 mol) in 100 ml of benzene was refluxed with 4-phenylthiosemicarbazide (*1c*) (0.10 mol) for 0.75 h. The reaction mixture was cooled in ice and the precipitate was filtered off. The precipitate was extracted with 1 M NaOH solution, the



Scheme 1. *a*, R = CH_3; *b*, R = H; *c*, R = Ph.

residue filtered off, washed with water yielding 32 % of light yellow crystals of 2-phenylamino-1,3,4-thiadiazole (*2c*), m.p. 171 – 172 °C.

2,4-Dihydro-4-phenyl-3*H*-1,2,4-triazole-3-thione (*3c*) was precipitated from the alkaline filtrate by acidification with glacial acetic acid. Yield 42 % of colourless crystals, m.p. 164 – 165 °C.

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Conformational Analysis. XVI. The Enthalpy and Entropy Difference Between Twist and Chair Forms of the 1,3-Dithian Ring

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Conformational equilibria of substituted six-membered heterocycles have been widely investigated and the conformational energies obtained have led to at least a qualitative understanding of the different forces and interactions in these compounds. 1,3-Dithians are especially suitable for studies of conformational equilibria since $-\Delta G^\circ$-values may be determined for a particular group at all three nonequivalent sites (2, 4/6 and 5) of the ring and moreover, acid-catalysed equilibration of epimeric derivatives normally occurs readily thus permitting the use of the reliable direct equilibrium method. For this purpose one must find models in which a given substituent biases one conformation for each isomer. In cyclohexanes the most commonly used models have a *t*-butyl group [1] ($-\Delta G^\circ \sim 24$ kJ mol^{-1}) located in position 4 with respect to the group studied. In 1,3-dithians, however, *cis*-2,5-dialkyl substituted derivatives are seldom biased [1,2] and hence unsuitable as direct models of the twist-chair equilibrium. Consequently, it is preferable to employ, *e.g.*, *r*-2-alkyl,*cis*-4,*cis*-6-dimethyl-(1) and *r*-2-alkyl,*trans*-4,*trans*-6-dimethyl-1,3-dithians (*2*) as model compounds [1–3] to determine the chair-twist enthalpy and entropy differences (Fig. 1). For this purpose epimeric 2-*t*-butyl-4,6-dimethyl-1,3-dithians were prepared in an acid-catalysed condensation reaction from *meso*-2,4-pentanedithiol and 2,2-dimethylpropanal.[1] Epimers were separated by preparative gas chromatography and then equilibrated at three temperatures using trifluoroacetic acid as catalyst. The samples were analysed by gas chromatography. The resulting equilibrium constants and thermodynamic parameters are shown in Table 1.

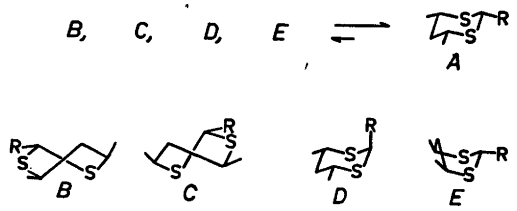


Fig. 1. The studied epimer equilibria. *1* (A) is *r*-2-alkyl,*cis*-4,*cis*-6-dimethyl- and *2* (B – E) *r*-2-alkyl,*trans*-4,*trans*-6-dimethyl-1,3-dithian. *a*, R = *t*-Bu; *b*, R = i-Pr.

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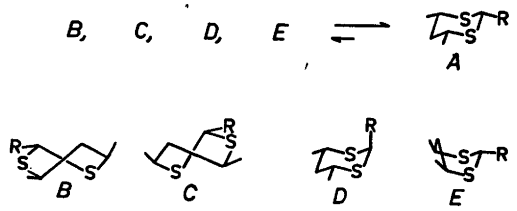


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Table 1. The results from the equilibration experiments (*2a*⇌*1a*). *K* is the equilibrium constant (*1a*/*2a*).

T/K	*K*	$-\Delta G°$/kJ mol^{-1}
321.2	77.1 ± 3.4	11.60
341.2	53.3 ± 1.2	11.28
361.2	36.7 ± 0.6	10.82
$-\Delta H°$/kJ mol^{-1}	17.9 ± 0.7	
$-\Delta S°$/J mol^{-1} K^{-1}	19.5 ± 2.0	

It has been pointed out that *r*-2-*t*-butyl,*trans*-4,*trans*-6-dimethyl-1,3-dithian (*2a* in Fig. 1) has two twist forms (B and C) which are free from extra nonbonded interactions due to the substituents.[2] The other possible conformations (D and E) may be excluded due to the axial *t*-butyl group (D) and to the availability of the much more stable twist forms (*cf.* E to B and C).[3] Eliel and Hutchins reported $-\Delta G° \sim 11.4$ kJ mol^{-1} for the equilibrium *2a*⇌*1a* at 342 K.[1] Owing to the experimental difficulties they were not able to determine the equilibrium constant as a function of temperature and hence the enthalpy and entropy differences remained unknown. Pihlaja [2] pointed out, however, that the free energy difference fits best to an equilibrium between the 1,4- (B) or 3,6-twist (C) form and a chair form (A) of the 1,3-dithian ring. The results presented herein confirm the postulate [2] that *2a* exists almost exclusively in the twist conformations B and C. In addition to $-\Delta G° = 11.2$ kJ mol^{-1} at 342 K which is very close to the value reported by Eliel and Hutchins [1] our results allow the estimation of $\Delta H°_{CT} = 17.9 \pm 0.7$ kJ mol^{-1} and $\Delta S°_{CT} = 19.5 \pm 2.0$ J mol^{-1} K^{-1} for the equilibrium between the 1,4- or 3,6-twist form and a chair form. This result is in close agreement with the averaged most probable values proposed earlier for the chair-twist equilibrium of the unsubstituted 1,3-dithian ring ($\Delta H°_{CT}$ 16.9 ± 1.2 kJ mol^{-1} and $\Delta S°_{CT}$ 19.0 ± 2.0 J mol^{-1} K^{-1}).[2]

To test the accuracy of the above result we may now evaluate the free energy difference for the equilibrium in Fig. 1 when R = i-Pr. The *r*-2-isopropyl,*trans*-4,*trans*-6-dimethyl derivative (*2b*) should include about 37 % of the twist forms and 63 % of the chair form (D) if the thermodynamic parameters for the chair-twist equilibrium are equal to those presented above and $-\Delta G°$ (*2ax*-i-Pr) has the value 9.7 kJ mol^{-1} derived in our previous report.[2] Hence $-\Delta H°$ for the epimer equilibrium at 342 K will be 12.7 kJ mol^{-1} and $-\Delta S° = 0.37 \times 19.5 + \Delta S_{mix}$("B + C" + D) = 7.2 + 5.5 = 12.7 J mol^{-1} K^{-1}. Thus $-\Delta G°$ (342) for the epimer equilibrium should be 8.4 kJ mol^{-1} in fair agreement with the value 8.2 kJ mol^{-1} reported by Eliel and Hutchins.[1] It is worth emphasizing that this treatment does not make any allowance for the possible differences in the rotational entropy of the isopropyl group in the various conformations (A, "B + C", D) but in the light of the above result these differences are likely to be small.

As a conclusion isomer *2a* exists predominantly and isomer *2b* appreciably in the twist form (B or C). Moreover, the values of the thermodynamic parameters from this and previous reports [1,2,4,5] confirm that 1,3-dithians may attain twist conformations relatively easily. In a forthcoming paper we shall try to clarify the contribution of the twist forms to the conformational equilibria in *cis*-2,5-dialkyl-1,3-dithians in some detail.[5]

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Correction to "Studies on the Kolbe Electrolysis. XII. * Complete Racemization of Optically Active Radicals from (—)-2-Methyloctadecanoate in a Mixed Coupling Reaction"

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On p. 907, in the left-hand column, fifth paragraph from above (line 27), the sentence beginning with "This permits us to conclude . . ." a deplorable error has sneaked in. As follows from the optical rotation data given in the paper, the word "retention" in this sentence should be replaced by "racemization".

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Phospholipase C from *Bacillus cereus*. Action on Some Artificial Lecithins

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The hydrolysis by phospholipase C from *B. cereus* of several lecithins of different fatty acyl chain length was examined. The enzyme showed significant activity towards monomolecularly dispersed short chain lecithins and the reaction obeyed normal Michaelis-Menten kinetics. Rate *vs.* substrate concentration curves obtained with dihexanoyl-, diheptanoyl- and dioctanoyllecithins showed marked discontinuities in the region of the known critical micelle concentrations for these substrates and distinctly higher rates were obtained just above these levels. Using these three lecithins at levels below their respective critical micelle concentrations, rate increases were noted if the reactions were allowed to proceed to a sufficiently great extent. The presence of deoxycholate in the reaction system had little or no effect on the rate of enzyme-catalysed hydrolysis of lecithins of fatty acyl chain length $\leq C_8$, but for fatty acyl chain lengths $\geq C_{10}$, significant rate increases occurred. The pH profile for the enzyme activity was also examined.

Phospholipase C (phosphatidylcholine choline phosphohydrolase, EC 3.1.4.3) from *Bacillus cereus* catalyses the hydrolysis of appropriate phospholipids at the phosphate moiety. Recently, studies have been carried out on the structure of the enzyme and on its active site.[1–3] As a part of a further investigation into the enzyme and its mechanism of action it was decided to try to obtain some simple kinetic data on the reaction. Normal substrates for phospholipase C are insoluble phospholipid structures and the use of such substrates would greatly complicate kinetic studies. Several synthetic lecithins with fairly short fatty acyl chains can be prepared and these, under certain conditions, exist in a monomolecularly dispersed form and as such are more suitable for use in kinetic investigations. A further point of interest is that whereas these lecithins are water-soluble at low concentrations, most of them are water-insoluble at high concentrations and form micelles. With phospholipase A_2 [4] and pancreatic lipase,[5] enzyme activity towards micellar substrates is considerably greater than towards monomolecularly dispersed (monomeric) substrates. With pancreatic lysophospholipase, however, there seems to be no preference for micelles over monomers.[6] Regarding the relative behaviour of phospholipase C towards micelles and monomers, no reports have been published apart from a very brief reference to a micelle effect by Zwaal and Roelofsen.[7] The present paper examines this micelle effect in more detail. In addition, some simple kinetic studies are reported together with the effect of pH on the reaction rate with a water-soluble lecithin substrate.

EXPERIMENTAL

Phospholipase C was prepared from the culture supernatant of *Bacillus cereus* by the method of Little *et al.*[8] with the small modification described.[2] *sn*-Glycero-3-phosphorylcholine (GPC) was prepared as the $CdCl_2$ adduct by methanolysis of 90 % egg lecithin (BDH).[9] Free GPC was obtained by allowing an aqueous solution of the $CdCl_2$ adduct to percolate through a column of mixed bed ion exchange resin consisting of Amberlites IRC-50 and IR-45. The eluent from the column was lyophilized and stored over P_2O_5.

Fatty acid anhydrides other than butanoic anhydride were synthesized [10] and recrystallized from acetone using low temperatures

where necessary. Butanoic anhydride was purchased from Fluka and fatty acids were obtained from Sigma.

Substrates. The various lecithins, *O*-(1,2-diacyl-*sn*-glycero-3-phosphoryl)choline, (3-*sn*-phosphatidylcholine) were prepared using free GPC with the molten anhydride constituting the reaction medium.[11] Ratios of GPC:fatty acid salt:fatty acid anhydride of 1:2:10, by weight, were used. Potassium salts of fatty acids were used except for the synthesis of dibutanoyllecithin, where the tetraethylammonium salt was employed. The various lecithins were purified from the reaction mixtures by chromatography on silicic acid (Mallincrodt CC-7) using established procedures. Lecithins of fatty acyl chain length $\leqslant C_7$ were further purified by chromatography on neutral alumina (Biorad AG-7). All lecithins used appeared as single spots when analysed by thin layer chromatography on silica gel H.

Chromatograms were developed using chloroform-methanol-acetic acid-water (65:25:10:4, by vol.) and visualized both by iodine vapour and 2,7-dichlorofluorescein. In addition, when the lecithins were incubated with phospholipase C, the enzyme eventually released > 95 % (typically 97 – 98 %) of the theoretically available acid when the latter was titrated with NaOH to pH 7.5.

Enzyme assays were carried out at 22 – 23 °C on Radiometer pH stat equipment. Except where the pH effect was studied, the end point was pH 7.5. 0.02 M NaOH was used as titrant and prepared by diluting a standard solution of the base (Titrisol, Merck). Under the conditions used, the nonenzymic rates were negligible.

Lecithins were added to a solution of NaCl (0.15 M) and subject to 4 – 6 one minute bursts of sonication whilst being maintained on ice. The suspensions of didodecanoyl- and ditetradecanoyllecithin in 0.15 M NaCl were distinctly turbid even after prolonged sonication. When the effect of deoxycholate was studied, the detergent was added after sonication.

RESULTS

1. *Hydrolysis of lecithins in solution.* In order to carry out a simple investigation of the kinetics of phospholipase C action, the enzyme activity was measured against lecithins with fairly short fatty acyl chains at concentrations where pure solutions are believed to occur. Relatively high rates of hydrolysis occurred especially with dihexanoyllecithin and it was found that the reactions followed normal Michaelis Menten kinetics. Thus, the reaction was first order in enzyme concentration and showed a tendency towards saturation at high substrate levels (see, for example, Fig. 1). From the data in Fig. 1, K_m values for phospholipase C of 37 mM with dibutanoyllecithin as substrate and 0.4 mM with dihexanoyllecithin were calculated. Maximum velocities, expressed as maximum molecular activities were

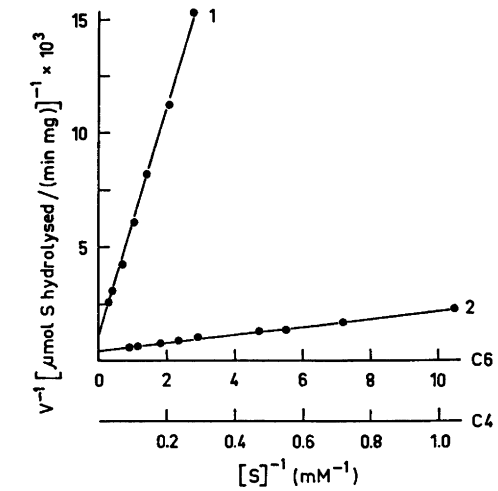


Fig. 1. Lineweaver-Burk plots showing the effect of substrate concentration on the rate of phospholipase C catalysed hydrolysis of dibutanoyllecithin (*1*) and dihexanoyllecithin (*2*). The lower of the [S]$^{-1}$ scales (0 – 1.0 mM^{-1}) refers to the reaction with dibutanoyllecithin.

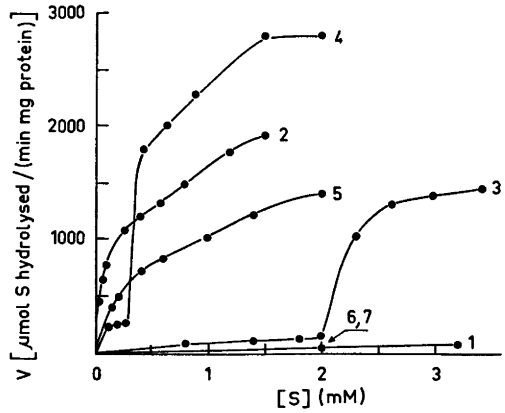


Fig. 2. Effect of substrate concentration on the rate of phospholipase C catalysed hydrolysis of different lecithins. *1*, dibutanoyllecithin; *2*, dihexanoyllecithin; *3*, diheptanoyllecithin; *4*, dioctanoyllecithin; *5*, didecanoyllecithin; *6*, didodecanoyllecithin; *7*, ditetradecanoyllecithin.

calculated to be 17 000 mol of dibutanoyllecithin hydrolysed/(min mol enzyme) and 50 000 mol of dihexanoyllecithin hydrolysed/(min mol enzyme).

2. *Hydrolysis of lecithin micelles.* In an attempt to study the possible micelle effect with phospholipase C, the activity towards a range of lecithins of different fatty acyl chain length was examined. The reaction rates were measured at different substrate concentrations up to about 3 mM (Fig. 2). In the absence of detergents, extremely low rates of reaction were obtained with didodecanoyl- and ditetradecanoyllecithins, whereas high rates occurred with dihexanoyl-, diheptanoyl-, dioctanoyl- and didecanoyllecithins. An interesting feature of Fig. 2 is the discontinuities obtained in the reaction rate *vs.* substrate concentration curves for diheptanoyl- and dioctanoyllecithins. The discontinuities occurred around 2 mM for the former substrate and around 0.3 mM for the latter, values which correspond well with the solubility limits for these lecithins.[6,4] Above these limits micelle formation occurs and it appears that phospholipase C from *B. cereus* has a significantly higher activity towards lecithin micelles than it does towards the corresponding monomers. Further support for this view was obtained by using dihexanoyllecithin as substrate at concentrations around its reported critical micelle concentration. Fig. 3 shows

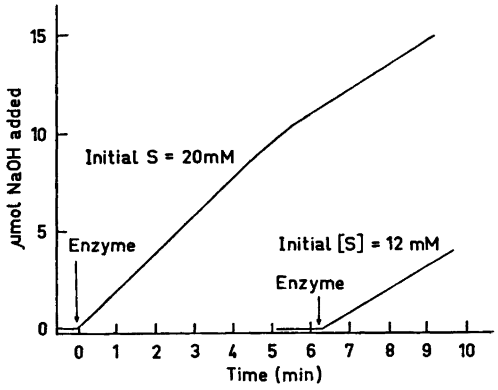


Fig. 3. Hydrolysis of dihexanoyllecithin. The figure shows the trace obtained from the pH stat recorder when enzyme was added to an initial volume of 5 ml of dihexanoyllecithin at pH 7.5. The initial substrate concentration was 20 mM or 12 mM as indicated in the figure.

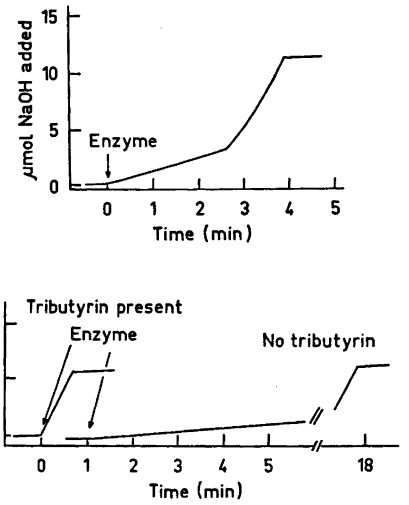


Fig. 4. Time course of hydrolysis of dioctanoyllecithin. Reactions were carried out in a volume of 40 ml at pH 7.5. pH stat recorder traces are shown. A: Hydrolysis of substrate at 0.28 mM initial concentration. B: Effect of tributyrin. The initial substrate concentration was 0.15 mM. A freshly sonicated dispersion of tributyrin to a final concentration of 1 mM was added to one sample just before addition of enzyme. The other sample of substrate received the same amount of enzyme but no tributyrin.

the results of an experiment in which the enzyme was incubated with 20 mM dihexanoyllecithin. It can be seen that initially a constant rate of reaction occurred. Later, the rate changed fairly suddenly and then the reaction continued at a constant but reduced rate. The remaining substrate concentration at which this rate change occurred was calculated after correcting for dilution as 13.3 mM, a value extremely close to the reported critical micelle concentration of 13.8 mM for this lecithin.[4] The reduced rate occurring after the rate change was equal to the initial rate obtained using a 12 mM solution of this lecithin. Consequently, it is unlikely that the rate change arose from enzyme inactivation or product inhibition. During the present work, another unusual rate effect was observed. Using concentrations of diheptanoyl- and dioctanoyllecithin below the critical micelle concentration resulted in relatively low initial rates. However, if the reaction was allowed to proceed further, marked increases in rate even-

Table 1. Effect of deoxycholate on the rate of hydrolysis of different lecithins. The activity of phospholipase C towards various lecithins in 0.15 M NaCl was measured in the presence and absence of 0.25 % (w/v) sodium deoxycholate at pH 7.5 and 22–23 °C. Substrate concentration, 2 mM.

Lecithin	Enzyme activity μmol S hydrolysed/(min mg enzyme)	
	−Deoxycholate	+Deoxycholate
Dihexanoyl-	1900	1350
Diheptanoyl-	150	150
Dioctanoyl-	2800	2200
Didecanoyl-	1400	2500
Didodecanoyl-	< 5	4800
Ditetradecanoyl-	55	5300

tually occurred and these elevated rates remained until the reaction stopped fairly abruptly at a point corresponding to total hydrolysis of the substrate (Fig. 4 A). The beginning of these rate increases coincided well with the onset of turbidity in the reaction mixture (results not shown). These rate increases could also be initiated if a sonicated suspension of tributyrin (1,2,3-tributanoylglycerol) was added to the reaction mixture. A further observation was that these elevated rates obtained below the critical micelle concentration were of a magnitude comparable with those obtained above the critical micelle concentration. These effects were most marked with deheptanoyl- and dioctanoyllecithins, but also occurred to a lesser extent with dihexanoyllecithin. No other lecithin used showed this behaviour. These rate increases occurring during the course of the reaction were abolished if 0.25 % deoxycholate was present in the reaction mixture. The presence of this detergent also had a marked effect on the initial rates obtained with some of the lecithins. Table 1 shows that with didodecanoyl- and ditetradecanoyllecithins, very large rate enhancements occurred when 0.25 % deoxycholate was present. A much smaller rate increase was obtained with didecanoyllecithin and the presence of the detergent had little or no effect on the rates occurring with lecithins of shorter fatty acyl chain length.

3. *Effect of pH on the rate of hydrolysis of a soluble lecithin.* Otnaess *et al.*[12] studied the effect of pH on the activity of phospholipase C towards lecithin micelles. A somewhat unusual pH profile occurred in which two peaks of enzyme activity, one around pH 6.5 and another around pH 8.5 were observed. To examine whether this double pH optimum also occurred with a monomolecularly dispersed substrate, the effect of pH on the rate of hydrolysis of a 5 mM solution of dihexanoyllecithin was examined. The results in Fig. 5 illustrate that in the pH range 5.5–10.0, the enzyme shows a single peak of activity around

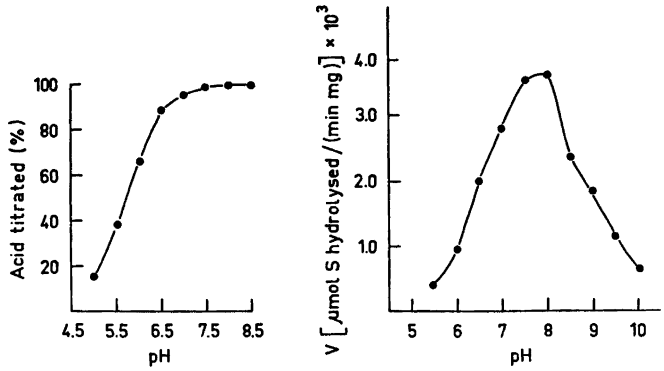


Fig. 5. Effect of pH on enzyme activity. The rate of hydrolysis of a 5 mM solution of dihexanoyllecithin was examined at different pH's by setting the end point on the pH stat to different pH values and measuring the rate of reaction. A: Titration efficiency at different pH values for phosphorylcholine in 0.15 mM NaCl. B: pH profile of phospholipase C activity. Activities are expressed as specific activities corrected for titration efficiency.

pH 7.5 – 8.0. This fits fairly well with the more alkaline of the two peaks reported by Otnaess *et al.*[12] With the soluble substrate there is clearly no evidence for an activity peak around pH 6.5.

DISCUSSION

It is apparent from the present results that phospholipase C from *B. cereus* shows relatively high rates of reaction with both monomolecularly dispersed and micellar substrates. Regarding the activity with monomolecularly dispersed substrates, the ratio of V_m/K_m for dihexanoyllecithin to the V_m/K_m value for dibutanoyllecithin is 303 under the reaction conditions used. Thus, the former lecithin is a clearly superior substrate to the latter. However, in the concentration range up to 2 mM, diheptanoyllecithin gave reaction rates which, although significantly greater than those obtained with dibutanoyllecithin, were considerably smaller than those shown with dihexanoyllecithin. It would therefore appear that there is no simple relationship between the length of the fatty acyl chain and the ability of a lecithin to serve as a substrate in the soluble form.

With three different lecithins, clearly defined discontinuities in the rate-substrate concentration curves were noted in the region of the reported respective critical micelle concentrations. The results strongly suggest that phospholipase C from *B. cereus* like pancreatic phospholipase A_2[4] and pancreatic lipase[5] shows a clearcut preference for a substrate in the micellar form over the same substrate in the monomolecularly dispersed or soluble form. In view of the apparent preference of the enzyme for micelles, the reaction rates obtained with solutions of dihexanoyllecithin are remarkably high. In fact, the enzyme shows a higher activity towards a 1.5 mM solution of this substrate than it does in the "standard" assay system for this enzyme which uses micelles of crude egg lecithin with detergent.[13,8]

Using solutions of dihexanoyl-, deheptanoyl- or dioctanoyllecithin, a pronounced rate increase was obtained during the reaction if the latter was allowed to proceed far enough. The reason for this behaviour is not completely clear, but three pieces of information may be pertinent: (a) the onset of the rate increase coincided with the onset of turbidity in the reaction mixture, (b) the stimulation could be induced by adding to the reaction system a sonicated dispersion of tributyrin, (c) the stimulation led to rates that were of a similar order to those occurring at lecithin levels above the critical micelle concentration. Turbidity in the present assay system arises from the aggregation of diglyceride produced during the hydrolysis and the present observations are compatible with the view that the monomolecularly dispersed lecithin molecules adsorb to the diglyceride aggregates to produce a type of micellar structure. The rate increase would then simply reflect the ability of micelles to serve as superior substrates. These stimulatory effects were not observed with dibutanoyllecithin, the least hydrophobic of the substrates used, which would be expected to have the smallest tendency to adsorb to diglyceride aggregates. The presence of deoxycholate in the reaction system abolished both the formation of turbidity and the rate increase. Other experiments involving deoxycholate provide further support for the view that the precise physical state of the substrate has a great effect on enzyme activity. This detergent at 0.25 % probably has little direct effect on the enzyme. Thus, with monomolecularly dispersed lecithins, the presence of 0.25 % deoxycholate had little or no effect on reaction rates. With micellar substrates the effect of the detergent on reaction rate was more complex. Deoxycholate (0.25 %) seemed to have a slight inhibitory effect on the rate of hydrolysis of dioctanoyllecithin, almost doubled the rate with didecanoyllecithin, increased the rate with ditetradecanoyllecithin some 100-fold and increased the rate with didodecanoyllecithin by around 1000-fold. With the latter two substrates, however, it was not possible to obtain optically clear dispersions in 0.15 M NaCl simply by ultrasonication. The enormous effects of the detergent may in these cases be due, in part at least, to the formation of superior dispersions. The pH profile of phospholipase C was also examined. Whereas Otnæss *et al.*[12] found two peaks of activity (at pH 6.5 and at pH 8.5) when using lecithin micelles, the present work with a soluble lecithin indicated a single peak of activity around pH 7.5 – 8.0. It is possible that the pH 6.5 peak reported by Otnæss *et al.*[12]

reflects some pH transition in the structure of the particular micelles used rather than an inherent property of the enzyme.

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Inactivation of Phospholipase C from *Bacillus cereus* by a Carboxyl Group Modifying Reagent

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Phospholipase C from *Bacillus cereus* was inactivated by incubation with either of the carboxyl reagents, a water-soluble carbodiimide plus a nucleophile or Woodward's reagent K. With the former reagent, the incorporation into the enzyme of the first mol of nucleophile caused a 4 – 5-fold increase in the K_m for dihexanoyllecithin with no significant effect on the V_m. The second mol of nucleophile incorporated caused no further change in K_m but destroyed most of the catalytic activity. Modification of the enzyme by carbodiimide plus nucleophile did not alter the relative activity of the enzyme towards micelles and monomolecularly dispersed solutions of diheptanoyllecithin. Furthermore, inactivation by this reagent did not significantly decrease the ability of the enzyme to bind to a substrate-based affinity gel. It was concluded that phospholipase C contains a single carboxyl group that is essential for catalytic activity. The enzyme also contains a total of 4 – 5 reactive/exposed carboxyl groups.

Recent studies of phospholipase C from *B. cereus* (phosphatidylcholine choline phosphohydrolase, EC.3.1.4.3) have indicated the presence in the enzyme of zinc atoms and lysine residues that are essential for catalytic activity.[1,2] As part of an examination of the enzyme's active site using amino acid-selective reagents, the influence of carboxyl-group modifying chemicals on the enzyme was investigated. Several enzymes have been shown to contain essential carboxyl groups and in some cases, *e.g.*, lysozyme, carboxyl groups appear to play a direct role in the catalytic reaction. With phospholipase C an active centre carboxyl could also be involved in substrate binding by electrostatic attraction of phosphatidylcholine. Water-soluble carbodiimides in the presence of suitable nucleophiles have been widely used as a means of selective modification of carboxyl side chains [3] and the results of a study of the effects of such reagents on phospholipase C are now presented.

MATERIALS AND METHODS

Materials. Woodward's reagent K (*N*-ethyl-5-phenylisoxazolium-3′-sulfonate) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were obtained from Sigma, St. Louis, Mo., USA and *N*-(2,4-dinitrophenyl)ethylenediamine.HCl (DNP-ethylenediamine) from Calbiochem AG, Lucerne, Switzerland. The concentration of this nucleophile and its extent of incorporation into the enzyme were investigated using an extinction coefficient for the 2,4-dinitrophenyl group (DNP) E^{M}_{360} of 15 000.

Phospholipase C was isolated from the culture supernatant of *B. cereus*.[2,5] Dihexanoyl- and diheptanoyllecithins [*O*-(1,2-diacyl-*sn*-glycero-3-phosphoryl)choline, 3-*sn*-phosphatidylcholine] were synthesized by the method of Cubero Robles and Van den Berg.[6,7] Sepharose-bound egg yolk lipoprotein was prepared using the method of Takahashi *et al.*[5,8]

Enzyme assays. Enzyme activity was assayed at 22 – 23 °C by measuring the rate of acid production either from pure lecithin substrates [7] or from crude egg yolk extract.[5,9] The assays were carried out by continuous titration using Radiometer pH stat equipment. Determinations of V_m and K_m were made using dihexanoyllecithin as substrate at pH 7.5. The kinetic constants were calculated from Lineweaver-Burk plots constructed from 8 – 10 rate measurements over a substrate concentration range 0.2 – 2 mM for each enzyme sample. Substrate was dissolved in 0.15 M NaCl. When the effect of enzyme modification on the micelle effect was studied, the rates of hydrolysis of diheptanoyllecithin in 0.15 M NaCl were measured. For each enzyme sample two different

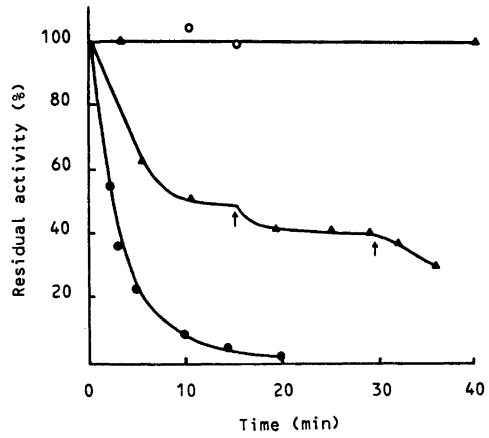


Fig. 1. Inactivation of phospholipase C by carboxyl reagents. ● enzyme incubated with EDC plus DNP-ethylenediamine. ○ control containing all but the diimide. ▲, 12 μM enzyme in 10 mM sodium phosphate buffer incubated with 2.6 mM Woodward's reagent K. The arrows indicate further additions of reagent to increase the final concentration by 2.6 mM. △, control containing no reagent. Enzyme activity was measured against egg yolk substrate and further experimental details are given in the Materials and Methods section.

substrate concentrations were used, 1.5 and 3 mM. The critical micelle concentration for this lecithin is approximately 2 mM.[10]

Inactivation conditions. Inactivations were carried out at 22 °C with the pH of the reaction mixture being maintained using a pH stat. For inactivation by the diimide, a pH of 4.75 was used and unless otherwise stated, the conditions used were: phospholipase C (40 μM), EDC (61 mM) and DNP-ethylenediamine (18 mM). The reaction medium was 0.15 M NaCl.

When the extent of DNP incorporation into the enzyme was to be measured, samples of inactivated enzyme were dialysed against 0.05 M sodium phosphate buffer, pH 6 (4 dialyses against 1 l portions of buffer) at 4 °C. Any precipitate was removed by centrifugation. Protein was determined by the method of Lowry *et al.* using bovine serum albumin as standard.[11]

RESULTS

Inactivation by carboxyl reagents. Incubation of phospholipase C with EDC in the presence of DNP-ethylenediamine resulted in rapid inactivation which was effectively complete after about 45 min (Fig. 1). Similar results occurred when the ethyl ester of glycine was used as nucleophile (results not shown). The inactivation was not reversed when the enzyme was dialysed for 5 h at 4 °C and pH 7 against 0.5 M hydroxylamine. When the enzyme was incubated with Woodward's reagent K, another carboxyl reagent,[12,13] loss of activity occurred, but further inactivation ceased after a few minutes. More extensive inactivation occurred upon the addition of more reagent (Fig. 1). These effects are consistent with the known instability of this reagent in aqueous solution.[14]

Stoichiometry of the inactivation. The remaining enzyme activity was measured as a function of the extent of enzyme modification by EDC plus DNP-ethylenediamine. In view of the warning given by Carraway and Koshland,[3] no attempt was made to quench the reaction with acetate buffer. Instead, the reaction was stopped by dialysis. As a consequence, it was difficult to obtain enzyme samples that were less than about 40 % inactivated. The data are shown in Fig. 2 and suggest that the incorporation of a single mol of DNP/mol of enzyme would cause total inactivation. It should, however, be noted that the straight line constructed in the figure passes through relatively few experimental points and that for extents of inactivation above 70 %, the data deviate strongly from the

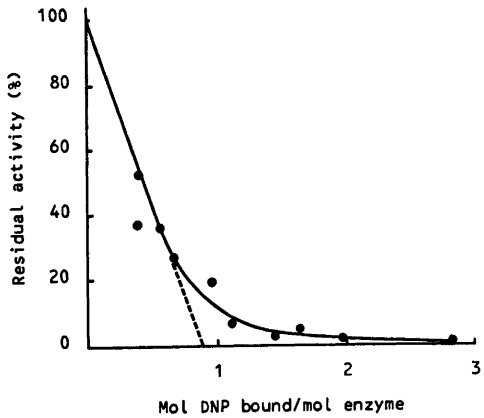


Fig. 2. Stoichiometry of the inactivation by EDC plus DNP-ethylenediamine. Portions of the reaction mixture were removed at different times and the remaining enzyme activity towards egg yolk substrate and the extent of DNP incorporation measured. For further details see Materials and Methods section.

straight line. Essentially similar results were obtained whether the activity was assayed by measuring the rate of acid production in crude egg yolk extract or by using the turbidimetric assay method.[15,1].

Kinetics of the modified enzyme. Changes in enzyme activity following chemical modification may arise from changes in the maximum velocity and/or changes in K_m. In view of the rather dubious linearity between the extent of inactivation and the amount of reagent incorporation into the enzyme, the kinetic parameters of the modified enzyme were examined and compared with those of the native enzyme. For this purpose, the water-soluble artificial substrate dihexanoyllecithin was chosen and used at concentrations well below its critical micelle concentration of about 14 mM.[10] In keeping with previous results, this compound served as a good substrate.[7] Lineweaver-Burk plots constructed from the rate data obtained for the native and for the modified enzyme suggest that the incorporation of one mol of DNP/mol of enzyme does not influence the maximum velocity but causes a marked increase in K_m (Fig. 3). The incorporation of 1.4 mol of DNP/mol of enzyme leads to little or no further change in K_m but is associated with a decrease in the maximum velocity. Further kinetic data are presented in Table 1, from which it would appear that the first mol of DNP incorporated into the enzyme is associated with a 4–5-fold increase in K_m, whereas the second mol of DNP to be incorporated leads to a near total loss of catalytic activity.

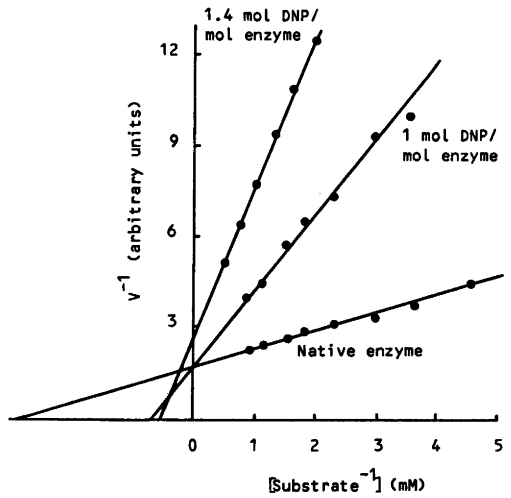


Fig. 3. Lineweaver-Burk plots for hydrolysis of dihexanoyllecithin by native and modified enzyme. The reaction rates have been corrected for differences in protein concentration between the samples.

Table 1. Effect of the extent of modification on the kinetic constants of phospholipase C. Dihexanoyllecithin was used as substrate.

DNP incorporation (mol DNP/mol enzyme)	Relative V_m (%)	K_m (mM)
0	100	0.4
0.77	87	0.8
1.0	100	1.7
1.4	62	2.0
2.14	5	1.7
3.14	< 1	–

Table 2. Influence of enzyme modification on the micelle effect. Diheptanoyllecithin was used as substrate.

DNP incorporation (mol DNP/mol enzyme)	Rate with micelles/ rate with monomers
0	6.4
1.4	6.8
2.14	6.0

Phospholipase C from *B. cereus* shows a markedly higher activity towards lecithin micelles than it does towards the same lecithin in a monomolecularly dispersed form.[7,16] The effect of modifying the enzyme by EDC plus DNP-ethylenediamine on this micelle effect was examined. The results (Table 2) clearly demonstrate that the stimulation in enzyme activity brought about by using micellar as opposed to monomolecularly dispersed substrates is not influenced by the incorporation into the enzyme of up to 2.14 mol of DNP/mol of enzyme.

Effect of modification on the binding of phospholipase C to an affinity gel. The substantial increase in K_m resulting from DNP incorporation into the enzyme could arise from, amongst other possibilities, an impairment of substrate binding. Such an effect might then lead to a

decrease in the tightness of enzyme binding to a substrate-based affinity gel. This affinity gel comprising egg yolk lipoprotein covalently bound to agarose is the basis of the enzyme purification procedure.[5] The gel binds the enzyme very tightly and fairly specifically and it is possible that the enzyme-gel interaction is of an enzyme-substrate nature, although other interactions cannot be ruled out. When a sample of modified enzyme containing 0.7 mol of DNP/mol of enzyme was applied to a column of this gel, the whole sample bound and required high concentrations of urea for its removal from the gel. Applying a sample of extensively modified and totally inactive enzyme containing 4.45 mol of DNP/mol of enzyme resulted in 97 – 98 % of the applied sample binding tightly to the gel and again requiring elution of the column with concentrated solutions of urea to remove the bound enzyme (results not shown). It is clear therefore that even quite extensive modification of the enzyme does not seriously diminish the binding to the gel.

Extent of reagent incorporation into the enzyme. The above results show that the incorporation of the first 2 mol of DNP into phospholipase C result in activity changes. In order to see how much DNP could be incorporated, the enzyme was incubated over long time periods with EDC in the presence of DNP-ethylenediamine. The results are shown in Fig. 4 and demonstrate that 4 – 5 mol of DNP/mol of enzyme appears to be the maximum extent of incorporation in the absence of denaturants. The extent of reaction was not limited by the availability of reagent since the addition of extra EDC caused little increase in the total incorporation.

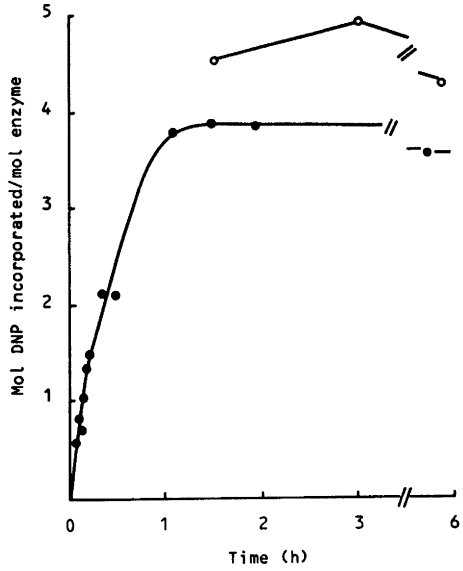


Fig. 4. Extent of DNP incorporation into phospholipase C. ● Enzyme (20 μM) was incubated with 60 mM EDC and 20 mM DNP-ethylenediamine. After different time periods, portions of the reaction mixture were removed and analysed for DNP content. ○, after 2 h incubation as above, extra EDC to give a concentration increase of 60 mM was added to the reaction mixture.

DISCUSSION

Incubation of phospholipase C under mild conditions with two chemically dissimilar reagents known to modify carboxyl groups caused rapid inactivation. The effect of one of these, EDC was examined in some detail. Under the reaction conditions used, the protein side chains which are most sensitive to modification by diimides are carboxyl, sulfhydryl and tyrosine groups.[3] The present enzyme appears to contain no free sulfhydryl group [17] and is not inactivated by sulfhydryl reagents.[18] Furthermore, it is unlikely that the inactivation involved tyrosine residues since hydroxylamine was unable to reactivate and also, tyrosine reagents do not cause inactivation.[18] The modification of lysine, histidine or aliphatic hydroxyl groups is very unlikely under the reaction conditions used [19] and the involvement of these side chains is made even less likely by the observation (results not shown) that the inactivation proceeded significantly less rapidly at pH 6 than it did at pH 4.75. It would therefore appear that the inactivation of phospholipase C by EDC plus nucleophile may be attributed to the modification of carboxyl groups. Kinetic analysis of the modified enzyme suggested that the inactivation observed arose partly from an effect on K_m and partly from a reduction in V_m. In particular, it seemed that when the enzyme was incubated with EDC plus DNP-ethylenediamine, the incorporation of the first mol of DNP led to a 4 – 5-fold increase in K_m with no significant effect on V_m.

Incorporation of the second mol of DNP caused little or no further change in K_m, but reduced the V_m to near zero. Thus, phospholipase C from *B. cereus* appears to contain a single carboxyl group that is essential for catalytic activity.

The present enzyme exhibits anomolous rate effects in the region of the critical micelle concentrations for short chain lecithins.[7] The extent of this micelle effect was not altered by exposure of the enzyme to EDC plus nucleophile. Thus, the activities towards micelles and monomers are reduced by equal proportions upon carboxyl group modification. This suggests that the two most reactive carboxyl groups in the enzyme are more involved with the catalytic centre than with any structure in the enzyme responsible for detecting micelles or lipid/water interfaces.

The first carboxyl group blocked by the carbodiimide is clearly not essential for catalytic activity. However, the associated 4 – 5-fold increase in K_m could arise from significant changes in the enzyme's ability to bind substrate. The failure of even quite extensive incorporation of DNP into the enzyme to decrease markedly the tightness of enzyme binding to a substrate-based affinity gel indicates that inactivation by the present reagent and the ability of the enzyme to bind to the affinity gel are independent processes and that the reactive carboxyls may not be involved in substrate binding. It should, perhaps, be noted that the gel bound substrate (egg yolk lipoprotein) and the monomeric dihexanoyllecithin used in the kinetic determinations are very dissimilar and the possibility that the enzyme binds the gel through some micelle recognition site should not be overlooked. The results do, however, demonstrate that the reactive carboxyl groups are not essential for the binding of the enzyme to the affinity gel.

The carboxyl groups whose modification results in activity changes are but two out of a total of 48 such residues in the enzyme.[17] Clearly, therefore, EDC modifies these two carboxyl groups with an extremely high degree of selectivity. Protein modification by water-soluble carbodiimides has been advocated as a method for determining the total numbers of exposed and buried carboxyl groups in a protein.[3] The present data suggest that in phospholipase C from *B. cereus*, 4 – 5 carboxyl residues are of very much greater reactivity towards EDC plus DNP-ethylenediamine than are the remaining ones. In the absence of protein denaturants, only 10 % of the carboxyl side chains are reactive towards the present reagent, so that a surprisingly high 90 % of the carboxyl groups appear to be hidden within the structure of the protein.

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Reactions between 2-Furanaldehyde and Grignard Reagents. I. 1,4- and 1,6-Additions of *t*-Butylmagnesium Chloride

RAINER SJÖHOLM

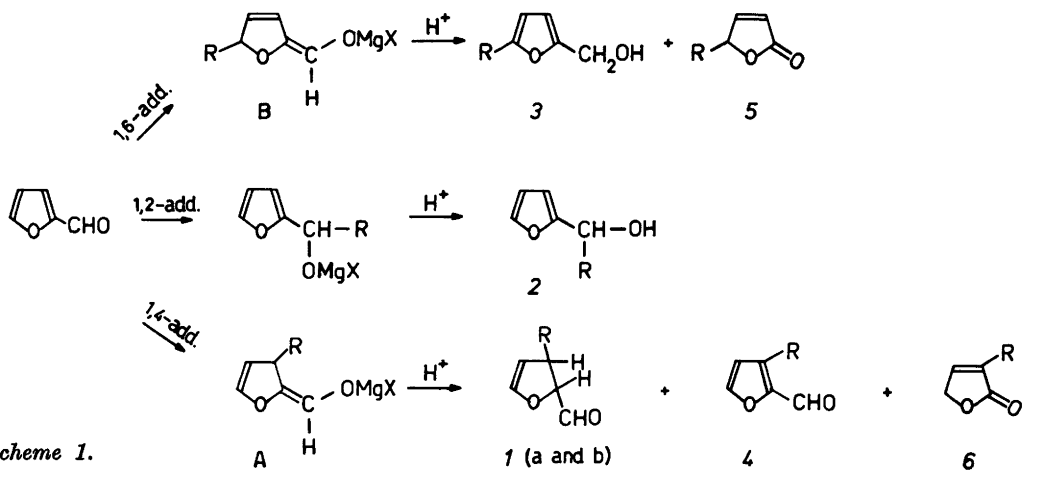
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When methylmagnesium iodide, ethylmagnesium bromide, isopropylmagnesium chloride, and isobutylmagnesium chloride react with 2-furanaldehyde, only 1,2-addition products are formed. *t*-Butylmagnesium chloride gives 1,4- and 1,6-addition products besides 1,2-addition. The 1,6-addition product rearranged easily and was isolated as 5-*t*-butylfurfuryl alcohol. Two lactones, 5-*t*-butyl-2-(5*H*)-furanone and 3-*t*-butyl-2-(5*H*)-furanone, were also isolated from the reaction mixture. The formation of the various products is discussed.

1,2-Addition of Grignard reagents to 2-furanaldehyde is a well-known reaction, which has been widely used for the preparation of various secondary furfuryl alcohols.[1]

Conjugate addition of Grignard reagents to the furan nucleus in 2-furanaldehyde, however, has not been previously reported. Gocmen *et al.*[2] could not detect any reaction products arising from conjugate addition, when they studied the reactions between 2-furanaldehyde and various Grignard reagents (methyl-, ethyl-, butyl- and phenylMgX). Fuson *et al.*[3,4] have shown that phenylmagnesium bromide adds in the 1,4-mode to furyl and benzofuryl ketones in which the carbonyl group is highly sterically hindered. They ascribed the 1,4-addition mainly to the steric effect. Berreby *et al.*,[5] however, reported a 30 % yield of conjugate addition in the reaction between 2-acetylfuran and benzylmagnesium chloride. In this case steric hindrance should be less important.

The 1,6- and 1,8-additions of *t*-butyl and benzyl Grignard reagents to diethyl furfurylidenemalonates [6–9] definitely show that steric hindrance cannot be the only factor determining whether addition of Grignard reagents to furan rings happens or not. These results seemed to justify a reinvestigation of the reactions between 2-furanaldehyde and Grignard reagents. Methylmagnesium iodide, ethylmagnesium bromide, isopropylmagnesium chloride, isobutylmagnesium chloride, and *t*-butylmagnesium chloride were chosen as the reagents. Except for the *t*-butyl reagent, the alkyl Grignard reagents gave only 1,2-adducts [*2*, R = CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$] (*cf.* Ref. 2). In addition to the 1,2-adduct *2*, which was the major product, the *t*-butyl reagent gave six products. The products were separated and purified by preparative gas chromatography. The identifications were performed by NMR and mass spectroscopy. The compound with the shortest retention time (*1a*) proved to be 3-*t*-butyl-2,3-dihydro-2-furanaldehyde formed by 1,4-addition of *t*-butylmagnesium chloride to 2-furanaldehyde. The ^1H NMR spectrum of *1a* showed that the *t*-butyl group is *trans* to the aldehyde group. The gas chromatogram showed a rather small peak on the slope of the peak of compound *1a*. This component (*1b*) was not isolated, but the mass spectrum was very similar to that of compound *1a*. On these presumptions, it seems possible that *1b* is the *cis* form of 3-*t*-butyl-2,3-dihydro-2-furanaldehyde. The preferential formation of the *trans* form is expected on steric grounds, assuming that both *1a* and *1b* are formed from the same precursor, namely the enolate A (Scheme 1) primarily formed in the 1,4-addition of *t*-butylmagnesium chloride to 2-furanaldehyde. However, no definite proof on the structure of 1b can be presented.



Scheme 1.

Compound *2* [R = C(CH$_3$)$_3$] was identified as 1-(2-furyl)-2,2-dimethylpropan-1-ol. This product is the result of 1,2-addition of the reagent to 2-furanaldehyde.

The formation of compound *3* represents a rather interesting 1,6-addition of *t*-butylmagnesium chloride to 2-furanaldehyde. In this case the primary product probably is the enolate B (Scheme 1). During the work-up of the reaction mixture the enol formed from this salt easily rearranges prototropically to the energetically more favourable structure, giving 5-*t*-butylfurfuryl alcohol. The mechanism is analogous to the one presented by Holmberg *et al.*[6] for the rearrangement of diethyl 2,3-dihydro-2-furylidenemethylmalonates under acidic conditions. The corresponding dihydro compound could not be detected by GLC.

The next compound on GLC proved to be 3-*t*-butyl-2-furanaldehyde (*4*). This compound is probably formed from *1* by some kind of oxidation.

The remaining compounds (*5* and *6*) proved to be lactones, namely 5-*t*-butyl-2-(5*H*)-furanone and 3-*t*-butyl-2-(5*H*)-furanone. Fuson *et al.*[3] have shown that lactones are formed when highly hindered benzofuryl ketones react with Grignard reagents under conditions not rigidly excluding air. In this work exclusion of air during the reaction did not reduce the amounts of lactones formed. Fuson also showed that a stream of oxygen passed through the reaction mixture after interruption of the reaction, increased the yield of lactones by oxidation of the enol formed when the reaction mixture was acidified. In the present work the mentioned procedure had no effect on the product distribution. This shows that in this case probably no enols are present after the interruption of the reaction as they immediately rearrange when formed. This indicates that the lactones are formed directly from the enolates.

When a great excess of Grignard reagent was used (10:1), the molar ratios of compounds *1* (*1a*+*1b*), *2*, *3*, *4*, *5*, and *6* were 29:63:2:2.5:0.5:3 (by GLC). It should be noted that compounds *5* and *6* probably are, at least primarily, 1,6- and 1,4-addition products and thus the molar ratios between 1,2-, 1,4- and 1,6-addition products are 63:34.5:2.5.

It may seem strange that no 3-*t*-butylfurfuryl alcohol, formed analogously to the formation of compound *3*, was detected. However, this could be explained by the steric strain that would be present in this compound (*cf.* Ref. 9). This steric strain arises from the proximity of the two bulky substituents in positions 2 and 3 in the furan ring. This and the fact that *1* represents a vinyl ether which is resonance stabilized explain the preferential formation of 3-*t*-butyl-2,3-dihydro-2-furanaldehyde.

Corresponding formation of 5-*t*-butyl-2,5-dihydro-2-furanaldehyde from the enolate B is not expected as the formation of *3* is accompanied by a considerable gain in resonance energy. Moreover, the rearrangement would lead to the formation of an energetically unfavourable structure.

EXPERIMENTAL

2-Furanaldehyde. Commercial 2-furanaldehyde was used (Riedel-de Haën, BRD) after purification by distillation (161 – 162 °C).

The reactions of 2-furanaldehyde with methylmagnesium iodide, ethylmagnesium bromide, isopropylmagnesium chloride and isobutylmagnesium chloride were performed by adding 2-furanaldehyde (20 mmol) dissolved in absolute diethyl ether (100 ml) to a stirred solution of 100 ml of 1 M (by titration) Grignard reagent. Stirring was continued for 1 h. The reaction mixture was worked up in the usual way using NH_4Cl solution. Each reaction gave only one product (by GLC and NMR), namely the α-substituted furfuryl alcohol [*2*, R = CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$]. The structures were confirmed by NMR and mass spectra.

The reaction between 2-furanaldehyde and t-butylmagnesium chloride was performed in the described way with one exception, the excess of Grignard reagent was greater (10:1). One reaction was performed under nitrogen and the ether used was distilled from benzophenone ketyl. However, the gas chromatogram (glass column 3 mm × 3 m; stationary phase 3 % OV-17 on Gas-Chrom Q; N_2 30 ml/min) of the resulting reaction mixture was identical with that of the reaction mixture from an experiment where no attempts to exclude air were made. In one experiment (under N_2) the reaction mixture was divided into two parts which were worked up separately. A stream of oxygen was passed through one of the ether solutions for 0.5 h. The gas chromatograms of the two solutions were identical.

The reaction products were separated and purified by preparative GLC (column 9.5 mm × 6.1 m; stationary phase 30 % SE-30 on Chromosorb W; He 200 ml/min).

3-t-Butyl-2,3-dihydro-2-furanaldehyde (1). The NMR spectrum clearly shows that *1* is a dihydrofuran aldehyde. The magnitude of the coupling between H-2 and H-3, assuming envelope conformation,[10] is in agreement with the value obtained for the *trans* form by the Karplus' rule. ^1H NMR (60 MHz, CCl_4): δ 4.3 (H-2, dd, *J* 4.3 and 1.4 Hz), 2.7 (H-3, ddd, *J* 4.3, 2.6 and 1.9 Hz), 4.8 (H-4, dd, *J* 2.7 and 2.6 Hz). 6.3 (H-5, dd, *J* 2.7 and 1.9 Hz), 9.5 (C*H*O, *J* 1.4 Hz), 0.9 [C(CH_3)$_3$, s].

MS[IP 70 eV; *m/e* (% rel. int.)]: 154 (15, M), 125 (2.5 [M – CHO]), 98 (16.8), 97 (7.5), 69 (43.8), 57 (100).

1-(-2-Furyl-)-2,2-dimethylpropan-1-ol [*2*, *R* = *C*(CH_3)$_3$]. This well-known substance was easily identified from NMR and mass spectra.

5-t-Butyl furfuryl alcohol (3). The NMR and mass spectra were compared with spectra of a synthesized sample[7] and were found to be identical.

3-t-Butyl-2-furanaldehyde(4). The NMR spectrum confirmed the aldehyde structure by showing a one-proton signal at δ 9.4. The substituent position was confirmed by the existence of an AB-system arising from H-4 and H-5. ^1H NMR (60 MHz, CCl_4): δ 6.1 (H-4, d, *J* 3.6 Hz), 7.0 (H-5, d, *J* 3.6), 9.4 (C*H*O), 1.3 [C(CH_3)$_3$, s].

MS [IP 70 eV; *m/e* (% rel. int.)]: 152 (26.3, M), 137 (100, M – CH_3), 123 (4.7, M – CHO), 109 (14.1), 95 (9.1).

5-t-Butyl-2-(5H)-furanone (5). This compound was synthesized by a method described in Ref. 11. Compound *5* and the synthesized compound gave identical NMR and mass spectra. ^1H NMR (60 MHz, CCl_4): δ 6.1 (H-3, dd, *J* 5.7 and 2.0 Hz) 7.6 (H-4, dd, *J* 5.7 and 1.3 Hz), 4.7 (H-5, dd, *J* 2.0 and 1.3 Hz), 1.1 [C(CH_3)$_3$, s].

MS [IP 70 eV; *m/e* (% rel. int.)]: 140 (1.5, M), 125 (4.0, M – CH_3), 97 (6.3), 84 (16.0), 57 (100).

3-t-Butyl-2-(5H)-furanone (6). ^1H NMR (60 MHz, CCl_4): δ 6.9 (H-4, t, *J* 1.8 Hz), 4.6 (H-5, d, *J* 1.8 Hz), 1.2 [C(CH_3)$_3$, s].

MS [IP 70 eV; *m/e* (% rel. int.)]: 140 (47.9, M), 125 (42.3, M – CH_3), 122 (13.4, M – H_2O), 111 (9.9, M – CHO), 107 (6.4), 97 (67.4), 95 (34.7), 79 (49.4), 57 (17.5), 41 (100).

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Charge Transfer Complexes From 1,2-Dithiolylium Salts, Electrical Conductivity and Structure

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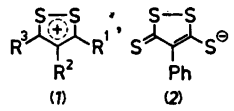
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It has been shown that the 4-phenyl-5-thioxo-1,2-dithiol-3-thiolate anion forms charge transfer salts with substituted 1,2-dithiolylium salts. Spectral data are given for a series of complexes. It is observed that the complex derived from the 3,5-diphenyl-1,2-dithiolylium ion has a relatively high d.c. conductivity. The structure of this complex *7* is determined by X-ray diffractometry.

The crystals of compound *7* belong to the orthorhombic space group *Pbca*. Cell dimensions are $a = 22.363(2)$, $b = 11.248(1)$, $c = 17.559(2)$ Å. Refinement by full-matrix least-squares calculations gave an R index of 0.068. Alternate packing of donor and acceptor molecules occurs in the axial directions with the highest packing densities. This explains the low conductivity of *7*.

A series of sulfur and selenium containing charge-transfer complexes related to the 1,3-dithiole series has been studied in the attempt to design organic conductors.[1–3]

Only a few charge-transfer complexes in the 1,2-dithiole series have hitherto been reported.[4,5] We have reported that 1,2-dithiolylium ions (*1*) form salts, which are charge transfer complexes, with 4-phenyl-5-thioxo-1,2-dithiole-3-thiolate anion.[5]



(*1*) (*2*)

The salts formed from the yellow cations and orange anion are dark-coloured compounds. It is not possible to observe any charge transfer bands in the UV-VIS spectrum of the complexes in the region 250 – 450 nm. If present, they are probably obscured by the intense absorption bands, *cf.* Fig. 1. However, it is evident that charge transfer has taken place as

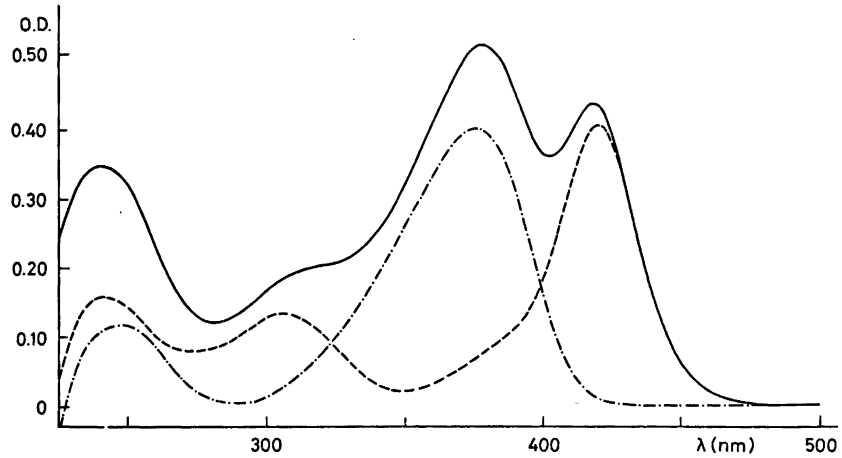


Fig. 1. VIS-spectrum of *7* ———, dimethylammonium 4-phenyl-5-thioxo-1,2-dithiole-3-thiolate – – – and 3,5-diphenyl-1,2-dithiolylium perchlorate —·— in CH_2Cl_2, $c = ca.\ 10^{-5}$ mol/l.

Table 1. 1,2-Dithiolylium-4-phenyl-5-thioxo-1,2-dithiole-3-thiolates.

	R^1	R^2	R^3	M.p./°C [a]	λ/nm(ε [b])					
1	4-$MeOC_6H_4$	H	H	154–155	310	(12 200);	427	(33 200)		
2	H	C_6H_5	H	110–111	325	(12 900);	432	(9800)		
3	C_6H_5	H	H	128–129	330	(13 600);	428	(14 500)		
4	4-BrC_6H_4	H	H	127–128	333	(14 700);	430	(13 300)		
5	4-FC_6H_4	H	H	128–129	333	(14 100);	430	(14 200)		
6	C_6H_5	H	CH_3	106–107	218	(16 000);	323	(12 600);	415	(8400)
7	C_6H_5	H	C_6H_5	214–215	381	(33 300);	420	(25 800)		
8	4-MeC_6H_4	H	C_6H_5	158–159	313	(13 900);	415	(39 800)		
9	4-$MeOC_6H_4$	H	C_6H_5	164–165	375	(18 900);	426	(47 300)		
10	MeS	H	C_6H_5	71–72	327	(14 700);	380	(17 200);	422	(18 200)
11	4-$MeOC_6H_4$	H	4-$MeOC_6H_4$	176–177	307	(13 000);	426	(23 400)		
12	C_6H_5	C_6H_5	H	112–113	303	(14 500);	428	(12 400)		
13	Me	Me	Me	179–180	300	(6800);	380	(7600);	419	(17 500)

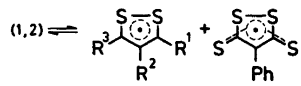
[a] All compounds show satisfactory elemental analysis. [b] 10^{-5} M solutions in CH_2Cl_2.

the extinction coefficient of the complex at a given wavelength is higher than the sum of the extinction coefficients of the starting ions at the same wavelength. This is further supported by a distinct shoulder in the absorption spectrum near 60000 nm, where none of the starting salts absorb.

It is found that the extinction coefficients are solvent dependent as they vary in the following order: $\varepsilon_{DMSO} > \varepsilon_{MeCN} > \varepsilon_{CHCl_3} > \varepsilon_{Dioxan}$ which is the same order as is found for the variation of the dielectric constant of the solvents, but no simple correlation between the extinction coefficient and the dielectric constant could be established.

The UV-VIS data of the complexes prepared are given in Table 1.

The charge transfer observed is probably due to the process shown in eqn. (1).



(1)

This assumption is supported by the observation that the complex derived from the 3,5-diphenyl-1,2-dithiolylium ion displays a weak ESR signal in the solid phase with an isotropic *g* value of 2.00. A *g* value of 2.003 has been observed for the electrochemically generated 3,5-diphenyl-1,2-dithiolyl radical in acetonitrile.[6]

The complex formed from the 3,5-diphenyl-1,2-dithiolylium ion has been found to have a specific d.c. resistance of 6×10^{12} ohm cm measured on a single crystal by the four probe technique along the crystallographic *b*-axis. The other complexes mentioned in Table 1 have a much higher resistance. This is consistent with the observation that the 3,5-diphenyl-1,2-dithiolylium ion is electrochemically reduced at a higher potential, −0.27 V,[6] than the 3-phenyl-1,2-dithiolylium and the 3,4-diphenyl-1,2-dithiolylium ions which are reduced at −0.45 and −0.46 V, respectively.[7] It has been assumed that electron-transfer calculated from solution redox potentials can be correlated to the conductivity in the solid phase.[8]

A prerequisite for an organic charge-transfer complex of the same type as TTF–TCNQ to be a good conductor is that the crystals are highly ordered, made up of segregated stacks of acceptor molecules and donor molecules, whereas mixing of acceptor and donor molecules in the stacks results in low conductivity.[8]

The crystal structure of *7* has its highest packing density in the directions of the *a* and *b* axes where the average distance between the 1,2-dithiole rings is 5.61 and 5.65 Å, respectively. The packing in these directions consists, however, of stacks with alternating donor and acceptor molecules. The packing in the direction of the *c* axis consists of isolated stacks of donor and acceptor molecules, but the

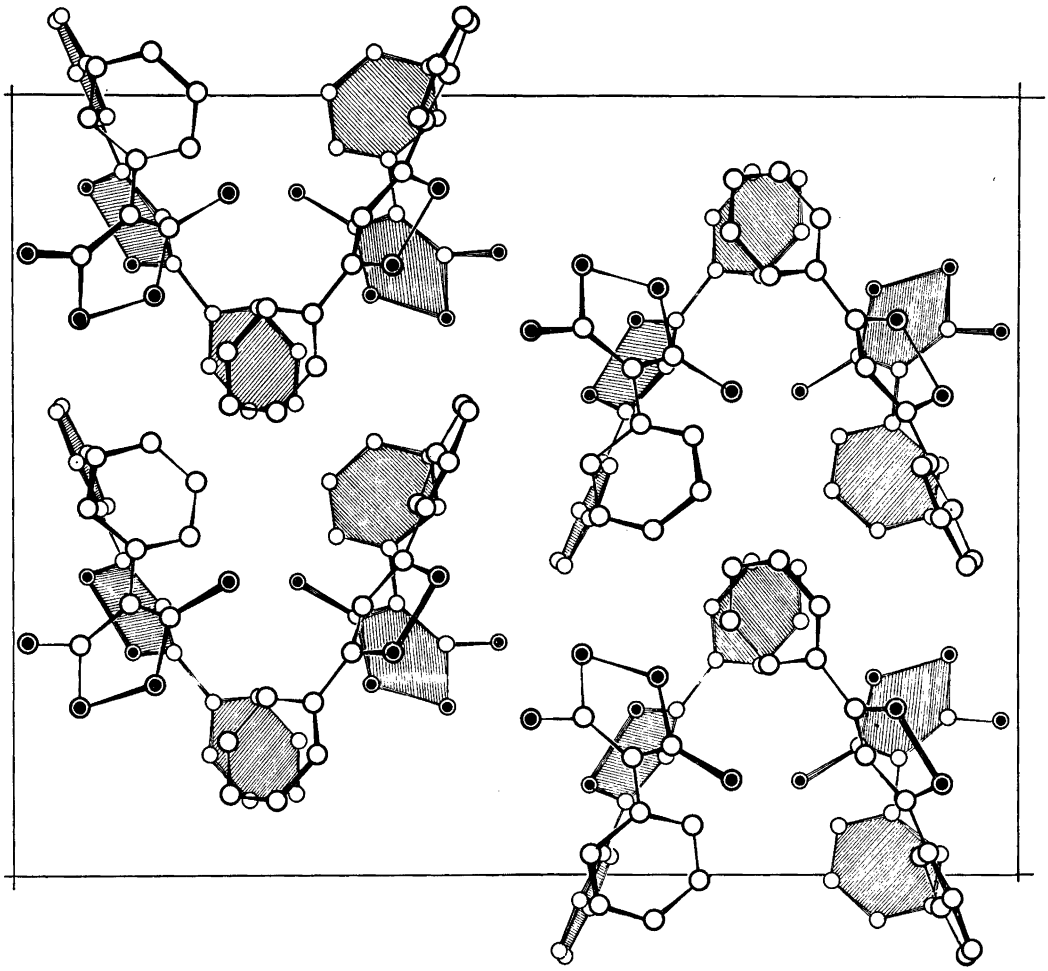


Fig. 2. The crystal structure of *7* as seen along the *b* axis.

distance between the dithiole rings here is 8.78 Å. This can be compared to the stacking distance between TTF molecules in TTF–TCNQ; this distance is 3.47 Å.[9] This explains the low conductivity of this complex.

From model considerations it is obvious that steric hindrance between H(52C) and H(4C) and H(36C) prevents the phenyl groups and the dithiole ring from being coplanar. As can be seen from the angles C(5C)–S(1C)–S(2C) and S(1C)–S(2C)–C(3C) (Table 3) the dithiole nucleus in the acceptor is not symmetrical, this is probably due to crystal effects rotating the phenyl groups out of the dithiole plane to various degrees (A,B = 16.8° and A,C = 24.8°, see Fig. 4) and thus the two phenyl groups do not contribute equally to the resonance of the system.

The structure of simple salts of the 3,5-diphenyl-1,2-dithiolylium ion has not been reported, but the results obtained for this complex are in good agreement with the structure published by Mason *et al.*[10] for bis(3,5-diphenyl-1,2-dithiolylium) tetrachloroferrate(II).

The dithiole part in the donor molecule has experimental, although not crystallographic, mirror symmetry and the phenyl group is rotated 55.93° out of the plane of the dithiole ring. Therefore, there is no mesomeric contribution from this phenyl group.

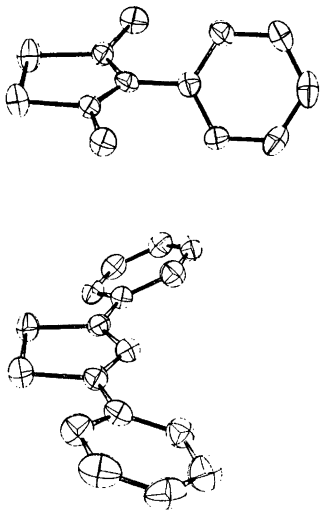
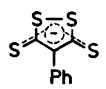


Fig. 3. Perspective drawing of the acceptor and donor molecules of *7*. Atoms are represented by ellipsoids enclosing 50 % of the thermal displacements.

Therefore, the correct form of the donor must be formulated as (*3*), and not as (*2*). The symmetrical form is also dominating in the dimethylammonium salt.[11] This is reflected in



(*3*)

the ^{13}C NMR spectrum of the salt which only displays two signals due to the dithiole carbons.

Table 2. Interatomic distances in Å. Estimated standard deviations in parentheses.

Atoms	Distance
S(1A) – S(2A)	2.065(2)
S(2A) – C(3A)	1.738(5)
C(3A) – C(4A)	1.410(7)
C(4A) – C(5A)	1.404(7)
C(5A) – S(1A)	1.735(5)
C(3A) – S(31A)	1.689(5)
C(5A) – S(51A)	1.697(5)
C(4A) – C(41A)	1.482(7)
C(41A) – C(42A)	1.397(7)
C(42A) – C(43A)	1.388(8)
C(43A) – C(44A)	1.385(9)
C(44A) – C(45A)	1.388(9)
C(45A) – C(46A)	1.393(8)
C(46A) – C(41A)	1.394(7)
S(1C) – S(2C)	2.017(2)
S(2C) – C(3C)	1.716(5)
C(3C) – C(4C)	1.374(7)
C(4C) – C(5C)	1.381(7)
C(5C) – S(1C)	1.713(5)
C(3C) – C(31C)	1.473(7)
C(5C) – C(51C)	1.468(7)
C(31C) – C(32C)	1.403(7)
C(32C) – C(33C)	1.374(7)
C(33C) – C(34C)	1.381(9)
C(34C) – C(35C)	1.378(9)
C(35C) – C(36C)	1.388(8)
C(36C) – C(31C)	1.402(7)
C(51C) – C(52C)	1.395(8)
C(52C) – C(53C)	1.386(8)
C(53C) – C(54C)	1.389(9)
C(54C) – C(55C)	1.366(9)
C(55C) – C(56C)	1.377(8)
C(56C) – C(51C)	1.404(7)

The H – C bond distances (Å) range from 0.89(6) to 1.10(7) (mean value 1.01).

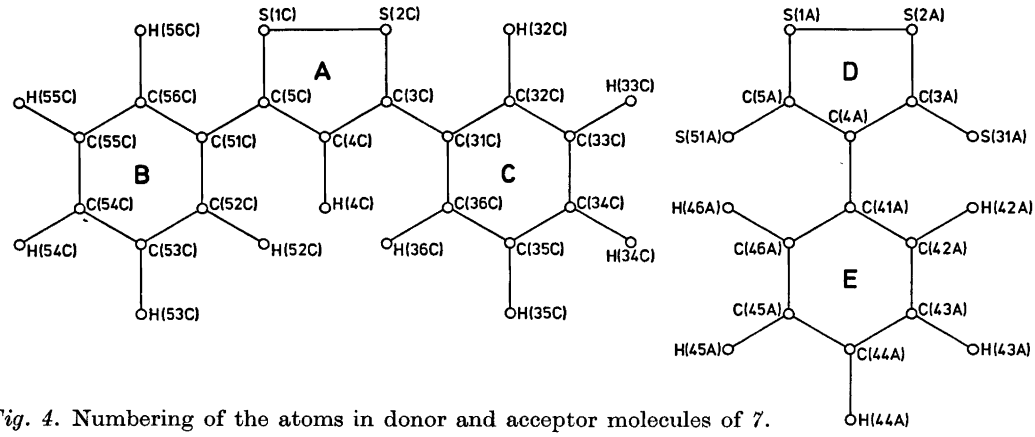


Fig. 4. Numbering of the atoms in donor and acceptor molecules of *7*.

EXPERIMENTAL

The compounds in Table 1 were prepared by mixing equimolar acetonitrile solutions of 1,2-dithiolylium perchlorates and dimethylammonium 4-phenyl-5-thioxo-1,2-dithiole-3-thiolate with stirring at room temperature; the precipitates were filtered after 5 min and then washed with acetonitrile and ether. The compounds obtained in this way were normally analytically pure.

The crystals used for the structure determinations were prepared by placing the two starting components in "the legs" of an H-shaped tube. The tube was carefully filled with acetonitrile and left for two weeks. After this time single crystals of the complex could be collected from the horizontal part of the H tube.

The electrical conductivity was found by four probe conductivity measurements using the contracting technique by Coleman.[12]

Accurate unit cell dimensions were determined by a least-squares refinement of data measured from a Guinier powder photograph taken with Cu$K\alpha_1$ radiation ($\lambda = 1.54050$ Å) and calibrated with quartz as an internal standard.

The space group *Pbca* (No. 61) was unequivocally established from the systematic absences. The three dimensional X-ray data were obtained using a three-circle Enraf-Nonius diffractometer and Zr filtered Mo$K\alpha$ radiation. A reflection was designated not observed if $I < 2\sigma(I)$. With this criterion 3880 out of 6316 (independently measured) reflections were regarded as observed. The temperature was 15 – 20 °C. A single reflection (14,0,0) was monitored every 10 reflections. Lorentz and polarization corrections were applied, but no extinction or absorption correction was made.

From the needle-shaped crystal a fragment measuring $0.5 \times 0.5 \times 0.75$ mm^3 was cut and mounted with the b axis parallel to the ϕ axis of the diffractometer, ω-scan was used. ω-scan range = 1.6°, ω-scan speed = 0.6° min^{-1}. The maximum value of sin θ/λ was 0.703 Å^{-1}.

All calculations were performed on an IBM 370/165 computer using the X-ray system.[13] The structure was solved by direct methods and the structural parameters were refined by full-matrix least squares. An E map computed with 616 phases revealed the 6 sulfur atoms and 12 carbon atoms. An electron density calculation based on the parameters from the identified E map atoms showed all the non-hydrogen atoms.

For the initial cycles of least-squares refinement, the single scale factor, individual isotropic temperature factors, and atomic coordinates for all non-hydrogen atoms were refined.

Position for the hydrogen atoms were calculated. The positional hydrogen parameters were refined in the last refinement cycles. The hydrogen atoms were given the same isotropic temperature factors as have the atoms to which they are bonded (initial individual isotropic refinement).

The quantity minimized in the final refinement cycles was $\sum w(|F_o| - |F_c|)^2$, unobserved reflections were not included. The weighting

Table 3. Bond angles in degrees with their estimated standard-deviations in parentheses.

Atoms	Angles (°)
C(5A) – S(1A) – S(2A)	95.6(2)
S(1A) – S(2A) – C(3A)	95.5(2)
S(2A) – C(3A) – C(4A)	115.8(3)
S(2A) – C(3A) – S(31A)	114.1(3)
S(31A) – C(3A) – C(4A)	130.0(4)
C(3A) – C(4A) – C(5A)	117.1(4)
C(3A) – C(4A) – C(41A)	121.2(4)
C(41A) – C(4A) – C(5A)	121.6(4)
C(4A) – C(5A) – S(1A)	115.9(4)
C(4A) – C(5A) – S(51A)	130.0(4)
S(51A) – C(5A) – S(1A)	114.1(3)
C(4A) – C(41A) – C(42A)	120.8(4)
C(4A) – C(41A) – C(46A)	120.7(4)
C(42A) – C(41A) – C(46A)	118.5(5)
C(41A) – C(42A) – C(43A)	120.6(5)
C(42A) – C(43A) – C(44A)	120.4(5)
C(43A) – C(44A) – C(45A)	119.8(5)
C(44A) – C(45A) – C(46A)	119.8(5)
C(45A) – C(46A) – C(41A)	121.0(5)
C(5C) – S(1C) – S(2C)	96.2(2)
S(1C) – S(2C) – C(3C)	95.2(2)
S(2C) – C(3C) – C(4C)	115.9(4)
S(2C) – C(3C) – C(31C)	116.5(4)
C(31C) – C(3C) – C(4C)	127.6(4)
C(3C) – C(4C) – C(5C)	117.7(5)
C(4C) – C(5C) – S(1C)	114.9(4)
C(4C) – C(5C) – C(51C)	127.3(5)
C(51C) – C(5C) – S(1C)	117.6(4)
C(3C) – C(31C) – C(32C)	121.5(4)
C(3C) – C(31C) – C(36C)	119.5(5)
C(32C) – C(31C) – C(36C)	118.9(5)
C(31C) – C(32C) – C(33C)	119.8(5)
C(32C) – C(33C) – C(34C)	121.0(5)
C(33C) – C(34C) – C(35C)	119.8(5)
C(34C) – C(35C) – C(36C)	120.3(5)
C(35C) – C(36C) – C(31C)	120.1(5)
C(5C) – C(51C) – C(52C)	120.4(5)
C(5C) – C(51C) – C(56C)	120.0(5)
C(52C) – C(51C) – C(56C)	119.5(5)
C(51C) – C(52C) – C(53C)	119.8(5)
C(52C) – C(53C) – C(54C)	120.3(5)
C(53C) – C(54C) – C(55C)	119.5(6)
C(54C) – C(55C) – C(56C)	121.7(5)
C(55C) – C(56C) – C(51C)	119.1(5)

The H – C – C bond angles (°) range from 111 (4) to 128 (4) (mean value 120).

Table 4. Distances of atoms (in Å) from least-squares planes. The planes are in the form $q_1x+q_2y+q_3z=q_4$. The coordinates x, y and z are expressed parallel to the orthorhombic axial directions a, b and c.[a]

Plane	q_1	q_2	q_3	q_4
A	0.73511	−0.46027	−0.49777	−0.64045
B	0.58899	−0.69741	−0.40831	−1.60566
C	0.91081	−0.07907	−0.40518	1.30052
D	0.58049	−0.63787	−0.50612	1.53814
E	0.34186	0.80608	0.48308	6.87636

S(1C)	.006	C(51C)	.013	C(31C)	.004	S(1A)	.004	C(41A)	−.006
S(2C)	−.009	C(52C)	−.012	C(32C)	−.005	S(2A)	−.002	C(42A)	.000
C(3C)	.010	C(53C)	.002	C(33C)	.006	C(3A)	−.001	C(43A)	.004
C(4C)	−.005	C(54C)	.007	C(34C)	−.006	C(4A)	.005	C(44A)	−.002
C(5C)	−.003	C(55C)	−.006	C(35C)	.005	C(5A)	−.007	C(45A)	−.004
*C(31C)	−.009	C(56C)	−.004	C(36C)	−.004	*S(31A)	.015	C(46A)	.008
*C(51C)	.024	*C(5C)	.077	*C(3C)	−.061	*S(51A)	−.021	*C(4A)	.007
*H(4C)	.11	*H(52C)	−.13	*H(32C)	.18	*C(41A)	.028	*H(42A)	−.01
		*H(53C)	−.03	*H(33C)	.14			*H(43A)	−.11
		*H(54C)	−.06	*H(34C)	−.06			*H(44A)	− 03
		*H(55C)	−.08	*H(35C)	−.17			*H(45A)	.06
		*H(56C)	−.01	*H(36C)	.08			*H(46A)	−.06

[a] Atoms marked with * were not included in the calculations of the least-squares planes.

Table 5. Final atomic coordinates with estimated standard deviations in parentheses.

Atom	x	y	z	Atom	x	y	z
S(1A)	.43463(7)	.22339(13)	.28092(8)	C(31C)	.0897(2)	.3628(4)	.0281(3)
S(2A)	.36007(7)	.12440(12)	.25269(9)	C(32C)	.0649(2)	.3019(5)	.9659(3)
S(31A)	.28476(6)	.14906(12)	.12080(8)	C(33C)	.0462(2)	.3641(6)	.9029(3)
S(51A)	.48599(7)	.42078(12)	.19942(8)	C(34C)	.0500(3)	.4866(6)	.9003(3)
C(3A)	.3449(2)	.2006(4)	.1688(3)	C(35C)	.0743(3)	.5479(5)	.9608(4)
C(4A)	.3845(2)	.2939(4)	.1507(3)	C(36C)	.0937(3)	.4872(5)	.0251(3)
C(5A)	.4312(2)	.3164(4)	.2022(3)	C(51C)	.1996(2)	.2846(5)	.2774(3)
C(41A)	.3772(2)	.3648(4)	.0802(3)	C(52C)	.2449(3)	.3694(5)	.2714(3)
C(42A)	.3237(2)	.4245(5)	.0652(3)	C(53C)	.2858(3)	.3828(6)	.3300(4)
C(43A)	.3173(3)	.4919(5)	.9995(3)	C(54C)	.2811(3)	.3142(6)	.3956(3)
C(44A)	.3638(3)	.5005(5)	.9476(3)	C(55C)	.2356(3)	.2337(6)	.4019(3)
C(45A)	.4173(3)	.4419(5)	.9618(3)	C(56C)	.1945(3)	.2171(6)	.3444(3)
C(46A)	.4239(2)	.3755(5)	.0282(3)	H(4C)	.176(3)	.417(6)	.144(4)
H(42A)	.288(3)	.417(6)	.105(4)	H(32C)	.069(3)	.214(6)	.964(4)
H(43A)	.281(3)	.526(6)	.982(4)	H(33C)	.037(3)	.316(6)	.864(4)
H(44A)	.355(3)	.555(7)	.901(4)	H(34C)	.032(3)	.526(6)	.850(4)
H(45A)	.455(3)	.454(6)	.922(4)	H(35C)	.073(3)	.631(6)	.971(4)
H(46A)	.458(3)	.331(6)	.036(4)	H(36C)	.114(3)	.531(6)	.066(4)
S(1C)	.11771(7)	.13506(12)	.21392(8)	H(52C)	.250(3)	.429(6)	.232(4)
S(2C)	.07408(7)	.16596(12)	.11530(8)	H(53C)	.320(3)	.449(6)	.324(4)
C(3C)	.1082(2)	.3000(4)	.0978(3)	H(54C)	.310(3)	.328(7)	.443(4)
C(4C)	.1480(2)	.3379(5)	.1522(3)	H(55C)	.227(3)	.186(6)	.450(4)
C(5C)	.1580(2)	.2646(4)	.2140(3)	H(56C)	.160(3)	.157(6)	.347(4)

scheme was $w = 1.0/1 + (F_o - B)/A)^2$ with $A = 80$ and $B = 40$. The resulting R index ($R = \sum||F_o| - |F_c||/\sum|F_o|$) was 6.8 %.

Atomic scattering factors were taken from *International Tables*.[14]

Crystal data. 3,5-diphenyl-1,2-dithiolylium-4-phenyl-5-thioxo-1,2-dithiole-3-thiolate ($C_{24}H_{16}S_6$) has a formula weight of 496.78. The crystals form black needles.

Space group *Pbca* (No. 61), $a = 22.363(2)$ Å, $b = 11.248(1)$ Å, $c = 17.559(2)$ Å, $Z = 8$, $D_m = 1.50$ g cm^{-3}, $D_c = 1.49$ g cm^{-3}. $\mu(MoK\alpha) = 6.65$ cm^{-1}.

A list of observed and calculated structure factors is available on request from Ole Simonsen, Kemisk Institut, Odense Universitet, Niels Bohrs Alle 75, DK-5000 Odense.

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Table 6. Thermal parameters μ_{ij} (Å)^2 with estimated standard deviations in parentheses. The expression used is: exp $[-2\pi^2(h^2a^{*2}U_{11} + \cdots + 2hka^*b^*U_{12} + \cdots)]$.

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S(1A)	.0549(9)	.0412(8)	.0402(7)	−.0024(6)	−.140(6)	.0082(6)
S(2A)	.0587(9)	.0351(7)	.0416(7)	−.0070(6)	−.0065(7)	.0089(6)
S(31A)	.376(7)	.0394(7)	.0445(8)	−.0029(6)	−.0029(6)	−.0053(6)
S(51A)	.0424(8)	.0429(8)	.0420(7)	−.0049(6)	−.0017(6)	−.0015(6)
C(3A)	.037(3)	.027(2)	.027(2)	.004(2)	.002(2)	.001(2)
C(4A)	.029(2)	.028(2)	.038(2)	.008(2)	.001(2)	.000(2)
C(5A)	.033(2)	.030(2)	.032(2)	.002(2)	.003(2)	.001(2)
C(41A)	.037(2)	.030(2)	.030(2)	.001(2)	−.002(2)	−.003(2)
C(42A)	.041(3)	.039(3)	.042(3)	.010(2)	−.003(2)	.002(2)
C(43A)	.049(3)	.047(3)	.048(3)	.008(3)	−.011(3)	.010(3)
C(44A)	.076(4)	.037(3)	.032(3)	.001(3)	−.012(3)	.003(2)
C(45A)	.058(4)	.051(3)	.029(2)	−.002(3)	.010(2)	.000(2)
C(46A)	.042(3)	.043(3)	.035(3)	.011(2)	.007(2)	.000(2)
S(1C)	.0503(8)	.0364(7)	.0416(8)	−.0069(6)	−.0041(6)	.0033(6)
S(2C)	.0445(8)	.0354(7)	.0426(7)	−.0099(6)	−.0045(6)	−.0008(6)
C(3C)	.028(2)	.034(2)	.036(2)	.000(2)	.000(2)	−.003(2)
C(4C)	.033(2)	.037(3)	.040(3)	.000(2)	−.001(2)	−.002(2)
C(5C)	.035(2)	.031(2)	.038(3)	.000(2)	.004(2)	−.005(2)
C(31C)	.032(3)	.032(2)	.040(3)	−.002(2)	.002(2)	.000(2)
C(32C)	.034(3)	.041(3)	.040(3)	−.006(2)	.001(2)	−.003(2)
C(33C)	.040(3)	.056(4)	.036(3)	−.007(3)	−.001(2)	−.002(3)
C(34C)	.041(3)	.056(3)	.039(3)	−.006(3)	−.001(2)	.010(3)
C(35C)	.045(3)	.035(3)	.063(4)	−.003(3)	−.008(3)	.007(3)
C(36C)	.046(3)	.041(3)	.043(3)	−.001(2)	−.008(2)	−.003(2)
C(51C)	.040(3)	.033(3)	.038(3)	.006(2)	.001(2)	−.002(2)
C(52C)	.046(3)	.038(3)	.040(3)	.002(2)	−.003(2)	−.006(2)
C(53C)	.058(4)	.052(4)	.046(3)	−.003(3)	−.004(3)	−.014(3)
C(54C)	.047(3)	.070(4)	.042(3)	.008(3)	−.016(3)	−.017(3)
C(55C)	.062(4)	.068(4)	.037(3)	.015(3)	−.005(3)	.003(3)
C(56C)	.045(3)	.053(3)	.041(3)	−.001(3)	−.002(2)	.001(3)

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The Crystal Structure of 1,4-Etheno-2-hydroxy-2,4a,6,9-tetramethyloctahydronaphthal-5-ene-3,7,8-trione

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The crystal structure of the title compound, $C_{16}H_{18}O_4$, has been determined from 2077 independent X-ray diffractometer reflexion data. The crystals are yellow and have monoclinic symmetry; the unit cell, with the dimensions $a = 13.067$ Å, $b = 8.463$ Å, $c = 13.950$ Å, $\beta = 116.23°$, contains four molecules. The structure was refined, in space group $P2_1/c$, to a final value of $R = 0.053$ by a full-matrix least-squares procedure.

The Diels-Alder product 1,4-etheno-2-hydroxy-2,4a,6,9-tetramethyloctahydronaphthal-5-ene-3,7,8-trione is an adduct of 2,4-dimethyl-*o*-quinol and 3,5-dimethyl-*o*-quinone, both formed upon periodate oxidation of 2,4-dimethylphenol. The structure is rather strained, having C(sp^3)–C(sp^3) bond lengths of 1.564, 1.553 and 1.580 Å for the bonds C(1)–C(8a), C(8a)–C(4a) and C(4)–C(4a), respectively.

Periodate oxidation of 2,4-dimethylphenol yields, among other compounds, the Diels-Alder product 1,4-etheno-2-hydroxy-2,4a,6,9-tetramethyloctahydronaphthal-5-ene-3,7,8-trione, which is a spontaneous adduct of the oxidation products 2,4-dimethyl-*o*-quinol (diene) and 3,5-dimethyl-*o*-quinone (philodiene).[1]

This investigation has been undertaken as part of a program concerned with the factors governing the highly stereospecific addition reactions of oxidized phenols. This matter has been discussed by, *e.g.*, Holmberg.[2] The specific aim of the X-ray analysis of the title compound was to establish the steric arrangement around carbon atom C(2) and to confirm the structure of the adduct.

EXPERIMENTAL

Unit cell dimensions were obtained, in least-squares refinement, from angular positions of 25 accurately centered reflexions on a computer-controlled Philips PW1100 diffractometer. Crystal data are: Space group $P2_1/c$, $a = 13.067(2)$, $b = 8.463(1)$, $c = 13.950(1)$ Å, $\beta = 116.23(2)°$, $V = 1384$ Å^3, $Z = 4$, $\varrho_{calc} = 1.32$ g cm^{-3}, $\mu = 7.80$ cm^{-1}. Intensity data were collected with the $\theta - 2\theta$ technique for $\theta \leq 70°$, with graphite monochromatized Cu$K\alpha$ radiation. Backgrounds were estimated by stationary counting at $\Delta\theta = \pm 0.75°$ from the reflexion maxima. The scan speed was 0.02° s^{-1}. Three monitor reflexions were measured every 60 min. No systematic intensity variation was noticed. Of the 2616 independent reflexions measured, 2077 were considered to be significantly above background, having $I_{net} \geq 4\sigma(I_{net})$, where $\sigma(I_{net})$ is based on counter statistics. Lorentz and polarisation factors were applied, but no correction for absorption.

STRUCTURE DETERMINATION AND REFINEMENT

The structure was solved by the automatic MULTAN direct phase determination procedure[3] using the 200 highest normalized structure factor magnitudes obtained from Wilson's method. The phase set having the highest reliability index revealed all non-hydrogen atoms.

Positional and thermal parameters were refined by the full-matrix least-squares method. A difference synthesis calculated at an R value of 0.09 displayed 16 of the 18 hydrogen atoms. The remaining two were positioned by chemical reasoning. Subsequent least squares refinements, using anisotropic temperature factors for the non-hydrogen atoms and fixed isotropic temperature factors for the hydrogens, gave a final R value of 0.053 for the 2077 reflexions.

Table 1. Positional and anisotropic thermal parameters ($\times 10^4$) of the non-hydrogen atoms. The B-values refer to the temperature factor expression $\exp[-(h^2B11+k^2B22+l^2B33+hkB12+hlB13+klB23)]$. Estimated standard deviations are given in parentheses.

ATOM	X	Y	Z	B11	B22	B33	B12	B13	B23
C(1)	2847(2)	1995(3)	7642(2)	51(2)	101(3)	34(1)	-7(3)	39(2)	8(3)
C(2)	2943(2)	2732(3)	8692(2)	56(2)	111(3)	38(1)	-7(3)	40(2)	-10(3)
C(3)	3016(2)	1342(3)	9435(2)	41(2)	145(3)	34(1)	-9(3)	29(2)	2(3)
C(4)	2884(2)	-259(3)	8915(2)	57(2)	115(3)	36(1)	-1(3)	42(2)	25(3)
C(4A)	1713(2)	-309(3)	7871(2)	52(2)	120(3)	35(1)	-22(3)	44(2)	-2(3)
C(5)	1645(2)	-1927(3)	7409(2)	66(2)	119(3)	45(2)	-29(4)	54(3)	5(3)
C(6)	1688(2)	-2341(3)	6501(2)	68(2)	125(3)	50(2)	-12(4)	60(3)	-15(3)
C(7)	1765(2)	-1103(3)	5808(2)	91(2)	154(4)	45(2)	-31(4)	75(3)	-24(4)
C(8)	1498(2)	573(3)	6013(2)	64(2)	137(4)	34(1)	-37(4)	33(2)	14(3)
C(8A)	1699(2)	1058(3)	7122(2)	48(2)	112(3)	37(1)	3(3)	36(2)	5(3)
C(9)	3839(2)	851(3)	7942(2)	47(2)	129(3)	44(2)	0(3)	44(2)	1(3)
C(10)	3845(2)	-295(3)	8591(2)	48(2)	127(3)	45(2)	21(4)	34(2)	10(3)
C(11)	3975(3)	3806(4)	9212(2)	89(2)	146(4)	55(2)	-75(5)	60(3)	-34(4)
O(12)	1926(2)	3591(2)	8460(2)	83(2)	165(3)	45(1)	67(3)	55(2)	-12(3)
O(13)	3194(2)	1548(2)	10356(1)	86(2)	183(3)	38(1)	-34(3)	54(2)	-14(3)
C(14)	720(2)	-126(4)	8173(2)	53(2)	190(5)	53(2)	-38(4)	58(3)	-19(4)
C(15)	1641(3)	-4022(3)	6154(3)	126(3)	132(4)	74(2)	-5(5)	120(4)	-25(5)
O(16)	1991(3)	-1336(3)	5057(2)	240(4)	207(4)	97(2)	-20(6)	251(4)	-22(4)
O(17)	1123(2)	1482(2)	5270(2)	154(3)	172(3)	43(1)	-28(4)	55(3)	30(3)
C(18)	4708(2)	1084(4)	7529(3)	66(2)	207(5)	70(2)	35(5)	87(3)	47(5)

Table 2. Positional parameters ($\times 10^3$) and isotropic temperature factors ($\times 10^2$) for the hydrogen atoms. Estimated standard deviations for the positional parameters are given in parentheses.

ATOM	X	Y	Z	B
H(C1)	285(2)	283(3)	715(2)	243
H(C4)	293(2)	-110(3)	937(2)	275
H(C5)	161(2)	-283(3)	786(2)	304
H(C8A)	109(2)	180(3)	702(2)	253
H(C10)	439(2)	-112(3)	883(2)	284
H1(C11)	388(3)	465(4)	878(2)	407
H2(C11)	397(3)	428(3)	985(2)	407
H3(C11)	462(3)	323(3)	935(2)	407
H(O12)	195(2)	392(3)	904(3)	364
H1(C14)	9(3)	-19(3)	761(2)	373
H2(C14)	73(2)	-109(3)	863(2)	373
H3(C14)	74(2)	92(4)	849(2)	373
H1(C15)	93(3)	-423(3)	549(3)	403
H2(C15)	229(3)	-423(3)	601(2)	403
H3(C15)	160(2)	-471(3)	665(2)	403
H1(C18)	505(3)	213(4)	774(2)	397
H2(C18)	445(3)	122(3)	686(3)	397
H3(C18)	527(3)	34(4)	779(2)	397

Hughes' weighting procedure [4] with $F_{o,min}=2.0$ was applied. Scattering factors for the non-hydrogen atoms were taken from the *International Tables for X-Ray Crystallography* [5] and that for hydrogen from Stewart, Davidson and Simpson.[6] The final positional and thermal parameters for the non-hydrogen atoms are given in Table 1, and those for the hydrogen atoms in Table 2. A list of the observed and calculated structure factors is available from the authors on request.

RESULTS AND DISCUSSION

A stereoscopic view of the molecule is given in Fig. 1, which shows that the addition has followed the *endo*-rule. The configuration around carbon atom C(2) is in harmony with that found in our X-ray investigations of related compounds. [7–10] The more polarizable hydroxyl substituent is oriented *anti* to the etheno bridge, in accordance with the results from Williamson *et al.*,[11] concomitantly leaving the bulkier methyl group to be directed away from the reaction center.

Intramolecular distances and bond angles involving the non-hydrogen atoms are given

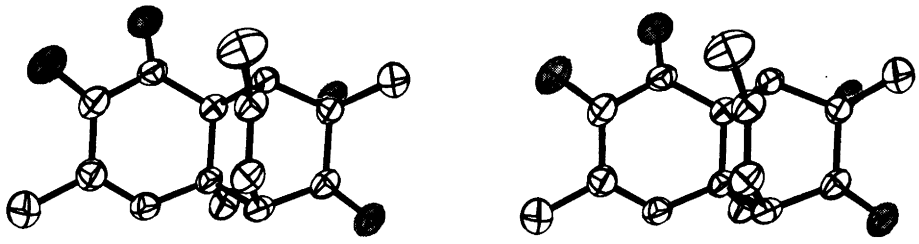


Fig. 1. A stereoscopic view of the molecule. Hydrogen atoms have been omitted. The oxygen atoms are represented by shaded ellipsoids.

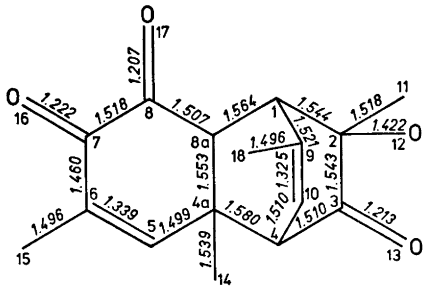


Fig. 2. Bond distances in the molecule. Standard deviations are estimated to be ≤ 0.003 Å.

in Fig. 2 and Table 3, respectively. The hydrogen atoms lie in chemically reasonable positions, with a mean C–H distance of 0.95 Å, in good agreement with values found by Stewart, Davidson and Simpson.[6] No correction of the measured distances for riding thermal motion has been applied. The lengthened bonds around C(4a) and C(8a) are consonant with observations in similar structures.[7–10] This effect is probably due to internal strain in the molecule.

The arrangement of molecules in the unit cell is given in Fig. 3. All intermolecular contacts are normal van der Waals distances.

Some general structural features found in molecules of this type will be given in a forthcoming article.

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Table 3. Bond angles (°) involving non-hydrogen atoms. Standard deviations are estimated to be $\leq 0.3°$.

C(2) – C(1) – C(8a)	107.7
C(2) – C(1) – C(9)	107.1
C(8a) – C(1) – C(9)	109.4
C(1) – C(2) – C(3)	106.5
C(1) – C(2) – C(11)	112.2
C(1) – C(2) – O(12)	108.4
C(3) – C(2) – C(11)	110.9
C(3) – C(2) – O(12)	108.4
C(11) – C(2) – O(12)	110.2
C(2) – C(3) – C(4)	113.6
C(2) – C(3) – O(13)	121.9
C(4) – C(3) – O(13)	124.4
C(3) – C(4) – C(4a)	109.0
C(3) – C(4) – C(10)	103.2
C(4a) – C(4) – C(10)	108.5
C(4) – C(4a) – C(5)	105.7
C(4) – C(4a) – C(8a)	108.6
C(4) – C(4a) – C(14)	109.6
C(5) – C(4a) – C(8a)	114.3
C(5) – C(4a) – C(14)	108.2
C(8a) – C(4a) – C(14)	110.4
C(4a) – C(5) – C(6)	128.8
C(5) – C(6) – C(7)	118.9
C(5) – C(6) – C(15)	122.8
C(7) – C(6) – C(15)	118.2
C(6) – C(7) – C(8)	117.2
C(6) – C(7) – O(16)	124.4
C(8) – C(7) – O(16)	118.3
C(7) – C(8) – C(8a)	120.0
C(7) – C(8) – O(17)	118.3
C(8a) – C(8) – O(17)	121.9
C(1) – C(8a) – C(4a)	110.0
C(1) – C(8a) – C(8)	108.1
C(4a) – C(8a) – C(8)	115.6
C(1) – C(9) – C(10)	113.6
C(1) – C(9) – C(18)	120.7
C(10) – C(9) – C(18)	125.7
C(4) – C(10) – C(9)	115.8

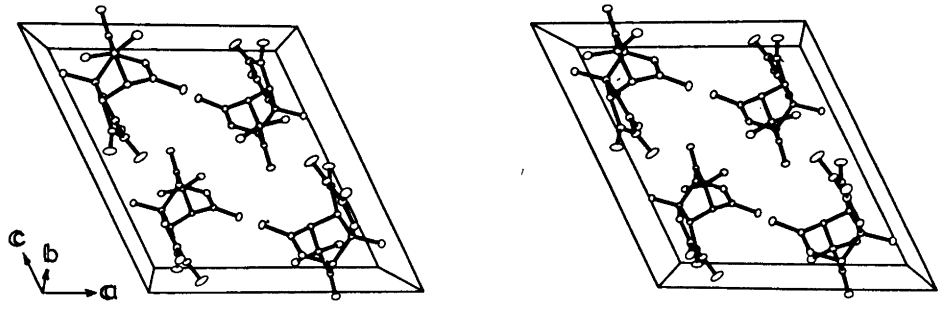


Fig. 3. A stereoscopic view of the molecular packing.

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Chemical Studies on Bryophytes. 18. Luteolin 7-*O*-Neohesperidoside-4′-*O*-sophoroside, another New Tetraglycoside from *Hedwigia ciliata*

BENGT-GÖRAN ÖSTERDAHL and GÖSTA LINDBERG

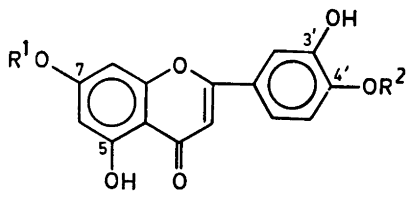
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A new luteolin tetraglycoside from the moss *H. ciliata* has been identified. The structure is luteolin 7-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside-4′-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside (*1*) as determined using spectroscopic methods, sugar sequence analysis by GLC-MS and hydrolytic experiments.

In a previous contribution,[1] the isolation and separation of twelve flavonoid compounds from the moss *H. ciliata* and the structural determination of one of these, a luteolin 7,4′-di-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (*2*), has been described. This paper reports the structure of another new luteolin tetraglycoside from the moss *H. ciliata*.

Acidic hydrolysis of *1* gave luteolin, glucose and rhamnose. UV spectral studies with diagnostic shift reagents show that the sugars are linked to the aglycone at the 7- and 4′-positions.[2] This was confirmed by permethylation followed by hydrolysis, whereby 5,3′-di-*O*-methylluteolin was obtained. The high R_F value in 15 % HOAc, and the elemental analysis indicated that *1* is probably a luteolin tetraglycoside.

Mass spectroscopy of permethylated *1* gave peaks indicating that *1* has both a rhamnoglucoside unit, *m/e* 393 and *m/e* 361, and a diglucoside unit, *m/e* 423 and *m/e* 391.[3] There are also peaks from a terminal glucose unit, *m/e* 219, *m/e* 187 and *m/e* 155, and peaks from a terminal rhamnose unit, *m/e* 189, *m/e* 157 and *m/e* 125.



	R^1	R^2
1	neohesperidosyl [a]	sophorosyl [b]
1a	neohesperidosyl [a]	β-D-glucosyl
1b	β-D-glucosyl	sophorosyl [b]
1c	neohesperidosyl [a]	H
1d	β-D-glucosyl	H
1e	H	sophorosyl [b]
2	neohesperidosyl [a]	neohesperidosyl [a]

[a] (2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosyl.
[b] (2-*O*-β-D-glucopyranosyl)-β-D-glucopyranosyl.

The ^{1}H NMR spectrum of the TMS ether of *1* showed that it is a luteolin tetraglycoside on the basis of the four sugar C-1 proton doublets at δ 5.52, 5.15, 4.86 and 4.55. The doublet at δ 1.21 and the signal at δ 4.86 ($J = 2$ Hz), indicate the presence of one α-rhamnose unit in 1.[2] The other three C-1 proton doublets ($J = 6$ Hz) must derive from glucose, since this coupling constant is characteristic for the C-1 proton of β-linked glucose.[4] Furthermore, the values of δ 5.52 and 5.15 for two of the C-1 protons indicate that two β-glucose units are linked to luteolin.

To establish the position of the interglycosidic linkages, a method described earlier [1,7–9] was used. After permethylation and hydrolysis,

Table 1. GLC-MS analysis data of the methylated alditol acetates. *T* values are relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

Alditol acetate	*T*	*T* [9]	Ratio of peak areas	Prominent fragments, *m/e*
1,5-Di-*O*-acetyl-2,3,4-tri-*O*-methyl-L-rhamnitol	0.47	0.46	1.2	175, 161, 131, 117, 115, 101, 89, 43
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	0.99	1.00	1.0	205, 161, 145, 129, 117, 101, 99, 87, 71, 45, 43
1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-glucitol	1.90	1.98	2.0	205, 189, 161, 145, 129, 101, 99, 87, 45, 43

the methylated sugars were separated from the aglycon by polyamide column chromatography, and then reduced and acetylated. GLC-MS analysis gave three different alditol acetates in the peak area ratio of 1:1:2 (Table 1). This analysis indicates that *1* has one rhamnose and one glucose as terminal sugar units and two glucose units substituted in the 2-position.

Enzymatic hydrolysis of *1* with β-glucosidase was very slow and gave luteolin 7-*O*-neohesperidoside [luteolin-7-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside] (*1c*), identified by UV spectral studies, sugar determination and partial hydrolysis. The slow enzymatic hydrolysis of *1* indicated a sophoroside [(2-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside] unit in the 4′-position.[5] These results suggest that *1* is a luteolin-7-*O*-neohesperidoside-4′-*O*-sophoroside. This was confirmed by identification of sophorose and neohesperidose after H_2O_2 oxidation of *1*,[6] and by identification of the products obtained on acid hydrolysis (Table 2). Enzymatic hydrolysis of the partially hydrolyzed products show that the three glucose units in *1* are β-D-glucose.

The optical rotation value, $[\alpha]_D^{25}$ −90.1° (*c* 1.34, H_2O), of *1* compared with the isolated luteolin 7,4′-di-*O*-neohesperidoside (*2*),[1] $[\alpha]_D^{25}$ −86.5° (*c* 1.59, H_2O), indicated that the rhamnose unit in *1* must be α-L-rhamnose, since the contribution of the rotation from an α-L-rhamnose is of nearly the same magnitude and in the same direction as with a β-D-glucose.[10]

Considering these data, the structure of *1* is proposed to be luteolin 7-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glycopyranoside-4′-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside.

EXPERIMENTAL

NMR, UV and mass spectra were recorded as described earlier.[1] Isolation and separation of the flavonoids from the moss *H. ciliata* were described in the previous paper of this series.[1] Solvent systems: BuOH − HOAc − H_2O, 6:1:2 (BAW), *t*-BuOH − HOAc − H_2O, 3:1:1 (TBA), BuOH − HOAc − H_2O, 4:1:5 top layer (BAW/4:1:5).

Luteolin 7-O-neohesperidoside-4′-O-sophoroside (1). Gel filtration on Sephadex G-25 with EtOH − H_2O (1:1) as eluent and recrystallization from EtOH − H_2O (1:1) gave 0.56 g of *1*. Drying at 110 °C/0.1 mmHg over P_2O_5 gave a very hygroscopic compound, m.p. 219 − 223 °C, $[\alpha]_D^{25}$ −90.1° (*c* 1.34, H_2O). Found: C 50.68; H 5.75. Calc. for $C_{39}H_{60}O_{25}$: C 50.98; H 5.49. UV (99.9 % MeOH): 269, 334; (+ $AlCl_3$): 277, 293 sh, 349, 379 sh; (+ $AlCl_3$ − HCl): 278, 292 sh, 344, 379 sh; (+ MeONa): 268, 375; (+ NaOAc): 266, 328; (+ NaOAc − H_3BO_3): 266, 330 nm. R_F values, see Table 2.

Acidic hydrolysis of *1* with 6 % HCl at 100 °C for 1 h gave luteolin, glucose and rhamnose. These were identified by co-chromatography with authentic samples.

Partial hydrolysis of *1* with 6 % HCl at room temperature for 10 days gave, besides luteolin and *1*, four intermediates; *1a*, *1b*, *1c*, and *1d*, where *1c* was obtained in a larger quantity than the others. The partially hydrolyzed products were separated by PC in 15 % HOAc and gel filtrated. R_F values, spot colour in presence of NH_3 in UV light, and results from partial acid hydrolysis of the intermediates are shown in Table 2.

Enzymatic hydrolysis was carried out at 37 °C in an acetate buffer solution (pH 5.0). *1* was slowly hydrolyzed to *1c*; only slight hydrolysis was observed after 24 h and it was not hydrolyzed completely even after 20 days; *1a* was rapidly hydrolyzed (< 2 h) to *1c*; *1b* was rapidly hydrolyzed (< 2 h) to *1e*, which was slowly hydrolyzed to luteolin. Finally, *1d* was rapidly hydrolyzed (< 2 h) to luteolin.

Table 2. R_F values and acidic hydrolysis data of *1* and its partially hydrolyzed products.

Compound		Spot colour NH_3/UV	R_F values [a] 15 % HOAc	BAW	TBA	Partial acidic hydrolysis products
1	Luteolin 7-*O*-neohesperidoside-4′-*O*-sophoroside	purple	0.63	0.14	0.08	*1a*, *1b*, *1c*, *1d* and luteolin
1a	Luteolin 7-*O*-neohesperidoside-4′-*O*-glucoside	purple	0.49	0.27	0.20	*1c*, *1d* and luteolin
1b	Luteolin 7-*O*-glucoside-4′-*O*-sophoroside	purple	0.33	0.13	0.07	*1d* and luteolin
1c	Luteolin 7-*O*-neohesperidoside	yellow	0.19	0.42	0.41	*1d* and luteolin
1d	Luteolin 7-*O*-glucoside	yellow	0.06	0.37	0.29	luteolin
1e	Luteolin 4′-*O*-sophoroside	purple	0.17	0.40	0.34	luteolin

[a] R_F values on 0.1 mm pre-coated cellulose TLC plates.

UV spectral data. 1a (99.9 % MeOH): 266, 270, 334; (+ $AlCl_3$): 266, 272, 293 sh, 349, 383 sh; (+ $AlCl_3$ – HCl): 266, 272, 293 sh, 345, 383 sh; (+ MeONa): 266, 269, 375; (+ NaOAc): 266, 269, 333; (+ NaOAc – H_3BO_3): 266, 269, 334 nm. *1b* (99.9 % MeOH): 266, 269, 334; (+ $AlCl_3$): 266, 276, 294 sh, 349, 381 sh; (+ $AlCl_3$ – HCl): 267, 276, 294 sh, 344, 381 sh; (+ MeONa): 267 sh, 272, 378; (+ NaOAc): 267, 270, 333; (+ NaOAc – H_3BO_3): 267, 270, 333 nm. *1c* (99.9 % MeOH): 253, 267, 348; (+ $AlCl_3$): 274, 295 sh, 332, 431; (+ $AlCl_3$ – HCl): 274, 295 sh, 361, 388; (+ MeONa): 268, 405; (+ NaOAc): 259, 360 sh, 405; (+ NaOAc – H_3BO_3): 259, 371 nm. *1d* (99.9 % MeOH): 252, 266, 269 sh, 347; (+ $AlCl_3$): 267 sh, 273, 295 sh, 333, 427; (+ $AlCl_3$ – HCl): 266, 273, 295 sh, 360, 385; (+ MeONa): 266, 269 sh, 403; (+ NaOAc): 257, 265, 269 sh, 403; (+ NaOAc – H_3BO_3): 257, 265 sh, 269 sh, 370 nm. *1e* (99.9 % MeOH): 269, 331; (+ $AlCl_3$): 277, 293 sh, 348, 385 sh; (+ $AlCl_3$ – HCl): 275, 293 sh, 344, 385 sh; (+ MeONa): 267, 357; (+ NaOAc): 274, 317 sh, 351; (+ NaOAc – H_3BO_3): 269, 332 nm.

H_2O_2 oxidation. Two mg of *1* was dissolved in 0.5 ml of 0.1 M NH_3 and two drops of H_2O_2 (35 %) was added. After 4 h at room temperature the solution was chromatographed on Whatman No. 1 paper in BAW/4:1:5 for 48 h with glucose as reference.[6] H_2O_2 oxidation of *1* gave sophorose, R_g value 0.59 (lit.[6] 0.58), and neohesperidose, R_g value: 0.83 (lit.[6] 0.87). On comparison *2* gave only neohesperidose with R_g value 0.84, as expected.

The TMS ether was prepared according to standard procedures.[2] ^1H NMR (100 MHz, CCl_4): δ 7.38 (H6′), 7.27 (H2′), 7.14 (H5′), 6.68 (H8), 6.33 (H6), 6.29 (H3), 5.52 (glucose H1, *J* 6 Hz), 5.15 (glucose H1, *J* 6 Hz), 4.86 (rhamnose H1, *J* 2 Hz), 4.55 (glucose H1, *J* 6 Hz), 4.10 – 2.90 (22 sugar H), 1.21 (rhamnose CH_3, *J* 6 Hz).

The permethyl ether was prepared with NaH, DMSO and CH_3I according to Hakomori's procedure.[6] The permethyl ether was purified by TLC on silica gel with acetone as eluent. MS [15 eV; *m/e* (% rel. int.)]: 423 (3), 399 (3), 394 (4), 393 (19), 392 (7), 391 (36), 390 (3), 385 (3), 370 (5), 362 (10), 361 (35), 360 (3), 329 (3), 328 (10), 315 (9), 314 (34), 313 (6), 300 (6), 219 (11), 218 (29), 209 (12), 204 (11), 195 (12), 190 (11), 189 (100), 188 (27), 187 (100), 181 (15), 157 (31), 155 (15), 145 (11), 131 (6), 129 (7), 127 (10), 125 (6), 116 (15), 111 (22), 101 (35), 99 (26), 89 (9), 88 (12), 87 (9), 75 (19), 74 (11), 73 (6), 72 (7), 71 (15), 59 (9). Only peaks larger than 6 % (3 % *m/e* 300 – 510) of the base peak are given.

Linkage analysis of sugar. The permethylated glycoside was hydrolyzed with 8 % H_2SO_4 at 100 °C for 1 h. The reaction mixture was then passed through a polyamide column (Woelm, 0.5 × 2 cm), packed in water. The acidic sugar solution was eluted with water until the eluate was neutral, the aglycone being eluted with CH_3OH. After neutralisation with $BaCO_3$, the aqueous sugar solution was reduced with $NaBH_4$ and acetylated with Ac_2O in pyridine as described earlier.[6] Retention times and prominent fragments in GLC-MS are given in Table 1. The isolated aglycone was identified as 5,3′-di-*O*-methylluteolin by R_F values and UV spectral data.

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Reactions of Parasorbic Acid. Synthesis of 2-Alkoxy-5,6-dihydro-α-pyrans and D,L-Osmunda Lactone

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Parasorbic acid (*1*) is reduced by diisobutylaluminiumhydride to the unsaturated sugar *2* which on heating or influence of light rearranges to the *trans*-aldehyde *3*. Reaction of *2* with alcohols or orthoformates leads to 2-alkoxy-5,6-dihydro-α-pyrans which are useful intermediates in general sugar synthesis. Epoxidation of the 2-isopropoxy derivative gives mainly the lyxo-isomer *7*. Treatment of *2* with base gives *cis-trans*-sorbic acid which in one step can be transformed into osmunda lactone *19* by hydroxylation with peracid. Parasorbic acid adds methanol and aziridine in a *trans*-fashion.

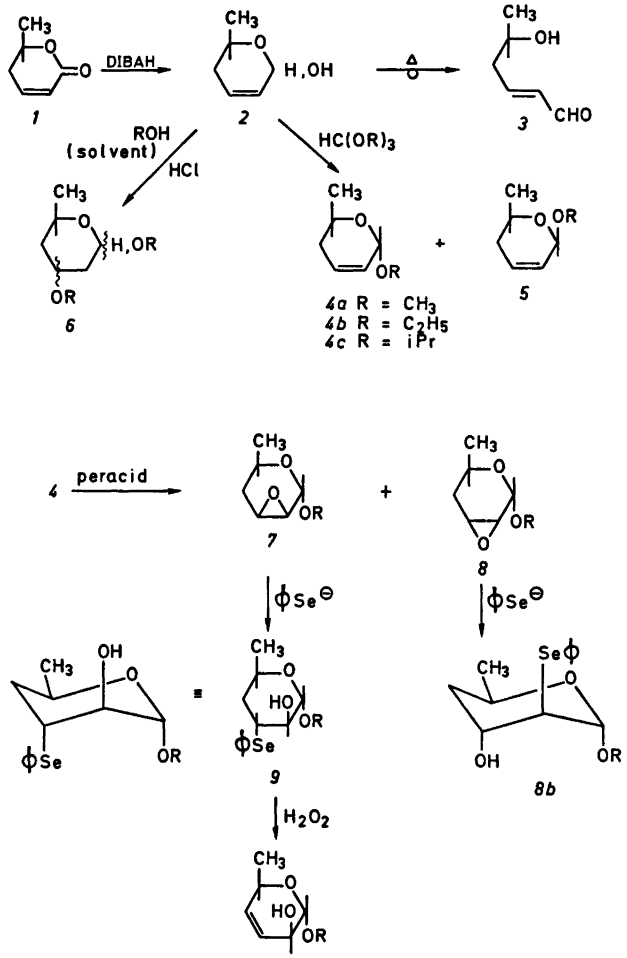
Parasorbic acid (*1*) was shown to be a useful intermediate for the preparation of some hexoses.[1] This work explores further reactions of *1* which lead to a variety of sugars.

Reduction with diisobutylaluminium hydride, DIBAH, converted *1* to 2-hydroxy-5,6-dihydro-pyran (*2*) which on distillation gave a mixture of products, the main component of which showed a rather strong carbonyl absorption at 1685 cm^{-1} in the IR. This was first believed to originate from free α,β-unsaturated *cis*-aldehyde but examination of the ^1H NMR spectrum showed the product to consist mainly of rearranged *trans*-aldehyde *3*, ($J_{2,3} = 16.0$ Hz). Inspection of the literature revealed that recently several similar rearrangements have been reported.[2–6] When *2* was left at room temperature for several weeks, it slowly rearranged to *3*, and a sample kept under the influence of light did so more rapidly than a sample kept in the dark. Even though the reaction is catalyzed by light and heat, an addition-elimination mechanism seems to be most plausible as evidenced by the addition reactions with methanol below. Because of its instability it was desirable to transfer *2* into the glycosides *4* and *5* which have been prepared by other routes and used for synthesis of 4,6-dideoxy sugars.[7–10]

Treatment of *2* with 2 % hydrochloric acid in methanol gave an anomeric mixture of *erythro*- and *threo*-isomers *6*. Ethanol gave the same result and it was not possible to prepare the glycoside by this procedure without adding alcohol across the double bond. When the *trans*-isomer *3* was treated in the same way, it also formed *6* which shows that Michael addition in this system is a facile reaction. Reaction of *2* with one equivalent of trimethyl or triethyl orthoformate gave the wanted glycosides, and later it was found that they could also be obtained more simply by reacting *2* with an equimolecular amount of alcohol in ether and with boron trifluoride etherate as catalyst. The anomeric effect favours the formation of the α-glycoside *4*.

GLC of a distilled sample of *4* showed only one main peak and minor impurities and the IR and ^1H NMR spectra were in agreement with data reported.[7] The ethyl and isopropyl glycosides *4b,c* were prepared similarly (Scheme 1).

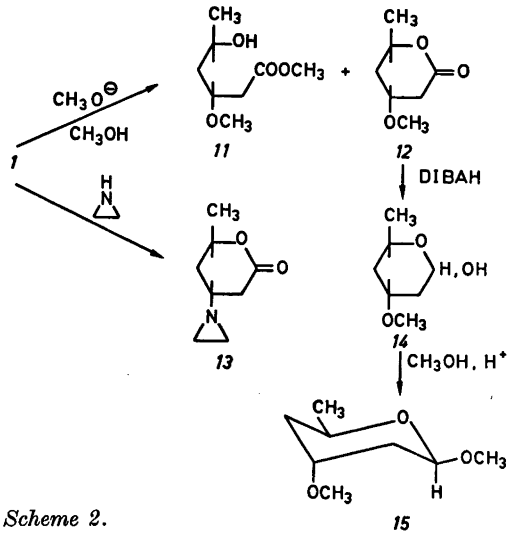
1 added methanol stereospecifically with simultaneous ring opening giving a mixture of the ester *11* and δ-lactone *12*. On acid treatment *11* cyclized to *12*. The ^1H NMR spectrum was in accordance with the *erythro*-structure. $J_{3e2ae} = 4.5$ Hz. Reduction of *12* and methylation gave predominantly β-glycoside, the conformation of which is represented by *15* ($_{1a2a}J = 9.5$, $J_{1a2e} = 2.6$, $J(^{13}C_1,H_{1a}) = 161$, $J(^{13}C_5H_{5a}) = 141.5$ Hz.



Scheme 1.

Aziridine added rapidly to *1* to give the *trans*-isomer *13* as proved by ^{1}H NMR (J_{3e2ae} = 5.1 Hz, H_5 shows both a – a and a – e couplings). GLC of the distilled product showed only one main peak and minor contaminations, *ca.* 10 % (Scheme 2). Epoxidation of *4a* and *4b* is reported to give mixtures of *ribo*, *8*, and *lyxo* derivatives *7* with increasing proportion of *7* with increasing size of R.[11] This was verified by our experiments and the isopropyl glucoside *4c* was therefore prepared and oxidized in order to obtain *7* in the purest possible state without resorting to chromatography. GLC of the product showed that *ca.* 10 – 15 % of the *ribo* isomer *8* was formed. This mixture was used in our further experiments directed towards the introduction of the 3,4 double bond. For that purpose we applied the phenyl selenide procedure.[12]

The epoxide opened in the expected direction and the selenium adduct *9* was obtained as the main product which on oxidation gave *10*, albeit in a poor yield. The corresponding methyl glycoside has been prepared earlier by a different route.[13] The structure and conformation of *9* was proved spectroscopically, J_{1e2e} = 2.3 Hz, $J_{^{13}C,H}$ = 165 Hz. $J_{^{13}C,H_5}$ = 144 Hz; H_3 appears as a quartet, $J_{2e3e} \sim J_{2e4ae} \sim$ 4.5 Hz and these data are only compatible with equatorial[14] H_1, H_3, and H_5 or slightly twisted conformation caused by 1,3 interaction at C_1 and C_3 formed by a *trans*-diaxial opening of the



Scheme 2.

epoxide ring at C_3. The selenium satellites that appeared in the ^{13}C NMR spectrum supported the assignment. By preparative TLC a small amount of an isomeric selenium adduct (*8b*) was also isolated, formed from the epoxide *8* by *trans* diaxial ring opening at C_2. The oxidative elimination of phenyl selenide was not performed at optimum conditions.

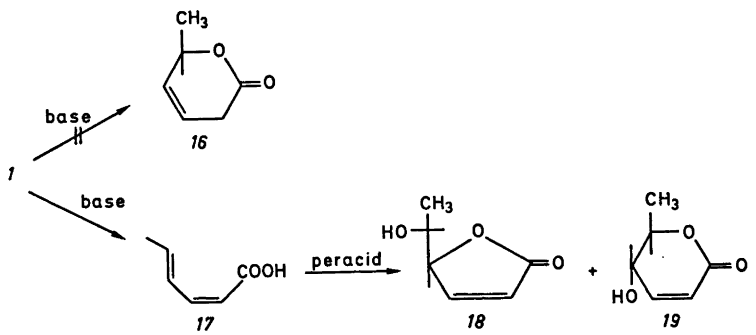
In order to make the application of parasorbic acid in sugar synthesis more useful, we tried to deconjugate its double bond. This would then easily make possible the preparation of several 2,6-deoxysugars and 6-deoxysugars after hydroxylations and reduction. To that effect *2* was treated with strong bases according to the method of Herrman *et al.*[15] However, *16* was not formed; instead 2-*cis*-4-*trans*-hexadienoic acid (*17*), an isomer of sorbic acid,[16,17] was obtained. This compound, which we had expected as an alternative product, is of utmost interest and can serve us in the same way as the desired compound *16*. Selective hydroxylation with peracid gave, *e.g.* directly the naturally occurring osmunda lactone (*19*) and its 5-membered ring isomer (*18*), 1:3. The ^1H NMR spectra of *18* and *19* were identical to those published earlier.[18] (Scheme 3).

EXPERIMENTAL

The melting and boiling points are uncorrected. The ^1H NMR couplings given are first order couplings.

2-Hydroxy-6-methyl-5,6-dihydro-α-pyran (2). Parasorbic acid (*1*) (5.6 g, 0.05 mol) was reduced with DIBAH (50 ml, 20 % toluene solution) at −25 °C under N_2. The reducing agent was added during a period of 1 h and after additional 2 h with stirring the temperature was allowed to rise to 0 °C; the excess of DIBAH decomposed by a sodium sulfate decahydrate: celite mixture below 20 °C. The organic phase was filtered and the residue washed with $CHCl_3$. The solution was dried over sodium sulfate and the solvent removed under vacuum to give 2-hydroxy-6-methyl-5,6-dihydro-α-pyran (*2*). It showed a weak carbonyl absorption at 1680 cm^{-1} that became more intense when the compound was left standing for a few weeks. The crude compound was directly used for the further reactions. ^1H NMR ($CDCl_3$): δ 1.23 (CH_3, d, J_{56} 6.2 Hz), 1.97 (H_4, m), 4.10 (H_5, sext., $J \sim 7$ Hz), 4.6 (OH, br.d), 5.39 (H_1, br.s), 5.79 (H_2, m), 6.00 (H_3, t, J_{34ae} 3.5 Hz, J_{23} 10 Hz).

On attempted distillation *2* gave the rearranged *trans*-aldehyde *3*, b.p. 87–89 °C/0.8 mmHg. IR (film): 1685 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.24 (CH_3, d, J_{56} 6.2 Hz), 2.51 (H_4, m, J_{34} 7.3 Hz, J_{45} 6.1 Hz, J_{24} 1.1 Hz), 4.04 (H_5, sext., J 6.1 Hz), 6.20 (H_2, ddt, J_{23} 16.0 Hz, J_{12} 7.8 Hz), 6.95 (H_3, dt).



Scheme 3.

2-Methoxy-6-methyl-5,6-dihydro-α-pyran (4a). Method A. To 2-hydroxy-6-methyl-5,6-dihydro-α-pyran (*2*) (1.14 g, 0.01 mol) and methanol (320 mg, 0.01 mol) in dry ether (20 ml) a few drops of BF_3/etherate were added. After keeping the mixture at room temperature for 45 min it was washed with sodium carbonate (10 %, 10 ml) and then with water. The ethereal solution was dried over sodium sulfate, the solvent evaporated under vacuum, and the residue distilled to give *4a*, b.p. 65–66 °C/55 mmHg. Yield: 900 mg, 70 %. The ^{1}H NMR and IR spectra agreed with the literature data.[7]

Method B. To *2* (2.85 g) and trimethyl orthoformate (3 g) in dry ether (30 ml), BF_3/etherate (0.5 ml) was added. The reaction mixture was kept at room temperature for 1 h. The ether solution was washed with sodium carbonate (10 %, 10 ml) and then with water, dried over sodium sulfate, and the solvent was removed under vacuum. The ^{1}H NMR spectrum of the residue was identical with that of the distilled product in Method A, yield: 75 %.

2-Ethoxy-6-methyl-5,6-dihydro-α-pyran (4b). Method A. B.p. 65–66 °C/35 mmHg yield: 70 %.

Method B. B.p. 65–66 °C/35 mmHg, yield: 75 %. The ^{1}H NMR spectrum of the ethyl glucoside shows the characteristic magnetic non-equivalence of the ethyl methylene group.

2-Isopropoxy-6-methyl-5,6-dihydro-α-pyran (4c), was prepared according to Method A using 3 mol of alcohol for 1 mol of *2* and the mixture was kept at room temperature for 1.5 h, b.p. 54–55 °C/15 mmHg, yield: 75 %. Calc. for $C_9H_{16}O_2$: C 69.23; H 10.26. Found: C 69.01; H 10.38. ^{1}H NMR ($CDCl_3$): δ 1.08, 1.19, and 1.22 (3 CH_3, d, J 6.2 Hz), 1.94 (H_4, m), 4.01 (H_{iPr}, hept.), 4.08 (H_5, sext.), 5.07 (H_1, br.s), 5.70 (H_2, m, J_{23} 10 Hz), 5.98 (H_3, t, J_{34ae} 3.4 Hz).

*Epoxidation of 2-isopropoxy-6-methyl-5,6-dihydro-α-pyran (4c) to isopropyl-2,3-anhydro-4,6-dioxy-α-*D,L*-lyxo-hexopyranoside.* To *4c* (1.56 g, 0.010 mol) in chloroform (40 ml) was added *m*-chloroperbenzoic acid (3.0 g, 0.014 mol, 80 %) and the mixture was stirred for 72 h at room temperature. The solid was filtered off, the filtrate washed with sodium carbonate (10 ml, 10 %) and water, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was distilled, b.p. 86–89 °C/13 mmHg. The yield of the isopropyl-2,3-anhydro-4,6-dioxy-α-D,L-*lyxo*-hexopyranoside (*7*) was 76 %. Calc. for $C_9H_{16}O_3$: C 62.75; H 9.35. Found: C 62.27; H 9.53. ^{1}H NMR ($CDCl_3$): δ 1.09 and 1.19 (iPr, d, non-equivalent, J 6 Hz), 1.23 (CH_3, d, J_{56} 6.3 Hz), 1.6–1.9 (H_4, m), 2.92 (H_2, d, J_{23} 3.8 Hz), 3.3 (H_4, m), 3.6–4.3 (H_5, iPr, m), 5.10 (H_1, s). The spectrum shows *ca.* 10–15 % contamination of the *ribo*-isomer *8* (H_4 gives a considerably broader AB-system and H_1 appeared as a satellite, d, at *ca.* 5.13).

Addition of methanol to parasorbic acid; trans-4-methoxy-6-methyltetrahydropyran-2-one (12). A mixture of parasorbic acid (*1*) (5.6 g) methanol (50 ml), and CH_3ONa (100 mg) was kept at room temperature for 5 h. The solution was concentrated to half of its volume, diluted with water, and extracted several times with ether. After drying over sodium sulfate the solvent was removed to give a residue (5 g, 70 %), a mixture of the lactone *12* and the open ester *11* (1:4).

4 g of the above mixture was treated with 2 M HCl (24 ml) at room temperature for 30 to 45 min and extracted with ether which was dried over sodium sulfate and evaporated. The residue gave pure *12*, b.p. 101–103 °C/1.8 mmHg; yield: 3 g, 87 %. Found: C 58.14; H 8.25. Calc. for $C_7H_{12}O_3$: C 58.33; H 8.33. IR (film): 1740 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.38 (CH_3, d, J_{56} 6.5 Hz), 1.64 (H_{4a}, ddd, J_{4ae} 14.5 Hz, J_{4a3} 3.6 Hz, J_{4a5} 10.8 Hz), 2.10 (H_{4e}, dt, $J_{4e3} \sim J_{4e5} \sim 3.5$ Hz), 2.68 (H_{2ae}, d, J_{2ae3} 4.5 Hz), 3.34 (OCH_3, s), 3.80 (H_3, q), 4.68 (H_5, ddq).

2-Hydroxy-4-methoxy-6-methyltetrahydropyran (14). DIBAH (toluene solution, 20 %, 9 ml) was added to a cooled solution (−25 to −30 °C) of 4-methoxy-6-methyltetrahydropyran-2-one (*12*) (1.44 g, 0.01 mol) in dry THF (15 ml) under N_2 with stirring during 30 min. After stirring the reaction for 2½ h at −25 to −30 °C, the temperature was allowed to rise to 0 °C and the complex was decomposed by adding a mixture of $Na_2SO_4.10H_2O$ and celite. The temperature was not allowed to exceed 25 °C. After filtration the solid was washed several times with hot $CHCl_3$. The combined organic solution was dried over sodium sulfate and the solvent removed under reduced pressure. The residue was distilled and gave 2-hydroxy-4-methoxy-6-methyltetrahydropyran *14* as an anomeric mixture, *ca.* 1:1, b.p. 78–80 °C/1 mmHg, yield: 800 mg, 57 %. Found: C 57.3; H 9.59. Calc. for $C_7H_{14}O_3$: C 57.75; H 9.56. A slight elimination of methanol occurred during the distillation. ^{1}H NMR ($CDCl_3$): δ 3.33 and 3.40 (OCH_3, s, of equal intensity), 5.05 (H_{1a}, J_{1a2a} 10 Hz, J_{1a2e} 2 Hz), 5.13 (H_{1e}, br.s).

2,4-Dimethoxy-6-methyltetrahydropyran (15). 2-Hydroxy-4-methoxy-6-methyltetrahydropyran (*14*) (2 g) was kept in methanolic hydrogen chloride (15 ml, 2 %) at room temperature for 30 min. The solvent was removed under reduced pressure and the residue distilled to give the glucoside *15*, b.p. 68–69 °C/12 mmHg, yield: 47 %. Found: C 60.02, H 10.19. Calc. for $C_8H_{16}O_3$: C 59.97; H 10.07. ^{1}H NMR ($CDCl_3$): δ 1.20 (CH_3, d, J_{56} 6.2 Hz; weak d at δ 1.15, J 6.3 Hz), 1.2–2.2 (H_2, H_4, m), 3.33 and 3.47 (OCH_3, s; the former s stronger), 3.5–4.2 (H_3, H_5, m), 4.61 (H_1, dd, J_{1a2a} 9.5 Hz, J_{1a2e} 2.6 Hz), 4.8 (H_1, anomer, weak).

trans-*4-Aziridino-6-methyltetrahydropyran-2-one (13).* To parasorbic acid (*1*) (1.6 g, 0.00145 mol) and aziridine (0.8 g, 0.00145 mol) was added potassium *t*-butylate (50 mg) as catalyst.

The mixture became warm and addition is complete after 15 min. Distillation gave pure *13*, 85 % yield, b.p. 120 °C/0.5 mmHg. ^{1}H NMR ($CDCl_3$): δ 1.21 and 1.77 ($N(CH_2)_2$, m), 1.36 (CH_3, d, J_{56} 6.3 Hz), 1.6–2.2 (H_3 and H_4, m), 1.61 (H_2, d, J_{2ae3} 5.1 Hz), 4.86 (H_5, m).

Osmunda lactone (19) and its isomer (18). *m*-Chloroperbenzoic acid (4.4 g, 85 %) was added to *cis,trans*-sorbic acid (*17*) (2.4 g) in methylene chloride (40 ml) at 0 °C. After 4 days the reaction mixture was filtered and the filtrate evaporated. The residue was shaken with cold water (3 × 6 ml), filtered, and the combined filtrates were evaporated *in vacuum*. The remainder consisted of a mixture of *19* and *18* (1:3) which was used for further reactions without purifications. The crude yield was *ca.* 90 %. The ^{1}H NMR spectra of *18* and *19* were identical with those published earlier.[18]

Opening of the epoxide ring of 7 *and* 8 *with phenylselenide, preparation of* 10. Diphenyl diselenide (0.9 g) was reduced by solid sodium borohydride (0.28 g) in dimethoxyethane (15 ml). The light yellow solution turned colourless on addition of methanol (0.2 ml). Crude *7* (0.74 g) was added and the mixture was stirred overnight, then heated at 60 °C for *ca.* 1 h, hydrolyzed with water (20 ml) and extracted with methylene chloride. Evaporation of the solvent gave a yellow oil that was purified by preparative TLC ($CHCl_3$). *9* was obtained as an oil that slowly crystallized, 0.50 g, m.p. 82–84 °C from carbon tetrachloride. Mw. 329.0. Found: M^+ 330 (^{80}Se), 328 (^{78}Se). ^{1}H NMR ($CDCl_3$): δ 1.17 and 1.28 (iPr, d, J_{78} 6.1 Hz), 1.21 (H_6, d, J_{56} 6.4 Hz), 1.6–2.3 (H_4, m), 3.41 (H_3, q, $J_{2e3e} \sim J_{2e4ae} \sim 4.5$ Hz), 3.8 (H_2, m), 3.98 (H_7, sept., 6.1 Hz), 4.3 (H_5, m), 4.80 (H_1, d, 2.8 Hz), 7.2–7.7 (5H, m). ^{13}C NMR ($CDCl_3$): δ 20.35 (C_6), 21.22 and 23.17 (C_8 and C_9), 34.54 (C_4), 42.12 (C_3, $^1J_{CH}$ 145 Hz, $^1J_{CSe}$ 70.0 Hz), 62.89 (C_5, $^1J_{CH}$ 143 Hz), 69.00 (C_7), 70.21 (C_2, $^2J_{CSe}$ 14.7 Hz), 97.27 (C_1, $^1J_{CH}$ 165 Hz).

A second band with a slightly higher R_F value proved to consist of pure *8b*, oil, 0.15 g. Mw. 329.0; found: M^+ 330 (^{80}Se), 328 (^{78}Se). ^{1}H NMR ($CDCl_3$): δ 1.12 (CH_3, *J* 6.1 Hz), 1.20 (2 CH_3, *J* 6.1 Hz), 1.5–2.3 (H_4, m), 3.37 (H_2, br.s), 3.98 (H_7, sept., *J* 6.1 Hz), 4.1 (H_5, m), 5.28 (H_1, br.s), 7.2–7.7 (5H, m). ^{13}C NMR ($CDCl_3$): δ 21.20 (C_6 and C_8), 23.34 (C_9), 36.06 (C_4), 46.36 (C_3, $^1J_{CSe}$ 67 Hz), 60.02 (C_5, $^1J_{CH}$ 144 Hz), 68.82 (C_3), 69.72 (C_7), 98.93 (C_1, $^1J_{CH}$ 170 Hz).

Oxidation of *9* (0.25 g) with hydrogen peroxide (1 ml, 15 %) in methylene chloride (5 ml, two phase system) overnight gave the pyranoside *10* (35 mg) together with another major product of unknown structure, still containing the phenyl selenide function. No starting material was present. *10* showed the following ^{1}H NMR data ($CDCl_3$): δ 1.21, 1.23, 1.28 (3 CH_3, *J* 6.2 Hz), 2.2 (OH, s), 3.76 (H_2, m), 4.01 (H_7, sept., *J* 6.2 Hz), 4.37 (H_5, br.q., J_{56} 7 Hz), 4.93 (H_1, br.s), 5.87 (H_3, H_4, br.s). MS: M^+ missing; 113, ($M^+ - O - iPr$), 84.

cis-trans-*Sorbic acid (17)* was prepared according to the method of Eisner *et al.*[16] An excess of 50 % sodium was used. The crude product, *17*, was used for the further reactions. Use of lithium diisopropylamide[15] in HMPT as base did not improve the yield.

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Tropylium Ions. Part VI.[1] Kinetics of Reactions of 3′,5′-Dialkyl-substituted 4′-Hydroxyphenyltropylium Ions in Aqueous Solution*

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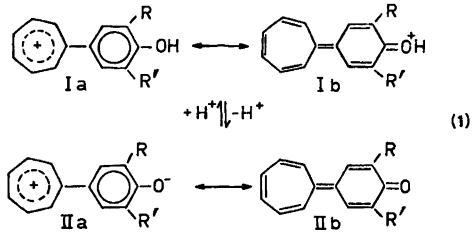
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The reactions of 3′,5′-di-*tert*-butyl-substituted and 3′,5′-dimethyl-substituted 4′-hydroxyphenyltropylium fluoroborate in aqueous solutions of different pH have been studied by conventional and stopped-flow spectrophotometry at 25 °C.

The pK_a values found for the dissociation of the two tropylium ions to 2,6-disubstituted 4-cycloheptatrienylidenecyclohexadienones were 4.48 and 6.26, respectively. The pK_R value for the second of the tropylium ions was 5.74.

The first-order rate constants measured from the disappearance of the substituted 4-cycloheptatrienylidenecyclohexadienones in different buffered solutions are minimum values. The rates are discussed in terms of reactions of the tropylium ions and the 4-cycloheptatrienylidenecyclohexadienones with water and hydroxide ion.

The reactivities and stabilities of the studied compounds are compared with those of the corresponding unsubstituted compounds.



(1)

Scheme 1.

As early as 1962, van Helden *et al.*[2] found that 4′-hydroxy-3′,5′-dimethylphenyltropylium chloride (the cation of which is I, R = R′ = Me) on heating in dry nitrogen or on basification with sodium hydrogen carbonate yielded a purple pigment (II, R = R′ = Me). The compound was rapidly oxidized to an insoluble material. At the same time and later on, the existence of the parent compound, 4-cycloheptatrienylidenecyclohexadienone (II, R = R′ = H), was observed in solution.[3–5] Attempts to prepare the compound in crystalline form proved unsuccessful, however. On the other hand, the compounds II where R or R and R′ are hydroxy,[4] methoxy,[4,8] *tert*-butyl,[6] bromo [7] or chloro [8] substituents have been reported.

During the course of our studies on tropylium ions we successfully used the stopped-flow technique to follow the reactions of 4′-hydroxyphenyltropylium ion (I, R = R′ = H) and 4-cycloheptatrienylidenecyclohexadienone (II, R = R′ = H) formed from it on basification.[9] To obtain quantitative information about the stabilizing effect of methyl and *tert*-butyl substituents we carried out the study now reported.

EXPERIMENTAL

Materials. 3′,5′-Dimethyl- and 3′,5′-di-*tert* butyl-substituted 4′-hydroxyphenyltropylidene were synthesized from 2,6-dimethylphenol and 2,6-di-*tert*-butylphenol, respectively, and 7-methoxytropylidene in acetic acid (*cf.* Ref. 6). The former was converted to 4′-hydroxy-3′,5′-dimethylphenyltropylium fluoroborate by boiling with an excess of triphenylmethyl fluoroborate in acetonitrile at 70 °C for 1 h. The product was precipitated by adding ethyl acetate and recrystallized from a mixture of acetonitrile and ethyl acetate; the crystals obtained decomposed at 205 °C, UV [water (log

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ε)]: 442 (4.21), 272 (4.85) nm (M^{-1} cm^{-1}). 4'-Hydroxy-3',5'-di-*tert*-butylphenyltropylium fluoroborate was synthesized analogously. It decomposed at 209 – 211 °C, UV [water (log ε)]: 443, (4.15), 270 (3.82) nm (M^{-1} cm^{-1}).

Buffer components were commercial chemicals used without further purification. Water was distilled from potassium permanganate solution under a nitrogen atmosphere.

Equilibrium constants. The pK_a values of the hydroxyphenyltropylium ions at 25 °C were calculated from the initial values of stopped-flow traces measured in different buffer solutions at the wavelength of the maximum absorbance of the substituted 4-cycloheptatrienylidenecyclohexadienones. As described earlier,[10] the pK_R value of the 4'-hydroxy-3',5'-dimethylphenyltropylium ion at 25 °C was measured at the wavelength of the maximum absorbance of the tropylium ion and calculated from the plot of $1/[H^+]$ against $1/(A - A_\infty)$. The ionic strength was 1.0×10^{-2} M.

Rate constants. The first-order rate constants were measured at 25 °C by following the disappearance of a 4-cycloheptatrienylidenecyclohexadienone either by conventional spectrophotometry or by stopped-flow technique, with tropylium ions in perchloric acid solutions mixed with the buffer solutions. The ionic strength was 1.0×10^{-2} M except where the pH was so high ($[OH^-] > 10^{-2}$) that this ionic strength was impossible.

Apparatus. The stopped-flow apparatus, of Gibson-Durrum type, has been described earlier.[10] The spectra were scanned with a Cary Model 16 spectrophotometer equipped with a specially designed thermostated cell holder. A Cahn Gram Electrobalance was used for weighing small amounts and a Radiometer PHM63 digital pH-meter for pH measurements.

RESULTS

At 25 °C the pK_a value of the fast proton-transfer reaction (1) was 6.26 for the 4'-hydroxy-

$$C_7H_6+C_6H_2RR'OH+H_2O \rightleftharpoons C_7H_6+C_6H_2RR'O^-+H_3O^+ \quad (1)$$

3',5'-dimethylphenyltropylium ion measured in dihydrogen phosphate – monohydrogen phosphate buffer solutions at 549 nm, and 4.48 for 4'-hydroxy-3',5'-di-*tert*-butylphenyltropylium ion measured in acetic acid – acetate buffer solutions at 510 nm.

The pK_R value of the slower reaction (2)

$$C_7H_6+C_6H_2RR'OH+2\ H_2O \rightleftharpoons HOC_7H_6C_6H_2RR'OH+H_3O^+ \quad (2)$$

could not be obtained for 4'-hydroxy-3',5'-di-*tert*-butylphenyltropylium ion as reaction (1) takes place before reaction (2) in the pH region necessary for measurements. Reaction (1) also interferes with the evaluation of the pK_R value for 4'-hydroxy-3',5'-dimethylphenyltropylium ion, but by working at 442 nm with acetic acid – acetate buffers having pH values from 3.90 to 5.17 we obtained a pK_R value of 5.74 at 25 °C.

The measured rate constants are given in Tables 1 and 2. The reaction of 2,6-di-*tert*-butyl-4-cycloheptatrienylidenecyclohexadienone was preceded by a faster reaction with decreasing absorbance. The rate constants for the latter, measured without taking any reverse reactions into account in the calculations, are given in Table 3.

The rate constant observed from the disappearance of a 4-cycloheptatrienylidenecyclo-

Table 1. First-order rate constants measured from the disappearance of 2,6-dimethyl-4-cycloheptatrienylidenecyclohexadienone in aqueous solutions at 25 °C.

Ionic strength/M	pH	Method [a]	k_{obs}/s^{-1}
NaOH as buffer			
2.0×10^{-1}	13.30	S	7.9
5.0×10^{-2}	12.69	S	2.1
2.5×10^{-2}	12.38	S	1.0
1.0×10^{-2}	11.60	S	1.9×10^{-1}
1.0×10^{-2}	11.18	S	5.7×10^{-2}
Carbonate as buffer			
1.0×10^{-2}	10.47	S	2.0×10^{-2}
	9.88	C	8.0×10^{-3}
Borate as buffer			
1.0×10^{-2}	9.60	C	2.5×10^{-3}
	9.28	C	2.0×10^{-3}
	8.82	C	1.6×10^{-3}
	8.62	C	1.8×10^{-3}
	8.33	C	2.0×10^{-3}
	7.94	C	4.1×10^{-3}
Phosphate as buffer			
1.0×10^{-2}	7.32	C	1.4×10^{-2}
	6.69	S	5.6×10^{-2}
	6.64	S	6.4×10^{-2}
	6.46	S	8.4×10^{-2}
	6.37	S	1.1×10^{-1}
	6.13	S	1.9×10^{-1}

[a] S = stopped-flow; C = conventional.

Table 2. First-order rate constants measured from the disappearance of 2,6-di-*tert*-butyl-4-tropylidenecyclohexadienone in aqueous solutions at 25 °C.

Ionic strength/M	pH	Method [a]	k_{obs}/s^{-1}
NaOH as buffer			
2.0×10^{-1}	13.30	S	1.5×10^{-2}
5.0×10^{-2}	12.69	C	2.9×10^{-3}
2.5×10^{-2}	12.38	C	9.3×10^{-4}
1.0×10^{-2}	11.60	C	2.9×10^{-4}
1.0×10^{-2}	11.18	C	1.8×10^{-4}
Carbonate as buffer			
1.0×10^{-2}	10.53	C	1.8×10^{-4}
	9.88	C	2.0×10^{-4}
Borate as buffer			
1.0×10^{-2}	9.28	C	1.6×10^{-4}
	8.60	C	1.2×10^{-4}
	8.28	C	1.6×10^{-4}
	7.72	C	1.5×10^{-4}
Phosphate as buffer			
1.0×10^{-2}	7.32	C	1.5×10^{-4}
	6.64	C	1.3×10^{-4}
	6.08	C	1.6×10^{-4}
Acetate as buffer			
1.0×10^{-2}	5.47	C	2.3×10^{-4}
	5.32	C	5.6×10^{-4}
	4.99	C	1.1×10^{-3}

[a] S = stopped-flow; C = conventional.

Table 3. First-order rate constants calculated from the fast initial decrease of the absorbance of 2,6-di-*tert*-butyl-4-cycloheptatrienylidene-cyclohexadienone in aqueous solutions at 25 °C. Method: Stopped-flow. Ionic strength: 1.0×10^{-2} M.

Buffer	pH	k_{obs}/s^{-1}
NaOH [a]	12.38	16
NaOH	11.18	9.6
Carbonate	10.47	8.3
Borate	8.60	4.2
Borate	7.75	3.4
Phosphate	7.32	2.5
Phosphate	6.64	1.4

[a] Ionic strength 2.5×10^{-2} M.

hexadienone for the reaction of the corresponding tropylium ion with water is given in eqn. (3)

$$k_{obs} = k_w/(1 + K_a/[H^+]) \quad (3)$$

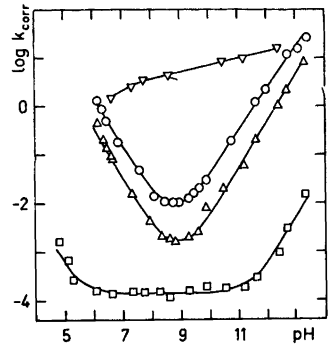


Fig. 1. The logarithms of the first-order rate constant measured from the disappearance of unsubstituted (○), 2,6-dimethyl-substituted (△) and 2,6-di-*tert*-butyl-substituted (▽ faster reaction, □ slower reaction) 4-cycloheptatrienylidenecyclohexadienones at 25 °C as functions of pH. The rate constants have been corrected by multiplying by $(1 + [H^+]/K_a)$.

and for the reaction of the tropylium ion with hydroxide ion in eqn. (4).

$$k_{obs} = k_{OH^-}[OH^-]/(1 + K_a/[H^+]) \quad (4)$$

The denominator $(1 + K_a/[H^+])$ in the equations becomes large in buffer solutions of high pH where the concentration of the tropylium ion is less than that of the 4-cycloheptatrienylidenecyclohexadienone.

The observed rate constant for the reactions of a 4-cycloheptatrienylidenecyclohexadienone is given in eqn. (5).

$$k_{obs} = (k'_H + [H^+] + k'_w + k'_{OH^-}[OH^-])/(1 + [H^+]/K_a) \quad (5)$$

The observed rate constants in the acidic range were corrected by multiplying by $(1 + [H^+]/K_a)$, after which the curves in Fig. 1 were drawn from the equation $k_{corr} = 2.5 \times 10^5 [H^+] + 8.0 \times 10^{-4} + 4.8 \times 10[OH^-]$ for dimethyl-substituted compounds and from the equation $k_{corr} = 4.7 \times 10[H^+] + 1.4 \times 10^{-4} + 4.5 \times 10^{-2} [OH^-]$ for di-*tert*-butyl-substituted compounds. The coefficients yielded the values of the rate constants collected in Table 4.

From eqn. (4) follows that $k_{OH^-} = k_{obs}(1 + K_a/[H^+])/[OH^-] \approx k_{obs}K_a/[H^+][OH^-] = k_{obs}K_a/K_w$. Thus k_{OH^-} is $8.0 \times 10^{-4} \times 5.50 \times 10^{-7}/1.01 \times 10^{-14} = 4.4 \times 10^4$ M^{-1} s^{-1} for dimethyl-substituted and $1.4 \times 10^{-4} \times 3.28 \times 10^{-5}/1.01 \times 10^{-14} = 4.6 \times 10^5$ M^{-1} s^{-1} for di-*tert*-butyl-substituted hy-

Table 4. Values of pK_a and the rate constants calculated for the reactions of substituted 4-cycloheptatrienylidenecyclohexadienones in aqueous solutions at 25 °C.

Substituents in 4-cycloheptatrienylidene-cyclohexadienone	λ_{max}/nm	pK_a	$k'_{H^+}/M^{-1}\,s^{-1}$	k'_w/s^{-1}	$k'_{OH^-}/M^{-1}\,s^{-1}$
None [a]	525	6.83	$< 1.6 \times 10^6$	5.8×10^{-3}	3.2×10^2
2,6-Dimethyl	549	6.26	$< 3.5 \times 10^5$	8.0×10^{-4}	4.7×10
2,6-Di-*tert*-butyl	510	4.48	4.7×10	1.4×10^{-4}	4.5×10^{-2}

[a] Ref. 9.

droxyphenyltropylium ion. The values of k_w were calculated from eqn. (3), using the k_{obs} values in Tables 1 and 2 measured in the most acidic range. The values are collected in Table 5.

The values for the unsubstituted compound differ slightly from the values reported earlier [9] as a result of the present more careful evaluation and of taking into account the effect of the ionic strength on the pK_a of dihydrogen phosphate ion.

The ratio k'_{H^+}/k'_w for di-*tert*-butyl-substituted 4-cycloheptatrienylidenecyclohexadienone is 3.4×10^5 M^{-1} (Table 4), and close to the value 3.0×10^5 M^{-1} measured recently for the corresponding reactions of 4-allydidene-2-methoxycyclohexadienone.[13] The same ratio also indicates that the value of k'_{H^+} for the two other compounds in Table 4 is too high and only an arithmetical result as the uncatalyzed hydrolysis of the corresponding tropylium ion is faster.

Critical examination of the rate constants in Table 5 is assisted by the fact that phenyltropylium ions display constant selectivities toward hydroxide ion and water;[10,12,14] *i.e.*, the ratio k_{OH^-}/k_w is constant, about 1.1×10^5 M^{-1}.[10] In any event, on the basis of the values of k_{OH^-} in Table 5, one can conclude that the values for dimethyl- and di-*tert*-butyl-substituted tropylium ions may not be unreasonably high.

DISCUSSION

The structure of a phenyltropylium ion can be described as a hybrid of canonical forms (*cf.* Scheme 1). Hence, the longest wavelength maximum of the UV spectrum has been found to be strongly dependent upon the substituents on the phenyl ring.[3] As *p*-hydroxy, *m*-*tert*-butyl and *m*-methyl substituents are electron repelling, they produce the bathochromic effects reported in Table 5. Surprisingly, the substituent effects on pK_R in trisubstituted compounds are additive, a feature which is not reflected in the values of the molar absorption coefficient owing to a buttressed substitution.[15]

The structure of a 4-cycloheptatrienylidenecyclohexadienone can likewise be assumed a hybrid of canonical forms (*cf.* Scheme 1). The

Table 5. Spectral data, values of pK_R and rate constants calculated for the reactions of substituted 4'-hydroxyphenyltropylium ions in aqueous solutions at 25 °C.

Substituents in phenyltropylium ion	λ_{max}/nm	$\varepsilon/M^{-1}\,cm^{-1}$	pK_R	k_w/s^{-1}	$k_{OH^-}/M^{-1}\,s^{-1}$
None [a]	350	1.3×10^4	4.84	1.0	7.9×10^4
4'-Hydroxy [b]	428	1.85×10^4	5.60	0.25	$< 8.5 \times 10^4$
3',5'-Dimethyl-4'-hydroxy	442	1.63×10^4	5.74	0.20	$\sim 4.4 \times 10^4$
3',5'-Di-*tert*-butyl-4'-hydroxy	443	1.40×10^4	—	—	$\sim 4.6 \times 10^5$
3'-Methyl [c]	373	1.31×10^4	4.91	0.78	8.7×10^4
4'-Methoxy [c]	426	1.91×10^4	5.61	0.27	4.3×10^4

[a] Ref. 12, at 23 °C. [b] Ref. 9. [c] Ref. 10.

longest wavelength maximum of the UV spectrum is 525 nm (2.36 eV) for the unsubstituted compound [9] and 510 nm (2.43 eV) and 549 nm (2.26 eV) for the compounds studied here. These values are indicative of a highly unsaturated, conjugated system. In estimating the relative weights of different canonical forms, the wavelength 605 nm (2.05 eV) calculated recently for 4-diphenylmethylidene-1-cycloheptatrienylidene-2,5-cyclohexadiene is pertinent.[16]

On the basis of the data accumulated and the above discussion, we propose that 4′-hydroxyphenyltropylium ions have an electronic structure close to I a and that 4-cycloheptatrienylidenecyclohexadienones have one close to II b.

The fast reaction observed immediately after the basification of di-*tert*-butyl-substituted 4′-hydroxyphenyltropylium ion solutions is noteworthy. Although we cannot at this time offer a clear interpretation of the reaction, we would call attention to the following: (1) the absorbance decreases during the reaction; (2) this decrease is independent of the pH; (3) the rate of the reaction is almost independent of the pH; (4) the reaction was found only in the case of the most stable 4-cycloheptatrienylidenecyclohexadienone studied here.

2,6-Dimethyl-substituted 4-cycloheptatrienylidenecyclohexadienone reacts with hydroxide ion 7 times slower and the 2,6-di-*tert*-butyl-substituted compound 7100 times slower than the unsubstituted compound. This stabilization can be ascribed, at least in the case of *tert*-butyl substituents, mainly to steric effects.

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Absolute Configurations of Alkylcitric Acids

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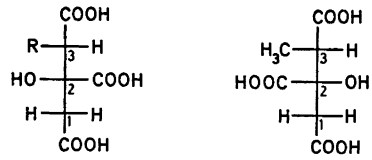
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The absolute configurations of five naturally occurring alkylcitric acids (*1*—*5*) have been determined. The relative configurations were elucidated by comparison with NMR spectra or GLC retention times of stereospecifically synthesised *R*,R** esters and *R*,S** esters (Fig. 1). The absolute configurations of *1*—*4* (2*S*,3*S*)† and *5* (2*R*,3*S*)† were determined by CD measurements on the natural acids and optically active synthetic acids (*12*—*15*) as their molybdate complexes. Previously reported molecular ellipticities for 2-alkylmalic acids must be corrected.

Five alkylcitric acids (*1*—*5*) have previously been isolated from or detected in natural sources and the determination of their absolute configurations is reported here. The methylcitric acids *1* and *5* are excreted in the urine of humans suffering from propionic acidemia, a rare disease leading to death in infancy.[1] A methylcitric acid has also been found in the culture of *Candida lipolytica* [2] and from the NMR and optical rotation data given [2] we conclude it to be acid *1*. (−)-Decylcitric acid (*2*) is a metabolite produced by a variant of *Penicillium spiculisporum*,[3,4] norcaperatic caid (*3*) is probably responsible for the toxicity of *Cantharellus floccosus*,[5] and the 1-monomethyl ester of the latter (caperatic acid [6]) is a common lichen acid. Agaricic acid (*4*) is a hexadecylcitric acid occurring in *Polyporus officinalis*.[7] Another representative of this structural class, butylcitric acid, has been assumed to be an intermediate in the biosynthesis of the fungal metabolite glauconic acid.[8]

In order to determine the stereostructures of the natural alkylcitric acids we have synthesised such compounds stereospecifically from the epoxides *6* and *7* (Fig. 2) as well as from their racemic modifications. The absolute configurations of *6* and *7* are known [9] since they have been correlated with the known [10–12] lactones of hydroxycitric acids. Evidence for a *trans* opening of the epoxide rings in *6* and *7* on reaction with lithium dialkylcuprate reagents comes from the fact that dimethyl epoxyfumarate, on reaction with lithium dimethylcuprate, gives *erythro*-3-methylmalic acid. Consequently, *6* should yield (2*S*,3*S*)-alkylcitric acids and *7* should yield (2*R*,3*S*)-alkylcitric acids as shown in Fig. 2.

The relative configurations of *2*—*4* were determined by ^{1}H NMR spectroscopy, using the synthetic *R*,R** esters and *R*,S** esters as reference materials; the spectral features used are depicted in Fig. 3. The trimethyl



1: R = CH_3
2: R = $C_{10}H_{21}$
3: R = $C_{14}H_{29}$
4: R = $C_{16}H_{33}$

5

Fig. 1. Absolute configurations of the natural alkylcitric acids. The formulae shown are to be regarded as Fischer projections although the lowest numbered chain members are not placed at the top.

† The numbering is that of 2-hydroxy-1,2,3-alkanetricarboxylic acids.

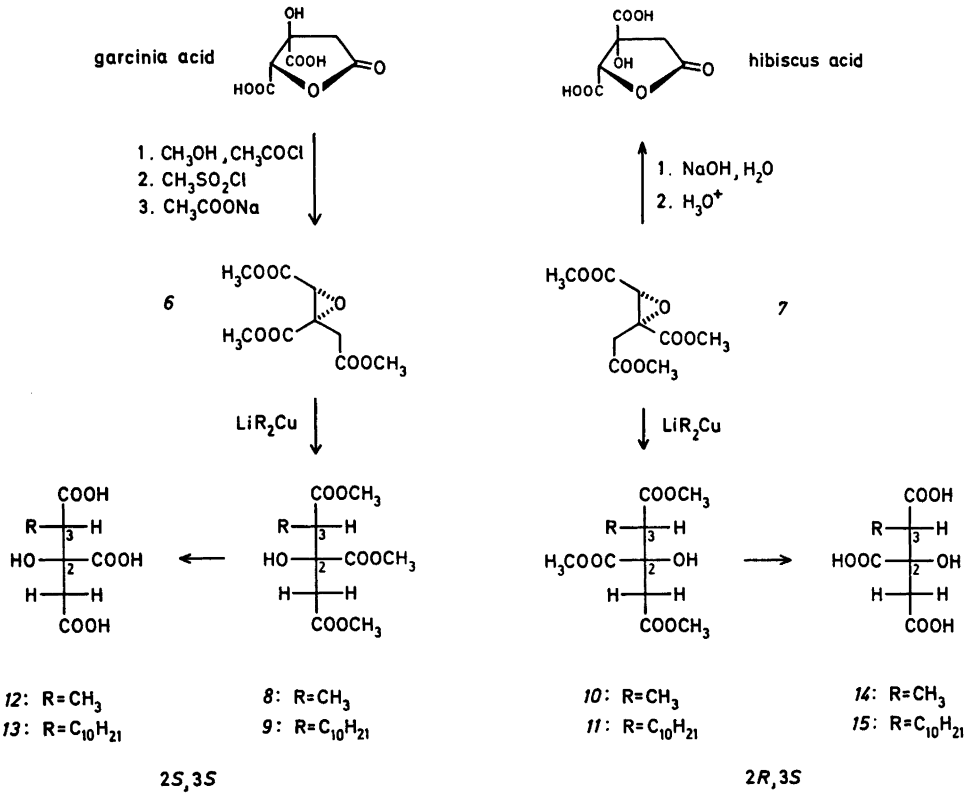


Fig. 2. Synthetic scheme for the correlation of alkylcitric acids with lactones of hydroxycitric acids. The formulae *8* – *15* are to be regarded as Fischer projections although the lowest numbered chain members are not placed at the top.

esters of *2* – *4* gave ^1H NMR spectra containing the wide-spread AB spectrum in Fig. 3a, demonstrating these acids to be either *R,R* or *S,S*. The NMR differences shown in Fig. 3 have been shown to be retained to a large extent in the spectra of the corresponding unesterified acids. The NMR results obtained here allow conclusions to be drawn about the configurations of alkylcitric acids described in the literature. Comparisons of the ^1H NMR data from our methylcitric acids with those given [2] for the methylcitric acid from *Candida lipolytica* indicate that the latter should belong to the *R*,R** pair, although differences in chemical shifts were apparent (see below). This conclusion is supported by comparison of the magnitude of the optical rotations of the trimethyl ester [2] of the *Candida lipolytica* acid with the rotations of *8* and *10* (see below). The NMR chemical shifts published for a crystalline methylcitric acid,[28] synthesised by a Reformatsky reaction,[18] clearly show that this acid is the *R*,S** isomer. The ethylcitric acid which is commercially available should on the same grounds be *R*,S**.[21] It is also possible to distinguish between diastereomeric methylcitric acids by GLC of their trimethyl esters. Using this technique the methylcitric acids obtained from the urine of a patient with propionic acidemia [1] were shown to be present as diastereomers in the approximate ratio 2:1. The retention times of the major and minor peaks were the same as those of the synthetic *R*,R** and *R*,S** isomers respectively.

The absolute configurations of *1* – *5* were studied by CD measurements on the natural and the synthetic acids *12* – *15* as molybdate (VI) complexes.[18] The acids *1* – *4* showed CD

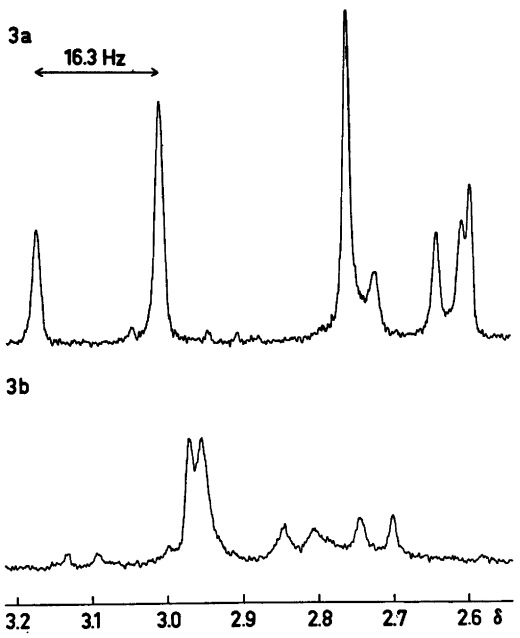


Fig. 3. NMR spectra ($CDCl_3$) of trimethyl esters of diastereomeric decylcitric acids. 3a, *9*; 3b, *11*.

spectra similar to those of *12*–*15*, therefore indicating that *1* is identical to *12*, and *2* is identical to *13*. These acids, as well as *3* and *4*, thus have the *2S,3S* configuration. In a similar investigation, *5* proved to be identical to *14* and is thus *2R,3S*.

Despite the fact that the configurations of *12* and *13* are *2S* and those of *14* and *15* are *2R*, the molybdate complexes of all these acids give CD spectra with similar Cotton effects. A comparison of these spectra with that of (*S*)-malic acid as its molybdate complex [13] indicates that the alkylcitric acids belonging to the *R*,R** pair bond to molybdenum by their carboxyl groups at C-1 and C-2, and that the acids belonging to the *R*,S** pair bond by their C-2 and C-3 groups.

It has been shown that the methyl- and decylcitric acids are biosynthesised from oxaloacetate and propionyl- or lauroyl-CoA.[1,14,15] The *2S* configuration of *1* and *2* requires attack by acyl-CoA at the *si* face of the keto carbonyl group of oxaloacetate; such an attack is analogous to that in the ordinary biosynthesis of citric acid, since the addition of acetyl-CoA to the carbonyl group occurs at its *si* face.[16] The co-occurrence of *1* and *5*, with *5* having the *2R* configuration, is remarkable since the biosynthesis of the latter requires a *re* attack on oxaloacetate. Addition of acetyl-CoA at the *re* face has, however, been found to occur in a few anaerobic bacteria.[17]

Alkylcitric acids have previously been synthesised from diethyl alkyloxaloacetates and Reformatsky reagents in yields ranging from 3 to 15 %.[16–20] By using instead the lithium ester enolate of (−)-menthyl acetate we obtained a 45 % yield of the mixed ester of ethylcitric acid, containing *R*,S** and *R*,R** isomers in the approximate ratio 10:1. In an analogous synthesis, methylcitric acid was obtained in a 30 % yield and only esters belonging to the *R*,S** pair could be detected (GLC, NMR). According to a CD investigation of the ethylcitric acids, the *2S,3R* isomer was formed in approximately 5 % enantiomeric excess.

Correction. The molecular ellipticities of 2-alkylmalic acids reported in two previous papers [22,23] are ten times too large.

EXPERIMENTAL

Epoxide ring opening reactions and isolations of the alkylcitric acids were performed as described previously.[23] The acidic hydrolysis procedure [23] gave a (*2S,3S*)-methylcitric acid which was partly (10 %) isomerized and so an alkaline method was used in this case for preparation of the free acid. The ester was treated with 0.5 M sodium hydroxide solution (25 °C, 4 days) and after washing with ether, the solution was passed through a Dowex 50W-X8 (H^+) column and evaporated to dryness. The crude acid obtained was investigated directly by CD and, after methylation with diazomethane, by GLC. All CD data given below refer to maxima or minima obtained for the alkylcitric acids in aqueous solutions (unless otherwise stated) (pH 3.4 – 3.7) containing molybdate(VI). Further CD conditions have been described previously.[23] Analytical GLC was performed on a Perkin-Elmer 900 instrument equipped usually with a JXR column (3 % on Gas-Chrom Q, 100 – 120 mesh, 0.2 × 180 cm); for the trimethyl methylcitrates an ECNSS-M column (3 % on Chromosorb W, 100 – 120 mesh) was used. Preparative GLC was performed on an Aerograph 1400 instrument using an ECNSS-M column (3 % on Chromosorb W, 100 – 120 mesh, 0.3 × 360 cm) for trimethyl methylcitrates and an SE-30 column (2 % on Chromosorb W, 60 – 80 mesh, 0.3 × 190 cm) for trimethyl decylcitrates. Optical rotations were measured on a Perkin-Elmer 141

polarimeter, NMR spectra were recorded on a Varian XL-100 instrument and mass spectra (GLC-MS) on a Varian MAT 311 spectrometer.

cis-*Epoxide* 6. (±)-*threo*-Trimethyl hydroxycitrate was prepared by osmium tetroxide hydroxylation of *trans*-trimethyl aconitate.[9] Distillation gave the dimethyl ester of (±)-garcinia acid which was then hydrolysed according to Martius *et al.*[24] Resolution of the racemic acid with chinchonine [9] gave, after one recrystallisation, a salt from which garcinia acid could be obtained (85 % optically pure). The *cis*-epoxide *6* was prepared from garcinia acid according to Guthrie *et al.*[9] and it gave $[\alpha]_D^{25}$ −27.9° (*c* 1.0, methanol) showing an 81 % optical purity; lit.[9] value: $[\alpha]_D^{25}$ −34.6° (*c* 1.07, methanol). ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 6 H), 3.72 (s, 3 H), 3.67 (s, 1 H), 3.25, 3.08, 2.83, and 2.66 (AB spectrum, 2 H). MS (*m/e*, relative intensity): M$^+$ − 31 = 201(8), 174(33), 173(46), 145(39), 141(56), 114(45), 113(100), 75(28), 59(87).

trans-*Epoxide* 7. The *trans*-epoxy acid was prepared by epoxidation of *trans*-aconitic acid.[9] Resolution with cinchonidine [9] gave, after one recrystallisation, a salt from which the (+)-epoxy acid could be obtained in 81 % optical purity. It showed $[\alpha]_D^{25}$ +51° (*c* 1.1, water) compared to the lit.[9] value $[\alpha]_D^{25}$ +63.1° (*c* 1.0, water). This acid was esterified with diazomethane to give *7*, $[\alpha]_D^{23}$ +133° (*c* 1.8, chloroform). ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 1 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 3.70 (s, 3 H), 3.11 (s, 2 H). MS (*m/e*, relative intensity): M$^+$ − 31 = 201 (11), 174 (9), 173 (92), 145 (36), 141 (100), 114 (9), 113 (97), 75 (47), 59 (98).

(2S,3S)-Trimethyl methylcitrate (*8*) was obtained in 60 % yield after preparative GLC. $[\alpha]_D^{22}$ +9.7° (*c* 0.3, methanol) for the sample being 81 % optically pure. Reported for the trimethyl ester of the methylcitric acid from *Candida lipolytica*: $[\alpha]_D^{20}$ +12° (*c* 1.5, methanol).[2] MS (*m/e*, relative intensity): M$^+$ = 248 (not observed), 189 (12), 157 (100), 115 (61), 101 (27), 88 (28), 59 (35). ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 1 H), 3.82 (s. 3 H), 3.70 (s, 3 H), 3.67 (s, 3 H), 3.14, 2.98, 2.88 and 2.72 (AB spectrum, 2 H), 2.85 (q, 1 H, J = 7 Hz), 1.20 (d, 3 H, J = 7 Hz). The corresponding *acid 12* showed (nm, $[\theta] \times 10^{-4}$): 275, −0.90; 249, +1.5; 233, −1.1.

(2R,3S)-Trimethyl methylcitrate (*10*) was also obtained in 60 % yield after preparative GLC: $[\alpha]_D^{22}$ −17.7° (*c* 0.75, methanol) for the sample being 81 % optically pure. MS: very similar to that of *8*. ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 1 H), 3.80 (s, 3 H), 3.68 (s, 6 H), 2.95 (q, 1 H, J = 7 Hz), 2.90 (s, 2 H), 1.26 (d, 3 H, J = 7 Hz). The corresponding *acid 14* showed (nm, $[\theta] \times 10^{-4}$): 280, −0.8; 249, +1.5; 233, −0.7; 218, +0.4; 205, −1.1.

(R,R*)-Trimethyl butylcitrate* was purified on a silica gel column with ether − light petroleum (2:3) as eluent. It was necessary to add a little diazomethane solution to the eluate since part of the ester had been hydrolysed on the column. MS (*m/e*, relative intensity): M$^+$ = 290 (not observed), 231 (8), 199 (100), 157 (59), 130 (28), 101 (43), 97 (30), 87 (50). ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 1 H), 3.80 (s, 3 H), 3.70 (s, 3 H), 3.66 (s, 3 H), 3.18 − 2.60 (m, 3 H, see Fig. 3), 1.90 − 0.70 (m, 9 H).

(2R,3S)-Trimethyl butylcitrate: $[\alpha]_{578}^{22}$ −13° (*c* 0.5, chloroform). MS: indistinguishable from that of the *R*,R** isomer. ^{1}H NMR ($CDCl_3$): δ 4.10 (s, 1 H), 3.79 (s, 3 H), 3.70 (s, 3 H), 3.69 (s, 3 H), 3.12 − 2.70 (m, 3 H, see Fig. 3), 1.80 − 0.70 (m, 9 H).

(2S,3S)-Trimethyl decylcitrate (*9*). The crude product was hydrolysed with 2 M sodium hydroxide in aqueous ethanol. After washing with ether the solution was acidified and the acid was extracted into ether. Esterification with diazomethane gave a product approximately 70 % pure (^{1}H NMR) (the presence of contaminating compounds only being revealed by too large a peak from decyl hydrogens). A sample of 90 % purity was obtained after preparative GLC. MS (*m/e*, relative intensity): M$^+$ = 374 (not observed) 315 (5), 283 (100), 241 (23), 214 (17), 163 (25), 101 (46), 87 (60), 74 (27), 69 (24), 59 (27), 55 (36). The ^{1}H NMR spectrum was indistinguishable from that of the trimethyl ester of the natural decylcitric acid,[4] except for the intensity of the signal from the decyl group. The *acid 13* showed (nm, $[\theta] \times 10^{-4}$): 275, −0.8; 250, +1.0; 230, −3.0.

(2R,3S)-Trimethyl decylcitrate (11). MS: indistinguishable from that of *9*. ^{1}H NMR ($CDCl_3$): δ 4.08 (s, 1 H), 3.78 (s, 3 H), 3.70 (s, 3 H), 3.69 (s, 3 H), 3.10 − 2.68 (m, 3 H, see Fig. 3), 1.80 − 0.70 (m, approximately 21 H). The *acid 15* showed in H_2O − THF (1:1) as solvent (nm, $[\theta] \times 10^{-4}$): 277, −0.30; 250, +0.12; 238, −0.32.

Investigation of natural acids. A crude mixture (4.9 mg) of methylcitric acids *1* and *5*, isolated from 40 ml of urine, was supplied by Dr. L. Sweetman (University of California, San Diego, USA). The acids were esterified with diazomethane and the esters were purified by preparative GLC on the ECNSS-M column at 170 °C. Two partially separated peaks were obtained which showed the sample to contain diastereomeric esters in the ratio 1:2, (retention times 14.8 and 16.4 min, respectively). The first eluted component (*R,S* or *S,R*) was obtained in more than 95 % purity and the second (*R,R* or *S,S*) in approximately 95 % purity. Found for *1* (nm, $[\theta] \times 10^{-4}$): 274, −1.1; 249, +1.5; 232, −1.6; 217, +1.4. Found for *5* (nm, $[\theta] \times 10^{-4}$): 280, −0.7; 249, +1.5; 232, −0.7; 222, +0.2; 207, −0.9. From the gas chromatographically determined relative configurations and these CD data it follows that *1* is identical to *12* and that *5* is identical to *14*.

The methylcitric acid from *Candida lipolytica* showed [2] in D_2O δ 1.17 (3 H, d), 2.61 (1 H, q),

2.73 (2 H, q, $J = 15.6$ Hz). Our acid *12* showed (in D_2O with sodium 3-(trimethylsilyl)propanesulfonate as internal reference): δ 1.20 (3 H, d), 2.90 (1 H, q), 2.91 and 3.17 (AB spectrum, 2 H, $J = 16$ Hz), whereas *14* showed δ 1.25 (3 H, d), ≈ 2.98 (1 H, obscured by the AB spectrum), 2.95 and 3.01 (AB spectrum, 2 H, $J = 16$ Hz). The spectra of these acids in alkaline D_2O solution did not show greater resemblance to that of the natural product than did the spectra of the acids. In our opinion, however, only the AB spectrum from *12* but not that of *14* resembles a 1:3:3:1 quartet. The specific rotation of the trimethyl ester of the natural acid is $[\alpha]_D^{20}$ +12° (*c* 1.5, methanol) and this value is in good agreement with that obtained for *8* (being 81 % optically pure): +9.7°. It differs clearly from that obtained for *10* (also 81 % optically pure): −17.7°.

(−)-*Decylcitric acid* [3,4] showed (nm, $[\theta] \times 10^{-4}$): 273, −2.4; 250, +2.3; 234, −3.2; 217, +3.0. With H_2O-THF (1:1) as solvent values of 285, −0.2; 249, +1.1; 225, −1.3; 210, +0.7 were obtained.

Norcaperatic acid (*3*),[5,25] obtained by hydrolysis of caperatic acid,[6] showed in H_2O-THF (1:1) (nm, $[\theta] \times 10^{-4}$): 282, −0.4; 247, +0.6; 225, −0.7.

Agaricic acid (*4*) was purchased from E. Merck and was recrystallised from ethanol. It showed in H_2O-THF (1:1) (nm, $[\theta] \times 10^{-4}$): 284, −0.3; 249, +1.2; 227, −1.5; 210, +0.8.

Trimethyl ethylcitrate was synthesised from diethyl ethyloxaloacetate and the lithium ester enolate of (−)-menthyl acetate in analogy with previously performed condensations with (−)-menthyl acetate.[22] Diethyl (−)-menthyl ethylcitrate was obtained in 45 % yield (NMR, piperonal as internal standard). Hydrolysis and subsequent methylation with diazomethane gave trimethyl ethylcitrate containing (*RS,SR*) and (*RR,SS*) isomers in the approximate ratio 10:1 (GLC, NMR). An analytical sample of the same composition was obtained by preparative GLC and showed $[\alpha]_{578}^{20}$ +2.1° (*c* 2.7, chloroform). $M^+ = 262$ (not observed), 203 (11), 171 (100), 129 (75), 112 (30), 111 (33), 87 (28), 59 (36). CD values obtained (nm, $[\theta] \times 10^{-4}$): 267, +0.2; 249, −0.4; 232, +0.2. These values show that the *2S,3R* isomer predominates over its enantiomer.

Dimethyl erythro-*3-methylmalate*. Dimethyl *trans*-2,3-epoxybutanedioate was prepared from the corresponding diacid [26] and diazomethane. It was then treated with lithium dimethylcuprate (as described for *6* and *7*), giving a product indistinguishable (GLC-MS) from an authentic sample of dimethyl *erythro*-3-methylmalate. The latter was obtained from the bis(*p*-bromophenacyl) ester [27] by alkaline hydrolysis, acidification and reaction with diazomethane. The *erythro* and *threo* isomers were well separated on the ECNSS-M GLC column.

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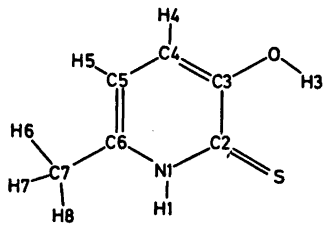
Crystal Structures of 3-Hydroxy-6-methylpyrid-2-thione and a Tetrahydrofuran Solvate of Its Disulfide

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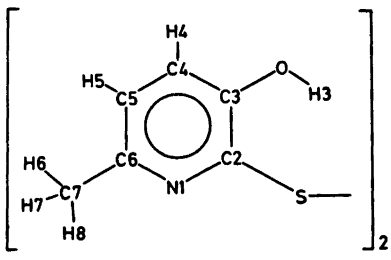
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The crystal structures of 3-hydroxy-6-methylpyrid-2-thione, and of a tetrahydrofuran solvate of its disulfide, have been derived from counter measurements and refined by least-squares calculations to $R=0.04$. In the thione some evidence is found for an intramolecular O—H···S hydrogen bond and neighbouring molecules are connected by N—H···S hydrogen bonds. In the disulfide the dihedral angle C—S—S—C is 96° and the angles between the pyridine rings and the S,S,C planes are 80 and 15°, respectively. This conformation is stabilized by an intramolecular O—H···N hydrogen bond. The tetrahydrofuran molecule is disordered.

Some of the structural features of the molecules were studied also by CNDO/2 methods.



1



2

The compound 3-hydroxy-6-methylpyrid-2-thione (*1*, hereafter called the thione) has imino-, thione- and hydroxyl groups in adjacent positions in the molecule. In connection with earlier crystallographic studies on *N*-hetero-aromatics [1,2] we wanted to examine the structure of this arrangement. Undheim and Lie have studied some of the reactions of the compound.[3,4] In the Michael additions to α-bromocrotonic acids *ortho*-hydroxyl derivatives, such as the thione, react relatively slowly. This was attributed to hydrogen bonding with sulfur.[3]

The thione is readily oxidized to the corresponding disulfide (2) and also this compound was investigated.

EXPERIMENTAL

Needle-shaped crystals of the thione, elongated along *a*, were obtained by recrystallization from an 1:1 alcohol-water mixture. Space group and preliminary unit cell dimensions were derived from Weissenberg- and precession photographs and refined by use of diffractometer measurements on 15 reflections.

Prismatic crystals of the disulfide were formed by slow evaporation from a tetrahydrofuran (THF) solution. Space group and unit cell dimensions were derived as for the thione, and the latter were refined by diffractometer measurements on 30 reflections.

Densities were measured by flotation. The density for the disulfide indicated that it had crystallized with one-half molecule of THF. The presence of THF in the crystals was confirmed mass spectrometrically.

CRYSTAL DATA

3-Hydroxy-6-methylpyrid-2-thione, C_6H_7NOS. Space group $P2_1/c$ (No. 14), $a=7.486(4)$ Å,

$b = 11.599(7)$ Å, $c = 15.499(7)$ Å, $\beta = 97.75(4)°$, $Z = 8$, $D_m = 1.395$ g cm^{-3}, $D_x = 1.406$ g cm^{-3}, $\mu(\mathrm{Mo}K\alpha) = 3.83$ cm^{-1}. There are two molecules (called A and B) in the asymmetric unit.

Bis-3-hydroxy-6-methyl-pyridyl-2,2'-disulfide hemitetrahydrofurane, $C_{12}H_{12}N_2O_2S_2 \cdot \frac{1}{2}C_4H_8O$. Space group $P2_1/n$ (No. 14), $a = 11.724(8)$ Å, $b = 13.380(4)$ Å, $c = 9.876(3)$ Å, $\beta = 95.14(3)°$, $Z = 4$, $D_m = 1.35$ g cm^{-3}, $D_x = 1.36$ g cm^{-3}, $\mu(\mathrm{Mo}K\alpha) = 3.44$ cm^{-1}.

DATA COLLECTION

The intensity measurements on the crystals of the thione and disulfide were, respectively, performed on an automatic Syntex $P\bar{1}$ and Picker diffractometer, using monochromatic Mo$K\alpha$ radiation (graphite monochromator) and the $2\theta - \theta$ scan technique. The thione crystal had dimensions $0.13 \times 0.22 \times 0.83$ mm and was mounted with its needle axis parallel to the ϕ-axis. 3828 reflections with $2\theta < 65°$ were measured, of which 2641 had intensities $I_o > 2.5\sigma(I_o)$ and were subsequently used in the refinements. For the disulfide the corresponding data are $0.37 \times 0.40 \times 0.55$ mm, 3531 measured reflections with $2\theta < 60°$ of which 2917 had $I_o > 2\sigma(I_o)$. The intensities of three test reflections did not vary significantly during the data collection. Corrections for absorption and extinction were not applied. This will cause errors in the temperature factors, but not significantly influence the positional parameters. The atomic form factors were those of Doyle and Turner,[5] except for hydrogen.[6]

STRUCTURE ANALYSES

In both structures the positions of the heavy atoms were derived from Patterson and Fourier syntheses. The THF molecules in the disulfide structure must occupy special positions and therefore be disordered. As the individual atoms could not be located on difference maps, the following method was used to derive their positions. Structure factors were calculated for different orientations of a THF molecule of assumed dimensions with $B = 5$ Å^2 and with its centre of gravity at $(\frac{1}{2},0,\frac{1}{2})$. The five ring atoms were all treated as carbon atoms and the THF hydrogen atoms neglected. Two orientations gave minima on an R versus orientation plot. THF molecules were placed in these orientations and tentatively given occupancy values of 0.3 and 0.2. Their positions were refined by translating the molecules around $(\frac{1}{2},0,\frac{1}{2})$. The description of the disorder thus obtained can probably be improved by considering more orientations and/or anisotropic thermal movements of the THF molecules.

The hydrogen atoms could not be located in the difference maps and were placed on the basis of stereochemical considerations. One of the methyl hydrogens and the hydroxyl hydrogen were assumed to lie close to the pyridine plane. For the thione the two possible hydroxyl hydrogen positions were tested by least-squares refinements and the position nearest to the sulfur atom gave a reasonable B-value, whereas the other gave B-values of 30 and 26 Å^2 for the two independent molecules.

The structures were refined by full-matrix least-squares calculations. The weighting schemes were based on standard deviations from counter statistics and an assumed 2 % fluctuation in diffractometer stability. Anisotropic temperature factors were applied to the ordered non-hydrogen atoms in both structures, isotropic ones to the hydrogen atoms.

It turned out to be necessary to refine the disordered portion of the disulfide structure in order to obtain reasonable parameters for the ordered hydrogen atoms. Positional, thermal (isotropic) and occupancy parameters for the individual atoms of the two THF molecules were therefore included in the least-squares full-matrix calculations. Overlap between atomic positions in the THF molecules caused difficulties during the refinement. In order to achieve convergence stepwise calculations had to be carried out. Some or all of the parameters of the THF molecules were varied, followed by refinement of the ordered portion of the structure, and so on. The hydrogen atoms of the methyl groups would not refine to reasonable positions, and are probably also disordered. In the THF part of the structure the atoms refined to stereochemically unreasonable positions (C–C bonds up to 0.2 Å off normal values), indicating that our model of the disorder is not satisfactory. We believe, however, that it is good enough to yield reliable parameters for the ordered atoms.

Table 1. Positional and thermal parameters ($\times 10^5$) for the non-hydrogen atoms in the thione. The temperature factor is $\exp[-(B11h^2+B22k^2+B33l^2+B12hk+B13hl+B23kl)]$.

	x	y	z	B11	B22	B33	B12	B13	B23
Molecule A									
S	37712 (9)	13784 (4)	- 8301 (3)	3823(17)	828 (4)	318 (2)	982(14)	-478 (8)	-110 (4)
O	34570(22)	38548(12)	- 4132 (9)	3399(40)	703(11)	440 (6)	324(34)	-213(26)	152(14)
N	51661(21)	14147(14)	8567 (8)	2116(35)	634(12)	327 (6)	15(35)	- 43(22)	31(14)
C2	44210(24)	20112(14)	1433(10)	1817(38)	683(13)	316 (6)	70(39)	120(25)	41(15)
C3	41948(26)	32022(15)	2664(11)	2018(40)	668(13)	368 (7)	-104(39)	117(27)	109(16)
C4	46968(31)	36914(19)	10625(12)	2992(55)	670(15)	477 (9)	-205(49)	30(35)	-129(19)
C5	54325(30)	30121(18)	17634(12)	2839(52)	870(17)	363 (8)	-632(50)	- 84(33)	-171(19)
C6	56632(25)	18665(16)	16600(10)	1863(40)	831(15)	318 (7)	-469(41)	3(26)	39(16)
C7	64230(45)	10427(24)	23605(15)	3095(69)	1031(22)	375 (9)	-436(68)	-518(40)	192(22)
Molecule B									
S	14478 (8)	-2848 (4)	12805 (3)	3208(15)	697 (4)	396 (2)	-109(13)	-505 (8)	70 (4)
O	17494(24)	12209(16)	27983 (8)	3604(44)	1131(15)	307 (5)	-444(44)	-229(24)	- 50(15)
N	886(21)	16886(13)	5560 (9)	2103(37)	729(12)	301 (6)	- 50(34)	42(24)	- 60(14)
C2	8189(24)	11163(15)	12770(10)	1767(37)	759(14)	316 (6)	-402(38)	101(26)	29(15)
C3	10598(27)	17751(17)	20530(10)	2102(42)	952(17)	309 (7)	-584(45)	104(27)	- 62(17)
C4	5919(32)	29060(20)	20484(13)	2915(57)	1001(19)	435 (9)	-295(54)	397(37)	-382(22)
C5	-1359(32)	34294(19)	12743(13)	2665(54)	805(18)	540(10)	206(50)	472(37)	-154(21)
C6	-4089(25)	28167(17)	5180(11)	1836(40)	778(15)	437 (8)	103(41)	297(29)	67(18)
C7	-12039(42)	32768(23)	-3457(16)	2760(62)	922(21)	522(11)	442(62)	120(44)	293(23)

The final values of the agreement indices are $R=0.040$, $R_w=0.038$ and $s=2.00$ for the thione, and $R=0.041$, $R_w=0.039$ and $s=3.45$ for the disulfide. The values of the goodness of fit (s) are slightly large for both structures, especially for the disulfide structure, but this has to be expected in the latter case.

The final parameters are given in Tables 1 – 4. Lists of observed and calculated structure factors will be supplied by the authors on request.

The calculations were carried out on a CD 3300 and on a CYBER-74 with programs described elsewhere.[7,8]

RESULTS AND DISCUSSION

The estimated standard deviations derived from the variance-covariance matrix are, as is well known,[9,10] often underestimated in structure analyses. A comparison of the values derived for corresponding bond lengths in molecules A and B in the thione, and residues I and II in the disulfide, indicates that this is the case in the present analyses. The e.s.d.'s given should probably be multiplied by a factor of about two.

The semiempirical theoretical calculations reported in this article have all been done by

Table 2. Positional ($\times 10^4$) and isotropic thermal parameters for the hydrogen atoms in the thione.

	Molecule A				Molecule B			
	x	y	z	B	x	y	z	B
H1	5285(24)	713(17)	790(11)	3.7 (4)	-14(26)	1344(16)	124(12)	4.0 (4)
H3	3291(34)	3401(21)	-820(15)	6.5 (6)	1917(31)	564(21)	2645(14)	5.6 (6)
H4	4577(28)	4480(20)	1130(13)	5.7 (5)	730(30)	3338(19)	2565(14)	6.2 (5)
H5	5724(29)	3343(18)	2314(14)	5.7 (5)	-492(28)	4191(20)	1243(12)	5.2 (5)
H6	6634(41)	1363(25)	2835(21)	8.7 (8)	-1635(33)	4031(23)	-287(14)	6.6 (6)
H7	7558(47)	693(28)	2279(19)	9.6 (9)	-2294(38)	2830(23)	-599(16)	7.7 (6)
H8	5701(37)	401(26)	2347(16)	7.7 (7)	-351(39)	3266(22)	-730(17)	7.6 (7)

Table 3. Parameters for the disulfide. Given as in Table 1, except for the coordinates for the tetrahydrofuran atoms (C10 – C19) which are multiplied by 10^4. G is the occupancy factor.

	x	y	z	B11	B22	B33	B12	B13	B23
Pyridyl I									
S	26250 (4)	30243 (3)	45742 (5)	557 (3)	364 (2)	1311 (6)	35 (5)	457 (7)	44 (6)
O	15326(14)	45255(11)	64632(15)	991(13)	681(10)	1428(19)	-474(19)	915(26)	-811(22)
N	4667(12)	24084(11)	44276(15)	589(11)	459 (8)	1066(17)	-82(15)	323(21)	-104(19)
C2	11806(13)	31158(12)	48704(16)	559(12)	368 (9)	872(18)	50(17)	252(23)	128(20)
C3	8420(15)	38294(13)	58639(18)	726(14)	456(10)	969(19)	-32(20)	379(27)	-76(23)
C4	-2893(18)	38003(16)	61723(23)	830(17)	618(13)	1442(27)	197(24)	747(35)	-353(31)
C5	-10043(18)	30840(17)	56217(23)	573(15)	760(15)	1602(29)	117(25)	588(33)	-150(34)
C6	-6136(15)	23862(15)	47415(20)	600(14)	619(12)	1326(24)	-97(21)	330(29)	-173(28)
C7	-13619(29)	15641(32)	41507(48)	778(22)	1217(27)	2608(67)	-736(39)	790(61)	-1411(73)
Pyridyl II									
S	27899 (4)	40700 (3)	31073 (5)	842 (4)	448 (3)	936 (5)	-343 (5)	451 (7)	-177 (6)
O	37554(12)	57521(10)	18592(13)	1022(12)	505 (8)	1098(16)	-169(16)	533(22)	159(18)
N	33559(12)	51988(10)	53355(15)	735(12)	406 (8)	1006(17)	-118(16)	167(23)	-75(19)
C2	33741(13)	51343(12)	40007(17)	537(12)	377 (9)	1010(20)	-23(16)	235(24)	-31(21)
C3	38158(14)	58817(12)	32160(18)	552(12)	426(10)	1122(21)	47(18)	293(25)	124(23)
C4	42805(16)	67023(14)	38992(22)	731(15)	407(10)	1458(27)	-195(20)	441(32)	90(26)
C5	42856(16)	67598(14)	52791(22)	732(15)	416(10)	1428(27)	-228(20)	15(31)	-156(26)
C6	38135(16)	60069(14)	59934(19)	751(15)	455(10)	1139(21)	-99(20)	19(28)	-182(25)
C7	37825(30)	60381(22)	74967(25)	1570(33)	684(16)	1187(27)	-567(40)	285(46)	-416(35)

Tetrahydrofuran

	x	y	z	G	B		x	y	z	G	B
C10	4604(15)	-740(10)	4095(17)	0.39(1)	11.5(3)	C15	5304(12)	-691 (9)	4588(15)	0.16(1)	2.7 (2
C11	4092(15)	441(12)	5224(16)	0.34(1)	7.2(2)	C16	5711(13)	607(13)	4580(15)	0.25(1)	5.5 (3
C12	5420(21)	884(12)	5034(25)	0.32(1)	10.1(5)	C17	4663(11)	948 (8)	4640(12)	0.30(1)	6.8 (2
C13	5961 (8)	234(11)	4820(11)	0.32(1)	4.7(2)	C18	3816(22)	237(24)	4832(27)	0.33(1)	16.0(10
C14	5488(14)	-775(10)	4235(15)	0.41(1)	9.5(4)	C19	4216(13)	-625(13)	4897(17)	0.22(1)	4.2 (2

use of the CNDO/2 approximation[11] with *d*-orbitals on the sulfur atoms. All of the structural parameters involving only non-hydrogen atoms have been taken directly from this work. For bond lengths of the type X – H standard values were used.

The thione

The bond lengths and angles are given in Table 5. There are no significant differences in bond lengths between the two independent molecules A and B, and the discussion will be based on mean values. The rings are essentially planar, but some of the atoms attached to them are significantly displaced. The sulfur atoms

Table 4. Positional ($\times 10^4$) and isotropic thermal parameters for the hydrogen atoms in the disulfide.

	Pyridyl I				Pyridyl II			
	x	y	z	B	x	y	z	B
H3	2203(21)	4615(17)	6096(23)	5.8 (5)	4031(25)	6359(25)	1436(30)	7.9 (7)
H4	-513(19)	4268(17)	6772(22)	5.4 (5)	4608(18)	7186(17)	3406(21)	4.8 (5)
H5	-1751(21)	3048(17)	5842(22)	5.6 (5)	4632(17)	7334(16)	5763(19)	4.8 (4)
H6	-1544(34)	1738(31)	3236(47)	10.4(11)	3306(32)	5574(33)	7845(36)	9.9(10)
H7	-2047(40)	1702(28)	4296(39)	10.7(10)	3022(43)	6277(39)	7627(46)	11.2(14)
H8	-1043(44)	1143(39)	3660(54)	14.0(15)	3967(29)	6714(27)	7849(33)	9.0 (8)

Table 5. Bond lengths (Å) and angles (°) for the thione.

	Molecule A	Molecule B		Molecule A	Molecule B
C2–S	1.689(2)	1.692(2)	S–C2–N1	122.9(1)	123.8(1)
C2–N1	1.359(2)	1.350(2)	S–C2–C3	121.5(1)	120.8(1)
C2–C3	1.408(2)	1.416(2)	N1–C2–C3	115.7(1)	115.4(2)
C3–O	1.353(2)	1.361(2)	C2–C3–O	119.2(2)	117.2(2)
C3–C4	1.364(3)	1.358(3)	O–C3–C4	120.4(2)	121.9(2)
C4–C5	1.394(3)	1.389(3)	C2–C3–C4	120.5(2)	120.9(2)
C5–C6	1.352(3)	1.363(3)	C3–C4–C5	120.1(2)	119.9(2)
C6–N1	1.356(2)	1.360(2)	C4–C5–C6	120.4(2)	120.7(2)
C6–C7	1.500(3)	1.489(3)	C5–C6–C7	125.6(2)	125.6(2)
			C7–C6–N1	116.7(2)	117.3(2)
N1–H1	0.83(2)	0.78(2)	C5–C6–N1	117.7(2)	117.1(2)
O–H3	0.82(2)	0.81(2)	C2–N1–C6	125.6(2)	125.9(2)
C4–H4	0.93(2)	0.94(2)	H1–N1–C2	116(1)	116(1)
C5–H5	0.93(2)	0.92(2)	H1–N1–C6	118(1)	118(1)
C7–H6	0.82(3)	0.94(3)	H3–O–C3	104(2)	105(2)
C7–H7	0.97(3)	1.00(3)	H4–C4–C3	120(1)	121(1)
C7–H8	0.92(3)	0.93(3)	H4–C4–C5	120(1)	119(1)
			H5–C5–C4	120(1)	123(1)
			H5–C5–C6	120(1)	117(1)
			H6–C7–C6	112(2)	110(1)
			H7–C7–C6	115(2)	112(1)
			H8–C7–C6	110(2)	110(2)
Intramolecular hydrogen bond			Intermolecular hydrogen bond		
H3···S	2.37(2)	2.32(2)	H1···S′	2.52(2)	2.60(2)
O···S	2.960(2)	2.914(2)	N1···S′	3.338(2)	3.346(2)
O–H3···S	129(2)	131(2)	N1–H1···S′	168(2)	162(2)
C2–S···H3	66(2)	67(2)			

are at the largest distance from the planes, 0.043 and 0.039 Å for molecules A and B, respectively.

The lengths of the C–S and C–O bonds, 1.691 Å and 1.357 Å, respectively, show that the molecule is the thione/hydroxyl tautomer, as expected. In accordance with other observations on heterocyclic compounds,[12] the extra-annular hydrogen atom attached to the nitrogen atom of the ring causes the C–N–C angle to be rather large.

CNDO calculations on the tautomers 6-methyl-2-mercaptopyridine and 6-methylpyrid-2-thione show that the thione form is stabilized relative to the mercapto form. The structures of the tautomers were taken to be similar to the disulfide and the thione, respectively. The calculated dipole moments of the two tautomers differ markedly, being 3.08 D for the mercapto and 7.00 D for the thione form. Jones and Katritzky [13] have found that in solution the tautomeric forms pyrid-2-thione and pyrid-4-thione are preferred by factors of *ca.* 10^4,[5] and 10^4 relative to the mercapto forms.

As far as we know the only other pyridinethione investigated by X-ray methods is α-thiopyridone.[14] This structure is, however, inaccurate ($\sigma \simeq 0.02$ Å). The structure most relevant for comparison with the present one would appear to be that of 5-chloro-pyridine-2-one.[15] The bond lengths and angles in the pyridine vary in the same manner in the two compounds. However, the χ^2-test [16] indicates that there are significant differences. It appears that substitution of the sulfur atom by oxygen causes changes in the ring bonds consistent with an increased "contribution" from V.B. structure in formula 1.

The angles between the C–CH_3 bond and the adjacent ring bonds are strikingly different, as is also found in the disulfide and in 2-carboxy-5-methyl-dihydrothiazolo[3,2-*a*]-pyridinium-3-carboxylate.[17]

The values of the angles between the C–O and C–S bonds and the adjacent ring bonds indicate an attraction between the hydroxyl and thione groups. The distances O···S are 2.960 and 2.914 Å, H···S 2.37 and 2.32 Å. This indicates the existence of an intramolecular hydrogen bond O–H···S between the hydroxyl and the thione groups.

CNDO calculations have been performed to roughly estimate the energy of the intramolecular hydrogen bond. A minimum of the energy is found when the hydrogen atom lies in the pyridine plane and points towards the sulfur atom. The calculated energy variation as a function of the dihedral angle (ϕ) between the pyridine plane and the plane defined by the atoms C3, O and H, keeping other structural parameters constant, can be closely approximated by an empirical function of the Morse potential type plus a cosine term:

$V(\phi)=D[1-\exp\{-a[r(\phi)-r_e]\}]^2+\frac{1}{2}V_2(1-\cos 2\phi)$ with $r(\phi)=[R-T\cos\phi]^{\frac{1}{2}}$, $r_e=2.310$ Å, $R=10.331$ and $T=5.031$.

D is the stabilization energy of the hydrogen bond, while the cosine term approximates the energy variation due to rotation of the hydroxyl group around the C–O bond. A least-squares calculation shows that D is relatively insensitive to the actual values of a and V_2. With $a=2.5$ Å^{-1} V_2 is 1.8 kcal/mol and $D=6.6$ kcal/mol. This value of V_2 is comparable with a value of 2.1 kcal/mol calculated for the compound 3-hydroxy-6-methylpyridine. Charge and π-bond order as calculated by the CNDO method for the thione are given in Fig. 1.

Intermolecular hydrogen bonds N–H···S link centrosymmetrically related molecules in pairs. The same type of hydrogen bonding is found for the structure of α-thiopyridone.[14] The molecules are nearly parallel to (100) and stack in columns along a. Molecules A and B alternate in the columns at distances between molecular planes of about 3.5 Å. There is an intercolumn contact of 2.89 Å between the oxygen atoms in molecules A and B.

The packing arrangement in the thione crystals is shown in Fig. 2.

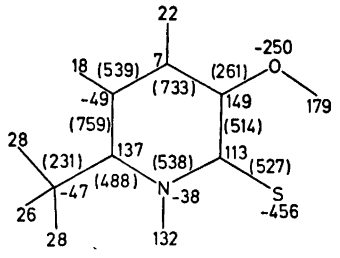


Fig. 1. Charge ($\times 10^3$) and π-bond order ($\times 10^3$, in parenthesis) for the thione.

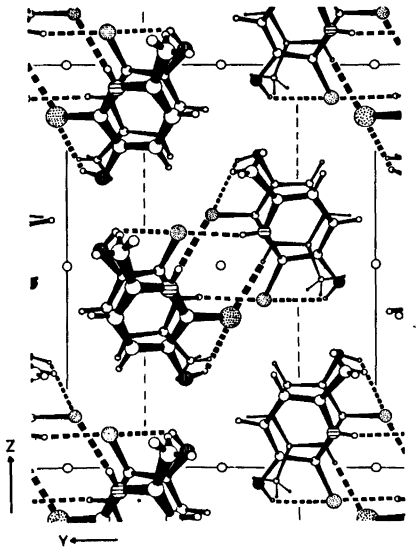


Fig. 2. The a projection of the thione structure. Broken lines indicate hydrogen bonds.

The disulfide

Bond lengths and angles are given in Table 6. The two pyridyls are denoted I and II, respectively. The pyridine rings are planar within error limits, but some of the extraannular atoms deviate significantly from the ring planes. The deviations are (Å) 0.010, 0.047, 0.054 and −0.14 for atoms S, O, C6, and H3, respectively, in pyridyl I, whereas the corresponding distances in pyridyl II are 0.027, −0.031, 0.00 and −0.13.

The dihedral angle C–S–S–C is 96.2(1)°, which is within the range of 90 ± 10° usually found for organic disulfides.[18,19] The orientation of the pyridine rings is unusual, as their planes form widely different angles, 14.8 and 79.8°, respectively, with the adjacent S,S,C planes. In the aromatic disulfides so far in-

Table 6. Bond lengths (Å) and angles (°) for the disulfide.

	Pyridyl I	Pyridyl II		Pyridyl I	Pyridyl II
C2–S	1.776(2)	1.780(2)	C2–S–S	105.2(1)	104.7(1)
C2–N1	1.343(2)	1.323(2)	S–C2–N1	115.8(1)	120.4(1)
C2–C3	1.382(2)	1.393(2)	S–C2–C3	121.5(1)	116.4(1)
C3–O	1.337(2)	1.347(2)	N1–C2–C3	122.5(1)	123.2(2)
C3–C4	1.388(3)	1.375(3)	C2–C3–O	124.5(2)	118.2(2)
C4–C5	1.355(3)	1.365(3)	O–C3–C4	118.4(2)	125.0(2)
C5–C6	1.381(3)	1.374(3)	C2–C3–C4	117.1(2)	116.8(2)
C6–C7	1.493(4)	1.489(3)	C3–C4–C5	120.1(2)	120.1(2)
C6–N1	1.331(2)	1.348(2)	C4–C5–C6	120.2(2)	120.4(2)
S–S	2.036(1)		C5–C6–C7	121.9(2)	122.6(2)
			C7–C6–N1	117.6(2)	117.3(2)
O–H3	0.90(3)	0.98(3)	C5–C6–N1	120.5(2)	120.1(2)
C4–H4	0.92(2)	0.92(2)	C2–N1–C6	119.7(2)	119.4(2)
C5–H5	0.92(2)	0.97(2)	H3–O–C3	115(1)	109(2)
C7–H6	0.94(4)	0.92(4)	H4–C4–C3	117(1)	118(1)
C7–H7	0.85(4)	0.97(4)	H4–C4–C5	123(1)	122(1)
C7–H8	0.85(4)	0.99(4)	H5–C5–C4	121(1)	120(1)
			H5–C5–C6	119(1)	120(1)
Intramolecular hydrogen bond			Intermolecular hydrogen bond		
H3(I)···N1(II)		1.78(3)	H3(II)···N1(I)		1.77(3)
O(I)···N1(II)		2.655(2)	O(II)···N1(I)		2.751(2)
O(I)–H3(I)···N1(I)		161(3)	O(II)–H3(II)···N1(I)		176(3)

vestigated these angles are approximately equal, being roughly either 0 or 90°. The arrangement found in the present structure is presumably due to the formation of an intramolecular hydrogen bond O–H···N between the two pyridyls.

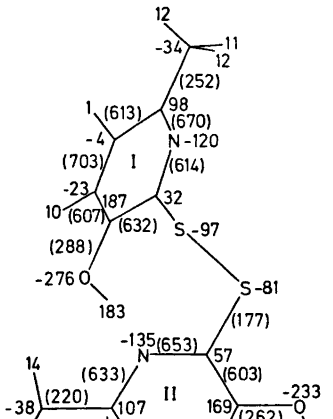


Fig. 3. Charge (× 10^3) and π-bond order (× 10^3, in parenthesis) for the disulfide.

The S–S bond is 2.036 Å, the S–C bonds 1.776 Å and 1.780 Å and the S–S–C angles 105.2 and 104.7°, respectively. In these respects

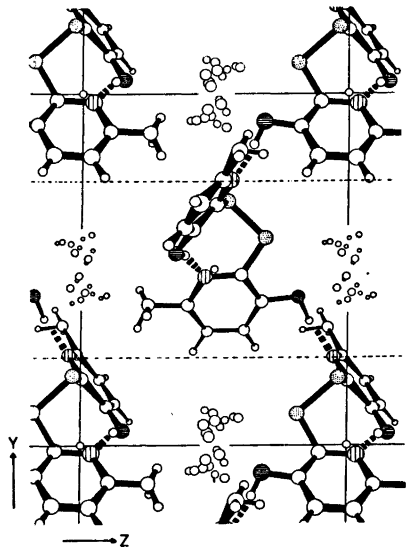


Fig. 4. The *a* projection of the disulfide. For clarity the mirrorplane-related molecules are not shown. The clusters of small circles indicate atoms in the THF molecules.

the disulfide belongs to the same type as, *e.g.*, diphenyl disulfide,[20] in which the angle between the phenyl and the S,S,C planes are 27 and 32°, respectively.

As to be expected, there are significant differences between the thione and the disulfide in the bond lengths and angles of the pyridine ring. The bonds N1 – C2, N1 – C6, C2 – C3 and C4 – C5 are shorter in the disulfide, and the bonds C3 – C4 and C5 – C6 are longer than corresponding ones in the thione, in accordance with the higher degree of delocalization of π-electrons in the pyridine part of the disulfide.

Charge and π-bond order in the disulfide calculated by use of the CNDO method are given in Fig. 3.

The disulfide molecules form helices along the screw axes and are connected within the helices by nearly linear O – H···N hydrogen bonds. The THF molecules occupy pockets of diameter roughly 4 Å and are in van der Waals contact with several atoms in surrounding disulfide molecules. This packing arrangement is shown in Fig. 4.

We wish to thank Professor S. Laland and Dr. K. Undheim for samples of the compounds.

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Indolizine Derivatives. VIII. 3-Acyloxyindolizines *via* Cyclization of Diethyl 2-Pyridylmethylenemalonate

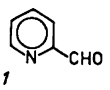
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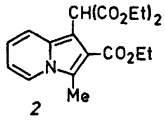
In boiling acetic anhydride the cyclization of diethyl 2-pyridylmethylenemalonate (*3*) gives ethyl 3-acetoxy-2-ethoxycarbonyl-1-indolizineacetate (*4*) in high yield. Use of [2-^{13}C]-labelled acetic anhydride affords 3-[2-^{13}C] acetoxy-1-[2-^{13}C] acetate enriched *4* (*4'*), suggesting that cyclization of the intermediate addition product of *3* and acetic anhydride occurs at the ester carbonyl group and involves an intramolecular ethoxy shift. Propionic anhydride yields diethyl 2-methyl-3-propionyloxy-1-indolizinemalonate (*9*) through reaction at the anhydride carbonyl.

Similar cyclizations in the presence of 1,3-dicarbonyl species gave pyrrolo[2,1,5-*cd*] indolizines *via* 3-acyloxyindolizines.

The cyclizations of 2-(2-pyridyl)methylene-1,3-diketones and -1,3-keto esters in acid anhydrides to yield 2-acylindolizines through reaction of the ketone carbonyl were discussed in Part VII of this series.[1] On the other hand, it was recently reported that the Perkin reaction of 2-pyridinecarbaldehyde (*1*) in the presence of diethyl malonate gives the indolizinemalonate *2*.[2] The next logical step was to study the reaction of 2-(2-pyridyl)methylene-1,3-diesters with an acid anhydride.



1

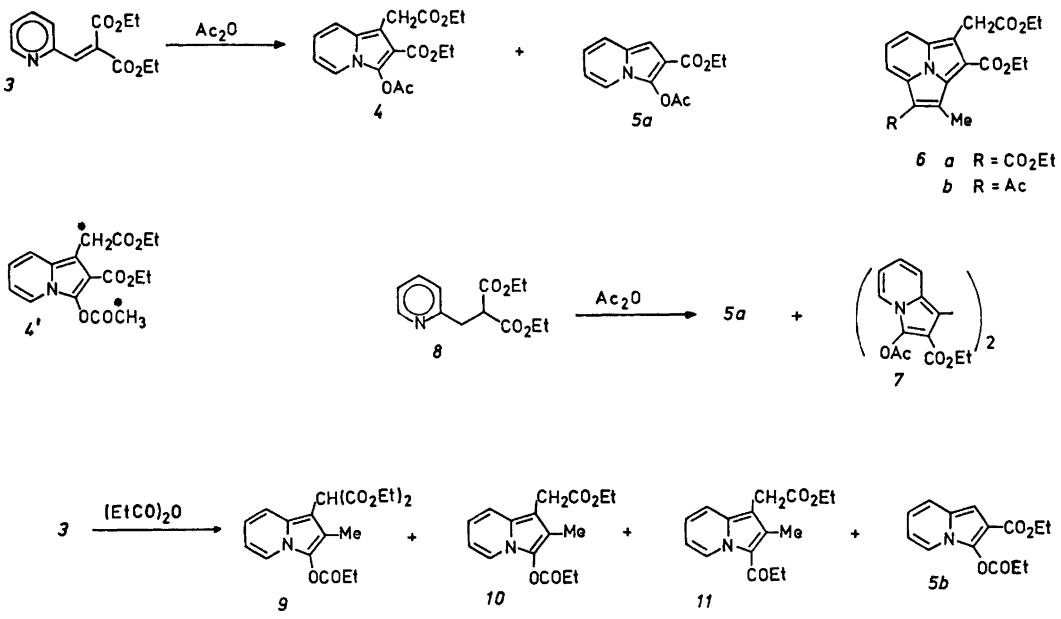


2

Thus, the present paper deals with the cyclization of diethyl 2-pyridylmethylenemalonate (*3*) with acetic and propionic anhydrides, as well as its reaction in the presence of some 1,3-dicarbonyl compounds. These novel conversions furnishing 3-acyloxyindolizines are described in detail. 3-Acyloxyindolizines can be used as starting materials in a versatile synthesis of the pyrrolo[2,1,5-*cd*]indolizine nucleus.[3] The reaction of *3* with ^{13}C-labelled acetic anhydride to solve the origin of the carbon atoms of the C-1 acetate group of *4* and to settle the question about the migrating group is included. The mechanisms are discussed.

Cyclization products

Diethyl 2-pyridylmethylenemalonate (*3*) showed no reaction with acetic anhydride below 100 °C, whereas at reflux temperature it was converted into the indolizineacetic ester *4* in 80 % yield. Some of the indolizine *5a* was also isolated (Scheme 1). The structure of *4* (like other new indolizine derivatives below) was assigned by comparison of its spectral data (UV, IR, NMR, MS) with those of related indolizines.[1,4] Thus, *4* was shown to be 1,2,3-trisubstituted indolizineacetic acid derivative (^{1}H NMR) and a 2-indolizinecarboxylic ester (UV, IR). Further, the formation of the cycloaddition compounds *6a* and *6b* from *4* with ethyl acetoacetate or 2,4-pentanedione, respectively, supported the presence of a 3-acyloxyindolizine partial structure.[3,4] The ^{13}C NMR spectrum of *4* was in accord with its formula. Cyclization of *3* with acetic anhydride/[2-^{13}C]-acetic acid gave the corresponding 3-[2-^{13}C]-acetoxy-1-[2-^{13}C]acetate enriched *4* (*4'*) as verified by ^{13}C NMR spectroscopy. The indolizine *5a* was the product, along with the dimer *7*, when diethyl 2-pyridylmethylmalonate (*8*) was treated with boiling acetic anhydride.



Scheme 1.

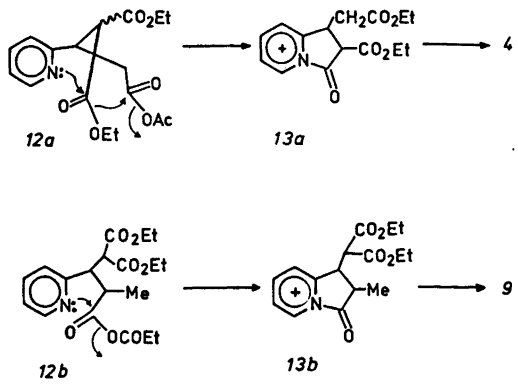
With propionic anhydride the diester *3* gave the indolizinemalonate *9* in 70 % yield at 130 °C. In refluxing propionic anhydride *3* was cyclized to a mixture of the indolizineesters *9*, *10*, *11* and *5b*, the malonate *9* being in slight majority (30 %). Heating *9* in propionic anhydride containing 20 % of propionic acid afforded a mixture of *10*, *11* and unchanged *9*.

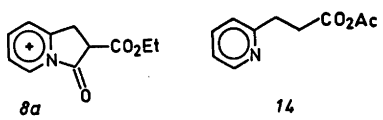
The diester *3* did not react with benzoic anhydride. Cyclization of *3* with acetic anhydride in the presence of ethyl acetoacetate gave the pyrrolo[2,1,5-*cd*]indolizine *6a* as the end product *via* *4* (TLC). 2,4-Pentanedione afforded similarly the pyrrolo[2,1,5-*cd*]indolizine *6b*, whereas acetic anhydride/diethyl malonate did not convert *3* further than to *4*.

Treatment of the diester *3* with acetic anhydride/potassium acetate in the presence of diethyl malonate gave the indolizinemalonate *2*, which has been shown to be the main product of the Perkin reaction of the aldehyde *1* in the presence of diethyl malonate.[2]

Mechanistical sequences

The formation of 2-acylindolizines from 2-(2-pyridyl)methylene-1,3-keto esters and acid anhydrides takes place exclusively *via* an intramolecular nucleophilic attack of the ring nitrogen atom on the side-chain ketone carbonyl group.[1] Hence, 2-(2-pyridyl)methylene-1,3-diesters would be expected to be quite unreactive. However, nucleophilic addition of acetic and propionic anhydrides probably changes diethyl 2-pyridylmethylenemalonate (*3*) into the intermediates *12a* and *12b*, respectively, which are able to undergo cyclizations. The reason for *3* remaining unchanged when heated with benzoic anhydride would be that no intermediate analogous to *12a* and *12b* can be formed.





8a *14*

The saturated diester *8* cyclizes only reluctantly, probably *via 8a*, to give *5a*,[5] the yield being moderate at its best. The much more facile cyclization of *12a* is readily understandable as being due to the simultaneous intramolecular transesterification (ethoxy shift) furnishing *4 via 13a*. This is strongly supported by isolation of the ^{13}C-labelled indolizine *4'* when *3* was treated with [2-^{13}C]-enriched acetic anhydride. Another, foreseeable possibility[3] involving the attack of the ring nitrogen on the anhydride carbonyl group and migration of one of the ethoxycarbonyl groups is out of the question.

Interestingly, cyclization of *12b* takes a different course, namely as a reaction at the anhydride carbonyl group to *9 via 13b*, although the saturated anhydride *14*[4] will not react at all in boiling acetic anhydride. At the present it is difficult to explain the divergent reactions of *3* with acetic and propionic anhydrides. Presumably, the methyl substituent prevents *12b* to attain the conformation essential for the attack on the ester carbonyl, while sterically enhancing conversion into *13b*, with *9* as the sole product.

The formation of *5a*, *5b* from *3*, and *11* from *9*, require a reduction step, occurring probably *via* a route proposed earlier for some analogous transformations,[1,4] as exemplified for the case of formation of *11* from *10 via 15* and *16*.

EXPERIMENTAL

The general conditions of related cyclizations, as well as separation procedures and instruments used, have been described in the earlier papers of this series.[1,4] The ^{13}C NMR spectra were measured for solutions in $CDCl_3$ with a JEOL JNM FX60 spectrometer through cooperation of Dr. E. Kantolahti at the laboratories of Kemira Oy.

Diethyl 2-pyridylmethylenemalonate (3).[6] From diethyl malonate (16.0 g, 0.10 mol) and 2-pyridinecarbaldehyde (10.7 g, 0.10 mol). Aldol formation catalyzed by 0.5 ml of $(Et)_2NH$ was accomplished at 20 °C within 2.5 h. Heating this aldol product in Ac_2O (50 ml) at 100 °C for 1 h effected the dehydration. Evaporation and recrystallization gave the malonate *3*, 19.5 g (78 %), m.p. (MeOH) 48 °C. Alternatively, the aldol was heated neat at 110 °C for 2 h to yield 82 % of *3*.

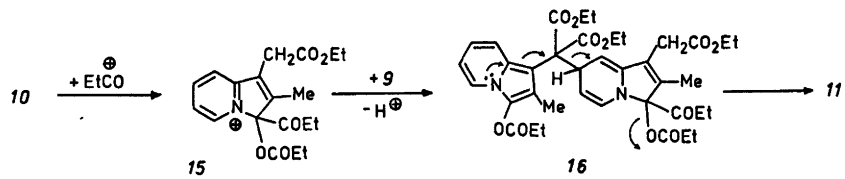
General procedure for cyclizations. The pyridyldiester was heated in an excess of acid anhydride (1/10, mol/mol), temperature and time given. After the reaction all volatile materials were removed *in vacuo*. The residue was fractionated, when necessary, by CC and the components purified by recrystallization from light petroleum (b.p. 40–60 °C) if not stated otherwise.

3 *with* Ac_2O. At refluxing temperature (5 h) *3* gave: *ethyl 3-acetoxy-2-indolizinecarboxylate* (*5a*),[5] yield 4 %, m.p. 77 °C; *ethyl 3-acetoxy-2-ethoxycarbonyl-1-indolizineacetate* (*4*), yield 78 %, m.p. 85 °C. Anal. $C_{17}H_{19}NO_6$: C, H, N. IR: 1780 (s), 1725 (s), 1715 (s), 1700 (s), 1690 (s) cm^{-1}. ^1H NMR: δ 7.47 (1 H, broad d, J 7 Hz), 7.26 (1 H, broad d, J 9 Hz), 6.70–6.20 (2 H, m), 4.22 (2 H, q), 4.02 (2 H, q), 3.91 (2 H, s), 2.38 (3 H, s), 1.32 (3 H, t), 1.20 (3 H, t). ^{13}C NMR: δ 14.2 ($2\times CH_3-CH_2-$), 20.3 (CH_3CO_2-), 30.6 ($Ar-CH_2-CO_2-$), 59.8 and 60.4 ($2\times CH_3CH_2-$), 104.2 and 105.6 (C-1 or C-2), 112.2, 117.0, 117.6 and 119.0 (C-5, C-6, C-7 or C-8), 125.2 (C-8a), 129.8 (C-3), 163.2, 167.5 and 171.1 ($3\times -CO-O-$). MS, *m/e*: 333 (M).

3 *with* $Ac_2O/2$-^{13}C *acetic acid.* To the preheated mixture of Ac_2O (30 ml) and [2-^{13}C]-acetic acid (1.0 ml, 90 %) 2.50 g (0.010 mol) of *3* was added and boiled for 4 h to afford 1.33 g (40 %) of *4'*. ^{13}C NMR: the peaks at δ 20.3 and 30.6 enriched (*ca.* five-fold as compared with those of *4*).

8 *with* Ac_2O. Heating *8*[5] (from *3* with H_2-Pd/C) with Ac_2O gave the indolizine *5a*[5] (22 %) and *diethyl 3,3'-diacetoxy-1,1'-biindolizine-2,2'-dicarboxylate* (*7*), yield 5 %, m.p. (light petroleum b.p. 40–60 °C/AcOEt) 177 °C. Anal. $C_{26}H_{24}N_2O_8$: C, H, N. MS, *m/e*: 492 (10, M).

3 *with* Ac_2O *and* Ac_2CH_2. *3* (5.0 g, 0.020 mol) and Ac_2CH_2 (3.0 g, 0.030 mol) were refluxed in Ac_2O (100 ml) for 15 h to give after aqueous work-up and recrystallization *ethyl 4-acetyl-2-ethoxycarbonyl-3-methyl-1-pyrrolo*[*2,1,5-cd*]*indolizineacetate* (*6b*), yield 3.6 g (51 %), m.p. (EtOH) 148 °C. Anal. $C_{20}H_{21}NO_5$: C, H, N.



^{1}H NMR: δ 8.32 (1 H, broad d, J 7 Hz), 7.90 (2 H, m), 4.49 (2 H, q), 4.40 (2 H, s), 4.18 (2 H, q), 3.16 (3 H, s), 2.81 (3 H, s), 1.47 (3 H, t), 1.25 (3 H, t).

3 *with* Ac_2O *and* $AcCH_2CO_2Et$. Similarly as above there was obtained *ethyl 2,4-diethoxycarbonyl-3-methyl-1-pyrrolo*[*2,1,5*-cd]*indolizineacetate* (*6a*), yield 24 %, m.p. (EtOH) 167 °C. Anal. $C_{21}H_{23}NO_6$: C, H, N.

3 *with* $(EtCO)_2O$. The reaction at reflux temperature (5 h) gave the following mixture: *ethyl 3-propionyloxy-2-indolizinecarboxylate* (*5b*), yield 5 %, m.p. 53 °C. Anal. $C_{14}H_{15}NO_4$: C, H, N; *ethyl 2-methyl-3-propionyloxy-1-indolizineacetate* (*10*), yield 21 %, m.p. 61 °C. Anal. $C_{16}H_{19}NO_4$: C, H, N. ^{1}H NMR: δ 3.57 (2 H, s), 2.11 (3 H, s); *diethyl-2-methyl-3-propionyloxy-1-indolizinemalonate* (*9*), yield 28 %, m.p. 81 °C. Anal. $C_{19}H_{23}NO_6$: C, H, N. ^{1}H NMR: δ 4.72 (1 H, s), 2.10 (3 H, s); *ethyl 2-methyl-3-propionyl-1-indolizineacetate* (*11*), yield 7 %, m.p. 70 °C. Anal. $C_{16}H_{19}NO_3$: C, H, N. IR: 1730 (s), 1720 (s), 1615 (s), 1605 (s) cm^{-1}. ^{1}H NMR: δ 9.93 (1 H, ddd, J 6.7, 1.2 and 1.0 Hz), 3.53 (2 H, s), 2.49 (3 H, s). The reaction at 130 °C (2.5 h) gave *9* in 72 % yield. Treatment of *9* with $(EtCO)_2O/EtCO_2H$ (1/8/2, mol/mol/mol) at 140 °C for 3 h afforded the following mixture: *9* (40 %), *10* (35 %) and *11* (5 %).

3 *with* $Ac_2O/KOAc$ *and* $CH_2(CO_2Et)_2$. 2.5 g of *3*, 3.2 g of $CH_2(CO_2Et)_2$ and 30 g of KOAc in 100 ml of Ac_2O were kept at 120 °C for 1 h producing after aqueous work-up and CC *diethyl 2-ethoxycarbonyl-3-methyl-1-indolizinemalonate* (*2*), yield 26 %, m.p. 72 °C. Anal. $C_{19}H_{23}NO_6$: C, H, N. ^{1}H NMR: δ 4.31 (1 H, s), 2.58 (3 H, s).

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The $^{13}C,H$ Coupling Constants in Structural and Conformational Analysis. Part I. The Correlation of $^2J(CH_3CH)$ and $^2J(HCH)$ in Certain Systems

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The geminal $^2J(CH_3CH)$ coupling constants were determined for a number of cyclic compounds. A linear correlation was found to exist between the corresponding $^2J(HCH)$ and $^2J(CH_3CH)$, the expression for the correlation being determined as $^2J_{CH} = 0.55 \times {}^2J_{HH} + 4.93$ from regression analysis. A correlation of the same linear type was detected also for those unsaturated acyclic compounds where the methyl group is attached to an sp^2 carbon, the correlation being here expressed as $^2J_{CH} = 0.58 \times {}^2J_{HH} + 2.86$. The restrictions of these correlations are also discussed.

The coupling constants between directly and indirectly bonded protons and carbon-13 have been studied by many authors.[1–4] The results indicate a clear relation to a number of structural and atomic properties of the compounds, such as bond lengths and angles, electronegativities of substituents, hybridization of the coupled atoms and additivity of substituent effects. It has also been noted that in general a linear relation exists between the corresponding J_{CH} and J_{HH} (and likewise between J_{CH} and J_{CC}), the ratio J_{CH}/J_{HH} being $+0.4$ to $+0.7$.[1–4] As pointed out earlier, one should determine J_{CC} and J_{CH} values of a system that can be related to a geometrically equivalent carbon-proton or proton-proton system, respectively.[3,4]

The present article is concerned with the two-bond coupling constants in systems where a hydrogen is being replaced by a methyl group and a comparison between $^2J_{HH}$ and $^2J_{CH}$ in the same geometrical surroundings is possible.

EXPERIMENTAL

All carbon data were measured with a JEOL FX-60 instrument at 15.04 MHz. The spectra were taken from concentrated samples (20–50 % w/v) in acetone-d_6 or in deuteriochloroform. A spectrum width of 500–1000 Hz was used in the observation of the non-decoupled methyl carbons and the data capacity was 8K. A proper S/N ratio was achieved in general by 1000–5000 accumulations. A gated decoupling technique (with NOE) was used to enhance the S/N ratio. The experimental error in the values of $^2J_{CH}$ couplings is considered to be ± 0.1 Hz in most cases. The compounds under examination were chosen as to allow a first order analysis of the spectra. In some cases (*cf.* below) first order methods are not strictly applicable but due to large relative chemical shifts of the protons (more than 6 times the corresponding J's in each case) the probable additional inaccuracy in some values is less than ± 0.1 Hz.

RESULTS

The data from the following cyclic compounds are collected in Table 1: 2-methyl-2-*tert*-butyl-4,6-dioxo-1,3-dioxane (*1*), *trans*-2,5-dimethyl-2-*tert*-butyl-4,6-dioxo-1,3-dioxane (*2*), 2-phenyl-4,6-dioxo-1,3-dioxane (*3*), *cis*-2-phenyl-5-methyl-4,6-dioxo-1,3-dioxane (*4*), 6-methyl-2-oxo-1,4-dioxane (*5*), *trans*-3,6-dimethyl-2-oxo-1,4-dioxane (*6*), *cis*-3,6-dimethyl-2-oxo-1,4-dioxane (*7*), 6-methyl-4-oxo-1,3-dioxane (*8*), *trans*-5,6-dimethyl-4-oxo-1,3-dioxane (*9*), 3,4-dihydro-2,3-dimethyl-2*H*-1,3-benzoxazine (*10*), 3,4-dihydro-*cis*-2,3,4-trimethyl-2*H*-1,3-benzoxazine (*11*), 3,4-dihydro-*trans*-2,3,4-trimethyl-2*H*-1,3-benzoxazine (*12*), *cis*-3,5-dimethyl-2-

Table 1. Observed geminal $J(HCH)$ and $J(CH_3CH)$ in the studied compounds.

Compound	Carbon	$^2J(HCH)$	Ref.	Compound	$^2J(CH_3CH)$
1	5	−21.4	14	*2*	−6.2
3	5	−18.5	14	*4*	−5.4
5	3	−17.7	15	*6*	−4.3
5	3	−17.7	15	*7* [a]	−4.2
8	5	−17.6	16	*9*	−4.6
10	4	−16.6	17	*11* [a,b]	−4.6
10	4	−16.6	17	*12* [b]	−4.7
6	5	−12.5	15	*14*	−2.3
15	6	−12.0	16	*18*	−2.4
19	6	−10.9	18	*9*	−1.3
13	6	−10.9	15	*14*	−1.6
16	2	−10.4	17	*11* [a,b]	$\lvert J\rvert < 1.5$ [c]
16	2	−10.4	17	*12* [b]	$\lvert J\rvert < 1.5$ [c]
17	2	−9 [d]	7	*23* [b]	$\lvert J\rvert < 0.5$ [c]
20	2	−6.1 [d]	7	*21*	+1.6
22	2	−5.4	19	*18*	+2.3
24	2	+1 [d]	7	*25*	+6.0

[a] Axial methyl group (at least partially). [b] The average orientation of the $N-CH_3$ group may be different from that of the reference compound. This can affect the relation of $^2J_{HH}$ and $^2J_{HC}$ (see Refs. 6–8). [c] Unresol ved signals due to other small long range couplings. These values were omitted from the regression analysis. [d] A "best value", see Refs. 6–7.

oxo-1,4-dioxane (*13*), *trans*-5,6-dimethyl-2-oxo-1,4-dioxane (*14*), 2,5,5-trimethyl-4-oxo-1,3-dioxane (*15*), 3,4-dihydro-3,4-dimethyl-2*H*-1,3-benzoxazine (*16*), tetrahydro-3-methyl-1,3-oxazine (*17*), *cis*-2,5,5,6-tetramethyl-4-oxo-1,3-dioxane (*18*), 5-methyl-4-oxo-1,3-dioxane (*19*), 1,3-dioxane (*20*), 2-methyl-1,3-dioxane (*21*), 5,5,6-trimethyl-4-oxo-1,3-dioxane (*22*), tetrahydro-2,3-dimethyl-1,3-oxazine (*23*), 1,3-dioxolane (*24*), 2-methyl-1,3-dioxolane (*25*).

The ^1H data were taken from reported results in the literature. Some of them are collected from unpublished works from this laboratory. As mentioned above the compounds were selected to allow a first order analysis of the carbon-13 spectrum; the methyl group is generally located on a carbon being situated between two hetero atoms or two fully substituted carbons (or carbonyls). Thus in most cases there are only two observable couplings in the CH_3 signal, a large $^1J_{CH}$ and the much smaller $^2J_{CH}$, both of which can directly be measured.

It is known that several factors influence the $^2J_{HH}$ value in cyclic and acyclic systems;[5–9] the interference by these factors was removed by a suitable choice of compounds, bearing in mind that the ring geometry should not change due to the substitution of a hydrogen by a methyl group. In most cases the reference compounds for ^1H data were selected so that only the methyl group under study was replaced by a hydrogen. In some cases this was not possible and another type of reference compound to give $^2J_{HH}$ was used (*cf.* Table 1). This may cause a minor inconsistency between the corresponding $^2J_{HH}$ and $^2J_{CH}$ values due to reasons mentioned above.

It is known that $^2J_{HH}$ can be of either sign, a negative one being more usual in sp^3 CH_2 groups. The $^2J_{HH}$ of methane is −12.4 Hz [5,10] which corresponds to a negative $^2J_{CH}$ in ethane.[11] Thus it is obvious that a $^2J_{CH}$ associated with a large negative $^2J_{HH}$ also is negative. It is also obvious that at a certain value of $^2J_{HH}$ the corresponding $^2J_{CH}$ coupling becomes positive because large positive $^2J_{HH}$ couplings are associated with a large positive $^2J_{CH}$ coupling, *e.g.* formaldehyde (+41 Hz) [12] *vs.* acetaldehyde (+26.7 Hz).[13] From Table 1 it is seen that the absolute value of $^2J_{CH}$ becomes gradually smaller as the absolute value of the corresponding $^2J_{HH}$ decreases but begins to increase again as $^2J_{HH}$ goes from *ca.* −6 Hz

Table 2. Values of $^2J(HCH)$ and $^2J(CH_3CH)$ over sp^2 carbons. $^2J(CH_3CH)$ values predicted from eqn. (1) are also shown.

Compound	$^2J_{HH}$	$^2J_{CH}$ obs.	$^2J_{CH}$ pred.	Ref.
Formaldehyde	+41	–	–	12
Acetaldehyde	–	+26.7	+27.5	13
3,5-Dinitrophenylhydrazone of formaldehyde	+11.6	–	–	20
of acetaldehyde	–	+9.1	+11.3	This work
Acrylic acid	+1.7	–	–	21
Crotonic acid	–	+3.9	+5.9	This work
Propenal	+1.0	–	–	22
trans-2-Butenal	–	+3.5	+5.5	This work
Acrylonitrile	+0.9	–	–	23
trans-Crotononitrile	–	+3.5	+5.4	This work
cis-Crotononitrile	–	+3.5	+5.4	This work

to *ca.* 0 Hz. Hence the $^2J_{CH}$ coupling must change sign somewhere around $^2J_{HH} = -9$ Hz.

As a first try, we have applied a linear regression analysis to the data of Table 1. This seems to be a rather good approximation since the correlation coefficient appears to be 0.991 suggesting, indeed, an approximately linear correlation:

$$^2J_{CH} = 0.551\ (\pm 0.020) \times {}^2J_{HH} + 4.929\ (\pm 0.292) \quad (1)$$

Eqn. (1) may be tested for, *e.g.*, formaldehyde and acetaldehyde, by substituting $^2J_{HH} = +41$ Hz [12] which gives $^2J_{CH} = +27.5$ Hz which fits well with observed value (26.7 Hz). However, the validity of eqn. (1) for these sp^2 carbon compounds is slightly fortuitous since in other sp^2 carbon compounds (Table 2) the agreement between the values of $^2J_{HA}$ and $^2J_{CH}$ is not as good as for formaldehyde *vs.* acetaldehyde. In fact, the examples of Table 2 subjected to a regression analysis yield another linear correlation (correlation coefficient 1.000):

$$^2J_{CH} = 0.578\ (\pm 0.006) \times {}^2J_{HH} + 2.863\ (\pm 0.111) \quad (2)$$

The slope of eqn. (2) is nearly the same as in eqn. (1) but the intercept is somewhat smaller.

CONCLUSIONS

The present study indicates that a linear correlation exists between corresponding $^2J_{CH}$ and $^2J_{HH}$ in similar geometrical surroundings, as could be anticipated from earlier results.[1–4] A behaviour like this is reasonable if the coupling mechanisms are similar, *i.e.* dominated by the Fermi contact term.

A relation between $^2J(HCH)$ and $^2J(CH_3CH)$ can be used to solve many kinds of problems. First, the $^2J_{CH}$ value of a methyl group in a compound having several different $CHCH_3$ fragments may be used as an aid in the spectral analysis: the corresponding $^2J_{HH}$ can usually be estimated (if it is not known) from known correlations with structure of this coupling constant,[5–8] and through eqn. (1) can the corresponding $^2J(CH_3H)$ coupling be calculated with a reasonable accuracy and can be compared with the observed coupling. A second application is to use the relation in conformational analysis because the $^2J_{HH}$ and $^2J_{CH}$ seem to behave similarly in a conformational sense. Finally, eqns. (1) and (2) provide a way to establish the signs of $^2J(CH_3CH)$ couplings provided the corresponding $^2J(HCH)$ is known and that the value of $^2J_{CH}$ is not too close to zero (or $^2J_{HH}$ too close to *ca.* −9 Hz).

It should be noted that the above correlations are limited to such systems where the central carbon is a part of a 5- or 6-membered (or larger) ring or an sp^3 carbon. Thus, for example, in the three-membered ring of propylene oxide, the predicted value of $^2J(CH_3CH)$ is +8.0 Hz [24] from eqn. (1) (and +6.1 Hz from eqn. 2), while the observed value is *ca.* +7 Hz (being between the values calculated for sp^2 and sp^3 carbons). Similarly, eqn. (1)

is not suitable for aliphatic acyclic compounds of the mono- or disubstituted ethane type; for example, the $^2J_{HH}$ of methane is -12.4 Hz [8,10] and the predicted value of $^2J_{CH}$ in ethane is -1.9 Hz, well removed from the observed coupling of -4.5 Hz.[11] Clearly, another type of correlation must be sought for these types of compounds.

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The Constituents of Conifer Needles. VI.* Phenolic Glycosides from *Pinus sylvestris*

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A β-D-glucopyranoside and an α-L-rhamnopyranoside of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol, an α-L-rhamnopyranoside, a β-D-xylopyranoside and a β-D-glucopyranoside of 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-hydroxyphenoxy]-1,3-propanediol, two β-D-glucopyranosides and one α-L-arabinofuranoside of (+)-isolariciresinol and a β-D-glucoside of (−)-seco-isolariciresinol as well as (+)-catechin and the 3′-*O*-β-D-glucopyranosides of quercetin and 2,3-dihydroquercetin, respectively, have been isolated and identified from needles of *Pinus sylvestris* L.

In previous parts of this series, low-molecular carbohydrates [2,3] and two new diterpene acids [4,5] isolated from the aqueous and methylene chloride soluble part, respectively, of the acetone extract of the needles of *Pinus sylvestris* L, have been studied. From the former fraction, using carbon-Celite chromatography, were also isolated two isomeric β-D-glucopyranosides of guaiacylglycerol,[3] the sugar not being linked to the phenolic group, as in coniferin, but to aliphatic hydroxyls in the glycerol side chain.

In subsequent preliminary studies at the Swedish Forest Products Research Laboratory, using fractionation on Sephadex LH-20 columns (eluent: water and aqueous ethanol) the latter glucosides, together with related glucosides of *p*-hydroxyphenylglycerol, were eluted as a group (which could be further fractionated on an anion-exchange column) after the saccharides.[6] Further studies on these glucosides are now in progress. After the monocyclic glycosides were eluted a group of compounds, which turned out to be dilignol glycosides, followed by a group of known phenolic extractives, of which dihydroquercetin (taxifolin) 3′-*O*-β-D-glucopyranoside seemed rather predominant. The fractionation of these two latter groups and the identification of a series of compounds thus obtained, have now been performed and are reported in this paper. The structural determination of two of the compounds, the 4′-*O*-β-D-glucopyranoside (*1*) and the 4′-*O*-α-L-rhamnopyranoside (*2*) of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol has recently been reported separately.[1] Some

Table 1. Fractionation of part (10.3 g) of the 2-butanone soluble products on Sephadex LH-20. Eluent: water.

Fraction No.	Elution volume/l	Weight/g	Main constituents (compound No.)
I	1.84	2.12	[a]
II	2.24	0.21	*4*
III	2.92	0.28	*4, 5*
IV	4.00	0.31	*1, 15*
V	5.28	0.27	*6, 7, 9, 10*
VI	7.60	0.69	*2, 9, 10*
VII	8.00	0.08	*2, 9*
VIII	9.80	0.19	*8, 17, 3*
IX	13.20	3.93	*17, 3*
X	16.90 [b]	0.21	*18, 19*
XI	19.70 [b]	0.03	*20*
XII	24.00 [c]	0.85	*21*

[a] Sugars and *p*-hydroxyphenyl- and guaiacylglycerol glucosides. [b] 50 % EtOH. [c] 96 % EtOH.

* Part V. See Ref. 1.

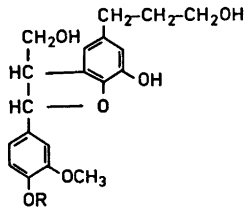
Table 2. Isolated and identified phenolic compounds.

Compound	Yields, % of the respective fraction: Ethyl acetate soluble	2-Butanone soluble	Water soluble	Total yield %: On dry weight of the needles
1	Trace	0.9	2.0	0.17
2	Trace	4.9	3.0	0.39
3	1.1	0.3	Trace	0.05
4	0.6	2.6	2.6	0.29
5	0.6	1.6	1.2	0.16
6	0.9	1.0	0.3	0.09
7	2.0	0.5	Trace	0.09
8	0.3	0.9	0.2	0.06
9	Trace	0.3	0.2	0.03
10	Trace	0.1	0.1	0.01
15		0.3	0.2	0.03
17	6.8	38.8	3.8	2.05
18	2.4	Trace		0.08
19	1.1	Trace		0.04
20	1.7	Trace		0.06
21	1.0			0.03
Total	18.5	51.9	13.4	3.57

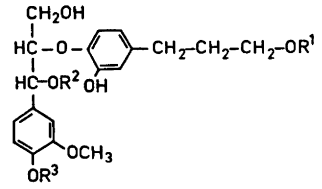
of our results on isolation and identification of the phenolic glycosides were recently briefly presented.[7]

RESULTS AND DISCUSSION

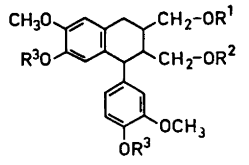
The needles collected in spring were extracted with acetone and divided in fractions of compounds soluble in light petroleum, ethyl acetate, 2-butanone (saturated with water) and water. The phenolic compounds from the three fractions were separated on Sephadex LH-20 columns (eluent: water and aqueous ethanol). Table 1 shows the order in which the compounds from the 2-butanone fraction were eluted. Refractionations were made on silicic acid columns. The compounds isolated and identified are presented in the scheme below, and their approximate yield from the three fractions in Table 2. The identification of compounds *1*, *2* and *3* were reported previously.[1] 2,3-Dihydroquercetin (*19*), quercetin (*20*) and their 3′-*O*-



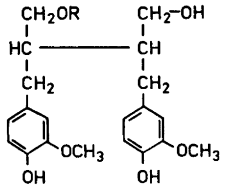
1 R = β-D-glucopyranoside
2 R = α-L-rhamnopyranoside
3 R = H



4 $R^1=R^3=H$ $R^2=\beta$-D-glucopyranoside
5 $R^1=R^2=H$ $R^3=\beta$-D-xylopyranoside
6 $R^2=R^3=H$ $R^1=\alpha$-L-rhamnopyranoside
7 $R^1=R^2=R^3=H$



8 $R^1=R^3=H$ $R^2=\alpha$-L-arabinofuranoside
9 $R^2=R^3=H$ $R^1=\beta$-D-glucopyranoside
10 $R^1=R^3=H$ $R^2=\beta$-D-glucopyranoside
11 $R^1=R^3=CH_3$ $R^2=H$
12 $R^2=R^3=CH_3$ $R^1=H$
13 $R^1=R^2=R^3=CH_3$
14 $R^1=R^2=R^3=H$



15 R = β-D-glucopyranoside
16 R = H

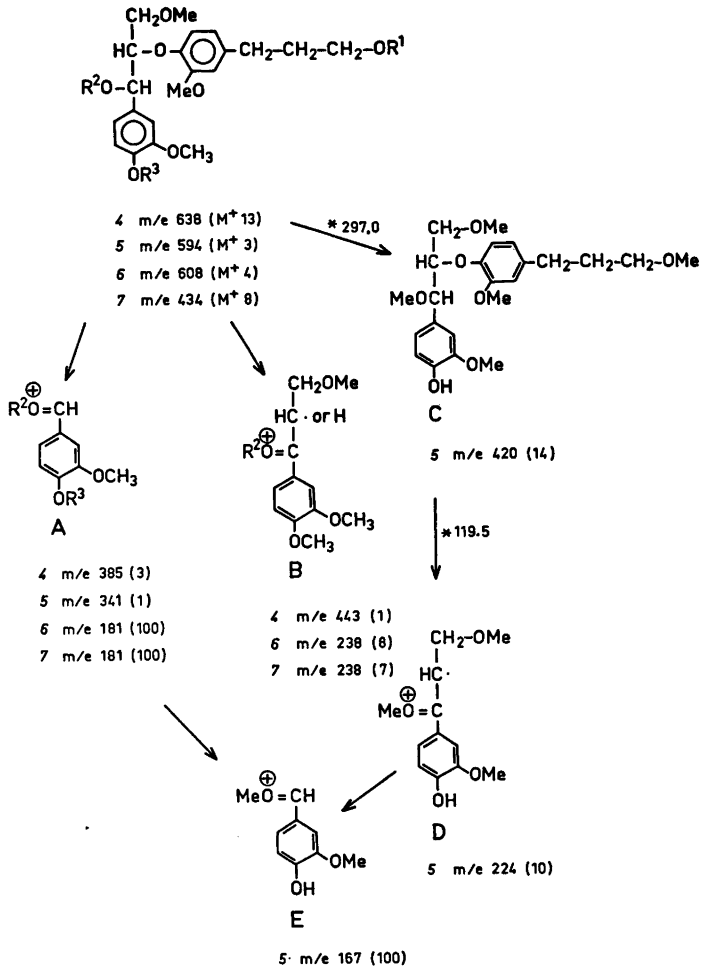


Fig. 1. Mass spectrometric fragments from methylated compounds *4*–*7*.

glucopyranosides *17* and *18* respectively, and (+)-catechin (*21*) were compared with authentic samples and found to be identical in all respects.

The sugar part of the glycosides (which all contain only one sugar unit as shown by NMR, of the free glycosides and their acetates, and by MS of their methyl ethers) were identified as β-D-glucopyranose (in *4*, *9*, *10* and *15*), β-D-xylopyranose (in *5*), α-L-rhamnopyranose (in *6*) and as α-L-arabinofuranose (in *8*); see Experimental.

Compounds *4*–*7* gave the same red colour with diazotized sulfanilic acid, indicating α-hydroxyvanillyl structures. Their ^{1}H NMR spectra were very similar, showing the presence of six aromatic protons, one benzylic proton (doublet around δ 4.9), one methoxyl group and six protons characteristic of a phenylpropanol side chain (broad triplet around δ 2.6 and multiplets around δ 1.8 and 3.5). Acidic hydrolysis of *4*, *5* and *6* made the aglycone decompose, but the sugars were identified as D-glucose, D-xylose and L-rhamnose, respectively. When hydrolyzed with a commercial enzyme preparation compound *4* and *5* gave the aglycone *7* while compound *6* was unaffected. The latter, however, was found to be identical with the *threo*-isomer of 1-(4-hydroxy-3-methoxyphenyl)-2-[2-hydroxy-4-(3-*O*-α-L-rhamnopyranoside propanol)-phenoxy]-1,3-pro-

panediol (TLC, NMR and MS of the acetate) previously isolated from *Thuja plicata*.[8]

The ^{1}H NMR spectrum of acetylated *4* showed, contrary to those of acetylated *5* and *6*, no downfield shift for the proton, indicating that the glucose in compound *4* is linked to the benzylic alcohol. The ^{1}H NMR spectrum of acetylated *5*, on the other hand, showed only one aromatic acetyl, indicating xylose, that is linked to a phenolic hydroxyl. The structures were further verified by mass spectra of the methylated glycosides and the aglycone (Fig. 1). All derivatives gave peaks corresponding to the expected molecular ions. Fragment A was most significant. The occurrence of *m/e* 385 and *m/e* 341 and absence of *m/e* 181 for compound *4* and *5*, respectively, proved the presence of a hexose-tetra-*O*-methyl and a pentose-tri-*O*-methyl residue in fragment A from *4* and *5*, respectively. The low intensity of *m/e* 341 (1 %) for compound *5*, having the xylose in a phenolic position, is expected, since such a glycosidic linkage is weaker than a linkage to an aliphatic hydroxyl group. Because of this, only MS of compound *5* showed the occurrence of fragment C, D and E, while compounds *4*, *6* and *7* gave fragment B. High resolution mass spectrometry of compounds *4*–*7* established their molecular formulas.

As revealed by the ^{1}H NMR spectrum, the aglycone *7* obtained from compound *4* by enzymatic hydrolysis has a *threo*-configuration in the glycerol chain, as was previously found in the guaiacylglycerol glycosides,[8] while the aglycone from compound *5* contains a mixture of the *threo*- and *erythro*-isomer in the ratio 1:2. Compound *6* has a *threo*-configuration. This was demonstrated in the acetylated compounds by the shifts and coupling constants of the benzylic α-protons, in accordance with Ludwig *et al.*[9] The glycosides *4*–*6* represent a notable example of three different sugars linked to hydroxyl groups in the same aglycone, but in three different positions. We looked carefully for the other combinations without finding any indication of their presence. The saturated state of the side chain and the fact that one of the aromatic rings is catecholic is a notable fact both for this group of compounds and for compounds *1*–*3*.

After hydrolysis, compounds *8*, *9* and *10* gave the same aglycone, (+)-isolariciresinol (*14*) (identical with authentic sample), and L-arabinose (from *8*) and D-glucose (from *9* and *10*). Methylation/hydrolysis studies showed sugar linkage to one of the two aliphatic hydroxyl groups. To verify the position of the arabinose linkage in compound *8*, the glycoside was methylated with methyl iodide in dimethylformamide and sodium hydride,[10] formolysed and hydrolysed. Chromatographic purification of the product yielded compound *11*. This trimethyl ether was not identical with the isomeric compound *12*, synthesized from conidendrin,[6] but they both gave the same product *13* when methylated. This proves that arabinose is linked to the α^3 hydroxyl in compound *8*.

Compound *9* after methylation and hydrolysis yielded a compound identical with the synthesized compound *12*. This proves that the glucose is linked to the α^3 hydroxyl. The structure of compound *10*, which was only obtained in admixture with *9*, was verified by the following findings: in NMR spectra of the mixture two anomeric glucose protons (ratio 2:3) could be seen; at δ 4.26 and 4.30 (CD_3OD), 4.76 and 4.95 (C_5D_5N), respectively, of which the latter must arise from *9*. Mass spectra of the methylated mixture showed an almost identical pattern with that of pure *9* including the molecular ion (*m/e* 620), and by formolysis and hydrolysis of that mixture compound *11* and *12* were obtained in the ratio 2:3, as revealed by the ^{1}H NMR spectra.

After hydrolysis compound *15* gave (−)-seco-isolariciresinol (*16*) (identical with an authentic sample) and D-glucose, and methylation with MeI/DMF, NaH yielded a heptamethyl ether. Since methylation with diazomethane followed by hydrolysis gave the dimethyl ether of *16* (identical with an authentic sample obtained by methylation of *16* with diazomethane) the glucose must be linked to one of the equivalent aliphatic hydroxyl groups.

The chromatographic behaviour of the compounds is shown in Table 3. The characteristic colour reactions on TLC with the three sprays used are, with one exception (compound *17*) almost identical for a glycoside and its aglycone. The anomeric configuration and ring size of the sugars in the glycosides *4*, *5*, *8*, *9*, *10*, and *15* were verified by ^{1}H NMR. The coupling constant of the anomeric proton in the D-glucopyranosides *4*, *9*, *10* and *15* and in

Table 3. Chromatographic mobilities (TLC in 2-butanone saturated with water) and colour reactions.

Compound	R_V[a]	Colour		
		Spray *a*	Spray *b*	Spray *c*
1	0.25	Dark red		Red violet
2	0.47	Dark red		Red violet
3	0.88	Dark red		Red violet
4	0.33	Red		Blue violet
5	0.40	Red		Blue violet
6	0.61	Red		Violet
7	0.88	Red		Blue violet
8	0.59	Brown		Red
9	0.36	Brown		Red
10	0.36	Brown		Red
14	0.86	Brown		Red
15	0.40	Green		Red violet
16	0.90	Green		Red violet
17	0.53	Yellow	Red violet	Orange yellow
18	0.51	Yellow brown	Green gray	Yellow
19	0.99	Yellow	Blue violet	Orange yellow
20	1.01	Pale yellow	Green gray	Yellow
21	0.97	Yellow	Green	Red orange

[a] Mobilities relative to that of vanillin.

the D-xylopyranoside *5* were all around 7 Hz thus indicating β-D-pyranosides. The coupling constant of the anomeric proton in the L-arabinofuranose *8*, was 2.0 Hz, indicating an α-L-arabinofuranoside.[11] Besides, the chemical shifts for the anomeric protons in compounds *8* and *9*, *10* and *15*, respectively, were very similar to those previously reported for methyl-α-L-arabinofuranoside and methyl-β-D-glucopyranoside,[11] respectively. The chemical shift and coupling constant for the anomeric proton in compound *5* were similar to those of authentic phenyl-β-D-xylopyranoside in C_6D_6N (δ 5.41, *J* 7.0 Hz).

To our knowledge compounds *1*–*5*, *7*, *8*, *11*–*13* and *15* have not previously been reported by other authors. Compounds *9* and/or *10* (the exact position of glucose not established) has previously been reported.[16]

EXPERIMENTAL

General

TLC was generally performed on Silica Gel HF_{254} (Merck) with 2-butanone, saturated with water, as solvent (mobilities in Table 3) and for further establishing of the purities in chloroform-methanol-water (70:30:5), ethyl acetate-methanol-water (130:17:13) and chloroform-isopropanol-water (60:25:2). Column chromatography was run on Sephadex LH-20, with water and water-ethanol as solvent and on silicic acid (Mallinckrodt, 100 mesh) with (*a*) water-saturated 2-butanone, (*b*) chloroform-methanol-water (70:25:3) and (*c*) ethyl acetate-acetone (1:1) as solvent. TLC-plates (after inspection in UV light) were sprayed with (*a*) 0.1 % diazotized sulfanilic acid in 10 % sodium carbonate followed by 50 % sulfuric acid, (*b*) 1 % iron(III) chloride in water and (*c*) anisaldehyde-sulfuric acid-ethanol (1:1:18), followed by heating (140 °C). Low and high resolution mass spectra were recorded at 70 *eV* or 30 *eV* on Varian MAT CH 7 and AEI 902 instruments, respectively, the latter spectra at the Institute of Medical Biochemistry, University of Gothenburg. The ^1H NMR spectra were recorded at 100 MHz on a Varian HA-100 D instrument, equipped with a Varian VFT-100 Fourier Transform System.

Isolation

Fresh needles (1.2 kg; water content 37 %) from *Pinus sylvestris* L, collected in spring, were boiled with acetone for 30 min. After filtration, the needles were milled and extracted continuously with acetone in a Soxhlet apparatus (2 × 24 h). After evaporation of the acetone, the combined acetone extracts were diluted with water (1.5 l) and extracted with light petroleum, b.p. 40–60 °C, (3 × 0.5 l),

ethyl acetate (3 × 0.5 l) and 2-butanone (3 × 0.5 l). The yields of the fractions after evaporation were: 60.4 g (light petroleum), 23.6 g (ethyl acetate), 31.1 g (2-butanone) and 48.7 g (water).

Part of the 2-butanone fraction (10.3 g) was added to a Sephadex LH-20 column, and eluted with water and ethanol/water; 12 fractions were collected (weights and contents are given in Table 1). The fractions were then fractionated on silicic acid columns with solvent *a*, except fractions III (solvent *b*), V and VII (solvent *c*)

In order to obtain pure samples of *6* and *8*, crude subfractions of the respective compound were treated with an enzyme (see below) which hydrolysed most interfering compounds but left *6* and *8* unchanged, followed by column separations (solvent *a*) yielding pure *8* and *6*, respectively.

The same procedure was used for the rest of the 2-butanone fraction, for the ethyl acetate fraction and for the water fraction. Table 2 gives the content of the compounds in the different fractions and their total approximate yield calculated on the dry weight of the needles (based on the weights of the fractions and visual TLC estimations). All the compounds isolated were chromatographically pure (TLC) in several solvent systems but attempts to crystallize any of the glycosides failed.

Hydrolysis

Compounds *4*, *5*, *9*, *10*, *15*, *17* and *18* were hydrolysed enzymatically with a commercial crude enzyme (cellulase C 36, Rohm and Haas Co.). Enzyme (about 5 mg) was added to the substance (50 mg) in water (50 ml), and the mixture was left at room temperature overnight. By extraction with ethyl acetate (4 × 25 ml), drying (Na_2SO_4) and evaporation, an almost pure aglycone was obtained from each glycoside. In the aqueous fraction the sugar was identified by optical rotation (the weights corresponded to one sugar unit per glucoside) and by GLC as its trimethylsilyl ether and as its alditol acetate;[12,13] D-glucose from compounds *4*, *9*, *10* and *15* and D-xylose from compound *5*. Compounds *6* and *8*, which were not affected by the enzyme, were hydrolysed with 1 M sulfuric acid for 3 h at 100 °C. Extraction as above gave (+)-isolariciresinol (identical with an authentic sample; IR, MS, NMR) and L-arabinose from compound *8*, but a complex of phenolic products and L-rhamnose from compound *6*.

Methylation

About 10 mg of each glycoside was methylated with methyl iodide in dimethylformamide and sodium hydride.[10] The *O*-methyl derivatives were purified on TLC (ethyl acetate as solvent). Pure derivatives were obtained from all glycosides except from compounds *17* and *18*, where decomposition occurred under the conditions used. The products obtained from the methylated glycosides after formolysis, hydrolysis, subsequent reduction and acetylation were studied by GLC/MS.[14,15] Thereby 2,3,-4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-glucitol was identified from compounds *4*, *9* and *10*, 2,3,-5-tri-*O*-methyl-1,4-di-*O*-acetyl-arabinitol from compound *8*, 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl-rhamnitol from compound *6* and 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl-xylitol from compound *5*, thus verifying the furanoside form of arabinose and the pyranoside form of the other sugars.

Compound 4 (amorphous), $[\alpha]_D^{20}$ −7.1° (*c* 3.0, EtOH). ^{1}H NMR (CD_3OD): δ 1.65 − 1.95 (2 H, m), 2.55 (2 H, broad t, *J* 7 Hz), 3.1 − 3.9 (10 H, m), 3.81 (3 H, s), 4.1 − 4.4 (1 H, m), 4.55 (1 H, d, *J* 7.0 Hz), 6.5 − 7.2 (6 H, m).

Acetylation (Ac_2O/pyr) of compound *4* yielded the octa-acetate (amorphous). ^{1}H NMR ($CDCl_3$): δ 1.80 (3 H, s), 1.91 (3 H, s), 1.95 (9 H, broad s), 2.02 (3 H, s), 2.24 (3 H, s), 2.26 (3 H, s), 1.8 − 2.4 (2 H, m), 2.62 (2 H, broad t, *J* 7 Hz), 3.4 − 3.7 (1 H, m), 3.81 (3 H, s), 3.6 − 4.3 (6 H, m), 4.52 (1 H, m), 4.7 − 5.2 (5 H, m), 6.7 − 7.1 (6 H, m).

Methylation (CH_3I/DMF, NaH) of compound *4* yielded the octamethyl ether (amorphous). MS, [IP 30 eV; *m/e* (% rel. int.)]: 638 (M, 14), 594 (1), 502 (1), 472 (6), 443 (1), 403 (1), 385 (3), 371 (2), 313 (2), 252 (1), 245 (3), 233 (1), 222 (25), 219 (13), 218 (12), 208 (11), 188 (11), 187 (100), 177 (10), 163 (17), 155 (12), 151 (14), 127 (9), 115 (9), 111 (30), 101 (35), 89 (16), 75 (17), 71 (16), 45 (45). Mol. wt., obs. 638.330, calc. for $C_{33}H_{50}O_{12}$ 638.330.

Compound 5 (amorphous). Mixture of *threo* and *erythro*-isomer in ratio 1:2. ^{1}H NMR (CD_3OD): δ 1.7 − 1.9 (2 H, m), 2.52 (2 H, broad t, *J* 7 Hz), 3.3 − 4.0 (9 H, m), 3.79 (3 H, s), 4.15 (1 H, m), 4.91 (2/3 H, d, *J* 5.0 Hz), 4.97 (1/3 H, d, *J* 6.0 Hz), 6.49 (2/3 H, dd, *J* 2.2 and 8.4 Hz), 6.54 (1/3 H, dd, *J* 2.2 and 8.4 Hz), 6.6 − 7.1 (5 H, m).

Acetylation (Ac_2O/pyr) of compound *5* yielded the heptaacetate (amorphous). ^{1}H NMR ($CDCl_3$): δ 1.95 (3 × 1/3 H, s), 1.97 (3 × 2/3 H, s), 2.02 (3 H, s), 2.04 (9 H, broad s), 2.09 (3 H, s), 2.15 (3 × 2/3 H, s), 2.25 (3 × 1/3 H, s), 1.8 − 2.3 (2 H, m), 2.62 (2 H, broad t, *J* 7 Hz), 3.47 (1 H, dd, *J* 7.6 and 12.0 Hz), 3.7 − 3.9 (1 H, m), 3.9 − 4.5 (4 H, m), 4.23 (1 H, dd, *J* 4.4 and 12.0 Hz), 4.67 (1 H, m), 4.9 − 5.3 (4 H, m), 5.97 (2/3 H, d, *J* 4.4 Hz), 6.02 (1/3 H, d, *J* 6.5 Hz) 6.75 − 7.15 (6 H, m).

As comparison, phenyl-β-D-tri-*O*-acetyl-xylopyranoside, available in this laboratory: ^{1}H NMR ($CDCl_3$): δ 2.07 (9 H, broad s), 3.52 (1 H, dd, *J* 7.6 and 12.0 Hz), 4.23 (1 H, dd, *J* 4.6 and 12.0 Hz), 4.9 − 5.3 (4 H, m), 6.9 − 7.4 (5 H, m).

Methylation (CH_3I/DMF, NaH) of compound *5* yielded the heptamethyl ether (amorphous) MS, [IP 30 eV; *m/e* (% rel. int.)]: 594 (M, 3), 420 (14), 239 (1), 224 (10), 222 (11), 193 (7), 179 (5), 175 (6), 174 (6), 168 (10), 167 (100), 151 (8), 137 (8), 125 (8), 101 (29), 99 (15), 45 (28). Mol. wt., obs 594.304, calc for $C_{31}H_{46}O_{11}$ 594.304.

Compound 6 (amorphous), $[\alpha]_D^{20}$ −58.1° (*c* 2.8, EtOH). ^1H NMR (CD_3OD): δ 1.24 (3 H, d, *J* 6.0 Hz), 1.7−2.0 (2 H, m), 2.59 (2 H, broad t, *J* 7 Hz), 3.4−3.9 (8 H, m), 3.79 (3 H, s), 4.0−4.2 (1 H, m), 4.63 (1 H, d, *J* 1.5 Hz), 4.91 (1 H, d, *J* 6.5 Hz), 6.55 (1 H, dd, *J* 2.0 and 8.4 Hz), 6.68 (1 H, d, *J* 2.0 Hz), 6.74 (1 H, d, *J* 8.4 Hz), 6.86 (1 H, dd, *J* 1.8 and 8.0 Hz), 6.94 (1 H, d, *J* 8.0 Hz), 7.01 (1 H, d, *J* 1.8 Hz).

Acetylation (Ac_2O/pyr) of compound *6* yielded the hepta-acetate (amorphous). ^1H NMR ($CDCl_3$): δ 1.21 (3 H, d, *J* 6.0 Hz), 1.95 (3 H, s), 1.99 (3 H, s), 2.05 (3 H, s), 2.06 (3 H, s), 2.15 (3 H, s), 2.26 (3 H, s), 2.30 (3 H, s), 1.8−2.3 (2 H, m), 2.66 (2 H, broad t, *J* 7 Hz), 3.4−4.3 (5 H, m), 4.6−4.8 (1 H, m), 4.71 (1 H, broad s), 5.06 (1 H, t, *J* 10.0 Hz), 5.24 (1 H, dd, *J* 1.8 and 3.5 Hz), 5.33 (1 H, dd, *J* 3.5 and 10.0 Hz), 6.06 (1 H, d, *J* 6.5 Hz), 6.8−7.0 (6 H, m).

Methylation (CH_3I/DMF, NaH) of compound *6* yielded the heptamethyl ether (amorphous). MS [IP 30 eV; *m/e* (% rel. int.)]: 608 (M, 4), 383 (7), 238 (7), 207 (7), 182 (11), 181 (100), 151 (20), 131 (12), 119 (12), 101 (21), 88 (17), 69 (62), 59 (11), 45 (40). Mol. wt., obs. 608.321, calc. for $C_{32}H_{48}O_{11}$ 608.320.

Compound 7. The *threo*-isomer obtained from compound *4* (amorphous). $[\alpha]_D^{20}$ +35.4°, (*c* 0.7, EtOH). ^1H NMR (CD_3OD): δ 1.4−1.9 (2 H, m), 2.55 (2 H, broad t, *J* 7 Hz), 3.47 (1 H, dd, *J* 5.0 and 11.5 Hz), 3.54 (2 H, t, *J* 6.5 Hz), 3.75 (1 H, dd, *J* 4.0 and 11.5 Hz), 3.81 (3 H, s), 3.9−4.2 (1 H, m), 4.91 (1 H, d, *J* 6.5 Hz), 6.55 (1 H, dd, *J* 2.2 and 8.2 Hz), 6.67 (1 H, d, *J* 2.2 Hz), 6.91 (1 H, d, *J* 8.2 Hz), 6.74 (1 H, d, *J* 8.0 Hz), 6.86 (1 H, dd, *J* 1.8 and 8.0 Hz), 7.01 (1 H, d, *J* 1.8 Hz). In the ^1H NMR spectra of the *erythro*-, *threo*-mixture of *7* obtained from compound *5* or isolated from fraction V (Table 1), slight differences could be seen between the two isomers when compared with the pure *threo*-isomer above, arising from the *erythro*-isomer; δ (CD_3OD): 3.9−4.3 (1 H, m), 4.86 (1 H, d, *J* 5.0 Hz), 6.50 (1 H, dd, *J* 2.2 and 8.2 Hz), 6.86 (1 H, d, *J* 8.0 Hz).

Acetylation (Ac_2O/pyr) of compound *7* *threo*-isomer yielded the pentaacetate (amorphous). ^1H NMR ($CDCl_3$): δ 1.92 (3 H, s), 2.02 (3 H, s), 2.04 (3 H, s), 2.23 (3 H, s), 2.27 (3 H, s), 1.9−2.4 (2 H, m), 2.63 (2 H, broad t, *J* 7 Hz), 3.80 (3 H, s), 4.0−4.2 (4 H, m), 4.5−4.8 (1 H, m), 6.05 (1 H, d, *J* 7.0 Hz), 6.8−7.0 (6 H, m). In the ^1H NMR spectra of acetylated (Ac_2O/pyr) isomer mixture mentioned above the *erythro*-isomer could be seen; δ ($CDCl_3$): 1.96 (3 H, s), 2.02 (3 H, s), 2.10 (3 H, s), 2.12 (3 H, s), 2.27 (3 H, s), 1.9−2.4 (2 H, m), 2.61 (2 H, broad t, *J* 7 Hz), 3.20 (3 H, s), 4.08 (1 H, dd, *J*, 4.0 and 12.0 Hz), 4.36 (1 H, dd, *J* 5.5 and 12.0 Hz), 4.5−4.8 (1 H, m), 6.01 (1 H, d, *J* 5.0 Hz), 6.8−7.0 (6 H, m).

Methylation (CH_3I/DMF, NaH) of compound *7* yielded the pentamethyl ether (amorphous) MS [IP 70 eV; *m/e* (% rel. int.)]: 434 (M, 8), 238 (7), 207 (7), 182 (12), 181 (100), 151 (12), 45 (12). Mol. wt., obs 434.230, calc. for $C_{24}H_{34}O_7$ 434.230.

Compound 8 (amorphous), $[\alpha]_D^{20}$ −36.8° (*c* 1.0, EtOH). ^1H NMR (CD_3OD): δ 1.75−2.25 (2 H, m), 2.81 (2 H, broad d, *J* 7 Hz), 3.13 (1 H, broad dd, *J* 2 and 10 Hz), 3.5−4.1 (9 H, m), 3.76 (3 H, s), 3.78 (3 H, s), 4.73 (1 H, d, *J* 1.8 Hz), 6.18 (1 H, s), 6.59 (1 H, dd, *J* 2.0 and 8.0 Hz), 6.64 (1 H, s), 6.67 (1 H, d, *J* 2.0 Hz), 6.75 (1 H, d, *J* 8.0 Hz).

Acetylation (Ac_2O/pyr) of compound *8* yielded the hexa-acetate (amorphous). ^1H NMR ($CDCl_3$): δ 2.05 (6 H, s), 2.07 (3 H, s), 2.08 (3 H, s), 2.20 (3 H, s), 2.28 (3 H, s), 1.8−2.4 (2 H, m), 2.90 (2 H, broad d, *J* 7 Hz), 3.14 (1 H, broad dd, *J* 2 and 10 Hz), 3.78 (1 H, broad dd, *J* 3 and 10 Hz), 3.76 (3 H, s), 3.79 (3 H, s), 4.0−4.5 (6 H, m), 4.88 (1 H, broad s), 4.90−5.05 (1 H, m), 5.07 (1 H, d, *J* 1.8 Hz), 6.42 (1 H, s), 6.6−6.8 (3 H, m), 6.96 (1 H, d, *J* 8.2 Hz).

Methylation (CH_3I/DMF, NaH) of compound *8* yielded the hexamethyl ether (amorphous). MS [IP 70 eV; *m/e* (% rel. int.)]: 576 (M, 8), 416 (2), 401 (27), 384 (10), 383 (100), 369 (17), 353 (12), 351 (18), 339 (12), 307 (7), 269 (6), 201 (5), 165 (6), 151 (24), 115 (5), 111 (5), 101 (27), 71 (13), 57 (13), 55 (10), 45 (26).

Compound 9 (amorphous) $[\alpha]_D^{20}$ +8.0° (*c* 0.9, EtOH). ^1H NMR (CD_3OD): δ 1.6−2.0 (1 H, m), 2.0−2.4 (1 H, m), 2.84 (2 H, broad d, *J* 7 Hz), 3.2−4.1 (11 H, m), 3.78 (3 H, s), 3.80 (3 H, s), 4.30 (1 H, d, *J* 7.5 Hz), 6.20 (1 H, s), 6.5−6.7 (2 H, m), 6.65 (1 H, s), 6.75 (1 H, d, *J* 8.0 Hz). ^1H NMR of the acetylated (Ac_2O/pyr) compound *9* as well as that of the acetylated compounds *9* and *10* mixture were in agreement with that of (+)-isolariciresinol-D-glucoside previously [16] reported.

Methylation (CH_3I/DMF, NaH) of compound *9* yielded the heptamethyl ether (amorphous). MS [IP 70 eV; *m/e* (% rel. int.)]: 620 (M, 20), 402 (14), 401 (49), 385 (12), 384 (49), 383 (100), 370 (11), 369 (39), 353 (23), 351 (16), 339 (22), 201 (10), 165 (11), 151 (46), 149 (34), 127 (12), 121 (12), 115 (10), 111 (16), 101 (44), 98 (14), 97 (14), 89 (18), 88 (65), 85 (18), 83 (19), 75 (48), 73 (24), 72 (45), 71 (54), 70 (18), 69 (34), 59 (14), 57 (78), 55 (37), 45 (75).

Compound 10 only obtained in admixture with compound *9*, showed a very similar ^1H NMR to that of pure *9*. Two anomeric sugar protons, however, could be seen at δ 4.28 and 4.30 (CD_3OD) or at δ 4.76 and 4.95 (C_5D_5N).

Compound 11. Crystals after storage, m.p. 141–155 °C; $[\alpha]_D^{20}$ −10.0° (*c* 1.0, $CHCl_3$); MS, [IP 70 eV; *m/e* (% rel. int.)]: 403 (25), 402 (M, 100), 370 (7), 353 (19), 340 (14), 339 (56), 325 (9), 324 (8), 321 (12), 312 (16), 299 (10), 269 (34), 201 (13), 189 (19), 151 (24), 45 (25). ^{1}H NMR ($CDCl_3$): δ 1.5–1.9 (1 H, m), 2.0–2.4 (1 H, m), 2.74 (2 H, broad d, *J* 7.5 Hz), 3.3–3.9 (4 H, m), 3.39 (3 H, s), 3.57 (3 H, s), 3.79 (3 H, s), 3.84 (3 H, s), 3.87 (3 H, s), 3.98 (1 H, broad d, *J* 10.4 Hz), 6.23 (1 H, s), 6.58 (1 H, s), 6.61 (1 H, d, *J* 1.7 Hz), 6.75 (1 H, dd, *J* 1.7 and 8.2 Hz), 6.81 (1 H, d, *J* 8.2 Hz).

Compound 12. Obtained from conidendrin [6] after methylation (CH_3I/DMF, NaH) and reduction ($LiAl_4$). Crystallized from ethanol, m.p. 140.5–141.0 °C, $[\alpha]_D^{20}$ +25.5° (*c* 2.8, $CHCl_3$). Anal. $C_{23}H_{30}O_6$: C, H. MS [IP 70 eV; *m/e* (% rel. int.)]: 403 (22), 402 (M, 84), 370 (12), 340 (30), 339 (100), 324 (10), 312 (20), 269 (24), 201 (16), 189 (18), 176 (13), 165 (13), 152 (10), 151 (42), 45 (78), 43 (19). ^{1}H NMR ($CDCl_3$): δ 1.8–2.1 (2 H, m), 2.6–3.1 (2 H, m), 3.25 (1 H, dd, *J* 5.2 and 10.0 Hz), 3.25 (3 H, s), 3.44 (1 H, dd, *J* 2.0 and 10.0 Hz), 3.56 (3 H, s), 3.80 (3 H, s), 3.83 (3 H, s), 3.88 (3 H, s), 3.6–3.9 (3 H, m), 6.19 (1 H, s), 6.60 (2 H, broad s), 6.70 (1 H, dd, *J* 2.0 and 8.0 Hz), 6.83 (1 H, d, *J* 8.0 Hz).

Compound 13. Obtained from compounds *11*, *12* and *14* by methylation (CH_3I/DMF, NaH). Crystallized from methanol, m.p. 110.4 –111.9 °C; $[\alpha]_D^{20}$ −8.5° (*c* 0.9, $CHCl_3$). Anal. $C_{24}H_{32}O_6$: C, H. MS [IP 70 eV; *m/e* (% rel. int.)] 417 (28), 416 (M, 100), 384 (11), 354 (14), 353 (49), 340 (22), 339 (45), 324 (12), 321 (21), 312 (27), 308 (10), 299 (10), 269 (28), 208 (18), 201 (22), 181 (10), 176 (15), 165 (11), 152 (10), 151 (39), 45 (63). ^{1}H NMR ($CDCl_3$): δ 1.7–2.0 (1 H, m), 2.00–2.35 (1 H, m), 2.85 (2 H, broad d, *J* 7.8 Hz), 3.09 (1 H, dd, *J* 3.3 and 9.5 Hz), 3.26 (3 H, s), 3.35 (3 H, s), 3.39 (1 H, dd, *J* 3.3 and 9.5 Hz), 3.46 (2 H, d, *J* 5.0 Hz), 3.58 (3 H, s), 3.80 (3 H, s), 3.83 (3 H, s), 3.87 (3 H, s), 4.00 (1 H, broad d, *J* 10.5 Hz), 6.26 (1 H, s), 6.62 (1 H, s), 6.63 (1 H, d, *J* 1.8 Hz), 6.70 (1 H, dd, *J* 1.8 and 7.8 Hz), 6.82 (1 H, d, *J* 7.8 Hz).

Compound 14. Identical (m.p., IR, MS, NMR) with an authentic sample of (+)-isolariciresinol, m.p. 115–116 °C (lit. value 112 °C;[17] 115–117 °C [16]): $[\alpha]_D^{20}$ +63.3° (*c* 2.0, $(CH_3)_2C{=}O$) (lit. value +61.7 °C;[16] +69.4 °C [17]) MS [IP 70 eV; *m/e* (% rel. int.)]: 361 (32), 360 (M, 100), 342 (12), 341 (10), 325 (11), 312 (18), 311 (97), 297 (11), 284 (27), 279 (12), 271 (16), 255 (12), 241 (29), 211 (10), 197 (10), 187 (14), 175 (27), 137 (33), 131 (10), 115 (15), 55 (12). ^{1}H NMR (CD_3OD): δ 1.6–2.2 (2 H, m), 2.77 (2 H, broad d, *J* 7 Hz), 3.4–3.9 (5 H, m), 3.75 (3 H, s), 3.78 (3 H, s), 6.20 (1 H, s), 6.51 (1 H, dd, *J* 2.0 and 8.0 Hz), 6.64 (1 H, s), 6.68 (1 H, d, *J* 2.0 Hz), 6.75 (1 H, d, *J* 8.0 Hz).

Compound 15 (amorphous), $[\alpha]_D^{20}$ −20.5° (*c* 3.2, EtOH). ^{1}H NMR (CD_3OD): δ 1.8–2.3 (2 H, m), 2.45–2.80 (4 H, m), 3.1–4.0 (10 H, m), 3.74 (6 H, s), 4.20 (1 H, d, *J* 7.0 Hz), 6.45–6.80 (6 H, m).

Methylation (MeI/DMF, NaH) of compound *15* yielded the heptamethyl ether (amorphous). MS [IP 30 eV; *m/e* (% rel. int.)]: 622 (M, 6), 386 (8), 355 (10), 247 (16), 233 (18), 203 (10), 187 (13), 177 (8), 167 (9), 165 (5), 152 (21), 151 (100), 127 (6), 111 (14), 101 (29), 89 (10), 88 (29), 75 (13), 71 (14), 45 (41).

Compound 16. Identical (NMR, MS, $[\alpha]_D^{20}$) with an authentic sample of (−)-seco-isolariciresinol (spectral data previously reported [18]).

Compound 17. Identical (IR, NMR) with an authentic sample of dihydroquercetin-3′-*O*-β-D-glucopyranoside. Amorphous white powder $[\alpha]_D^{20}$ −32.0° (*c* 0.6, H_2O) (lit.[19] value −23°). ^{1}H NMR (CD_3OD): δ 3.3–4.0 (6 H, m), 4.55 (1 H, d, *J* 12.0 Hz), 4.98 (1 H, d, *J* 12.0 Hz), 5.88 (1 H, d, *J* 2.0 Hz), 5.93 (1 H, d, *J* 2.0 Hz), 6.88 (1 H, d, *J* 8.0 Hz), 7.10 (1 H, dd, *J* 2.0 and 8.0 Hz), 7.39 (1 H, d, *J* 2.0 Hz). The anomeric sugar proton was hidden under solvent peak at δ 4.8–5.0.

Compound 18. Identical (m.p., IR) with quercetin-3′-*O*-β-D-glucopyranoside, synthesized from compound *17*.[19] Crystallized from aqueous acetone, m.p. 237.0–239.0 °C (lit.[19] value 216 °C).

Compounds 19, 20 *and* 21 were identical (TLC, m.p., IR) with authentic samples of dihydroquercetin (taxifolin), quercetin and (+)-catechin, respectively, available in this laboratory.

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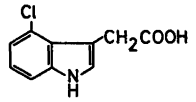
Short Communications

Preparation of Chlorinated 3-Indolylacetic Acids

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The interest in chlorinated 3-indolylacetic acids as plant hormones has increased since 4-chloro-3-indolylacetic acid [1] (*1*), its methyl ester,[2,3] its aspartic acid amide,[4] and two derivatives of 4-chlorotryptophan [5] were isolated from immature green pea seeds. Many other plants may contain similar hormones. When fed $^{36}Cl^-$ at least



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ten cultivated species incorporate radioactivity into compounds migrating close to 4-chloro-3-indolylacetic acid methyl ester on thin layer chromatograms.[6]

Published procedures of preparation are tedious or require rare chemicals or equipment.[7–10] Therefore, simplified procedures giving similar or better yields were adopted. Five new compounds were synthesized. 4-Chloro-3-indolylacetic acid and 6-chloro-3-indolylacetic acid were made from the corresponding indoles by refluxing 3-diethylaminomethyl-chloroindoles with sodium cyanide overnight instead of four days.[9,11] The other compounds were prepared by a two-step Fischer indole synthesis from 3-formylpropionic acid (from glutamic acid) and the appropriate phenylhydrazine in pyridine/HCl [12] avoiding distillation and saponification of the ethyl esters.[7,8] A few compounds could also be prepared in one step from a phenylhydrazine and α-oxoglutaric acid in pyridine/HCl,[13] but this method was not generally applicable. The results are shown in Table 1.

All new compounds showed satisfactory elemental analyses (Novo microanalytical laboratory). Infra-red spectra (Perkin Elmer Spectrophotometer 221, KBr) were very similar to the spectra of 5-bromo- and 5-fluoro-3-indolylacetic acids.[14] The electron impact mass spectra (Varian MAT CH5) gave the following values of *m/e* for chloro-3-indolylacetic acids: 209 (M^+), 164 (100 %), 128, 129 and 101; for 7-bromo-3-indolylacetic acid: 253 (M^+), 208 (100 %), 128, 129 and 101; for dichloro-3-indolylacetic acids: 243 (M^+), 198 (100 %), 162, 163, 127, 128 and 99; for 5-chloro-7-methyl-3-indolylacetic acid: 223 (M^+), 178 (100 %), 142 and 115; all in good agreement with the mechanism of fragmentation proposed for 4-chloro-3-indolylacetic acid methyl ester.[15]

4-Chloro-3-indolylacetic acid. To a solution in ethanol (225 ml) of crude 3-diethylaminomethyl-4-chloroindole [16] (22.2 g) prepared from 4-chloroindole (15 g, 0.1 mol; Zion Chemical Products, POB 51 Yavne, Israel) was added a solution of NaCN (0.5 mol) in water (50 ml). The mixture was refluxed for 3 h and 100 ml distilled to remove diethylamine. Water (50 ml) was added and reflux continued for 16 h. The mixture was concentrated to 75 ml and water (80 ml) was added. The precipitate of nitrile + amide was filtered off. From the filtrate crude 4-chloro-3-indolylacetic acid (4.4 g) was isolated after acidification (hood!) with conc. HCl.

A mixture of nitrile + amide in ethanol (50 ml) and NaOH (20 g) in water (70 ml) was refluxed for 6 h. After concentration to about 60 ml, addition of water (80 ml) and filtration, more crude acid (10.6 g) was isolated after acidification with conc. HCl.

Crude 4-chloro-3-indolylacetic acid (15 g) was reprecipitated with conc. HCl from 40 ml of 10 % NaOH, recrystallized from water (1500 ml, a red insoluble fraction discarded) and from ethyl acetate:hexane 1:3. Yield 8.4 g (40 %), m.p. 184 – 187 °C.

Fischer indolization. To a fresh solution (60 °C) of 3-formylpropionic acid prepared from glutamic acid [17] (0.1 mol) was added the appropriate phenylhydrazine.HCl (0.05 mol, Aldrich) dissolved in 30 % acetic acid. The pH was adjusted to 4 with NaOH (5 M). After cooling the precipitate was collected and dissolved in pyridine (75 ml). Conc. HCl (100 ml) and 85 % H_3PO_4 (25 ml) were added and the mixture refluxed for 16 h protected from light under N_2.

The reaction mixture was diluted with water (600 ml), the tars filtered off, and the filtrate extracted repeatedly with ether. The combined ether fractions (600 ml) were washed with water and the indolylacetic acid extracted back into 0.5 M NaOH (200 ml).

Table 1. Yields, corrected melting points and ^1H NMR spectral data.[13] δ measured in CD_3COCD_3 on a Jeol JNM-PMX 60 instrument at 60 MHz. All compounds had broad peaks between δ 8 and 11 (−COOH and >NH).

3-Indolylacetic acid derivative	Starting material phenylhydrazine.HCl	Yield %	M.p. °C (dec.)	δ s (−CH_2−)	m (arom.)	
4-Chloro-	(4-Chloroindole)	40	184–187 [a]	4.00	7.0–7.4 (4 H)	
6-Chloro-	(6-Chloroindole)	38	182–184 [b]	3.76	6.9–7.7 (4 H)	
5-Chloro-	4-Chloro-	28	156–159 [c]	3.76	7.0–7.7 (4 H)	
7-Chloro-	2-Chloro-	23	163–165 [d]	3.77	6.9–7.7 (4 H)	
4,6-Dichloro-	3,5-Dichloro-	7	210–214	3.97	7.0 (1 H) 7.4 (2 H)	
4,7-Dichloro-	2,5-Dichloro-	9	214–217	4.00	7.1 (2 H) 7.4 (1 H)	
5,7-Dichloro-	2,4-Dichloro-	22	202–204 [e]	3.76	7.2 (1 H) 7.4 (1 H)	7.6 (1 H)
6,7-Dichloro-	2,3-Dichloro-	11	170–175	3.76	7.1–7.6 (3 H)	
5-Chloro-7-methyl-	4-Chloro-2-methyl-	4	178–180	3.73 [f]	6.9 (1 H) 7.3 (1 H)	7.5 (1 H)
7-Bromo-	2-Bromo-	7	180–182	3.77	6.8–7.7 (4 H)	

Literature [8] m.p.'s: [a] 185–187 °C, [b] 187–188 °C, [c] 158–160 °C, [d] 164–165 °C, [e] 194–197 °C. [f] 5-Chloro-7-methyl-3-indolylacetic acid also had a peak at δ 2.46 (s, $-CH_3$).

After boiling the indolylacetic acid was precipitated with conc. HCl (pH 1). If possible, the soapy tars separating first were filtered or decanted off, before crystals appeared. Recrystallization twice from water, water/ethanol, toluene, or ethyl acetate/hexane.

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On the Preparation of Dibenzylidenesuccinic Acid by the Stobbe Condensation

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The synthesis of dibenzylidenesuccinic acid (*1*) from benzaldehyde and diethyl succinate *via* the Stobbe condensation is a well-known procedure.[1] However, when the literature directions were followed, the crude product was invariably a dark-brown syrup from which it was difficult, and in some cases even impossible, to isolate the crystalline acid. Yields were low, less than 20 %, as was indeed noted previously.[1,2]

The bases used in the early work were sodium alkoxides or sodium metal. The often superior base, sodium hydride, introduced by Johnson [3] with excellent results in the condensation of ketones, had not been tried before in the synthesis of *1*. In the hope of finding a cleaner and higher-yielding synthetic procedure for *1*, needed for studies of the electrohydrocyclization reaction,[4] a series of condensations under different conditions was undertaken with this base. The experiments were performed on a 0.01 molar scale in five solvents; hexane, toluene, diethyl ether, dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Sodium ethoxide in ethanol was included for a comparison with the conventional method. The results are summarized in Table 1, the most striking feature of which is that yields are the same and relatively high in the three nonpolar solvents and considerably lower in the two polar ones (DMF and DMSO) where substantial amounts of tar were formed. Only a small temperature effect is noticeable and in contrast to a previous report,[4] higher temperatures seem to be favourable.

Table 1 further shows the relative product composition of the different runs. The main byproducts are monobenzylidenesuccinic acid (*2*) and benzoic acid (*3*). The run in refluxing toluene was performed twice, showing good interexperimental reproducibility.

Benzoic acid (*3*) is a prominent product in most cases which means that part of the aldehyde is removed from the reaction. It may be formed either by air oxidation or a Cannizzaro reaction, the latter possibility being suggested by Stobbe.[1a] This was confirmed by the results obtained in a run carried out in a nitrogen atmosphere. Benzyl alcohol, the second product from the Cannizzaro reaction, was detected in this case too. The amount of *3* was similar to that formed in an air atmosphere (see entry 1, Table 2). One presumably has to accept *3* as an unavoidable byproduct since it is difficult to see how one could suppress the Cannizzaro reaction. There is obviously no need for an inert atmosphere as has been prescribed in some published procedures.[3,5] The fact that ketones work so much better than aldehydes in the Stobbe condensation may depend on their lack of sensitivity to oxidative side-reactions.

The amount of *2* in the product mixture is usually between 15 and 20 %. One reason for the fact that *2* does not react further to give *1* might be the loss of aldehyde in the Cannizzaro reaction. Two experiments were therefore performed to see if a doubled molar ratio of base and aldehyde to ester would be advantageous (entries 2 and 3, Table 2). A consequence of the large amount of aldehyde used is then that the product mixture consists of predominantly *3*. The ratio of *1*/*2* is unchanged in the two cases. The yield of *1* has, however, been increased from 41 to 60 % when cold ether was used as a solvent. In refluxing toluene, the yield is the same (56 *vs.* 52 %).

The effect of doubling only the ratio of base

Table 1. Yield of products from the Stobbe condensation of benzaldehyde and diethyl succinate, using sodium hydride as the base.

Solvent	Absolute yield [a] of *1*/%		Relative product composition/%							
	Reflux temperature [b]	Low temperature [c]	Reflux temperature [b]				Low temperature [c]			
			1	*2*	*3*	Others	*1*	*2*	*3*	Others
Hexane	54	43	54	17	11	18	50	13	23	14
Toluene	52	44	52; 51	14; 17	5; 5	29; 27	55	12	20	13
Diethyl ether	50	41	55	16	13	16	41	15	30	14
Dimethyl sulfoxide	10	7 [d]	29	20	20	31	8	26	10	56
Dimethylformamide	22	5	48	14	16	22	6	23	31	40
Ethanol [e]	5	7	10	26	37	27	13	10	57	20

[a] Determined by GLC after esterification with methanol. [b] Reflux time, 3 h. [c] Kept at 3 °C for 48 h and room temperature for 24 h. [d] Kept at room temperature for 72 h. [e] Sodium ethoxide as base.

Table 2. Yield of products from the Stobbe condensation of benzaldehyde and diethyl succinate using sodium hydride as the base, under different reaction conditions.

Entry No.	Reaction conditions (see also text)	Absolute yield of *1*/%	Relative product composition/%			
			1	*2*	*3*	Others
1	Under N_2 in ether at 3 °C	46	51	14	19	16
2	4.4 mol of PhCHO and NaH/2 mol of ester; in ether at 3 °C	60	34	8	49	9
3	Same as 2 but in refluxing toluene	56	40	10	31	19
4	4.4 mol of base/2 mol of ester in refluxing toluene	40	42	15	15	27
5	Normal addition of components; hexane, 20 °C	45	57	8	10	25
6	Inverse addition of components; hexane, 20 °C	14	19	1	13	67
7	NaH, 60 % in mineral oil, instead of 100 %; refluxing toluene	38	39	16	8	37
8	Crown ether added; refluxing toluene	38	44	19	11	26

to ester was also studied. The effect (entry 4, Table 2) was to lower the yield of *1* and increase the occurrence of side-reactions, while the amount of *2* was unaffected.

In normal practice, a mixture of the ester and aldehyde is added to the base. Therefore there is a large excess of base in the beginning of the reaction and, as just noted, such conditions are unfavorable. In another variation of the reaction conditions, inverse mixing of the reactants was employed. Solid sodium hydride was then added to a solution of diethyl succinate and benzaldehyde in hexane. A drastic reduction in the yield of *1* is noted (entries 5 and 6, Table 2) contrary to expectations.

As is seen from entry 7, Table 2, the use of 60 % sodium hydride in mineral oil instead of the 100 % powder lowers the yield of *1*. The addition of dibenzo-6-crown-18 did not improve the yield of *1* (entry 8, Table 2).

Summarizing, the Stobbe condensation of benzaldehyde and diethyl succinate to give dibenzylidenesuccinic acid, using sodium hydride as the base, is favored in nonpolar solvents at higher temperatures. There is no need to use an inert atmosphere, since the process removing aldehyde from the Stobbe condensation is the Cannizzaro reaction.

Experimental. GLC analyses were performed on a Hewlett-Packard 5830A instrument equipped with a 2 m × 3 mm 3 % OV-17 on Chromosorb W column.

The standard procedure for performing the Stobbe reaction was as follows: Diethyl succinate (1.76 g, 10 mmol) and freshly distilled benzaldehyde (2.34 g, 22 mmol) were mixed and added to a slurry of 100 % sodium hydride (0.54 g, 22 mmol) in the appropriate solvent (20 ml). Absolute ethanol (*ca.* ten drops) was added. The solution was either refluxed for 3 h or kept at 3 °C for 48 h and then at room temperature for 24 h. The solvent was evaporated, the residue refluxed with 10 ml of 0.1 M aqueous KOH for 1 h, and the resulting solution extracted twice with ether. After acidification of the aqueous layer, the organic material was extracted with three portions of ether. The combined ether extracts were dried, and the ether evaporated. The mixture of acids (*1* – *3*) was then esterified with methanol/sulfuric acid in the usual way.

Deviations from this procedure are indicated in Table 2. In the experiment with dibenzo-6-crown-18, this compound was added in an amount equimolar with sodium hydride.

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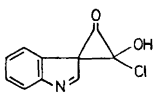
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On the Mechanism of Decarbonylation of Indole-3-glyoxyloyl Chloride

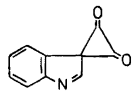
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We have recently observed [1] the thermal decarbonylation of an intermediate cyclopropanone in the Favorskii rearrangement of 3-(α-haloacyl)-indoles. Since certain arylglyoxyloyl chlorides are known [2–4] to undergo thermal decarbonylation, it appeared to us that a similar mechanism involving intermediates such as *1* and *2* might operate here. Little is known



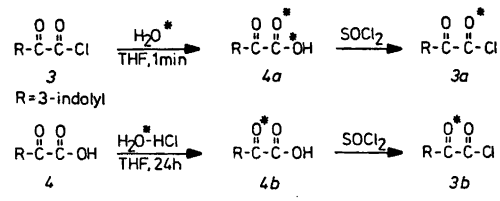
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2

about the mechanism of such decarbonylation reactions and therefore we decided to study the thermal decarbonylation of indole-3-glyoxyloyl chloride *3*. The results presented here from decarbonylation of the specifically ^{18}O labelled compounds *3a* and *3b*, indicate that the main path does not involve any three-membered intermediate.

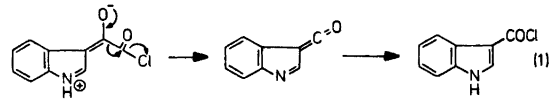
The compounds *3a* and *3b* were prepared as shown in Scheme 1.



Scheme 1.

The decarbonylation of *3a* or *3b* was performed in diglyme [$(CH_3OCH_2CH_2)_2O$] or tetrachloroethane at 120 °C. The indole-3-carbonyl chloride and the carbon monoxide formed were analysed for their content of ^{18}O. The results of the decarbonylations of *3a* and *3b* are given in Table 1. On decarbonylation, *3a* gave carbon monoxide with a high enrichment of ^{18}O, whereas *3b* gave carbon monoxide with a low percentage of ^{18}O. The content of ^{18}O found in the indole-3-carbonyl chloride which was isolated as a cross-check in some of the experiments, is in agreement with the values obtained from measurements on carbon monoxide. Thus the results show that the carbon monoxide formed by the decarbonylation of indole-3-glyoxyloyl chloride mainly comes from the COCl group.

The fact that the carbon monoxide formed has its origin in the COCl group rather than in the keto group rules out * *1* and *2* as main intermediates, which would have required a total (*1*) or partial (*2*) origin of the carbon monoxide in the keto group. A possible main path, consistent with the failure to detect radicals in the decarbonylation of phenylglyoxyloyl chloride,[4] is formation of the indolenine compound *5*, followed by decomposition to the ketene *6* (eqn. 1). A radical mechanism cannot be excluded but seems unlikely since only



(1)

5 *6*

* The slight systematic deviation from the expected amount of ^{18}O in the products, assuming the carbonyl in the COCl group is lost, appears to indicate a minor involvement of three-ring intermediates *1* or *2*. In fact, the figures from Table 1 from decarbonylation of *3b* in tetrachloroethane are consistent with approximately 25 % involvement of a path *via* the intermediate *2*.

Table 1. Decarbonylation of indole-3-glyoxyloyl chloride.

Compound[d]	Starting material ^{18}O enriched[a,c] (%) RCO*COCl	RCOCO*Cl	Products ^{18}O enriched[b,c] (%) RCO*CO*Cl	RCO*Cl	CO*
3b[e]	16.8	4.2	1.0		6.0
3a[e]	4.2	20.6	1.4		21.0
3b[f]	15.1	2.0	0.3	10.2	3.4
3b[f]	32.4	5.6	2.7		10.9
3b[f]	30.3	7.4	3.1	27.0	12.6

[a] Determined from the acid before reaction with $SOCl_2$ by mass spectrometry. [b] Determined by mass spectrometry; the carbon monoxide was separated from N_2 and O_2 on a *GC* column connected to the mass spectrometer. [c] The estimated errors in Table 1 vary from ± 0.1 for the small values to ± 0.5 for the large values. [d] R = 3-indolyl. [e] Performed at 120 °C in diglyme. [f] Performed at 120 °C in tetrachloroethane.

aliphatic glyoxyloyl chlorides appear [4] to decarbonylate *via* a radical chain.

Experimental. General. Mass spectra and IR spectra were recorded on an LKB-9000 and a Perkin-Elmer 421 spectrometer, respectively. Gas chromatographic separations were carried out at 50 °C using a 1.8 m × 3 mm column packed with molecular sieves (5A). ^{18}O enriched H_2O with a content of 20 % and 40 % ^{18}O was used.

Indole-3-glyoxyloyl chloride 3 was prepared from indole and oxalyl chloride according to Speeter and Anthony.[5]

4a. 3 (207 mg, 1 mmol) was hydrolysed by ^{18}O enriched H_2O (19 μl) in THF (1 ml) at 0 °C. After 1 min the solvent was rapidly evaporated *in vacuo*. The produced indole-3-glyoxalic acid [m.p. 218 °C (acetonitrile) lit.[6] 216 °C] was analysed for its ^{18}O content by mass spectrometry.

4b. Indole-3-glyoxylic acid (unlabelled, 189 mg) ^{18}O enriched H_2O (92 μl) and 8 μl conc. HCl was stirred in THF (1 ml) for 24 h at room temperature. After the reaction was completed the solvent was removed *in vacuo* and the acid analysed for its ^{18}O content (*MS*).

3a and *3b*. The appropriate labelled indole-3-glyoxylic acid *4a* or *4b* (189 mg) was treated with $SOCl_2$ (250 μl) in a mixture of THF (3 ml) and ether (2 ml) for 20 h at room temperature. After this time the solvent and excess $SOCl_2$ were removed *in vacuo*.

Decarbonylation of 3a *and* 3b was performed at 120 °C as described in Ref. 3, using diglyme or tetrachloroethane as solvent.

Acknowledgements. We thank The Swedish Natural Science Research Council for financial support.

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Chemical Synthesis and Disproportionation of *N*-Hydroxytyrosine

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In spite of the various routes reported for the chemical synthesis of *N*-hydroxyamino acids [1–3] they remain difficult to obtain either because of instability, poor yields, or limited applicability of each of the methods. *N*-Hydroxyamino acids have been established as components of several naturally occurring compounds [4,5] and have also, although the experimental data are weak, been postulated to be involved in the biosynthesis of several classes of secondary plant products.[6] We were particularly interested in testing *N*-hydroxytyrosine as an intermediate in the biosynthesis of the cyanogenic glucoside dhurrin and this report describes its synthesis and characterization.

Experimental. N-Hydroxytyrosine was synthesized by a modification of the method described by Ahmad.[3] *p*-Hydroxyphenylpyruvic acid (20 mmol) and hydroxylamine hydrochloride (30 mmol) were dissolved in a mixture of 35 ml of H_2O, 25 ml of EtOH and 45 ml of 1 M NaOH. Sodium cyanoborohydride (35 mmol) was added and pH kept at 4 by the addition of 1 M HCl. After reaction at room temperature for 24 h an additional 35 mmol of sodiumcyanoborohydride were added. After 60 h the reaction was stopped by the addition of concentrated HCl to pH ~0. The reaction mixture was evaporated to dryness at 30 °C in a rotary evaporator. The yellow residue was suspended in 50 ml of EtOH and insoluble inorganic material removed by filtration. The ethanol extract was evaporated to dryness and analytically pure *N*-hydroxytyrosine was obtained as white crystals in 74 % yield by recrystallizing the residue from hot water (Found: C 54.64; H 5.70; N 7.11. Calc. for $C_9H_{11}NO_4$: C 54.82; H 5.62; N 7.10). M.p. 226–228 °C (decomp.). MS [IP 70 eV, solid probe, 140 °C]: 197 (M^+), 107 (base peak). Potentiometric titration: $pK_1 = 2.52$ and $pK_2 = 5.26$.

Results and discussion. N-Hydroxyamino acids described earlier have shown considerable discrepancies in both physical and chemical properties.[3] The main criteria used for identification and purity has been elemental analysis, providing only little information on the nature of the impurities present. In this study NMR analysis was found very suitable for analyzing

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Table 1. Chemical shifts of *N*-hydroxytyrosine and related compounds.

	δ
N-Hydroxytyrosine	6.77 and 7.11 (2 doublets, 4 arom. H, J ~8 Hz), 4.26 (d of d, 1 H, J ~6 Hz), 3.20 (m) [a]
Tyrosine	6.79 and 7.14 (2 doublets, 4 arom. H, J ~8 Hz), 4.08 (app. t., 1 H, J ~6 Hz), 3.18 (m) [a]
p-Hydroxyphenylpyruvic acid oxime	6.63 and 7.06 (2 doublets, 4 arom. H, J ~8 Hz), 3.76 (s, 1 H)
p-Hydroxyphenylacetaldoxime	6.69 and 7.03 (2 doublets, 4 arom. H, J ~8 Hz), 6.74 (5, 1 H, J ~5), 3.56 (d, 2 H, J ~5)

[a] Overlaps HCD_2OD signal.

reaction mixtures and products obtained (Table 1). Smaller quantities of *N*-hydroxytyrosine and possible contaminants are best analyzed by gas chromatographic separation of their trimethylsilyl derivatives as recently described.[7] These two analytical methods were extensively used during this study and should have rather general applicability. The recrystallized *N*-hydroxytyrosine sample obtained contained no impurities when analyzed by either of these two methods. The yields obtained by the unmodified method of Ahmad [3] involving reduction at higher pH for a shorter time period were much lower (30–40 %) and the product obtained difficult to purify because of incomplete reduction of *p*-hydroxyphenylpyruvic acid oxime under these conditions. When the reaction mixture subsequently was acidified, this compound decomposed resulting in the requirement of additional purification steps *via* ion exchange chromatography.

When refluxed under nitrogen, α-*N*-hydroxyamino acids are reported to disproportionate to the corresponding α-amino acid and α-ketoacid oxime.[8] Although phenylpyruvic acid oxime has been isolated in low yield as a disproportionation product of *N*-hydroxyphenylalanine, the assumption of the involvement of an α-ketoacid oxime in this disproportionation reaction of *N*-hydroxyamino acids is generally based on the formation of equimolar amounts of α-amino acid and carbon dioxide. The latter is assumed to be formed from the rapid conversion of the α-ketoacid oxime to the homologous lower nitrile.[8] However, when *N*-hydroxytyrosine was refluxed under nitrogen, equimolar quantities of tyrosine and *p*-hydroxyphenylacetaldoxime were formed with only traces of *p*-hydroxyphenylacetonitrile and no *p*-hydroxyphenylpyruvic acid oxime present. Reflux of *p*-hydroxyphenylacetaldoxime [9] resulted in a similar small amount of *p*-hydroxyphenylacetonitrile while reflux of *p*-hydroxyphenylpyruvic acid oxime resulted in nearly complete conversion to *p*-hydroxyphenylacetonitrile. It is therefore concluded that *p*-hydroxyphenylpyruvic acid oxime is not an intermediate in the disproportionation of *N*-hydroxytyrosine and the earlier postulated involvement of α-ketoacid oximes in the disproportionation of *N*-hydroxyamino acids when refluxed under nitrogen based solely on carbon dioxide production [8] thus would appear to be questionable.

Acknowledgements. This investigation was supported by Grant 511-3988 from the Danish Natural Science Research Council and by a Fulbright-Hays Act Fellowship (to B.L.M.) and by NSF grant BMS-7411997 and USPHS grant GM-05301 (to E.E.C). Dr. D. S. Seigler, Department of Botany, University of Illinois, Urbana, is thanked for recording the NMR spectra.

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Studies of Hydrogen Bonding. Part XXVIII.* Hydrogen Bond Association of Phenol with 5,5-Dimethyl-2-oxo-1,3,2-dioxaphosphorinanes and Diethylphosphonates

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The association constant and enthalpy of formation of the equilibrium between phenol and twelve 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanes and six diethylphosphonates have been determined. It has been found that the association constant, K_{ass}, enthalpy of association, ΔH, half band width, $\nu_{\frac{1}{2}}$, of the complexed O–H bond, P=O bond moment, μ_{PO}, and excess charge density, Δ_q, on the phosphoryl oxygen give linear relationships with the O–H frequency shift, $\Delta\nu_{OH}$. The calculated P=O bond moment in the axial and equatorial position gives a linear correlation with the experimental P=O stretching frequency. Furthermore, the square root of the additional dipole moment, $\Delta\mu^{\frac{1}{2}}$, due to hydrogen bond formation, correlates linearly with the association constant, the O–H frequency shift, and with the half band width. These findings indicate a close relationship between band width of the complexed O–H bond and the strength and polarity of the hydrogen bonded complex.

Since we previously [2] have carried out conformational studies of 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanes, we found it of interest to extend our study to include the dipole moment and structure of the complex formed with phenol and further to correlate the hydrogen bond data with P=O bond moment, excess charge density on the phosphoryl oxygen and with the square root of the additional dipole moment, $\Delta\mu^{\frac{1}{2}}$, *i.e.* with the difference between the dipole moment of the complex and the vector sum of the individual moments of the components.

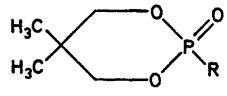
* For Part XXVII see Ref. 1.

EXPERIMENTAL

Materials. Phenol was purified by several recrystallizations from light petroleum and the white needles obtained were dried over phosphorus pentoxide in a desiccator. Carbon tetrachloride and benzene were purified chromatographically by using Molecular Sieve Type 4 Å and basic aluminium oxide. Carbon disulfide (Merck Uvasol) was used without further purification. The phosphonates and dioxaphosphorinanes, not commercially available, were prepared by literature methods.[2] The proton acceptors were redistilled or recrystallized immediately before use, and the purity was checked by IR and mass spectroscopy.

Infrared measurements. All measurements were carried out with a Perkin-Elmer 225 or a UNICAM SP 200 G spectrophotometer. The ground state stretching vibration of the proton donor O–H band was used for the determination of the association constants. Quartz absorption cells (Hellma No. 110, QI) of 10.00 mm path length were used. The spectra were run at temperatures of 25, 35 and 50 °C immediately after preparing the solutions. The concentration of phenol was kept at about 0.0025 M and the concentration range of the proton acceptors was 0.0020 – 0.0100 M. The solutions were made up by weighing, and the molarity of each solution was calculated at various temperatures from the change in density with temperature of carbon tetrachloride or of benzene. The details of the measurements have been published previously.[3,4] The K_{ass}, ΔH, $\Delta\nu_{OH}$ and $\nu_{\frac{1}{2}}$ values of the complexes between phenol and the phosphorinanes and the phosphonates tabulated in Tables 1 and 2 are the mean value of five separate determinations and have been corrected for the change in density of the solvent with temperature. The $\Delta\nu_{OH}$ values are estimated to be accurate within $\pm 3-5$ cm^{-1}, ΔH values within ± 2 kJ and K_{ass} values within 10 %.

Table 1. Spectral data for 1:1 hydrogen-bonded complexes between phenol and various 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanes (1) in carbon tetrachloride.



R	$\Delta\nu_{OH}$/ cm^{-1}	K_{ass}/ M^{-1}	$-\Delta H$/ kJ mol^{-1}	$-\Delta S$/ J K^{-1} mol^{-1}	$\nu_{\frac{1}{2}}$/ cm^{-1}	Temp/ °C
Cl	182	33.0 26.5 16.5	23.4	49.4	122	25 35 50
CCl_3	220	40.4 26.6 18.8	24.7	50.6	145	25 35 50
OC_6H_5	245	80.9 54.5 34.5	26.8	53.1	158	25 35 50
SCH_3	257	79.2 57.7 34.2	26.8	53.1	168	25 35 50
H	259	75.2 52.6 32.5	26.8	54.0	169	25 35 50
OCH_3	277	135.5 89.0 55.0	28.5	55.2	176	25 35 50
$C(C_6H_5)_3$	332	206.2 143.0 75.3	31.4	57.7	214	25 35 50
C_6H_5	333	240.1 149.4 92.7	30.1	55.2	213	25 35 50
CH_3	336	295.4 205.0 112.5	31.4	57.3	215	25 35 50
$CH_2C_6H_5$	337	317.8 211.8 121.2	31.0	55.7	217	25 35 50
$N(CH_3)_2$	356	352.6 233.0 131.0	31.8	57.3	221	25 35 50
$C(CH_3)_3$	359	322.6 232.1 128.5	32.2	59.0	222	25 35 50

Dipole moment measurements. The dipole moment measurements were carried out in carbon tetrachloride or in benzene at 20 ± 0.05 °C. The dielectric constants were measured with an internally thermostated, heterodyne-beat dipolmeter, type DMO 1, manufactured by Wissenschaftlich-Technische Werkstätten, GmbH, Germany. The thermostated dielectric cell, type DFL, of 20 ml capacity, had gold-coated interior plates. The refractive indices were measured at 20 °C with a Bausch & Lomb Precision Refractometer, 33-45-02-01. The den-

Table 2. Infrared data for the interaction of phenol with various diethylphosphonates, $(C_2H_5O)_2P(O)R$. Comparison of hydrogen bond data, dipole moments and electron densities. Solvent carbon tetrachloride.

R	ν_{PO}/cm^{-1} in CS_2	μ_a/D in CCl_4	Δq/ electrons [a]	ν_{OH}/ cm^{-1}	K_{ass}/ M^{-1}	$-\Delta H$/ kJ mol^{-1}	$-\Delta S$/ J K^{-1} mol^{-1}	$\nu_{\frac{1}{2}}$/ cm^{-1}	Temp/ °C
					71.9		51.5		25
CCl_3	1279	3.26	−0.5218	244	51.0	25.9		157	35
					32.3				50
					141.0		53.1		25
SCH_3	1262	2.67		294	98.0	28.0		183	35
					58.6				50
					130.1		54.0		25
H	1260	3.11	−0.3625	298	95.3	28.5		184	35
					54.1				50
					296.1		54.8		25
$C(C_6H_5)_3$	1247	2.86		338	178.3	30.1		218	35
					105.2				50
					380.4		57.3		25
CH_3	1243	2.74	−0.4008	357	249.5	31.8		223	35
					141.1				50
					455.9		57.7		25
$N(CH_3)_2$	1253			367	294.6	32.6		234	35
					170.3				50

[a] Δq, Calculated excess charge density on the P=O oxygen by the CNDO/2 method (details will be published elsewhere).

sity of carbon tetrachloride, benzene and of the solutions was determined by using a 20 ml capacity pycnometer. All solutions were prepared by weighing and they were handled in a dry-box. For the dipole moment measurements the weight fraction range of phenol was $4\times10^{-4}-30\times10^{-4}$ and of proton acceptor $8\times10^{-4}-60\times10^{-4}$.

The experimentally observed dielectric constants, ε_2, the specific volumes, v_2, and the square of the refractive indices, n_2^2, of the carbon tetrachloride and benzene solutions of proton donor and of acceptor were found to be lineary dependent on their weight fractions, w_2. The equations employed were:

$$\varepsilon_2 = \varepsilon_1 + \alpha w_2 \quad (1)$$

$$v_2 = v_1 + \beta w_2 \quad (2)$$

$$n_2^2 = n_1^2 + \gamma w_2 \quad (3)$$

The dielectric constant, ε_1, the specific volume, v_1, and the square of the refractive index, n_1^2, correspond to the values of pure carbon tetrachloride or of pure benzene at 20 °C. The α, β and γ values were obtained by the method at least squares. The total polarization, $P_{2\infty}$, and the molar refraction, R_D, at infinite dilution were calculated by using the method of Sharpe and Walker.[5] The dipole moment, μ, was calculated [5] from eqn. (4).

$$\mu = 0{,}01281\ (P_0T)^{\frac{1}{2}} \quad (4)$$

P_0 is the orientation polarization derived from $P_0 = P_{2\infty} - R_D$ (it is assumed that $R_D = P_E + P_A$). The probable error in the dipole moment, μ, was estimated not to exceed 0.05 D for each compound examined. The μ_a values for the phosphorinanes are tabulated in Table 3 (for details see Ref. 2), and for the phosphonates in Table 2.

To evaluate the dipole moment of the hydrogen bonded complex we need to know the concentration of the free proton donor, and the concentration of the free acceptor and of the hydrogen bonded complex in the reaction mixture (see eqn. 5).

$$C_6H_5OH + O{=}P\!\!<\!\!\!\!> \rightleftharpoons C_6H_5OH\cdots O{=}P\!\!<\!\!\!\!> \quad (5)$$

By using the K_{ass} values obtained by IR measurements (see Table 4), the weight fraction of

Table 3. Experimental P=O stretching frequency, ν_{PO}, and dipole moment, μ_a, and CNDO/2 calculated P=O bond moment, μ_{PO}, and excess charge density, Δq, on the P=O oxygen for various 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanes, *1*.

R	ν_{PO}/cm^{-1} in CS_2	in KBr	$\mu_{a/D}$ in C_6H_6	$\mu_{PO/D}$ ax	eq[b]	Δq/electrons [d]
Cl	1321 1296	1287	6.49	10.40	10.72	−0.3492 (eq)
CCl_3	1310 1286	1286	6.18	10.44	10.74	−0.4196 (eq)
OC_6H_5	1322 1297	1285	5.71 [a]	9.24 (ga)[c]	9.32	−0.3289 (eq/ga)
SCH_3	1286 1268	1273	5.65	10.77 (ga)	10.46	−0.4173 (eq/ga)
H	1310 1281		5.94	9.74	9.68	−0.3333 (eq)
OCH_3	1306 1273	1287	5.41	10.04 (ga)	9.96	−0.3541 (eq/ga)
$C(C_6H_5)_3$	1254	–	–	–	–	–
C_6H_5	1285 1259	1275	5.73	10.57	10.66	−0.3763 (ax)
CH_3	1252	1244	4.91	10.78	–	−0.3790 (ax)
$CH_2C_6H_5$	1275 1255	1246	5.25	–	–	–
$N(CH_3)_2$	1259	1238	4.24	10.75	–	−0.3923 (ax/ga)
$C(CH_3)_3$	1250	1247	4.13	10.88	–	−0.3988 (ax)

[a] From ref. 35. [b] ax and eq. mean P=O in axial or in equatorial position (for details see Ref. 2). [c] ga means *gauche* conformation (see Ref. 2). [d] Calculated charge density on P=O oxygen in the more stable (lowest energy) conformation (see Ref. 2).

Table 4. Total polarization, $P_{x\infty}$, molar refraction, R_D, parameters α_x, β_x, γ_x, and dipole moments, μ_x, and additional dipole moments $\vec{\Delta\mu}$ ($\vec{\Delta\mu}=\vec{\mu_x}-\vec{\mu_d}-\vec{\mu_a}$) of the complex formed between phenol and various phosphoryl compounds at 20 °C.[a] The dipole moment increment due to charge transfer, $\vec{\Delta\mu}_{CT}$, and to electrostatic attraction $\vec{\Delta\mu}_{ind}$ ($\vec{\Delta\mu}=\vec{\Delta\mu}_{ind}+\vec{\Delta\mu}_{CT}$).

Proton acceptor	Solvent	K_{ass}/M^{-1}	α_x	β_x	γ_x	$P_{x\infty}$/cm^3	R_D/cm^3	$\vec{\mu_x}$/D	$\vec{\Delta\mu}$/D	$\vec{\Delta\mu}_{ind}$/D	$\vec{\Delta\mu}_{CT}$/D
1, R=CCl_3	C_6H_6	19	16.754	−0.9363	0.0593	1141.98	25.31	7.33	0.19	0.07	0.12
1, R=CH_3	C_6H_6	140	17.944	−0.6834	−0.1661	903.58	27.53	6.51	0.33	0.12	0.21
$(C_2H_5O)_2P(O)CCl_3$	CCl_4	85	9.091	0.1316	0.1400	410.71	78.02	3.99	0.23	0.09	0.14
$(C_2H_5O)_2P(O)H$	CCl_4	153	15.294	0.2780	0.0682	433.80	59.41	3.98	0.28	0.11	0.17
$(C_2H_5O)_2P(O)CH_3$	CCl_4	423	13.710	0.5600	0.1276	440.03	83.62	4.14	0.37	0.14	0.23

[a] Weight fraction range of phenol, $4\times10^{-4}-30\times10^{-4}$; and of acceptor, $8\times10^{-4}-60\times10^{-4}$. K_{ass} measured at 20 °C.

the different species in the mixture could be calculated. To obtain the dielectric constant, ε_x, of the hydrogen-bonded complex, the contribution due to free phenol, ε_d, and that of the free proton acceptor, ε_a, have to be subtracted from the observed value, *i.e.*, $\varepsilon_x = \varepsilon_{obs} - (\varepsilon_d + \varepsilon_a)$. The ε_d and ε_a values could easily be obtained from the curves $(\varepsilon_d - \varepsilon_l)$ *vs.* w_d and $(\varepsilon_a - \varepsilon_l)$ *vs.* w_a, respectively (w_d = weight fraction of phenol, w_a = weight fraction of proton acceptor). Similarly, the v_x and n_x^2 values could be obtained. It was found, just as for the proton donor and the acceptors in CCl_4 or in C_6H_6, that the plots of dielectric constant, ε_x, specific volume, v_x, and square of the refractive index, n_x^2, of the hydrogen-bonded complex against the weight fraction showed no deviation from linearity. The dipole moments, μ_x, of the following hydrogen bonded complexes phenol/dioxaphosphorinanes (R = CCl_3, CH_3, see Table 4) in benzene and phenol/diethylphosphonates (R = CCl_3, H, CH_3) in carbon tetrachloride have been determined experimentally at 20 °C. The polarization data α_x, β_x, γ_x, $P_{x\infty}$, R_D and $\vec{\mu}_x$ of the hydrogen-bonded complexes are given in Table 4.

Calculations of μ_{PO}. CNDO/2 calculations of the P=O bond moments were carried out and by using the method of Santry and Segal.[6] The calculated μ_{PO} values when P=O is in the axial or in the equatorial position, and the R group (R = $-OC_6H_5$, $-SCH_3$, $-OCH_3$ or $-N(CH_3)_2$ is in the *gauche* conformation (see Ref. 2), are tabulated in Table 3. Calculations of the total energy of the various conformations of the phosphoryl compounds have been published elsewhere.[2]

Calculations of $\vec{\Delta\mu}$. The formation of a hydrogen bond, $X-H+B \rightleftharpoons X-H\cdots B$, brings about displacements of the electrons and the nuclei which results in a difference between the dipole moment of the hydrogen bonded complex and the vector sum of the individual moments of the components. This difference can be expressed in terms of a dipole increment, $\Delta\mu$, defined by the vector equation (6).

$$\vec{\Delta\mu} = \vec{\mu}_x - \vec{\mu}_d - \vec{\mu}_a \qquad (6)$$

where $\vec{\mu}_x$, $\vec{\mu}_d$ and $\vec{\mu}_a$ are the dipole moment of the hydrogen bonded complex, of phenol (1.47 D in benzene and 1.502 in carbon tetrachloride [8] at 20 °C) and of the phosphoryl compounds, respectively. The value of $\vec{\Delta\mu}$ can only be computed from experimental values of μ_x, μ_d and μ_a if the angles between these vectors are given, *i.e.*, we have to assume a geometrical configuration of the complex. In the calculation of the $\vec{\Delta\mu}$ value from experimental data [9,10] one neglects the change of dipole moment which can appear in the outside parts of the $-H\cdots B$ bridge. This may be questionable, in particular for groups like $\ddot{N}-P=O$ and $\ddot{O}-P=O$ where unshared electron pairs may operate. However, IR as well as ^1H NMR measurements show that the changes observed outside the H-bond are negligible compared to the changes observed in the $-H\cdots B$ part.[9] Nevertheless, by comparing a series of hydrogen bonded complexes with similar structural characteristics, the evolution of $\vec{\Delta\mu}$ values corresponding to the various spatial structures allow us to predict the most probable structures.[11,12] The evaluation of the additional dipole moment, $\vec{\Delta\mu}$, acting along the hydrogen bond, is based on the following equation, $\vec{\mu}_x = \vec{\mu}_a + \vec{\mu}_d + \vec{\Delta\mu}$ which when squared, on introducing an azimuthal angle ϕ yields:

$$(\mu_x^2)_\phi = \mu_a^2 + \mu_d^2 + \Delta\mu^2 + 2\Delta\mu\mu_d \cos\beta_d + 2\Delta\mu\mu_a \cos\beta_a + 2\mu_d\mu_a \cos\beta_d \cos\beta_a + 2\mu_d\mu_a \sin\beta_d \sin\beta_a \cos\phi$$

Since the phenol part of the complex can rotate around the O−H bond, the average value of $\cos\phi \approx 0$. This yields $\mu_x^2 = \mu_d^2 + \mu_a^2 + \Delta\mu^2 + 2\Delta\mu\mu_d \cos\beta_d + 2\Delta\mu\mu_a \cos\beta_a + 2\mu_d\mu_a \cos\beta_d$

The value of $\Delta\mu$ from the above equation becomes:

$$\Delta\mu = \sqrt{\mu_x^2 - \mu_d^2 \sin^2\beta_d - \mu_a^2 \sin^2\beta_a} - \mu_d \cos\beta_d - \mu_d \cos\beta_a$$

The value of the angle β_d between the resultant dipole moment and the O−H direction in the phenol molecule (see Fig. 1) has been calculated [10] to be 27°. The angle β_a between the P=O vector and the resultant dipole moment of the phosphoryl acceptors has been estimated in the following way. The equation of the P=O vector is tabulated from a COORD program devised by Dewar.[13] The direction of the total dipole moment, β_a, of the various phosphoryl compounds is known from the CNDO/2 calculation.[2] Simple mathematics are then used to calculate the angle β_a between the two vectors. The results of the calculated β_a values of the

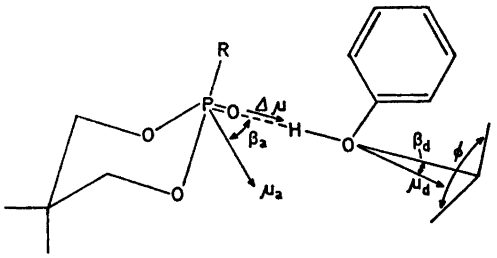


Fig. 1. Hydrogen bonding between phenol and 1,3,2-dioxaphosphorinane showing the various angles used to calculate $\vec{\Delta\mu}$.

hydrogen bonded complexes phenol/dioxaphosphorinanes are 46.2 and 19.9° for R = CCl_3 and CH_3, and the angles of the phenol/diethylphosphonates are 71.0, 46.3 and 66.7° for R = CCl_3, CH_3 and H, respectively. The $\Delta\mu$ values for the various hydrogen-bonded complexes are tabulated in Table 4.

Charge-transfer in H-bonds. Following Ratajczak [14,15] it is to be expected that the observed enhancement of the dipole moment of hydrogen bonded complexes might be due to two main contributions, (a) to an induced dipole moment due to mutual electrostatic attraction,

$$X^{\delta-}-H^{\delta+}\cdots{}^{\delta-}B,$$

and (b) to a dipole moment due to charge transfer (electron delocalization),

$$X^{\delta-}-H\cdots B^{\delta+}$$

Therefore, it can be written,

$$\vec{\Delta\mu} = \vec{\Delta\mu}_{ind} + \vec{\Delta\mu}_{CT}$$

An approximate estimate of $\vec{\Delta\mu}_{ind}$ can be evaluated according to the method of Frank,[16] hence the dipole moment, $\Delta\mu_{CT}$, due to electron delocalization can be obtained. The method of Frank leads to the following relationship [9]

$$\Delta\mu_{ind} = 2\alpha\mu_b(\varepsilon+2)/3\varepsilon r^3$$

where ε is the dielectric constant of the solvent,[17] α is taken to be the polarizability of the O–H bond of phenol,[18] μ_b is the inducing dipole moment approximated by the dipole moment of the lone-pair orbital of the oxygen atom ($\mu_b = 2$ D), and r is the distance between the dipole and the polarizable center. This was taken to be the distance between the O atom and the center of the O–H bond. The distance r was estimated from an empirical correlation between this quantity and $\Delta\nu_{OH}$.[19,20] The $\vec{\Delta\mu}_{ind}$ and $\vec{\Delta\mu}_{CT}$ values thus obtained are given in Table 4. The $\Delta\mu_{ind}$ values are between 0.07–0.14 D and values of $\Delta\mu_{CT}$ between 0.12–0.23 D, depending upon the system. A linear plot of ΔH against $\Delta\mu^2$, predicted theoretically,[9] can be shown to exist by using the values in Tables 1 and 4.

RESULTS AND DISCUSSION

As can be seen from Tables 1–3, the measured values for 2-oxo-1,3,2-dioxaphosphorinanes and diethylphosphonates fall onto the same straight line correlating K_{ass} with $\Delta\mu_{OH}$, and ΔH with $\Delta\nu_{OH}$, *i.e.*, there is no marked difference in hydrogen bonding ability between the phosphorinanes and the phosphonates in relation to $\Delta\nu_{OH}$. In both cases the equations correlating log K_{ass} with $\Delta\nu_{OH}$ are given by,

$$\log K_{ass}^{25} = 0.0062\ \Delta\nu_{OH} + 0.378 \text{ (at 25 °C)}$$

$$\log K_{ass}^{35} = 0.066\ \Delta\nu_{OH} + 0.084 \text{ (at 35 °C)}$$

$$\log K_{ass}^{50} = 0.056\ \Delta\nu_{OH} + 0.174 \text{ (at 50 °C)}$$

If comparison is made, however, between 2-oxo-1,3,2-dioxaphosphorinanes and diethylphosphonates containing the same P–R group, we see that the phosphonates, in general, have a lower P=O stretching frequency shift, larger K_{ass} and $\Delta\nu_{OH}$ values with phenol than the phosphorinanes, *e.g.*, the phosphorinane containing the P-CCl_3 group (ν_{PO}, 1310, 1286; K_{ass}[28], 40; ν_{OH}, 220) should be compared with $(C_2H_5O)_2POCCl_3$ (ν_{OH}, 1279 cm^{-1}; K_{ass}[25], 72 M^{-1}; ν_{OH}, 224 cm^{-1}). The main reason for this might be that the O-P-O angle is found to be smaller in the diethylphosphonates [21] than in the 1,3,2-dioxaphosphorinanes.[22,23] As a consequence, different hybridization may occur on the phosphorus atom and thus cause a lower electron density on the P=O oxygen in the latter compound (see Δq in Tables 2 and 3).

A conformational study of the 5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanes by means of dipole moments, ionization potentials and

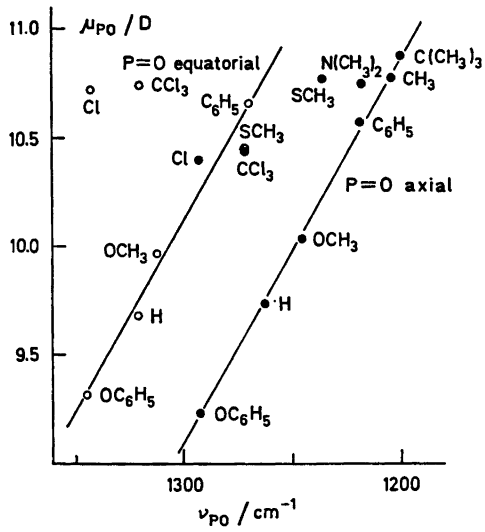


Fig. 2. Correlation of the P=O bond moment, μ_{PO}, calculated by the CNDO/2 method with IR frequency ν_{PO} in axial (●) and equatorial (○) position of the various phosphorinanes.

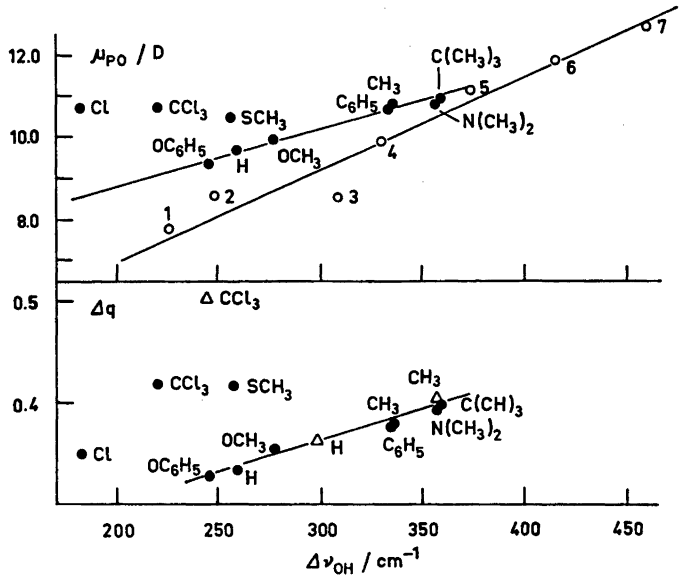


Fig. 3. CNDO/2 calculated P=O bond moment, μ_{PO}, and excess charge density, Δq, on P=O oxygen *vs.* $\Delta\nu_{OH}$ for the association of phenol with 1,3,2-dioxaphosphorinanes (●) and diethylphosphonates (△). Open circles are μ_{PO} data calculated from IR intensity measurements of the P=O band,[24]

(1) $(C_6H_5O)_3PO$,
(2) $(C_6H_5O)_2(CH_3O)PO$,
(3) $(CH_3O)_3PO$,
(4) $(C_2H_5O)_3PO$,
(5) $(C_2H_5O)_2(CH_3)_2NPO$,
(6) $(C_6H_5)_3PO$,
(7) $[(CH_3)_2N]_3PO$.

CNDO/2 calculations has been published.[2] In this work we have calculated by the CNDO/2 method the P=O bond moments, μ_{PO}^{ax} and μ_{PO}^{eq} (P=O in the axial or equatorial position) and found that they form, with the exception of R=Cl or CCl_3, a fairly good linear relationship when plotted against observed P=O stretching frequencies in the axial and equatorial positions (Fig. 2). Following Majoral [23] we have associated the band with the lowest μ_{PO} value with a conformer with the P=O group in the axial position. Since we have found that the CNDO/2 calculated μ_{PO}^{ax} and μ_{PO}^{eq} correlate with the two bands which appear in the region 1320 – 1250 cm^{-1}, our findings substantiate the conclusion drawn by Majoral that the two bands may be assigned to P=O stretching frequencies.

We have also found, as shown in Fig. 3, that the P=O bond moments, μ_{PO}, calculated by the CNDO/2 method (μ_{PO}^{eq} values for R=Cl, CCl_3, OC_6H_5, SCH_3, H, OCH_3 and C_6H_5, and μ_{PO}^{ax} for R=$N(CH_3)_2$, $C(CH_3)_3$ and CH_3) give, with the exception of R=Cl, CCl_3 or SCH_3, a linear relation with $\Delta\nu_{OH}$. A similar relationship has also been found to exist by using the μ_{PO} values reported by Schulze and Müller [24] as shown in Fig. 3. Our CNDO/2 calculated P=O bond moments are in fairly good agreement with those calculated from infrared P=O bond

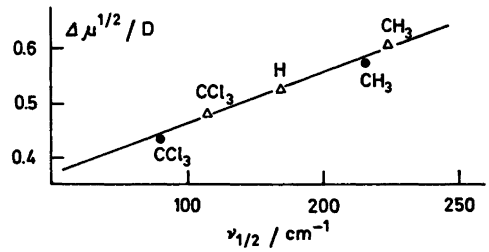


Fig. 4. Half band width, $\nu_{\frac{1}{2}}$, of the O–H band *vs.* the square root of the dipole moment, $\mu^{\frac{1}{2}}$, for the association of phenol with phosphorinanes (●) and diethylphosphonates (△).

intensities of Schulze and Müller. In contrast, however, Mauret and Fayel [25] have reported μ_{PO} values of order 3–4 D for similar phosphoryl compounds. The great deviation of 2-oxo-1,3,2-dioxaphosphorinanes and diethylphosphonates containing the groups R = Cl, CCl_3 or SCH_3 from the straight line correlating μ_{PO} with $\Delta\nu_{OH}$ and ν_{PO} (see Figs. 2 and 3), may be due to the fact that the parameters in the CNDO/2 program do not satisfactorily account for the interaction of the *d*-orbitals.[26] It is well known,[6,26] that the atomic parameters of the CNDO/2 method have been found to be unsatisfactory for charge density and dipole moment calculations for the second row atoms. To our knowledge there have not previously been reported any systematic CNDO/2 calculations of the charge density, Δq, on phosphoryl oxygen. Wagner [27] has, however, by using the internally consistent LCAO-MO method, reported Δq values which are not consistent with our results. It is also interesting to note that we have found a linear correlation between Δq and $\Delta\nu_{OH}$ for the association of the phosphoryl compounds with phenol. The Δq values of 2-oxo-1,3,2-dioxaphosphorinanes and diethylphosphonates fall onto the same straight line (Fig. 3). No linear correlation has been found between Δq and the Taft inductive substituent constant σ^* for the group R. This should imply that the electron density on the phosphoryl oxygen is not entirely controlled by the inductive effects of the substituents groups. In this connection it should be mentioned that alkaline hydrolysis of a series of organohalophosphorus compounds indicates that the electron density on the phosphorus atom is influenced by mesomeric effects, *e.g.*, there is an increase in the activation energy of approximately 16.7 and 25.1 kJ mol^{-1} in passing from dialkyl to dialkoxy and from dialkyl to bisdialkylamino groups,[28–30] respectively. Consequently, it is reasonable to assume that the unshared electron pair on the nitrogen, and to a smaller extent that on the oxygen atom, is conjugated with a 3*d* orbital of the phosphorus atom and hence reduces its fractional positive charge. This observation is also in accordance with the data obtained from bond lengths [31] and spectroscopic studies.[32,33] What may be questionable is to what extent the mesomeric effects are transmitted through the P = O group and hence affect the electron density on phosphoryl oxygen. Lack of linearity between Δq and σ^*, however, indicates strongly that the resonance effect plays an important part.

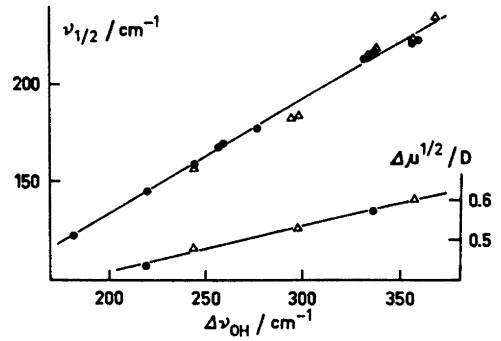


Fig. 5. Half band width, $\nu_{\frac{1}{2}}$, of the O–H band and the square root of the additional dipole moment, $\Delta\mu^{\frac{1}{2}}$, *vs.* $\Delta\nu_{OH}$ for the association of phenol with phosphorinanes (●) and diethylphosphonates (△).

The $\vec{\Delta\mu}$ values have been determined by using experimentally obtained μ_x, μ_d, μ_a values and a CNDO/2 calculated β_a value (see Fig. 1). In Fig. 4 we have shown that there exists a linear relationship between the square root of the additional dipole moment $\Delta\mu^{\frac{1}{2}}$ and half band width $\nu_{\frac{1}{2}}$. This finding is very important and demonstrates clearly that the greater the half band width the greater is the enhancement of the dipole moment. We have also found, as shown in Fig. 5, a linear correlation between $\Delta\mu^{\frac{1}{2}}$ and $\Delta\nu_{OH}$ (or ΔH). This indicates, since the extent of the proton transfer reaction in this system is negligible,[34] that the dipole increment is mainly determined by the strength of the H-bond formed. If the method of Frank [16] with included modifications [9] gives reliable $\vec{\Delta\mu}_{ind}$ values, we can see from Table 4 that the main factor responsible for the enhancement of polarity observed in the $\diagdown\!\!\diagup P{=}O\cdots H{-}OC_6H_5$ system is the change of electron distribution due to an electron-transfer effect. As can be seen from Tables 1 and 4 this electron delocalization effect is linearly related to the H-bond strength, *i.e.*, the stronger the H-bond the greater is the electron delocalization effect.

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Two- and Three-bond $^{13}C—^{1}H$ Couplings in Some Carbohydrates

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From the proton coupled ^{13}C NMR spectrum of 1,6-anhydro-β-D-galactopyranose, measured at 67.89 MHz, the two and three bond C—H coupling constants with values larger than ±2 Hz were obtained. Some long-range C—H couplings of a few other hexose derivatives were also measured. The three bond couplings show a Karplus type of dependence. The two bond couplings have values which are determined by the orientation of the oxygen atoms directly connected to the coupling path.

With modern pulsed Fourier NMR spectrometers proton coupled ^{13}C NMR spectra of carbohydrates are readily obtained. The numerically large one-bond $^{13}C—^{1}H$ couplings can in most cases be measured directly from these spectra.[1–3] The much smaller two- and three-bond $^{13}C—^{1}H$ couplings have, on the other hand, mostly been obtained from proton NMR spectra of ^{13}C-enriched carbohydrates.[4–11] Lately, however, a few long-range $J(^{13}C—^{1}H)$ values have been measured from natural abundance ^{13}C NMR spectra of carbohydrates;[9,10] but in no case has a proton coupled ^{13}C NMR spectrum of a carbohydrate been completely analysed.

In the proton coupled ^{13}C NMR spectra measured previously at 22.63 MHz[1–3] a large number of long-range couplings were observed, but the assignment of these couplings was difficult. We have now measured some carbohydrates at 67.89 MHz; under these conditions it was possible to obtain two- and three-bond $^{13}C—^{1}H$ couplings for virtually all carbon atoms in a number of cases. In the present paper we report the analysis of the spectrum of 1,6-anhydro-β-D-galactopyranose (*1*). In addition some spectral data of a few other hexose derivatives are reported. Chemical shifts and some coupling constants of *1* and of other 1,6-anhydro-hexopyranoses have been reported recently.[10,12]

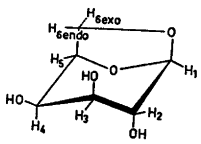
The two galactose derivatives *1* and *2* were chosen because their ^{13}C as well as their ^{1}H NMR spectra are rather well dispersed at 63.4 kG, and second order effects are therefore less likely.

In Table 1 the $^{2}J(^{13}C—^{1}H)$ and $^{3}J(^{13}C—^{1}H)$ values of 1,6-anhydro-β-D-galactopyranose (*1*) are presented. The signs of these coupling constants were not determined, but are assumed to be in agreement with those measured by Perlin *et al.*[9] Coupling constants not shown are not necessarily zero, but may have values between ±2 Hz. Almost all assignments could be made through selective proton decoupling. Some selectively deuterated derivatives of *1* were, however, also studied in order to confirm the assignments.

In Fig. 1A is shown the signal of C1 in the proton coupled spectrum of *1*. Selective irradiation of H5 or $H6_{endo}$ produced the spectra shown in Fig. 1B and C, respectively; it is seen that C1 couples with H5 and $H6_{endo}$. Other decoupling experiments showed that C1 couples with H3, but not with H2, H4, or $H6_{exo}$.

Fig. 2B shows the signals of C2, C3, and C5 in the proton coupled spectrum of *1*. Selective decoupling of H1 produced the spectrum depicted in Fig. 2A, showing that the signals of all three carbons were simplified and, hence, that they couple to H1. The same result was obtained from a proton coupled spectrum of the 1-deuterio analogue of *1* (Fig. 2C).

Similar decoupling experiments confirmed the assignments shown in Table *1*. In addition the 4-deuterated and the 6,6-dideuterated analogues of *1* were prepared and their proton coupled ^{13}C NMR spectra were studied.



C-1

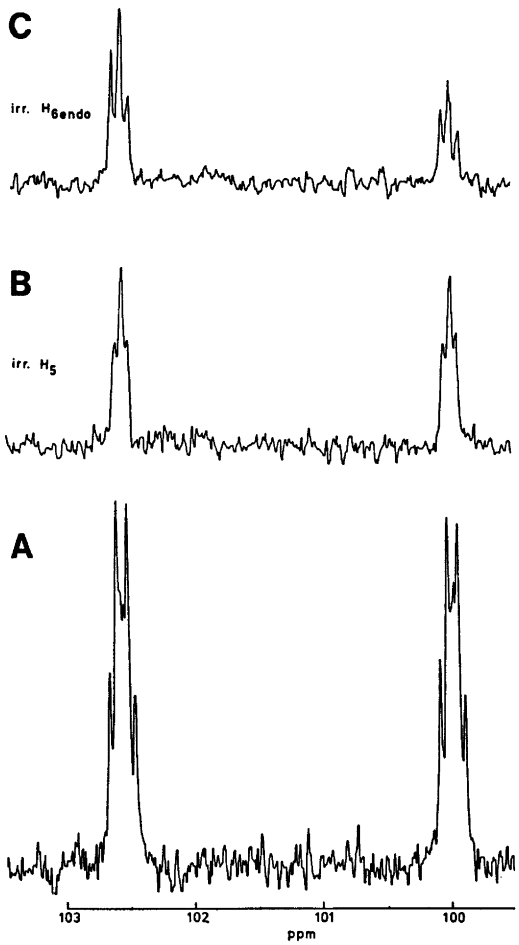


Fig. 1. The ^{13}C NMR signal of C1 of 1,6-anhydro-β-D-galactopyranose (A) Fully proton coupled signal; (B) and (C) selective decoupling of H5 and $H6_{endo}$, respectively.

It may be seen from Table *1* that ^{3}J(C−C−C−H) values of *ca.* 4−5 Hz are found in the cases where the dihedral angle is close to 180°. When the dihedral angle is close to 60° (or 120°) no coupling is observed. This is similar to previous results in carbohydrates [5,7,10,11] and in other compounds.[13] A somewhat larger ^{3}J(C1−H5) values (6.1 Hz) was found, possibly because an oxygen atom is part of the coupling path in this case.[5]

The three bond couplings observed in methyl α-D-galactopyranoside (*2*) and in 3-*O*-acetyl-1,2-5,6-di-*O*-isopropylidene-α-D-glucofuranose (*4*) seem to show the same dependence of the dihedral angle. In the glucofuranose derivative (*4*) J_{C1H3} was found to be 4.6 Hz, slightly lower than the values found for the pyranoses. In agreement herewith the C1−C2−C3−H3 angle of *4* was *ca.* 140°, measured from a model. In 3-*O*-acetyl-1,2-5,6-di-*O*-isopropylidene-α-D-allofuranose no coupling was observed between C1 and H3, corresponding to a dihedral angle of 100°, measured on a model of this compound.[5,7]

In addition to the three bond couplings discussed above a number of two bond couplings were also found (Table 1). Two bond $^{13}C-H$ couplings have been measured in some carbohydrates [7–11] and in a few cases their signs have been determined. For $^{2}J_{CH}$ values angular dependence is probably not important; but orientation of oxygen atoms around the coupling path will affect the coupling constant. Thus J_{C1H2} values varied from −6 to +5.5 Hz, depending on the orientation of the two oxygen atoms at C1 relative to the C2−H2 bond.[7,9] When the "resultant" of the C1−O1 and C1−O5 bonds is pointing away from the C2−H2 bond the coupling constant becomes more positive.[9]

We find that the rules of Perlin *et al.*[7–11] explain the two bond couplings of C3, C4, C5, and C6 in *1* and *2*. However, the J_{C1H2} and J_{C2H1} values are not in agreement with these rules. Thus C2 of methyl α-D-galactopyranoside (*2*) couples to H3 (−5.0 Hz), but not to H1 although O2 is *gauche* to both H3 and H1.[10] Similarly, C2 of methyl tri-*O*-acetyl-α-D-rhamnopyranoside (α-*3*) does not couple to H1 in spite of the fact that O2 is *gauche* to H1. The β-anomer (β-*3*), on the other hand, has a J_{C2H1} value of +9.0 Hz (the sign of this coupling constant was determined by selective proton decoupling).

The J_{C1H3} and J_{C2H1} values may be related to the orientation of the oxygen atoms, which are connected to the coupling path, if all three

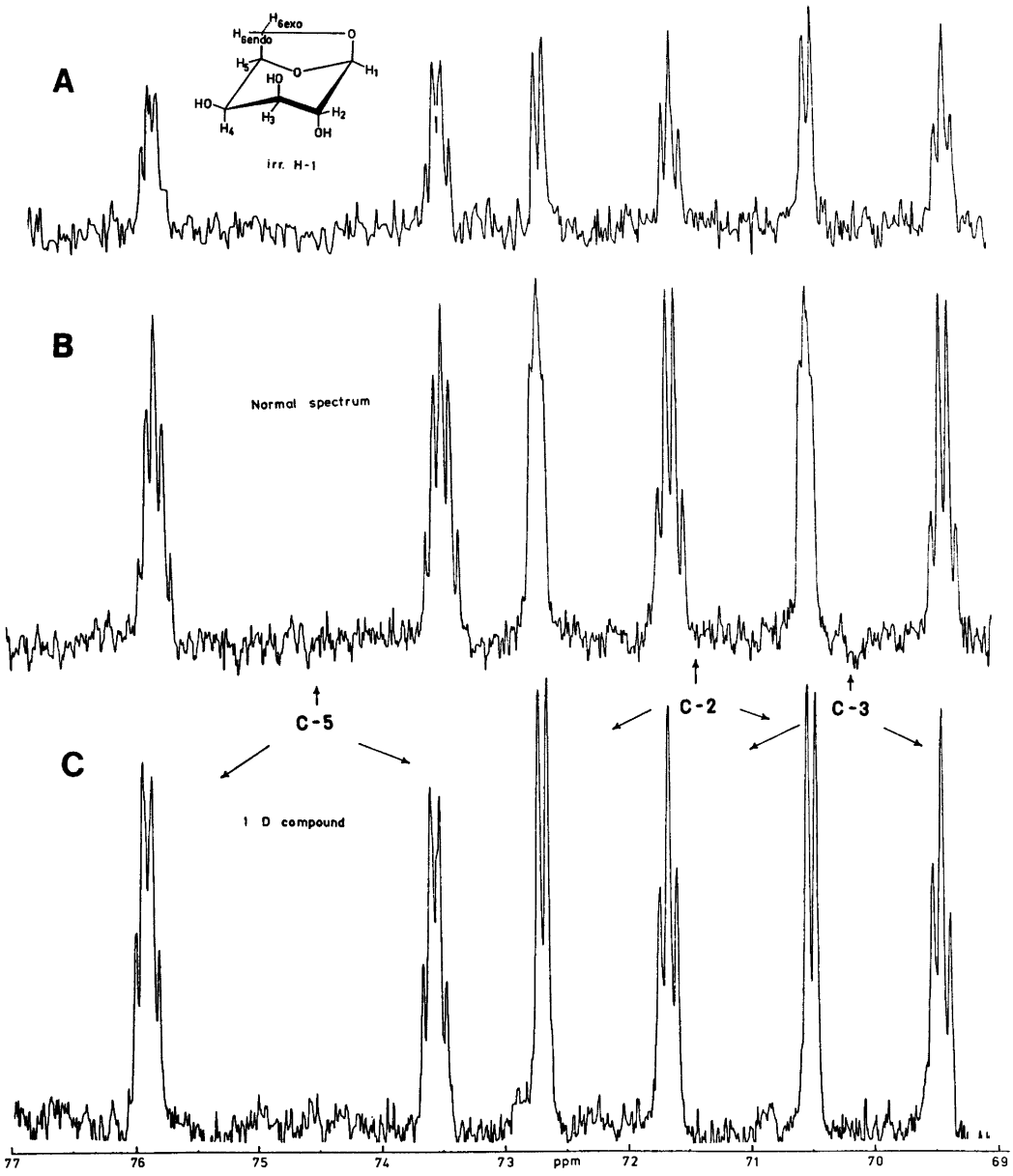


Fig. 2. The ^{13}C NMR signals of C2, C3, and C5 of 1,6-anhydro-β-D-galactopyranose. (B) Fully proton coupled spectrum; (A) Selective decoupling of H1; (C) The 1-deuterio analogue.

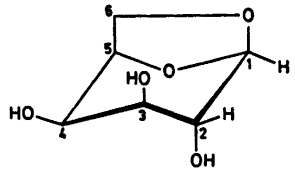
oxygens (O1, O5, and O2) are considered. In a Newman projection, viewed from ^{13}C to ^{12}C, the C–O bonds are projected on an axis *trans* to the ^{12}C–H bond and the sum of the three projections are then related to the coupling constant.

In *5* is shown a Newman projection of *β-3*, viewed from C2 to C1. If the projection of the C2–OAc bond on an axis *trans* to the C1–H1 bond is given the value +1, then the projections of C1–O1 and C1–O5 will each be +0.5 (cosine of 60°) and the sum will be +2. This

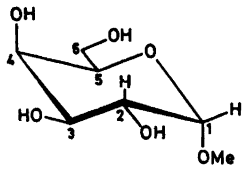
Table 1. $^{2}J(^{13}C-^{1}H)$ and $^{3}J(^{13}C-^{1}H)$ values of 1,6-anhydro-β-D-galactopyranose (*1*) in deuterium oxide solution. Couplings not shown are not observable.

Carbon	^{2}JCH values		^{3}JCH values and dihedral angles[d]		
C1			+3.7[a] H3 170°	+3.7[a,b] $H6_{endo}$ 120°	+6.1[a] H5 170°
C2	−3.2[a,b] H1	−4.1[a,b] H3			
C3	−4.6[a] H2		+4.6[a,b] H1 180°	+4.6[a] H5 180°	
C4	−3[c] H5	−3[c] H3	+4[c] H2 180°	+3[c] $H6_{exo}$ 170°	
C5	−4.5[b] H4	−4.5[c] $H6_{exo}$	+5[a,b] H1 180	+4.5[a] H3 180	
C6	−2.8 H5		+5.2[a,b] H1 160°	+5.2[a,b] H4 180°	

[a] By selective decoupling. [b] By deuteration. [c] From line width. [d] Measured from Dreiding models.

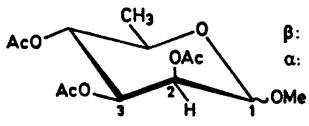


1



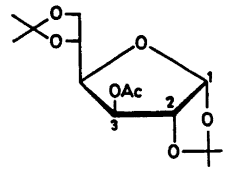
2

C2H1 - 0 C2H4 + 5.0
C2H3 - 5.0 C3H1 + 5.0
C3H2 - 5.0 C5H1 + 6.0
C3H4 - 5.0
C6H5 - 5.0



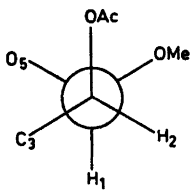
3

β: C2H1 + 9.0
α: C2H1 - 0

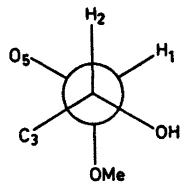


4

C1H2 + 5.5
C2H1 + 5.5
C3H2 - 4.0
C2H3 - 2.8
C1H3 + 4.6



5



6

corresponds to a $J_{C_3H_1}$ value of +9.0 Hz. A similar projection (*6*) of methyl α-D-galactopyranoside (*2*) gives a sum of +0.5 (+0.5 from C1 − O5 and C1 − OMe and −0.5 from C2 − O2), corresponding to a $J_{C_3H_1}$ value of *ca.* 0 Hz. The same values are found for α-*3*.

These projection sums and similar sums corresponding to the $J_{C_1H_2}$ data of Perlin *et al.*[9] are plotted against the coupling constants in Fig. 3. The plot indicates that a sum of +0.5 corresponds to a coupling of *ca.* 0 Hz; a sum of +1 should give a small positive coupling, in many cases probably too small to observe. A sum of +1.5 is found for $J_{C_3H_1}$ and $J_{C_1H_2}$ of the furanose *4*, corresponding to couplings of +5.5 Hz.

3-*O*-Acetyl-1,2-5,6-di-*O*-isopropylidene-α-D-allofuranose has the same sum and $J_{C_3H_1} = 5.1$ Hz and $J_{C_1H_2} = +4.5$ Hz.

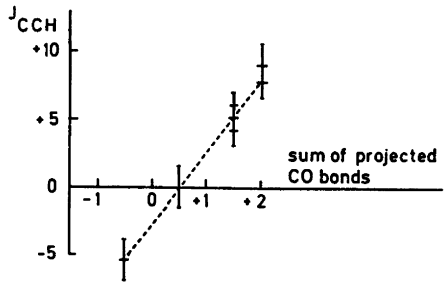


Fig. 3.

One point, representing $J_{C_2H_1} = -3.2$ Hz of *1*, is apparently falling outside in Fig. 3. The sum plotted for this coupling is +0.5 assuming a regular chair conformation for *1*. The conformation may, however, be distorted and this would result in a different sum.

The projection sum corresponding to $J_{C_2H_1}$ will be +0.5 for all α-pyranoses or pyranosides in the $^4C_1$ conformation, irrespective of the configuration at C2; a $J_{C_2H_1}$ value of *ca.* 0 Hz may therefore be expected. The same value is found for β-pyranoses ($^4C_1$ conformation) with the *R*-(D-)-configuration at C2. Those β-pyranoses which have the *S*-(L-)-configuration at C2 will have a projection sum of +2 and a large positive $J_{C_2H_1}$ value is found for *β-3* and also for β-D-mannopyranose ($J_{C_2H_1} = +8.0$ Hz).

The projection sum corresponding to $J_{C_1H_2}$ is +1 for α- and β-D-pyranoses and pyranosides ($^4C_1$) with C2 in the *S*-(L-)-configuration (e.g. D-mannose and D-rhamnose) and α-D-pyranoses with *R*-(D)-configuration at C2. Walter *et al.*[12] observed a small (~1.6 Hz) coupling in β-D-[1-^{13}C]-mannopyranose whereas C1 of the α-anomer only coupled to H1. No coupling was observed between C1 and H2 in α-D-glucopyranose.[9] In β-D-pyranoses with *R*-(D)-configuration at C2 the projection sum is −0.5 and in agreement herewith $J_{C_1H_2}$ values of *ca.* −5 Hz are found for β-D-gluco- and β-D-allopyranose.[9]

Similar sums were also calculated for the two bond C−H couplings involving only two oxygen atoms. In most of the compounds discussed above, the sum is zero, corresponding to a coupling constant of −3 to −5 Hz. In a few cases the sum is +1.5 which corresponds to a coupling of ~0 Hz. The data are insufficient to document a relation equivalent to that of Fig. 3; the rules of Perlin *et al.*[7–11] are, however, applicable when only two oxygen atoms are connected to the coupling path.

EXPERIMENTAL

Spectra were measured at 67.89 MHz on a Bruker HX-270S instrument. Compounds *1* and *2* were measured in deuterium oxide solution, *3* and *4* in deuteriochloroform. Spectra were measured with a spectral width of 6000 Hz using 32 K of computer memory for spectrum accumulation, corresponding to a digital resolution of 0.4 Hz. Gated decoupling was used for fully proton coupled spectra, decoupling time 3 s, pulse width 13 μs ($\alpha = 90°$). For selective proton decoupling a weak, continuous field was applied at the appropriate proton frequency. The latter frequency was found by measuring a ^{1}H spectrum of the same sample (10 mm sample tube) using the proton decoupler coil of the ^{13}C-probe head as transmitter-receiver coil

Preparation of the selectively deuterated derivatives of *1* will be described elsewhere.

The Bruker HX-270S instrument was provided by the Danish National Science Research Council.

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Dioxolanylium Ions Derived from Carbohydrates. III. Reaction of Arabinopyranoside Derivatives with Nucleophiles

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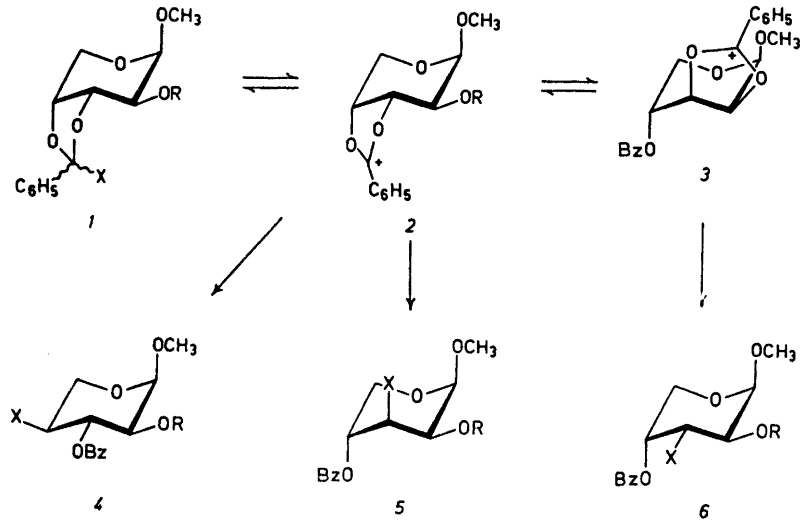
Treatment of methyl 2-*O*-benzoyl- (or 2-*O*-*p*-toluenesulfonyl-) 3,4-*O*-benzylidene-β-D-arabinopyranoside with triphenylmethyl fluoroborate gave 3,4-benzoxonium ions (*2a* or *2b*). The reaction of these benzoxonium ions with a number of nucleophiles has been studied. With chloride, bromide, iodide, thiocyanate, tosylate, and trifluoroacetate *trans* opening of the benzoxonium ion took place. With fluoride, acetate, azide, amides, cyanide, and methoxide orthoacid derivatives were formed; these on subsequent reaction with water underwent *cis* opening to give hydroxy benzoates.

In the preceding papers of this series [1,2] it was shown that benzylidene derivatives of carbohydrates in certain cases can be converted into benzoxonium ions by using triphenylmethyl fluoroborate as a hydride abstractor. The resulting benzoxonium ions reacted with water to give *cis* hydroxy benzoates; with bromide ion *trans* bromo deoxybenzoates were obtained. A *trans* opening of dioxolanylium ions with nucleophiles may provide a method for introduction of functional groups into carbohydrates. The reaction of benzoxonium ions with a variety of nucleophiles has therefore been investigated with pentopyranose derivatives as substrates.

An equilibrium mixture of the two benzoxonium ions *2a* and *3* in acetonitrile solution was prepared as described previously.[1] Treatment of this mixture with chloride or iodide ion gave a mixture of the 4-halo-L-xylose and 3-halo-D-arabinose derivatives (*4a* and *6a*, respectively). The ratio *4a*:*6a* was 6:1 in case of the chlorides and 1:2 with the iodides while bromide ion (previously reported [1]) gave the two products in approximately equal amounts, signifying a difference in reactivity of the two ions *2a* and *3* towards the different halide ions.

Treatment of the ions *2a* and *3* with fluoride ion did not give any fluoro deoxybenzoate. After hydrolysis of the reaction mixture the hydroxy benzoate *6a* (X = OH) was obtained. This could mean that the ion *2a*, (or *3*) did not react with fluoride. However, a ^{1}H NMR spectrum of the solution, measured shortly after addition of sodium fluoride, showed that the characteristic low field signals of the benzoxonium ion [1] had disappeared. The ^{1}H NMR spectrum gave no further information, but a ^{19}F NMR spectrum showed a signal at 53 ppm upfield from fluoroform which indicated that an orthoacid fluoride (*1a*, X = F) was formed.[3] This compound was stable for at least 24 h at room temperature, but decomposed when heated overnight at 70 °C. The hydroxy benzoate *6a* (X = OH) was probably formed by hydrolysis of *1a* (X = F).

Reaction of the ions *2a* and *3* with *p*-toluenesulfonate ion resulted in *trans* opening of the benzoxonium ion and formation of the L-xylose derivative *4a* (X = OTs) and the D-arabinose derivative *6a* (X = OTs) in approximately equal amounts. Treatment of the mixture of *2a* and *3* with cyanide or with methanol gave, on the other hand, only the orthoacid derivatives *1a* (X = CN) and *1a* ($X = OCH_3$), respectively. Two epimers may arise in each case; but in both cases one epimer was strongly favoured, probably the *endo* phenyl compound.[4] The orthoacid ester *1a* ($X = OCH_3$) was completely converted into *6a* (X = OH) on treatment with methanol and aqueous acid.



a: R = Bz
b: R = Ts

In order to simplify the reaction mixtures a number of experiments were carried out with methyl 3,4-*O*-benzylidene-2-*O*-*p*-toluenesulfonyl-β-D-arabinopyranoside (*1b*, X=H). On treatment with triphenylmethyl fluoroborate it gave the benzoxonium ion *2b*, which was characterized through its ^{1}H and ^{13}C NMR spectra. Since no neighbouring group effect is expected from the 2-*O*-*p*-toluenesulfonyl group only one benzoxonium ion is present in this case.

Reaction of *2b* with water gave exclusively the axial 4-*O*-benzoate *6b* (X=OH).[5] With bromide ion *2b* predominantly gave the 4-bromo-L-xylose derivative *4b* (X=Br) and only a small amount of the 3-bromo-D-lyxose derivative *5b* (X=Br), indicating a preference of *ca.* 10:1 for attack at C4 relative to C3. Treatment of the ion *2b* with thiocyanate ion for 5 h followed by hydrolysis gave the hydroxy benzoate *6b* (X=OH) only. When *2b* was allowed to react with thiocyanate for 5 days *trans* opening took place and the 4-thiocyanato-L-xylose and 3-thiocyanato-D-lyxose derivatives, *4b* and *5b* (X=−SCN), respectively, were isolated. The distinction between thiocyanate and isothiocyanate structures was based on IR spectroscopy.[6] Reaction of *2b* with trifluoroacetate ion also resulted in *trans* opening of the benzoxonium ion to give *4b* ($X=CF_3COO-$) which was directly hydrolysed to the *trans*-hydroxy benzoate *4b* (X=OH). The latter sequence provides an indirect method for *trans* opening of dioxolanylium ions with water.

In attempts to introduce a nitrogen function into the pyranose ring, the benzoxonium ion *2b* was treated with azide and phthalimide ion and with acetamide. In no case, however, did *trans* opening of the ion take place. Addition of azide ion to the acetonitrile solution of *2b* caused all ^{1}H NMR signals of the latter to disappear at once. The resulting product was stable for at least 5 days at room temperature and was converted to the hydroxy benzoate *6b* (X=OH) upon addition of water. The NMR spectrum of the primary product, formed from *2b* and azide ion was not well resolved, and there is therefore no direct evidence for an orthoacid type of compound (*1b*, $X=N_3$). However, the formation of *6b* (X=OH) on hydrolysis indicates that such a compound was indeed formed.

Treatment of *2b* with acetamide gave two unstable *N*-acetylated orthoamides (*1b*, $X=CH_3CONH-$) which were isolated and characterized by ^{1}H NMR spectroscopy. On hydrolysis they yielded *6b* (X=OH).

Reaction of *2b* with sodium fluoride gave an unstable orthoacid fluoride (*1b*, X = F). This was not hydrolysed, but instead treated with trimethyl phosphite to give two epimeric phosphonium derivatives [*1b*, X = $-P^+(OCH_3)_3$], isolated as the phosphonates *1b* [X = $-P(O)(OCH_3)_2$] after further treatment with bromide ion. ^{13}C NMR spectra of the latter products exhibited the large one-bond $^{31}P-^{13}C$ coupling in the low field signal of the "benzylic" carbon. The same two phosphonates were obtained by direct treatment of *2b* with trimethyl phosphite followed by reaction with bromide.

It is generally found that reaction of dioxolanylium ions with acetate ion leads to *trans* opening.[7] Such reactions have usually been carried out with anhydrous acetic acid as the solvent.[8,9] However, treatment of the benzoxonium ion *2b* with sodium acetate in acetonitrile solution did not result in *trans* opening, even after 5 days at room temperature. When the reaction was studied by ^{13}C NMR spectroscopy it was found that the low field signals of C3 and C4 (84 and 87 ppm, respectively) of the ion *2b* shifted to *ca.* 74 ppm immediately after acetate ion was added. This indicates that an orthoacid derivative (*1b*, X = OAc) is formed and in agreement herewith the hydroxy benzoate *6b* (X = OH) was obtained on hydrolysis. The reaction of other benzoxonium ions with acetate will be discussed in the following paper.

EXPERIMENTAL

Thin layer chromatography (TLC) was performed on Silica gel PF_{254} (Merck); for preparative work 1 mm layers were used on 20 × 40 cm plates. Compounds were visualised by UV light. Melting points are uncorrected. Optical rotations were measured in chloroform solution on a Perkin Elmer 141 instrument. ^1H NMR spectra were measured on Bruker HXE-90 and HX-270 instruments, ^{19}F and ^{31}P spectra on HXE-90, and ^{13}C NMR spectra on a Bruker WH-90 instrument as previously recorded. All spectra were measured in deuteriochloroform solutions unless otherwise specified. Chemical shifts of ^{31}P spectra are relative to phosphoric acid.

Methyl 3,4-O-benzylidene-2-O-p-toluenesulfonyl-β-D-arabinopyranoside (1b). Crude methyl 3,4-*O*-benzylidene-β-D-arabinopyranoside (from 4.92 g of methyl β-D-arabinopyranoside)[1] was treated overnight with *p*-toluenesulfonyl chloride (7.5 g) in pyridine (25 ml). Precipitation of the tosylate with water and recrystallization from ethanol gave 6.7 g (55 %) of *1b* as a mixture of diastereomers,[10] m.p. 103 – 122 °C.

Treatment of benzoxonium ions with nucleophiles. General procedure. The benzoxonium ions (*2* and *3*) were prepared by treatment of the benzylidene compound with triphenylmethyl fluoroborate in acetonitrile solution as described previously.[1] To this solution was added 3 – 5 molar equivalents of the appropriate nucleophile (dried over phosphorus pentoxide or conc. sulfuric acid) and the mixture was stirred at room temperature for the time specified under the individual nucleophiles. The reaction mixture was then stirred for 5 min with 10 ml of saturated, aqueous $NaHCO_3$ and extracted with chloroform. The chloroform solution was washed with water, dried and concentrated. Preparative TLC using ether-pentane (1:1) as the eluent (unless otherwise specified) gave triphenylmethane, moving with the solvent front, followed by triphenylcarbinol and the products.

Ions 2a *and* 3 *from methyl 2-O-benzoyl-3,4-O-benzylidene-β-D-arabinopyranoside* (1a, X = H). *Fluoride ion.* The benzylidene compound (*1a*, X = H) (747 mg) was converted to the benzoxonium ion and stirred with 209 mg of sodium fluoride for 2 h. Work-up as described above gave 517 mg (66 %) of methyl 2,4-di-*O*-benzoyl-β-D-arabinopyranoside (*6a*, X = OH), m.p. 140 – 142 °C, identical with the product described previously.[1]

Chloride ion. The benzoxonium ion from (*1a*, X = H) (593 mg) was stirred for 6 h with tetraethylammonium chloride (1.0 g). Work-up and chromatography gave three products. The fastest-moving fraction gave 412 mg (63 %) of methyl 2,3-di-*O*-benzoyl-4-chloro-4-deoxy-α-L-xylopyranoside (*4a*, X = Cl), m.p. 120 – 121 °C after recrystallization from cyclohexane, $[\alpha]_D^{20}$ −142° (*c* 1.6). Anal. $C_{20}H_{19}ClO_6$: C, H, Cl. ^1H NMR: δ 5.15 (H1), 5.12 (H2), 5.95 (H3), 4.16 (H4), 3.93 (H5 and H5′); J_{12} = 3.5 Hz, J_{23} = 10.0, J_{34} = 9.5, J_{45} = 7.5, $J_{45'}$ = 9.5.

The second fraction (71 mg, 11 %) consisted of methyl 2,4-di-*O*-benzoyl-3-chloro-3-deoxy-β-D-arabinopyranoside (*6a*, X = Cl) as a syrup, $[\alpha]_D^{20}$ −237° (*c* 2.2). Anal. $C_{20}H_{19}ClO_6$: C, H, Cl. ^1H NMR: δ 5.17 (H1), 5.57 (H2), 4.76 (H3), 5.57 (H4), 4.06 (H5), 3.98 (H5′); J_{12} = 3.4 Hz, J_{23} = 11.1, J_{34} = 3.3, J_{45} = 1.6, $J_{45'}$ = 1.9, $J_{55'}$ = 13.1.

A third fraction (43 mg) was impure methyl 2,4-di-*O*-benzoyl-3-chloro-3-deoxy-β-D-lyxopyranoside (*5a*, X = Cl), only characterized through its ^1H NMR spectrum: δ 4.88 (H1), 5.72 (H2), 4.51 (H3), 5.47 (H4), 4.51 (H5), 3.67 (H5′); J_{12} = 2.7 Hz, J_{23} = 4.0, J_{34} = 6.3, J_{45} = 3.3, $J_{45'}$ = 5.6, $J_{55'}$ = 12.5.

A small amount (32 mg, 4 %) of *6a*, X = OH was also isolated.

Iodide ion. The benzoxonium ion from *1a*, X = H (424 mg) was stirred with potassium

iodide for 5 h. Work-up and chromatography gave three products.

The fast-moving fraction (143 mg, 25 %) gave methyl 2,3-di-*O*-benzoyl-4-iodo-4-deoxy-α-L-xylopyranoside (*4a*, X = I), m.p. 140 – 141 °C after recrystallization from ethyl acetate-pentane, $[\alpha]_D^{21}$ −105° (*c* 1.0). Anal. $C_{20}H_{19}IO_6$: C, H, I. ^{1}H NMR: δ 5.22 (H1), 5.11 (H2), 6.01 (H3), 4.23 (H4), 4.13 (H5), 3.94 (H5′); J_{12} = 3.4 Hz, J_{23} = 9.7, J_{34} = 10.1, J_{45} = 12.0, $J_{45'}$ = 4.4, $J_{55'}$ = 10.5.

The second fraction (224 mg, 39 %) gave methyl 2,4-di-*O*-benzoyl-3-iodo-3-deoxy-β-D-arabinopyranoside (*6a*, X = I) as a syrup, $[\alpha]_D^{25}$ −289° (*c* 2.3). Anal. $C_{20}H_{19}IO_6$: C, H, I. ^{1}H NMR: δ 5.10 (H1), 5.59 (H2), 4.94 (H3), 5.52 (H4), 4.09 (H5), 3.90 (H5′); J_{12} = 3.3 Hz, J_{23} = 11.7, J_{34} = 3.2, J_{45} = 1.3, $J_{45'}$ = 1.7, $J_{55'}$ = 12.9.

The third fraction (26 mg, 5 %) was methyl 2,4-di-*O*-benzoyl-3-deoxy-3-iodo-β-D-lyxopyranoside (*5a*, X = I), m.p. 134 – 136 °C after recrystallization from ethyl acetate-pentane. Anal. $C_{20}H_{19}IO_6$: C, H. ^{1}H NMR: δ 4.92 (H1), 5.41 (H2), 4.64 (H3), 5.58 (H4), 4.57 (H5), 3.68 (H5′); J_{12} = 2.5 Hz, J_{23} = 4.0, J_{34} = 6.1, J_{45} = 3.4, $J_{45'}$ = 6.1, $J_{55'}$ = 12.5.

In addition 41 mg (9 %) of *6a*, X = OH was isolated.

p-*Toluenesulfonate ion*. Benzoxonium ion from 628 mg of *1a* (X = H) was stirred overnight with 1.1 g of tetraethylammonium *p*-toluenesulfonate. Work-up and chromatography gave 473 mg (51 %) of a mixture (45:55) of two products. Crystallization from chloroform (5 ml)-pentane (10 ml) yielded 191 mg (21 %) of methyl 2,3-di-*O*-benzoyl-4-*O*-*p*-toluenesulfonyl-α-L-xylopyranoside (*4a*, X = TsO), m.p. 172 – 173 °C, $[\alpha]_D^{21}$ −112° (*c* 1.8). Anal. $C_{27}H_{26}O_9S$: C, H, S. ^{1}H NMR: δ 5.10 (H1), 5.24 (H2), 6.04 (H3), 4.91 (H4), 4.05 (H5), 3.86 (H5′); J_{12} = 3.5 Hz, J_{23} = 9.9, J_{34} = 9.3, J_{45} = 6.4, $J_{45'}$ = 10.6, $J_{55'}$ = 11.0.

Preparative TLC of the mother liquor (ether-pentane 3:1) gave 55 mg of a mixture and 177 mg (19 %) of methyl 2,4-di-*O*-benzoyl-3-*O*-*p*-toluenesulfonyl-β-D-arabinopyranoside (*6a*, X = TsO), m.p. 127 – 130 °C. The product was identical with a sample prepared by tosylation of methyl 2,4-di-*O*-benzoyl-β-D-arabinopyranoside [1] with *p*-toluenesulfonyl chloride in pyridine, m.p. 131 – 132 °C, $[\alpha]_D^{21}$ −220° (*c* 1.0). Anal. $C_{27}H_{26}O_9S$: C, H, S. ^{1}H NMR in a 1:1 mixture of benzene-d_6 and acetone-d_6: δ 5.16 (H1), 5.57 (H2), 5.35 (H3), 5.50 (H4), 4.00 (H5), 3.90 (H5′); J_{12} = 3.3 Hz, J_{23} = 10.2, J_{34} = 3.4, J_{45} = 1.4, $J_{45'}$ = 2.0, $J_{55'}$ = 13.3.

Cyanide ion. Benzoxonium ion from 536 mg of *1a* (X = H) was stirred overnight with 1.0 g of finely powdered potassium cyanide. Chromatography yielded 317 mg (55 %) of methyl 2-*O*-benzoyl-3,4-*O*-cyanobenzylidene-β-D-arabinopyranoside (*1a*, X = CN). Crystallization from ether and recrystallization from ethyl acetate-pentane gave a product with m.p. 130 – 131 °C, $[\alpha]_D^{23}$ −194° (*c* 1.1). Anal. $C_{21}H_{19}NO_6$: C, H. N. IR: 2235 cm^{-1} (CN). ^{1}H NMR in benzene-d_6: δ 4.87 (H1), 5.36 (H2), 4.68 (H3), 4.29 (H4), 3.83 (H5), 3.48 (H5′); J_{12} = 3.4 Hz, J_{23} = 7.8, J_{34} = 6.3, J_{45} < 0.5, $J_{45'}$ = 3.0, $J_{55'}$ = 13.9.

Methanol. Benzoxonium ion from 520 mg of *1a* (X = H) was poured into 0.5 ml of methanol in pyridine (5 ml) and stirred for 5 min. The mixture was diluted with methylene chloride (25 ml) and worked up as described above. Chromatography gave two products. Both the fast-moving (45 mg, 8 %) and the slow-moving fraction (324 mg, 58 %) gave ^{1}H NMR spectra which corresponded to an orthoester. ^{1}H NMR (fast moving fraction): δ 5.04 (H1), 5.65 (H2), 4.67 (H3), 4.23 (H4), 4.11 (H5), 3.92 (H5′); J_{12} = 3.4 Hz, J_{23} = 7.7, J_{34} = 6.3, J_{45} < 0.5, $J_{45'}$ = 2.8, $J_{55'}$ = 13.5. Slow-moving in benzene-d_6: δ 4.90 (H1), 5.29 (H2), 4.78 (H3), 4.48 (H4), 3.95 (H5), 3.68 (H5′); J_{12} = 3.4 Hz, J_{23} = 7.4, J_{34} = 6.1, J_{45} < 0.5, $J_{45'}$ = 2.9, $J_{55'}$ = 13.6.

The mixed fractions were heated to 50 °C for 5 min with methanol (2 ml) containing 1 drop of 0.1 N hydrochloric acid. This caused a quantitative conversion to *6a* (X = OH), m.p. 147 – 149 °C.

The ion 2b *from 3,4-O-benzylidene-2-O-p-toluenesulfonyl-β-D-arabinopyranoside* (1b) was prepared as described above in acetonitrile solution and reacted with a number of nucleophiles. NMR spectra were measured on a solution of *2b* in acetonitrile-d_3. ^{1}H NMR: δ 4.92 (H1), 4.89 (H2), 6.05 (H3), 5.97 (H4), 4.51 (H5), 4.20 (H5′); J_{12} = 4.0 Hz, J_{23} = 6.5, J_{34} = 7.8, $J_{45} \simeq 0$, $J_{45'}$ = 2.8, $J_{55'}$ = 15.5. ^{13}C NMR: 95.6 ppm (C1), 74.1 (C2), 84.4 (C3), 87.5 (C4), 55.1 (C5), 182.4 (—C+).

Reaction with water. The benzoxonium ion *2b*, prepared from 633 mg of *1b* (X = H) as described above, was stirred for 5 min with aqueous $NaHCO_3$. Work-up as described above and crystallization from ether-pentane gave 454 mg (69 %) of methyl 4-*O*-benzoyl-2-*O*-*p*-toluenesulfonyl-β-D-arabinopyranoside (*6b*, X = OH), m.p. 110 – 113 °C. Two recrystallizations from ethanol gave the pure product, m.p. 114 – 115 °C, $[\alpha]_D^{25}$ −204° (*c* 1.8). Anal. $C_{20}H_{22}O_8S$: C, H, S. ^{1}H NMR: δ 4.93 (H1), 4.79 (H2), 4.29 (H3), 5.43 (H4), 3.91 (H5), 3.82 (H5′); J_{12} = 3.5 Hz, J_{23} = 9.8, J_{34} = 3.7, J_{45} = 1.5, $J_{45'}$ = 1.7, $J_{55'}$ = 13.4.

Bromide ion. Benzoxonium ion *2b* from 562 mg of *1b* (X = H) was stirred with tetraethylammonium bromide for 4 h. The crude product was crystallized from chloroform to give 376 mg (56 %) of methyl 3-*O*-benzoyl-4-bromo-4-deoxy-2-*O*-*p*-toluenesulfonyl-α-L-xylopyranoside (*4b*, X = Br), m.p. 161 – 163 °C. Three recrystallizations from ethyl acetate-pentane gave a product with m.p. 164 – 165 °C, $[\alpha]_D^{25}$ −62° (*c* 2.4). Anal. $C_{20}H_{21}BrO_7S$: C, H, Br, S. ^{1}H NMR: δ 5.04 (H1), 4.51 (H2), 5.73

(H3), 4.0–3.8 (H4,H5,H5′); $J_{12}=3.6$ Hz, $J_{23}=9.7$, $J_{34}\simeq 10$.

Chromatography of the material in the mother liquors gave an additional 32 mg of *4b* (X=Br), 62 mg (11 %) of *6b* (X=OH), and 31 mg (5 %) of methyl 4-*O*-benzoyl-3-bromo-3-deoxy-2-*O*-*p*-toluenesulfonyl-β-D-lyxopyranoside (*5b*, X=Br). This product was only identified through its ^{1}H NMR spectrum: δ 4.77 (H1), 5.01 (H2), 4.16 (H3), 5.32 (H4), 4.43 (H5), 3.66 (H5′); $J_{12}=3.4$ Hz, $J_{23}=3.9$, $J_{34}=4.6$, $J_{45}=2.2$, $J_{45'}=3.5$, $J_{55'}=13.0$, $J_{35'}=1.2$.

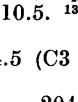
Thiocyanate ion. Ion *2b* from 570 mg of *1b* (X=H) was stirred for 5 days with potassium thiocyanate (1.1 g). The crude product was crystallized from chloroform to give 212 mg (33 %) of methyl 3-*O*-benzoyl-4-deoxy-4-thiocyanato-2-*O*-*p*-toluenesulfonyl-α-L-xylopyranoside (*4b*, X=−SCN), m.p. 216–217 °C. Recrystallization from ethyl acetate-pentane gave a product with m.p. 217–218 °C, $[\alpha]_D^{25}$ −46° (*c* 1.4). Anal. $C_{21}H_{21}NO_7S_2$. C, H, N, S. IR: 2155 cm^{-1} (−SCN). ^{1}H NMR: δ 4.99 (H1), 4.62 (H2), 5.64 (H3), 3.38 (H4), 3.97 (H5 and H5′); $J_{12}=3.6$ Hz, $J_{23}=9.7$, $J_{34}=10.6$, $J_{45}=8.1$, $J_{45'}=9.2$, $J_{55'}\simeq 12$.

Chromatography of the material in the mother liquor gave three products. The fast-moving fraction (115 mg, 18 %) was methyl 4-*O*-benzoyl-3-deoxy-3-thiocyanato-2-*O*-*p*-toluenesulfonyl-β-D-lyxopyranoside (*5b*, X=−SCN), m.p. 142–143 °C. Recrystallization from ethyl acetate-pentane gave a product with m.p. 143–144 °C, $[\alpha]_D^{25}$ −188° (*c* 1.4). Anal. $C_{21}H_{21}NO_7S_2$: C, H, N, S. IR: 2155 cm^{-1} (−SCN). ^{1}H NMR: δ 4.81 (H1), 5.04 (H2), 3.61 (H3), 5.29 (H4), 4.23 (H5), 3.80 (H5′); $J_{12}=3.0$ Hz, $J_{23}=5.0$, $J_{34}=4.3$, $J_{45}=1.5$, $J_{45'}=1.7$, $J_{55'}=13.5$, $J_{35'}=1.7$.

The next fraction was impure *4b* (X=−SCN) (23 mg, 4 %), m.p. 185–210 °C, and the third fraction gave 118 mg (20 %) of the hydrolysis product *4b* (X=OH).

Trifluoroacetate ion. The ion *2b* from 485 mg of *1b* (X=H) was stirred overnight with sodium trifluoroacetate (1.0 g). Water (10 drops) was then added and after 2 days at room temperature the mixture deposited 285 mg of crystals, m.p. 200–203 °C. The mother liquor was then worked up as described above. Crystallization from ethyl acetate-pentane gave an additional 95 mg of compound with m.p. 204–206 °C. The combined products were recrystallized from ethyl acetate-pentane to give 286 mg (57 %) of methyl 3-*O*-benzoyl-2-*O*-*p*-toluenesulfonyl-α-L-xylopyranoside (*4b*, X=OH), m.p. 213–214 °C, $[\alpha]_D^{25}$ −136° (*c* 1.3). Anal. $C_{20}H_{22}O_8S$: C, H, S. ^{1}H NMR in acetone-d_6: δ 4.94 (H1), 4.61 (H2), 5.59 (H3), ~4.0 (H4), 3.76 (H5), 3.63 (H5′); $J_{12}=3.7$ Hz, $J_{23}=10.0$, $J_{34}=8.7$, $J_{45}=5.5$, $J_{45'}=10.8$, $J_{55'}=10.9$.

Trimethyl phosphite. Benzoxonium ion *2b* from 570 mg of *1b* (X=H) was treated with 0.5 ml of trimethyl phosphite for 5 min. Tetraethylammonium bromide (1.0 g) was then added and the mixture was stirred overnight. Work-up followed by preparative TLC (ethyl acetate-chloroform 1:1) yielded two diastereomeric methyl 3,4-*O*-(dimethylphosphono-benzylidene)-2-*O*-*p*-toluenesulfonyl-β-D-arabinopyranosides [*1b*, X=−P(O)(OCH$_3$)$_2$]. The fast moving product (197 mg, 27 %) was a syrup, $[\alpha]_D^{20}$ −74° (*c* 1.4). Anal. $C_{22}H_{27}O_{10}PS$: C, H, P. ^{1}H NMR: δ 4.62 (H1), 4.06 (H2), 4.63 (H3), 4.94 (H4), 4.12 (H5), 3.93 (H5′); $J_{12}=3.6$ Hz, $J_{23}=7.8$, $J_{34}=5.6$, $J_{45}<0.5$, $J_{45'}=1.8$, $J_{55'}=13.8$, $J_{PH_3}=1.3$, $J_{POCH_3}=10.2$, ^{13}C NMR: δ 97.0 (C1), 77.8 (C2), 73.6 (C3), 76.7 (C4), 57.7 (C5), 106.8 ( C-P); $J_{CP}=206$ Hz, $J_{P(OCH_3)_2}=7$. ^{31}P NMR: −14.7 ppm.

The slow moving product (218 mg, 30 %) slowly crystallized. It was recrystallized from ethyl acetate-pentane, m.p. 143–144 °C, $[\alpha]_D^{20}$ −101° (*c* 1.9). Anal. C, H, P. ^{1}H NMR: δ 4.82 (H1), 5.11 (H2), 4.47 (H3), 3.93 (H4), 4.11 (H5), 3.84 (H5′); $J_{12}=3.6$ Hz, $J_{23}=7.5$, $J_{34}=6.0$, $J_{45}<0.5$, $J_{45'}=2.8$, $J_{55'}=13.4$, $J_{PH_3}\simeq 0.5$, $J_{POCH_3}=10.5$. ^{13}C NMR: 97.5 ppm (C1), 78.6 (C2), 74.5 (C3 and C4), 57.4 (C5), 106.7 ( C−P); $J_{CP}=204$ Hz, $J_{P(OCH_3)_2}=7.5$. ^{31}P NMR: −15.7 ppm.

Fluoride ion. Benzoxonium ion *2b* from 531 mg of *1b* (X=H) was stirred for 7 h with sodium fluoride (200 mg). Treatment with trimethyl phosphite and tetraethylammonium bromide and work-up as described above gave 103 mg (15 %) of the fast moving fraction and 177 mg (26 %) of the slow-moving fraction. NMR spectra proved the identity with the products described above.

Azide ion. Benzoxonium ion 2b from 514 mg of *1b* (X=H) was stirred for 5 days with sodium azide (1.0 g). Hydrolysis and crystallization gave 351 mg (66 %) of *6b* (X=OH), m.p. 113–114 °C. The product was identical (^{1}H NMR, mixed m.p.) with that described above.

Phthalimide. Benzoxonium ion *2b* from 555 mg of *1b* (X=H) was stirred for 24 h with potassium phthalimide (1.0 g). Hydrolysis and crystallization gave 227 mg (39 %) of *6b* (X=OH), m.p. 112–114 °C.

Acetamide. To the ion *2b*, from 562 mg of *1b* (X=H), was added acetamide (500 mg) and the solution was kept overnight. Hydrolysis followed by preparative TLC (ether-pentane 4:1) gave four fractions. The fast-moving fraction gave 174 mg (30 %) of *6b* (X=OH), m.p. 113–116 °C. The next fraction, 103 mg, m.p. 190–195 °C, was probably *N*-tritylacetamide as seen from its ^{1}H NMR spectrum. The third fraction (73 mg, 11 %) was one of the diastereomeric *N*-acetylated orthoacid amides *1b* (X=CH$_3$CONH−). ^{1}H NMR (270 MHz): δ 4.71 (H1), 5.09 (H2), 4.40 (H3), 4.15 (H4), 4.09

(H5), 3.82 (H5′), 1.91 (H_3CCO), 7.04 (*NH*); $J_{12}=3.3$ Hz, $J_{23}=6.9$, $J_{34}=5.6$, $J_{45}\simeq 0$, $J_{45'}=2.7$, $J_{55'}=13.5$.

The last fraction (158 mg, 25 %) was the other isomer of *1b* (X = CH_3CONH-). ^1H NMR (270 MHz): δ 4.72 (H1), 4.82 (H2), 4.54 (H3), 4.12 (H4), 4.13 (H5), 3.91 (H5′), 1.89 (H_3CCO), 6.48 (*NH*); $J_{12}=2.9$ Hz, $J_{23}=6.8$, $J_{34}=6.0$, $J_{45}\simeq 0$, $J_{45'}\simeq 2$, $J_{55'}=13.6$,

When kept in deuteriochloroform solution for 1 – 2 days both products equilibrated to the same 1:1 mixture of diastereomers. Each of the two products was boiled for 5 min with methanol (20 ml) containing 1 drop of 0.1 N HCl. The solutions were then evaporated and the residues were crystallized from ether to give two products with m.p. 108 – 114 and 110 – 114 °C, respectively. The combined products were recrystallized from ethanol-water to give *6b* (X = OH), m.p. 114 – 115 °C, undepressed in admixture with the sample described above.

Acetate ion. Benzoxonium ion *2b* from 487 mg of *1b* (X = H) was stirred with anhydrous sodium acetate for 5 days. Work-up and chromatography gave, besides triphenylmethane and triphenylcarbinol, only one fraction (380 mg). ^1H NMR spectra showed that this fraction consisted of *6b* (X = OH) mixed with two minor components which were not identified. They contained no acetoxy groups.

In a separate experiment the ion *2b* was prepared in acetonitrile-d_3 solution and anhydrous sodium acetate was added. The solution thus obtained probably contained *1b* (X = OAc). ^{13}C NMR: 96.3 ppm (C1), 78.6 (C2), 74.6 (C3), 73.8 (C4), 56.6 (C5).

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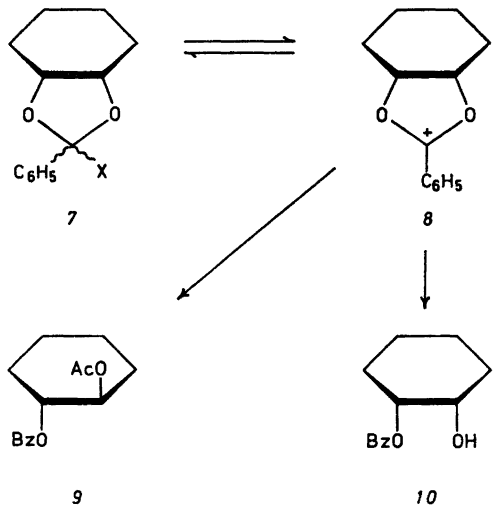
Dioxolanylium Ions Derived from Carbohydrates. IV. Reaction of Ribofuranose Derivatives with Nucleophiles

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Reaction of methyl 5-*O*-benzoyl- (or 5-*O*-methoxybenzoyl-) 2,3-*O*-benzylidene-β-D-ribofuranoside (*1*) with triphenylmethyl fluoroborate gave a 2,3-benzoxonium ion (*2*) which was in equilibrium with a 3,5-ion (*3*), derived from D-xylofuranose. Treatment of this ion with *p*-toluenesulfonate led to *trans* opening with formation of *p*-toluenesulfonylated D-xylofuranose derivatives. Reaction of *2* with acetate or azide ion gave orthoacid derivatives (*4*) which on subsequent hydrolysis underwent *cis* opening.

In a preceding paper [1] the preparation of benzoxonium ions derived from D-arabinopyranose and their reactivity towards a series of nucleophiles were described. In the present paper this investigation is extended to benzoxonium ions derived from D-ribofuranose.



Treatment of methyl 5-*O*-benzoyl-2,3-*O*-benzylidene-β-D-ribofuranose (*1a*) with triphenylmethyl fluoroborate in acetonitrile yields the benzoxonium ion *2a*. This ion has previously been shown to undergo *cis* opening with water.[3] With bromide ion it gives a 3-bromo-3-deoxy-D-xylofuranose derivative with *trans* opening.[2]

When *2a* was treated with anhydrous sodium acetate a reaction took place at once as seen from the ^{13}C NMR spectrum (a large upfield shift of the signals of C2 and C3). On hydrolysis of the reaction mixture only methyl 2,5- and 3,5-di-*O*-benzoyl-β-D-ribofuranosides were isolated. This indicates that *2a* reacts with acetate to give the orthoacid acetate *4a* (X = OAc) which on hydrolysis undergoes *cis* opening. A similar result was obtained when *2a* was treated with azide ions.

Treatment of the ion *2a* with *p*-toluenesulfonate ion led to *trans* opening and formation of the 3-*O*-tosyl derivative *5*. However, in addition to this product the 5-*O*-tosylate (*6*) was obtained. The latter product cannot arise from the ion *2a*, but must be formed by attack at C5 of the dioxanylium ion *3a*. The amount of dioxanylium ion *3a*, present in equilibrium with *2a*, is small because it could not be observed in the ^1H and ^{13}C NMR spectra of *2a*. Obviously, low concentration of *3a* does not exclude formation of substantial amounts of *6* because the reactivity of *3a* may be higher than that of *2a*.

It has been observed previously that benzoxonium ions are stabilized by a *p*-methoxy-group.[2,4] In an attempt to observe the ion *3* methyl 2,3-*O*-benzylidene-5-*O*-*p*-methoxybenzoyl-β-D-ribofuranoside (*1b*) was treated with trityl fluoroborate in acetonitrile. This gave a

mixture of the cyclic ions *2b* and *3b* containing 16 % of the latter ion as estimated from ^1H and ^{13}C NMR spectra. Hydrolysis of this mixture, followed by deacylation, yielded methyl β-D-xylofuranoside (18 %) and methyl-β-D-ribofuranoside (82 %).

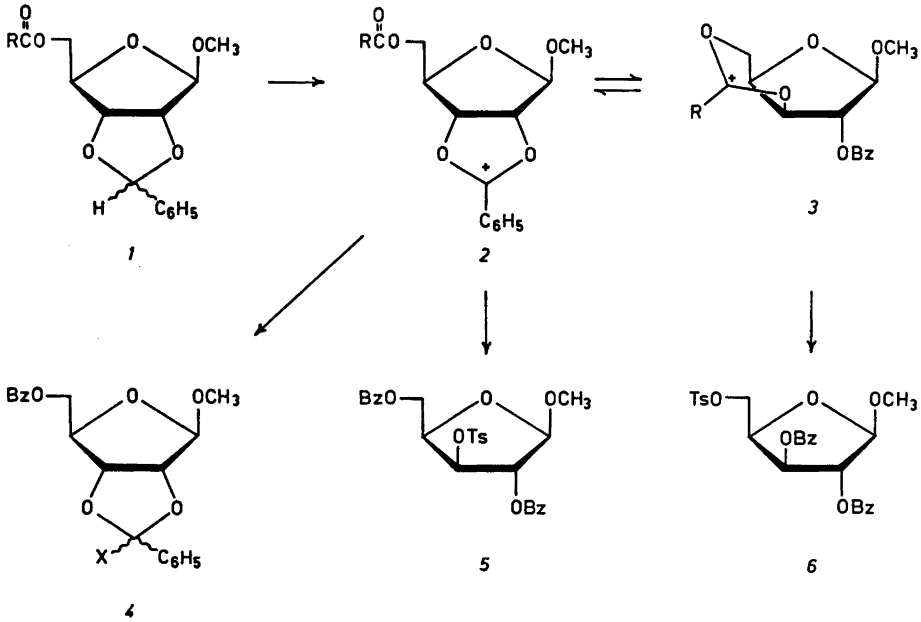
The fact that *2a* and the benzoxonium ion discussed in the preceding paper,[1] do not undergo *trans* opening with acetate ion is somewhat surprising in view of the work of Winstein *et al.* on the acetoxonium ion derived from *cis*-1,2-cyclohexanediol.[5] However, Winstein *et al.* used acetic acid as a solvent at *ca.* 75 °C whereas acetonitrile was used at room temperature in the present work. Carbohydrate benzoxonium ions are not stable at elevated temperatures and it is therefore not possible to compare their reactivity with the results obtained by Winstein *et al.*

It was, however, of interest to see how the benzoxonium ion *8* behaved when treated with acetate ion in acetonitrile. A solution of the ion *8* in acetonitrile was therefore prepared by treatment of 1,2-*O*-benzylidene-*cis*-cyclohexanediol (*7*, X=H) with triphenylmethyl fluoroborate. In a series of experiments *8* was treated with sodium acetate and hydrolysed after varying periods of time. This showed that at room temperature more than 1 month is required for complete *trans* opening of *8* to give *9*. After a short reaction time the *cis* monobenzoate *10* was the main product. When the reaction was followed by ^{13}C NMR spectroscopy it was found that *8* was immediately converted into an intermediate compound on treatment with acetate; the latter compound subsequently rearranged to the *trans* acetate benzoate *9*. Since the intermediate product gave the *cis* monobenzoate *10* on hydrolysis it is probably an orthoacid acetate (*7*, X=OAc).

EXPERIMENTAL

For details about thin layer chromatography, optical rotations, and NMR spectra see the preceding paper.

Preparation of benzoxonium ions. The ions were prepared in acetonitrile solutions as described previously.[2,3] To the solution of the ion were then added 3–5 molar equivalents of the appropriate nucleophile (dried over phosphorus pentoxide or conc. sulfuric acid) and the mixture was stirred at room temperature for the time specified. The mixture was then stirred for 5 min with aqueous $NaHCO_3$ and extracted



a: R = C_6H_5

b: R = *p*-$CH_3OC_6H_4$

with chloroform. The chloroform solution was washed with water, dried and concentrated. Preparative TLC (ether-pentane (1:1) unless otherwise specified) gave triphenylmethane, moving with the solvent front, followed by triphenylcarbinol and the products.

Benzoxonium ion 2a *from methyl 5-O-benzoyl-2,3-O-benzylidene-β-D-ribofuranoside (*1a*).* A solution of this ion (*2a*) was prepared in acetonitrile-d_3. ^{13}C NMR: 106.1 ppm (C1), 93.9 and 93.2 (C2 and C3), 82.0 (C4), 63.2 (C5), 179.3 (>C$^+$−).

Reaction with acetate ion. Benzoxonium ion *2a* from *1a* (483 mg) was stirred with anhydrous sodium acetate (1.0 g) for 24 h. Hydrolysis and chromatography as described above gave 41 mg of unchanged *1a* and 311 mg of a mixture of methyl 2,5- and 3-5-di-*O*-benzoyl-β-D-ribofuranoside.[3] The products were identified by ^{1}H NMR spectroscopy.

In another experiment the ion *2a* was prepared in acetonitrile-d_3 and treated with anhydrous sodium acetate to give a solution of the orthoacid acetate *4a* (X = OAc). ^{13}C NMR: 108.2 ppm (C1), 86.4, 83.3, and 83.3 (C2, C3, and C4), 64.4 (C5).

Reaction with p-*toluenesulfonate ion.* Benzoxonium ion *2a* from 614 mg of *1a* was stirred with tetraethylammonium tosylate (1.0 g) for 3 days. Hydrolysis and chromatography gave, besides unchanged *1a* (50 mg) and methyl 2,5- and 3,5-di-*O*-benzoyl-β-D-ribofuranoside, 323 mg (36 %) of a mixture of two tosylates in about equal amounts. Re-chromatography (using chloroform as an eluent) gave two products.

The fast-moving fraction gave 132 mg of methyl 2,3-di-*O*-benzoyl-5-*O*-*p*-toluenesulfonyl-β-D-xylofuranoside (*6*), m.p. 86 – 88 °C, identical with an authentic specimen described below. The slow-moving fraction, 72 mg, m.p. 118 – 128 °C, consisted of methyl 2,5-di-*O*-benzoyl-3-*O*-*p*-toluenesulfonyl-β-D-xylofuranoside (*5*). Recrystallization from ethyl acetate-pentane and from ethanol-water gave a product with m.p. 127 – 129 °C, identical with an authentic specimen (see below), $[\alpha]_D^{25}$ +16° (*c* 1.1). Anal. $C_{27}H_{26}O_9S$: C, H, S. ^{1}H NMR : δ 5.01 (H1), 5.38 (H2), 5.28 (H3), 4.84 (H4), 4.62 (H5), 4.61 (H5′); J_{12} = 0.8 Hz, J_{23} = 2.2, J_{34} = 5.7, J_{45} = 5.2, $J_{45'}$ = 6.7.

Reaction of methyl β-D-xylofuranoside [6] (526 mg) with *p*-toluenesulfonyl chloride (721 mg) in pyridine (10 ml) for 8 h at 0 °C and 16 h at 25 °C gave 469 mg (43 %) of crude methyl 5-*O*-*p*-toluenesulfonyl-β-D-xylofuranoside. This was benzoylated with benzoyl chloride in pyridine to give 704 mg of a syrupy product. Preparative TLC (chloroform) gave 527 mg (68 %) of *6* as a syrup which crystallized on standing, m.p. 86 – 87 °C, $[\alpha]_D^{25}$ +35° (*c* 2.4). Anal. $C_{27}H_{26}O_9S$: C, H, S. ^{1}H NMR: δ 5.08 (H1), 5.43 (H2), 5.72 (H3), 4.84 (H4), 4.38 (H5), 4.33 (H5′); J_{12} < 0.5 Hz, J_{23} = 1.4, J_{34} = 5.8, J_{45} = 6.3, $J_{45'}$ = 6.5. $J_{55'}$ = 10.2.

Benzoylation of methyl 2-*O*-benzoyl-β-D-xylofuranoside [7] (468 mg) with benzoyl chloride (0.13 ml) in pyridine (10 ml) for 2 days at 0 °C gave a syrup. Preparative TLC (ether-pentane 3:1) yielded 234 mg (40 %) of methyl 2,5-di-*O*-benzoyl-β-D-xylofuranoside and 62 mg of recovered 2-*O*-benzoate. Reaction of the 2,5-dibenzoate with *p*-toluenesulfonyl chloride (500 mg) in pyridine (5 ml) for 3 days at room temperature and work-up in the usual way yielded 317 mg of *5*, m.p. 125 – 127 °C after recrystallization from ethanol-water.

*Methyl 2,3-O-benzylidene-5-O-*p*-methoxybenzoyl-β-D-ribofuranoside (*1b*).* Crude methyl 2,3-*O*-benzylidene-β-D-ribofuranoside [2] (from 1.64 g of methyl β-D-ribofuranoside) was treated with *p*-methoxybenzoyl chloride (2.0 g) in pyridine (10 ml). Work-up in the usual way gave 3.2 g of crude *1b*, m.p. 69 – 75 °C. Recrystallization from ethyl acetate-pentane gave 1.8 g (47 %) of a mixture of diastereomers, m.p. 72 – 75 °C. Anal. $C_{21}H_{22}O_7$: C, H.

*Benzoxonium ion from methyl 2,3-O-benzylidene-5-O-*p*-methoxybenzoyl-β-D-ribofuranoside (*1b*).* A solution containing the ions *2b* and *3b* was prepared in acetonitrile-d_3 as described above and spectral data were obtained from this solution. ^{1}H NMR of *2b*: δ 5.62 (H1), 6.27 and 6.64 (H2 and H3), 5.23 (H4), 4.63 (H5), 4.52 (H5′), 3.46 (glycosidic OCH_3), 3.87 (arom. OCH_3); $J_{12} \simeq 0$ Hz, J_{23} = 6.8, $J_{34} \simeq 0$, J_{45} = 6.3, $J_{45'}$ = 6.8, $J_{55'}$ = 11.8 ^{1}H NMR of *3b* (only the two methoxy-signals could be identified): 3.14 (glycosidic OCH_3), 4.01 (arom. OCH_3). ^{13}C NMR of *2b*: 106.1 ppm (H1), 93.9 and 93.3 (C2 and C3), 82.2 (C4), 62.9 (C5), 182.2 (>C$^+$). ^{13}C NMR of *3b*: 105.8 (C1), 70.5 (C2), 77.7 (C3), 83.2 (C4), 72.1 (C5).

Reaction with water. A mixture of the ions *2b* and *3b*, prepared from *1b* (530 mg), in acetonitrile solution was stirred for 5 min with aqueous $NaHCO_3$. Work-up as described above gave a crude mixture which was deacylated with sodium methoxide in methanol. The solution was neutralized with CO_2 and evaporated to dryness. The residue was suspended in water and extracted with chloroform. The aqueous solution was evaporated to dryness and dissolved in deuterium oxide and the ^{13}C NMR spectrum was recorded. Except for a small signal from carbonate the spectrum showed only the signals of methyl β-D-ribofuranoside (82 %) and methyl β-D-xylofuranoside (18 %).[8] The relative amounts were estimated from the ratio of peak intensities of carbons 1, 2, 3, and 5.

*Reaction of 2-phenyl-4,5-tetramethylenedioxolanylium ion with acetate. cis-*1,2-*O*-Benzylidenecyclohexane [9] (500 mg) was treated with a 10 – 20 % excess of triphenylmethyl fluoroborate for 15 min at room temperature in acetonitrile (10 ml). Anhydrous sodium acetate (1.0 g) was then added and the mixture was stirred at room temperature for the time specified below. The mixture was then stirred for 5 min

with aqueous $NaHCO_3$ and extracted with chloroform. The chloroform solution was dried and concentrated. Preparative TLC (ether-pentane 1:2) gave triphenylmethane (with the solvent front), triphenylcarbinol, *trans*-2-acetoxycyclohexanol benzoate (*9*), and *cis*-2-benzoyloxycyclohexanol (*10*).[10] Three experiments were carried out:

1. 5 h, *9*: 5 – 10 %, *10*: 77 %
2. 5 days, 9: 27 %, 10: 50 %
3. 25 days, 9: 73 %, 10: 16 %

The syrupy *9* was characterized through its NMR spectrum. Besides it was deacylated with sodium methoxide in methanol to give *trans*-1,2-cyclohexanediol, m.p. 102 – 103°C, undepressed in admixture with an authentic sample.[9,10]

In a separate experiment the benzoxonium ion *8* was generated in acetonitrile-d_3 solution. ^{13}C NMR: 88.3 ppm (C1, C2), 23.8 (C3, C6), 16.1 (C4, C5), 181.4 ($\rangle C^+$). A ^{13}C NMR spectrum measured shortly after addition of anhydrous sodium acetate gave the following values: 75.1 (C1, C2), 25.3 (C3, C6), 19.1 (C4, C5).

Microanalyses were performed by Novo Microanalytical Laboratory. The pulsed Fourier spectrometers were provided by the Danish National Science Research Council.

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Voltammetry in Benzene and Chlorobenzene. The Behaviour of Ions of Aromatic Compounds in Nonpolar Media

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By employing minute working electrodes, it was found possible to measure well defined redox potentials of aromatic hydrocarbons in the nonpolar solvents, benzene and chlorobenzene. Both solvents were found to have large accessible anodic and cathodic potential ranges. Anions appear to be exceptionally stable in benzene, the reversible formation of a trianion-radical being observed. Both the anion radical and dianion of perylene were formed reversibly under cyclic voltammetric conditions in benzene while the cation radical was found to adsorb strongly to the electrode. The latter behaviour was rationalized in terms of stronger ion-pairing between the anions and supporting electrolyte cations taking place stabilizing the anions while similar strong interactions involving the cation radicals are absent. Due to the inertness of chlorobenzene, its wide voltammetric potential range, and the ready solubility of tetrabutylammonium salts in the solvent, chlorobenzene was suggested to be an excellent solvent for the study of reversible oxidation and reduction of aromatic compounds.

Voltammetric studies are generally confined to solvents of relatively high dielectric constants. Exceptions to this rule can be found, for example ethers such as tetrahydrofuran and 1,2-dimethoxyethane have found limited use. The latter are exceptional in that the ethereal oxygens provide sites for coordination of cations and thus they are good solvents for many salts which can be used as supporting electrolytes. Studies in the ethereal solvents have revealed much information regarding the ion-pairing properties of anions derived from aromatic compounds.[1]

The present investigation was initiated in order to determine the behaviour of organic ions generated at electrodes in nonpolar solvents which have no special solvating features for either anions or cations. The ultimate goal would be to be able to do electrochemistry in a saturated hydrocarbon solvent such as hexane. However, a stringent requirement for a voltammetric solvent is that it is capable of dissolving supporting electrolyte salts, a property not shown by hexane. Thus, it was necessary to choose benzene, a somewhat more polar and less inert hydrocarbon, as the least polar solvent likely to have the necessary features for voltammetric studies. Here, we report results of voltammetric studies on redox reactions of aromatic hydrocarbons in benzene and chlorobenzene.

RESULTS AND DISCUSSION

Voltammetry in benzene. Tetrahexylammonium perchlorate was found to be sufficiently soluble in benzene for voltammetric studies. Attempts to measure voltammograms for the reduction of perylene (PE) in benzene containing Hex_4NClO_4 (up to 0.5 M) at a commercial platinum disk electrode (area ≈ 25 mm^2) failed; no current which could be ascribed to the reduction of PE was observed. However, measurements made using electrodes fabricated by polishing the cross section of fine platinum wire embedded in soft glass (see experimental section) resulted in well defined voltammetric peaks for the two consecutive one electron reductions of PE.

The dependence of the voltammetric characteristics for the reduction of PE in benzene

Table 1. Reduction of perylene in benzene/hex_4NClO_4 or DMF/Bu_4NBF_4 at a micro mercury electrode.

$[Hex_4NClO_4]$/M	ν/mVs^{-1} [a]	ΔE_p^1/mV [b]	ΔE_p^2/mV [b]	$\Delta E^\circ_{1,2}$/mV
0.1	61.8	*ca.* 350	*ca.* 320	695
	156	370	320	695
	309	390	315	685
	618	415	310	690
0.15	61.8	180	200	590
	156	200	200	595
	309	205	195	610
	618	215	200	620
0.2	61.8	*ca.* 150	160	580
	156	140	160	590
	309	135	155	600
	618	135	160	605
0.25	61.8	140	*ca.* 150	580
	156	120	130	600
	309	110	130	605
	618	100	125	605
0.3	61.8	120	*ca.* 150	595
	156	95	120	600
	309	90	115	605
	618	85	115	600
0.2 [c]	61.8	100	110	570
	156	90	100	570
	309	85	90	570
	618	70	75	570

[a] Voltage sweep rate. [b] Difference in potential of oxidation and reduction peaks on cyclic voltammograms. [c] DMF/Bu_4NBF_4.

upon supporting electrolyte concentration and voltage sweep rate is shown in Table 1. The symbols, ΔE_p^1 and ΔE_p^2, refer to the potential differences between the oxidation and reduction peaks measured from cyclic voltammograms for the first and second charge transfers to PE, respectively. The differences in ΔE° for the two charge transfers, referred to as $\Delta E^\circ_{1,2}$ in Table 1 were estimated from the differences in peak potentials for the consecutive reductions. The electrode used was constructed from a 0.03 mm diameter platinum wire and the exposed surface was covered with Hg just prior to making the measurements. Voltammetric data for the reduction of PE in dimethylformamide (DMF) containing Bu_4NBF_4 (0.2 M), a well defined system,[2] at the same micro electrode is included for comparison at the end of Table 1. At an electrolyte concentration of 0.1 M, the peak separations for both charge transfers are very large (>300 mV) indicating a major contribution of uncompensated resistance. The same effect, although to a lesser degree, was observed when the salt concentration was 0.15 M with the peak separation increasing with increasing voltage sweep rate in the expected manner. Curiously, the effect of increasing the voltage sweep rate reversed upon increasing the electrolyte concentration to 0.2 M; *i.e.* increasing the sweep rate resulted in decreasing the peak separation for the first charge transfer. The same trend continued with further electrolyte concentration increases, with the effect of uncompensated resistance becoming relatively unimportant.

Cyclic voltammetry theory requires that ΔE_p for a reversible one electron transfer at a planar electrode be equal to 58 mV at 25 °C.[3]

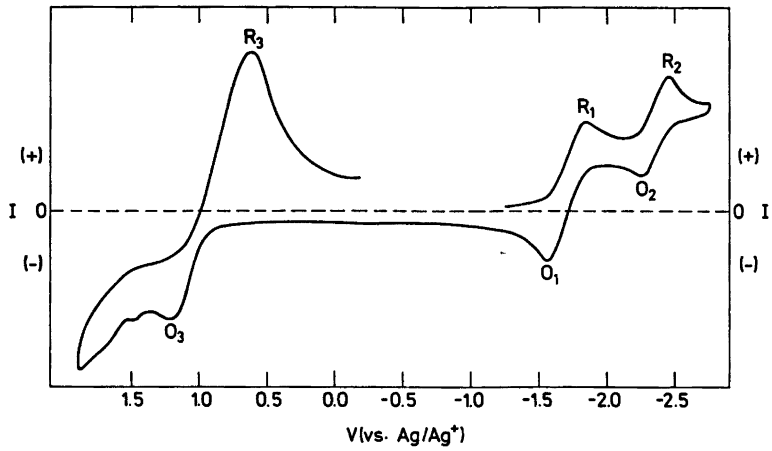


Fig. 1. Cyclic voltammograms showing consecutive reductions (R_1 and R_2) and the oxidation (O_3) of perylene in benzene containing tetrahexylammonium perchlorate (0.2 M) at a micro platinum electrode (diameter = 0.03 mm). Voltage sweep rate: 156 mV/s.

The theoretical relationships are followed for the reduction of PE in DMF at planar electrodes.[2] The voltammetric behaviour of the PE in DMF system using the micro electrode of this study is informative. Firstly, the peak separations (as high as 110 mV) are much greater than the theoretical values and secondly, the ΔE_p values which should be independent of sweep rate were found to decrease with increasing sweep rate. The latter forces us to the conclusion that the theoretical relationships for the very small electrodes do not hold since the edge effect becomes very important and modes of mass transport other than planar linear diffusion are operative.

The most frequently desired data from voltammetric studies are potential differences. The data for the reduction of PE in benzene can readily be assessed in the latter respect. It has been found that ΔE° for PE in DMF ($Bu_4NBF_4 = 0.2$ M) is equal to 565 mV.[2] The same system using the micro electrode gives an acceptable value of 570 mV. Little change in ΔE° for PE in benzene was observed at electrolyte concentrations greater than 0.15 M and a value of the order of 590 mV, was found very similar to that in DMF in spite of the great difference in the two solvents. We conclude that the potential differences that can be measured in benzene are reliable estimates.

Another aspect of the voltammetric behaviour of PE in benzene is illustrated by the cyclic voltammogram shown in Fig. 1. The reduction is characterized by two reversible redox couples, $R_1 - O_1$ and $R_2 - O_2$, involving the anion radical and the dianion, respectively. On the other hand, the oxidation producing the cation radical is accompanied by severe adsorption (O_3) and a very large reduction peak (R_3), at which the adsorbed cation radical is reduced from the electrode surface, is observed on the reverse sweep. The shape of the voltammogram shows that both the anion radical and the dianion remain in solution upon formation while the cation radical never leaves the electrode surface. Although there are indications that the cation radical is further oxodized on the electrode, no discrete oxidation to the dication can be discerned from the voltammogram. The fact that the anion radical and dianion appear to behave normally even in the nonpolar solvent is indicative that ion-pairing with the Hex_4N^+ ions allows the aromatic anions to go into solution. On the other hand, ion-pairing between the aromatic cations and ClO_4^- ions is probably far less important and thus the cations remain on the electrode.

The unusual stability of aromatic anions in benzene solution is further demonstrated by the voltammetric behaviour of 1,2-bis(9-anthryl)ethane (*1*) shown in Fig. 2. The expected

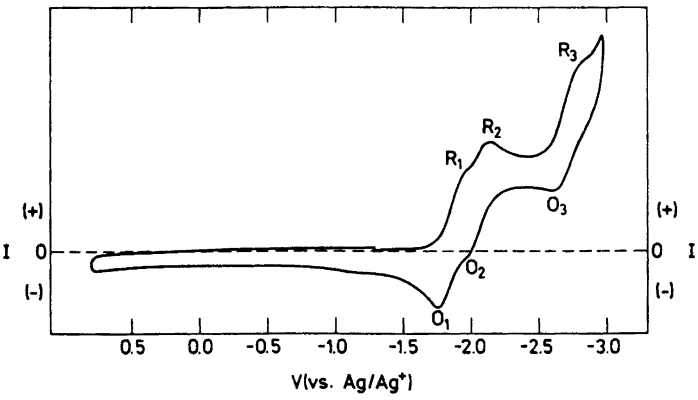
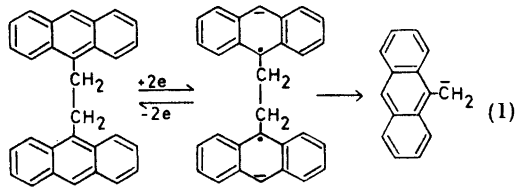


Fig. 2. Cyclic voltammogram showing the consecutive reductions of 1,2-bis(9-anthryl)ethane in benzene containing tetrahexylammonium perchlorate (0.2 M) at a micro platinum electrode (diameter = 0.03 mm). Voltage sweep rate: 156 mV/s.

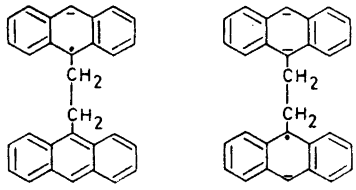
reaction, which occurs readily in more polar media,[4] is that illustrated by eqn. (1). Formation of the dianion diradical (*2*) was expected



(1)

1 *2* *3*

to be accompanied by the spontaneous cleavage of the saturated C – C bond and the generation of the carbanion (*3*). However, this process is not observed at all under the conditions of the experiment. Two closely spaced one electron transfers to give first the anion radical (*4*) and then the dianion diradical (*2*) were followed at more negative potential (~700 mV) by a further reversible one electron transfer to give the trianion radical (*5*).



4 *5*

The saturated C – C bond in the free dianion diradical (*2*) is expected to be severely weakened by the mutual repulsion of the negative charges in the two anthracene moieties. The weakening of the ethane linkage by charge repulsion would in turn be neutralized to a degree by ion-pairing. The fact that the bond remains intact even upon formation of the trianion (*5*) indicates that the extent and strength of ion-pairing in this medium are indeed very great.

The observation of the reversible formation of *5* also points out the very negative cathodic limit of the benzene – Hex_4NClO_4 system. The reduction peak, R_3, which appears at about – 2.8 V. *vs.* SCE is the most negative reversible reduction of an aromatic system that we are aware of. In general the more negative potential necessary to form an anion, the more reactive the intermediate will be.

Voltammetry in chlorobenzene. The more common electrolytic supporting electrolyte, tetrabutylammonium fluoroborate was found to be sufficiently soluble in chlorobenzene for voltammetric studies. The effect of voltage sweep rate and supporting electrolyte concentration for the reduction of PE in chlorobenzene at a micro platinum electrode (diameter = 0.03 mm) is shown in Table 2. In this case the inverse voltage sweep rate relationship, due to the edge effect of the small electrode, was observed at Bu_4NBF_4 greater than 0.1 M. The effect of uncompensated resistance appears

Table 2. Voltammetric reduction of perylene in chlorobenzene at a micro platinum electrode.[a]

Voltage sweep rate/ mVs^{-1}/Bu_4NBF_4	ΔE_p/mV [b] 0.05 M	0.1 M	0.15 M	0.20 M	0.25 M	0.30 M
618	280	100	85	80	75	80
309	260	110	90	95	80	95
156	250	120	110	100	105	–
61.8	250	150	150	125	120	120

[a] Constructed from a platinum wire, 0.03 mm diameter. [b] Peak potential difference of the reduction and oxidation segments of the first reduction step measured on cyclic voltammograms.

to be important only at very low supporting electrolyte concentration (0.05 M).

The lack of reactivity of chlorobenzene to aromatic hydrocarbon anion and cation radicals as well as the favorable anodic and cathodic potential ranges is illustrated by the cyclic voltammogram for oxidation and reduction of anthracene shown in Fig. 3. Both redox couples are shown to be reversible and the potential range is seen to cover at least 4.0 V. The difference in E° for the oxidation and reduction of anthracene in chlorobenzene under the conditions of this study was found to be equal to 3.32 V as compared to 3.34 V reported recently for the E° difference in acetonitrile.[5]

CONCLUSIONS

When very small electrodes are employed, the nonpolar solvents benzene and chlorobenzene are suitable voltammetric solvents. The theoretical relationships of cyclic voltammetry do not hold for the small electrodes due to diffusion toward and electron transfer at the edges of the electrode which constitute a significant portion of the active area. The potentials measured at the electrodes are, however, significant and it was shown that they compare favourably with those measured at large electrodes in polar solvents.

The use of these new nonpolar solvents should provide valuable information about the effect of ion association on the stability of electrode generated intermediates. That anions undergo strong ion-pairing in benzene was demonstrated by the stability of the trianion radical (*5*) which was observed to be formed reversibly at the very negative potential, −2.8 V.

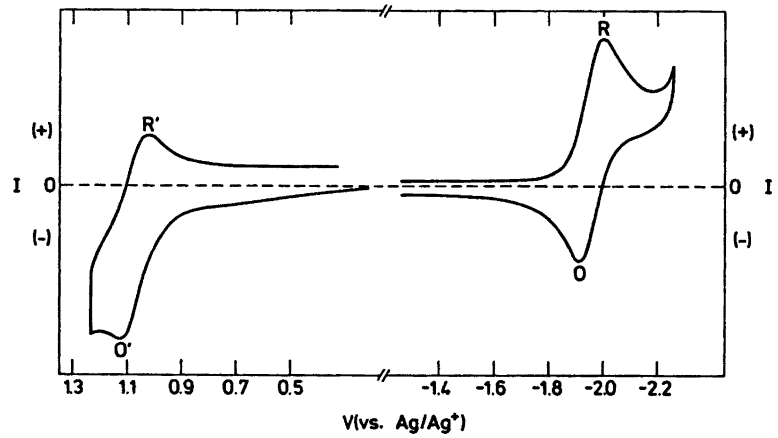


Fig. 3. Cyclic voltammogram for the oxidation and reduction of anthracene in chlorobenzene containing tetrabutylammonium fluoroborate (0.2 M). Voltage sweep rate: 309 mV/s.

EXPERIMENTAL

Tetrahexylammonium perchlorate (Hex_4NClO_4) was prepared by mixing an aqueous solution of Hex_4NBr with an equimolar solution of perchloric acid (70 %). The precipitated salt was dissolved in dichloromethane and washed first with $NaHCO_3$ solution and then with water. After removal of most of the dichloromethane under reduced pressure, the salt was precipitated by the addition of dry ether. The salt was collected and dried over P_2O_5; m.p. 102 – 103 °C. Tetrabutylammonium fluoroborate (Bu_4NBF_4) was prepared in a similar manner from Bu_4NHSO_4 and fluoroboric acid (35 %).

Benzene, BDH Analar, was used as received. Clorobenzene (Kebo) was fractionally distilled before use. Distillation of the solvent did not improve the voltammetric potential range but decreased the residual anodic current.

Micro electrodes were fabricated by sealing fine platinum wire (0.03 mm diameter) into the end of a soft glass tube, then cutting and polishing the end first with emory cloth and finally on a ground glass surface. The polishing procedure was found to be very critical to the performance of the electrodes and repeated treatment was often necessary to obtain the best response. It appears that, owing to the fineness of the wire, minor imperfections in the sealing process markedly affect the performance of the electrodes. Careful polishing removes the portion of the electrode where defects exist. Mercury plating of the polished electrodes was accomplished by electrolytic deposition from an aqueous $Hg(ClO_4)_2$ solution. Examination of these electrodes under a magnifying glass indicated that the mercury coatings were uniform. The effective area of the coated electrodes was estimated by voltammetry to be about twice that of the polished platinum surface.

Voltammetric measurements were conducted in an undivided cell of about 10 ml capacity. The counter electrode was a platinum wire and an Ag|0.1 M $AgNO_3$|MeCN electrode was used as the reference. A Luggin capillary for the reference was found not only to be unnecessary but also to introduce noise into the circuit. The appropriate amount of supporting electrolyte was weighed into the cell together with a small quantity of neutral alumina [2,6] and the solvent was introduced through a short column packed with neutral alumina. The substrate was then added and the solution was deaerated using a stream of dry nitrogen. Due to the small currents associated with the use of the micro electrodes, typically of the order of about 5×10^{-2} μA, electrical screening of the cell was necessary to prevent excessive noise pick up. Once efficient screening had been achieved, noise free voltammograms could be recorded even at the highest sensitivity of the equipment which was 4×10^{-2} μA per cm on the recorder chart. A three electrode voltammetry unit of the type previously described [7] was used along with a Watanabe WX 442 recorder. Our experience indicates that measurements with even smaller electrodes are feasible provided that the constructional difficulties of the micro electrodes can be overcome.

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Cathodic Cleavage of *ortho*-Alkyl Substituted Alkyl Aryl Sulfones

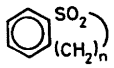
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The cathodic cleavage of methyl *o*-tolyl sulfone, methyl *o-tert*-butylphenyl sulfone, and benzyl *o-tert*-butylphenyl sulfone has been studied both in methanol and *N*,*N*-dimethylformamide solution at a mercury cathode. Whereas methyl phenyl sulfone undergoes exclusively alkyl-sulfur bond cleavage under these conditions, the *ortho* substituted methyl aryl sulfones also show aryl-sulfur bond cleavage to an extent that increases with the bulkiness of the *ortho* substituent. The cleavage mode is interpreted in terms of the preferred conformations of the sulfones. Benzyl *o-tert*-butylphenyl sulfone gives exclusively toluene and *o-tert*-butylbenzenesulfinate ion, probably owing to the stability of the incipient benzyl radical.

The current-time dependence of the methyl aryl sulfone electrolyses suggests that an indirect reduction mechanism is operating. The tetraalkylammonium cation of the supporting electrolyte is assumed to act as a mediator in the overall charge transfer.

The cathodic cleavage at mercury of some cyclic sulfones having the general formula



$n = 1-4$

has been studied by Simonet and one of the present authors.[1] In contrast to methyl phenyl sulfone, which cleanly undergoes alkyl-sulfur cleavage to yield methane and benzenesulfinate ion,[2,3] the main ($n = 1$, 4) or exclusive ($n = 2$, 3) reaction for the cyclic sulfones is aryl-sulfur cleavage. The cleavage mode is thought to be governed by the orientation of the sulfonyl group with respect to the aromatic ring.[1] The preferred conformation of a representative cyclic sulfone ($n = 2$) and of methyl phenyl sulfone, respectively, is shown in Fig. 1. In the Koch and Moffitt terminology,[4] the cyclic compound is a Case I sulfone, and the other one represents Case II.[5]

It was pointed out to us by Dr. G. Jeminet (Université de Clermont-Ferrand, France) that an *ortho* alkyl substituted methyl phenyl sulfone might provide an interesting case for comparison. Inspection of Stuart-Briegleb models of methyl *o*-tolyl sulfone and methyl *o-tert*-butylphenyl sulfone suggested that these compounds might approach Case I. If the conformation is the decisive factor governing the cleavage mode, the *ortho* substituted methyl phenyl sulfones would be expected to show aryl-sulfur cleavage.

RESULTS

Synthesis of starting materials. The preparation of methyl *o*-tolyl sulfone followed a literature procedure.[6] Methyl *o-tert*-butylphenyl sul-

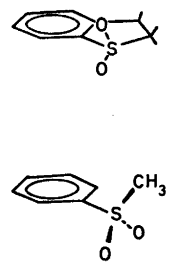
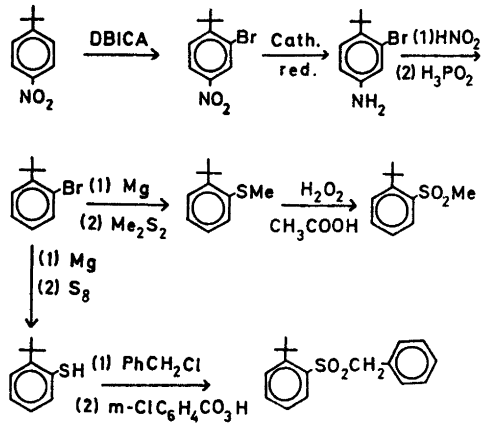


Fig. 1. Preferred conformations of 2,3-dihydrobenzo[*b*]thiophene-1,1-dioxide, a Case I sulfone, and of methyl phenyl sulfone, a Case II representative.



Scheme 1. Synthetic routes to benzyl and methyl *o-tert*-butylphenyl sulfone. DBICA = Dibromoisocyanuric acid.

fone was synthesized *via* a lengthy but straightforward route, illustrated in Scheme 1. Some steps in the synthesis were improved compared to earlier methods. Bromination of *p*-nitro-*tert*-butylbenzene was made with dibromoisocyanuric acid [7] instead of the expensive silver sulfate–bromine mixture.[8] Reduction of 2-bromo-4-nitro-*tert*-butylbenzene to the corresponding amine was carried out electrochemically rather than with iron and hydrochloric acid.[9] In the next-to-the-last step, a Grignard reagent was allowed to react with a disulfide. This reaction is known in the literature [10] but appears to have been seldom used.

Table 1. Polarographic half-wave potentials of some sulfones, recorded in DMF solution, tetrabutylammonium iodide 0.1 M. Sulfone concentration 10^{-3} M.

Sulfone	$E_{1/2}$/V [a]
Methyl phenyl	−1.73 [b]
Methyl *o*-tolyl	−1.73
Methyl *o-tert*-butylphenyl	−1.65
Benzyl phenyl	−1.60
Benzyl *o-tert*-butylphenyl	−1.43

[a] Potential *vs.* Ag,AgI/0.1 M Bu_4NI, DMF. [b] Taken from Ref. 2 and reconfirmed in this work.

The electrolysis results prompted the inclusion of a third compound in the present study, namely, benzyl *o-tert*-butylphenyl sulfone. This compound was prepared as shown in Scheme 1.

Polarography. The half-wave potentials, recorded in DMF solution containing tetrabutylammonium iodide, 0.1 M, are listed in Table 1. The sulfone concentration was in all cases 10^{-3} M.

Electrolyses. The compounds were electrolyzed both in methanol and DMF solution. The starting amount in each run was 2–5 mmol. The sulfinate ions formed upon cathodic cleavage were qualitatively and quantitatively determined *via* ^{1}H NMR spectroscopy. The results are presented in Table 2. For methyl *o*-tolyl sulfone and methyl *o-tert*-butylphenyl sulfone, the electricity consumption was always

Table 2. Product yields and conditions of electrolysis of some *ortho*-substituted sulfones.

Sulfone	Temp. °C	Supp. electrolyte	Cath. pot. Volt	Per cent cleavage alkyl-sulfonyl	Per cent cleavage aryl-sulfonyl	Ratio alkyl-sulfonyl/ aryl-sulfonyl
Methyl *o*-tolyl	10	MeOH, 0.05 M TMAC [a]	−2.2 [c]	83	18	4.6
	25	DMF, 0.05 M TEAP [b]	−1.8 [d]	93	7	13.3
	65	DMF, 0.05 M TEAP [b]	−1.8 [d]	93	7	13.3
Methyl *o-tert*-butylphenyl	10	MeOH, 0.05 M TMAC [a]	−2.2 [c]	7	91	0.08
	25	DMF, 0.05 M TEAP [b]	−1.85 [d]	7	91	0.08
	70	DMF, 0.05 M TEAP [b]	−1.85 [d]	16	84	0.19
Benzyl *o-tert*-butylphenyl	10	MeOH, 0.5 M TMAC [a]	−2.0 [e]	− [g]	0	
	10	MeOH, 0.1 M LiCl	−2.1 [f]	86	0	

[a] TMAC Tetramethylammonium chloride. [b] TEAP Tetraethylammonium perchlorate. [c] *vs.* Ag,AgCl/0.05 M Cl^-. [d] *vs.* Ag,AgI/0.1 M I^-. [e] *vs.* Ag,AgCl/0.5 M Cl^-. [f] *vs.* Ag,AgCl/0.1 M Cl^-. [g] Not quantitatively determined.

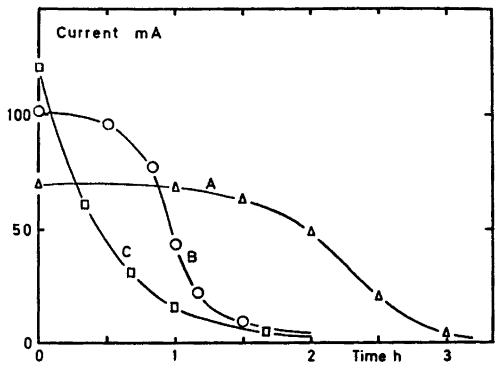


Fig. 2. Current-time dependence of sulfone electrolyses in methanol-tetramethylammonium chloride. Curve A, methyl *o*-tolyl sulfone, B, methyl *o-tert*-butylphenyl sulfone, C, benzyl *o-tert*-butylphenyl sulfone.

2 F mol^{-1} within a few per cent. With benzyl *o-tert*-butylphenyl sulfone, the current dropped to the background value after 1.5 – 1.7 F mol^{-1}. The current-time dependence was recorded for all runs in methanol. Fig. 2 illustrates the behaviour for the three sulfones. Simple exponential decay is only shown by benzyl *o-tert*-butylphenyl sulfone (curve C), whereas the methyl sulfones give an initially constant current (curves A and B).

An experiment to test the influence of changing the concentration of supporting electrolyte, not included in Table 2, was also performed. In a run with methyl *o*-tolyl sulfone in methanol, doubling the concentration of tetramethylammonium chloride caused a 33 % increase of the initial current.

DISCUSSION

Cleavage mode. Whereas methyl phenyl sulfone is exclusively cleaved between the methyl and sulfonyl groups,[1,3] the introduction of an *ortho* methyl group brings about some aryl – sulfonyl cleavage (see Table 2). With an *ortho tert*-butyl group, aryl – sulfonyl cleavage becomes the major mode.

The increasing trend towards aryl – sulfonyl cleavage as the bulkiness of the *ortho* substituent increases is believed to be caused by stereoelectronic effects of the type discussed in Ref. 2. It must be remembered, however, that steric repulsion between the *ortho* substituted aryl group and the methanesulfonyl group will generally facilitate the breaking of the bond between them. Since the conformations of the *ortho* substituted sulfones are not independently known, it is impossible to assess the relative importance of the two factors which determine the cleavage mode. The chemical shifts of the methyl protons in the ^1H NMR spectra are too similar to allow any conclusions and might not be sensitive to conformational changes. They are, for phenyl, *o*-tolyl, and *o-tert*-butylphenyl methyl sulfone, respectively, δ ($CDCl_3$) 3.06, 3.06, and 3.11.

In the benzyl *o-tert*-butylphenyl sulfone, the cleavage takes place exclusively between the benzyl and the sulfonyl groups. Here the relative stability of the incipient benzyl radical explains the cleavage mode. Inspection of the polarographic half-wave potentials clearly bears this out. For benzyl and methyl *o-tert*-butylphenyl sulfone they differ by 0.22 V (Table 1), the benzyl sulfone being more easily reduced. For benzyl phenyl sulfone and methyl phenyl sulfone, the difference is less, 0.13 V. Steric effects may account for the discrepancy of the two differences.

Electrolysis of benzyl *o-tert*-butylphenyl sulfone provides a synthesis of *o-tert*-butylbenzenesulfinic acid (obtained as a salt). This compound has not been found in the literature and might be difficult to synthesize by any other route. For example, an attempted addition of sulfur dioxide to *o-tert*-butylphenylmagnesium bromide failed. Nor did *o-tert*-butylphenyllithium and sulfur dioxide yield the desired acid.

A close inspection of Table 2 reveals that the alkyl – sulfonyl/aryl – sulfonyl ratio for methyl *o*-tolyl sulfone is different in methanol and DMF. Also, there is a temperature effect on the ratio for methyl *o-tert*-butylphenyl sulfone in DMF, but not for methyl *o*-tolyl sulfone. We refrain from trying to explain these effects.

Current-time dependence. Typically, in electrolytic runs carried out at constant electrode potential, the current decreases exponentially with time. This first-order kinetic behaviour is shown by benzyl *o-tert*-butylphenyl sulfone (Fig. 2, curve C). The instantaneous current is proportional to the bulk concentration of electroactive substrate, and the usual and most simple interpretation is that electrons are

transferred directly from the electrode to the substrate. The reaction scheme is

$$S + e^-_{electrode} \longrightarrow S^{\cdot -} \longrightarrow R^{\cdot} + A^-$$

$$R^{\cdot} + e^-_{electrode} \longrightarrow R^-$$

$$R^- + H^+ \text{ (from the medium)} \longrightarrow RH$$

Whether the species $S^{\cdot -}$ is an intermediate or not is presently being investigated. In the mechanism above, S is sulfone, $R^{\cdot}$ alkyl radical, and A^- sulfinate anion.

A quite different behaviour is shown by the two other sulfones studied in this work (Fig. 2, curves A and B). The reaction rate is zero-order with respect to sulfone for a substantial part of the run, and at the end becomes first-order. This behaviour strikingly demonstrates a phenomenon recently brought to attention by Lund, Michel and Simonet,[11] namely, the participation of the supporting electrolyte in the reaction. A necessary condition for this is that the reduction potential of the substrate is not too far from that of the supporting electrolyte. The half-wave potential of benzyl *o-tert*-butyl-phenyl sulfone is too positive to allow the supporting electrolyte to participate.

A mechanism that will account for the observed kinetics is the following one:

$$Q^+ + e^-_{electrode} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Z \qquad (1)$$

$$Z + S \xrightarrow{k_2} Q^+ + R^{\cdot} + A^- \qquad (2)$$

$$R^{\cdot} + e^-_{electrode} \xrightarrow{k_3} R^- \qquad (3)$$

$$R^{\cdot} + Z \xrightarrow{k_4} R^- + Q^+ \qquad (4)$$

$$R^- + H^+ \text{ (from the solvent)} \xrightarrow{k_5} RH \qquad (5)$$

In the above scheme, equilibrium (1) represents the reversible, potential-dependent transfer of an electron from the cathode to the supporting electrolyte, yielding a species Z, "tetraalkylammonium radical" or "tetraalkylammonium amalgam". This equilibrium is far to the left. In reaction (2), charge is transferred from Z to the sulfone to regenerate the cation of the supporting electrolyte, Q^+, and bring about cleavage of S into a radical $R^{\cdot}$ and a sulfinate ion A^-. Reversible formation of a radical anion $S^{\cdot -}$ followed by rapid cleavage would also be possible.

The radical $R^{\cdot}$ is further reduced, either *via* electron transfer from the electrode; (3), or by Z; (4), and finally, the resulting carbanion R^- is protonated by the solvent to form the corresponding hydrocarbon; reaction (5).

If we first assume that the conversion of $R^{\cdot}$ to R^- follows the homogeneous path (4) rather than the heterogeneous one, (3), we obtain

$$d[Z]/dt = k_1[Q^+] - k_{-1}[Z] - k_2[Z][S] - k_4[Z][R^{\cdot}] \qquad (6)$$

We also have

$$d[R^{\cdot}]/dt = k_2[Z][S] - k_4[Z][R^{\cdot}] \qquad (7)$$

Making the steady-state approximations that the time derivatives of [Z] and $[R^{\cdot}]$ may be equalled to zero leads to

$$[Z] = k_1[Q^+]/(k_{-1} + 2k_2[S]) \qquad (8)$$

The reaction rate is given by the following expression:

$$-d[S]/dt = k_1k_2[S][Q^+]/(k_{-1} + 2k_2[S]) \qquad (9)$$

If, on the other hand, reaction (3) rather than (4) is operating, the term $2k_2[S]$ in the denominator of (9) will be replaced by $k_2[S]$.

In the beginning of an electrolytic run, when [S] is large, it may be possible to neglect k_{-1} besides $2k_2[S]$ or $k_2[S]$, depending on the relative magnitude of the rate constants. This leads to

$$-d[S]/dt = k_1[Q^+]/2 \text{ or } k_1[Q^+], \text{ respectively.} \qquad (10)$$

The rate depends only on $[Q^+]$. Towards the end of the run, when [S] will have dropped to a low value, we may instead neglect the term $2k_2[S]$ or $k_2[S]$, respectively, and we obtain

$$-d[S]/dt = k_1k_2[S][Q^+]/k_{-1} \qquad (11)$$

Eqns. (10) and (11) correspond to the zero- and first-order parts of the runs.

It is, in principle, possible to make a detailed mathematical analysis of the curves A and B, Fig. 2. However, a further complication must be considered in the present work. In the course of electrolysis, two cations are transported through the ion exchange membrane for each molecule of sulfone reacting. This causes $[Q^+]$ to increase in the cathode com-

partment, and since the initial concentrations of Q^+ and S differ by less than a factor of 3, the concentration of Q^+ can by no means be regarded as a constant. In fact, a close inspection of curve A reveals a slight upward drift in the beginning of the run, caused by an increase of $[Q^+]$.

The runs in DMF showed the same kinetic behaviour as described above for the methanol experiments, *i.e.*, for methyl *o*-tolyl sulfone and methyl *o-tert*-butylphenyl sulfone, the current remained constant for a large part of the reaction.

Eqn. (10) shows that the reaction rate (*i.e.*, the current) should be proportional to $[Q^+]$ for reactions being initially zero order with respect to [S]. In an experiment with methyl *o*-tolyl sulfone in methanol, the concentration of tetramethylammonium chloride was changed from 0.05 to 0.1 M. This caused a 33 % increase of the current, rather than the predicted 100 %. This apparent discrepancy is resolved by consideration of an ion-pair dissociation equilibrium for the supporting electrolyte. The dissociation constant for tetraethylammonium chloride in methanol is 0.1,[12] and that of tetramethylammonium chloride should be smaller. It then follows from Ostwald's dilution law that the concentration of dissociated quaternary ions will increase roughly as the square root of the stoichiometric concentration of the salt. It is assumed that only the free ions partake in the electrochemical mechanism postulated above.

EXPERIMENTAL

Syntheses. Melting points have been determined on a Kofler Hot Stage microscope. Pressures are given in SI units. 1 Pa = 7.5×10^{-3} mmHg.

Methyl o-tolyl sulfone was prepared from *o*-thiocresol (Fluka AG).[6] Yield 83 %, m.p. 54–56 °C, lit.[6] 55–57 °C.

2-Bromo-4-nitro-tert-butylbenzene. A solution of 38.5 g (0.2 mol) of 4-nitro-*tert*-butylbenzene [13] in 100 ml of ice-cold 92 % sulfuric acid was first prepared. A suspension of 28.7 g (0.1 mol) of dibromoisocyanuric acid [7] in 300 ml of ice-cold 92 % sulfuric acid was added at such a rate that the temperature did not rise above 25 °C. During the addition and 2 h afterwards, the reaction mixture was mechanically agitated and cooled in an ice-bath. It was then poured into 2 l of ice-water, and the solid material was collected on a suction filter, washed with water and allowed to dry. Continuous extraction with ether in a Soxhlet apparatus separated the product from the ether-insoluble isocyanuric acid. Evaporation of the ether solution to dryness and recrystallization of the residue from 95 % ethanol gave 35.6 g (69 % yield) of 2-bromo-4-nitro-*tert*-butylbenzene, m.p. 94 °C, lit.[8] 94 °C.

o-Bromo-tert-butylbenzene. A solution of 25.8 g (0.1 mol) of the preceding compound in 300 ml of 95 % ethanol and 15 ml of conc. hydrochloric acid was placed on top of a 5 mm layer of mercury in a thick-walled 1 l beaker. Electrical contact with the mercury was made *via* a short piece of platinum sealed in glass. The anode, a piece of lead sheet, was immersed in 10 % sulfuric acid in a ceramic cup serving as a diaphragm and placed inside the beaker. The cathode surface was mechanically agitated and the cell was cooled in water during the electrolysis. A current of 8 A was fed through the cell. The reduction required 16.2 Ah theoretically and in fact, when this amount had been introduced, a vigorous hydrogen evolution started at the cathode. In order to ensure complete reduction, the current was gradually diminished to zero during 30 min in such a way that the hydrogen evolution was barely visible. The catholyte was separated from the mercury and evaporated to dryness *in vacuo*, giving a quantitative yield of 4-*tert*-butyl-3-bromoaniline hydrochloride as a white, crystalline mass. This was redissolved in 250 ml of water and 20 ml of conc. hydrochloric acid and diazotized with a solution of 7.9 g (0.1 mol) of sodium nitrite in 20 ml of water. The temperature was kept below −2 °C by cooling in a Dry Ice–acetone bath. Without undue delay, 250 ml of 50 % phosphinic acid was added and the solution kept overnight at 5 °C. The product was isolated through extraction with ether, washing with 2 M sodium hydroxide solution to remove phenolic impurities, drying over anhydrous sodium sulfate and distillation. The yield was 15.7 g or 74 %, b.p. 88–92 °C/1 kPa, lit.[9] 96–98 °C/1.6 kPa.

Methyl o-tert-butylphenyl sulfone. A Grignard reagent was prepared [9] from 6.4 g (0.03 mol) of *o*-bromo-*tert*-butylbenzene. A solution of 3 ml (appr. 0.03 mol) of dimethyl disulfide in 10 ml of absolute ether was added, causing a mildly exothermic reaction and the gradual appearance of a gray precipitate. After a reflux period of 5 h, enough 2 M hydrochloric acid was added to dissolve the precipitate, and the resulting two phases were separated. The ether phase was washed with water, sodium carbonate solution and water, dried over anhydrous sodium sulfate and filtered. Vacuum distillation gave 2.7 g (50 %) of a colorless oil, b.p. 108–110 °C/930 Pa. This sulfide was dissolved in 15 ml of glacial acetic acid, and 8 ml of 30 % aqueous hydrogen peroxide (appr. 0.08 mol) was added. The reaction mixture was refluxed

for 30 min and poured onto ice. The solid material which precipitated was collected and recrystallized from 50 % ethanol to give 2.7 g (85 %) of colorless crystals, m.p. 69–69.5 °C. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.60 (9 H, s), 3.11 (3 H, s), 7.25–7.75 (3 H, m), 8.10–8.35 (1 H, m).

o-tert-*Butylthiophenol*. A Grignard reagent was prepared [9] from 10.7 g (0.05 mol) of *o*-bromo-*tert*-butylbenzene. Elemental sulfur, 1.6 g (0.05 gram atom), was added in portions and the mixture was kept at reflux for 3 h. About 0.2 g of lithium tetrahydridoaluminate was added in order to reduce any disulfide formed, and 40 ml of 5 M hydrochloric acid was added. After separation of the two phases, the ether phase was washed with water and conc. sodium chloride solution and dried over anhydrous sodium sulfate. Distillation gave 3.3 g (40 %) of product, b.p. 115–118 °C/2.4 kPa, lit.[14] 99 °C/1.3 kPa.

Benzyl o-tert-*butylphenyl sulfide*. A solution of 1.2 g (0.03 mol) of sodium hydroxide in 2 ml of water was added to a solution of 3.3 g (0.02 mol) of *o-tert*-butylthiophenol and 2.5 g (0.02 mol) of benzyl chloride in 25 ml of methanol. A moderately exothermic reaction took place. The mixture was left overnight at room temperature and then evaporated *in vacuo*. The remaining oil was freed from inorganic solids by dissolution in ether, washing with water, drying over Sikkon ® and reevaporation *in vacuo*. The residue was 5.1 g (100 %) of the sulfide, a colourless oil.

Benzyl o-tert-*butylphenyl sulfone*. Of the preceding sulfide, 3.0 g (0.012 mol) was dissolved in 60 ml of dichloromethane. *m*-Chloroperbenzoic acid (Fluka AG, 85 % grade), 5 g (0.025 mol peracid), was added in portions over a period of 5 min. After another 10 min, the solution was washed with 1 M sodium hydroxide solution to remove acids, dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield 3.5 g of colourless oil (theoretical yield of sulfone 3.4 g). After 6 months at 5 °C the oil had not crystallized. IR (film): bands at 1130, 1155, and 1320 cm^{-1}. Sulfones are reported [15] to have strong bands at 1175 and 1325 cm^{-1}. Sulfoxides have a strong band in the 1000–1050 cm^{-1} region,[15] in which no strong absorption of the present sample was observed. ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.60 (9 H, s), 4.40 (2 H, s), 7.0–8.0 (9 H, multiplet). The sulfoxide most probably would have shown an AB pattern for the benzyl group, but a singlet was observed.

Purification of supporting electrolytes and solvents. Tetramethylammonium chloride (Fluka AG) was recrystallized from a mixture of methanol and ether and dried *in vacuo*. Tetraethylammonium perchlorate was prepared from the bromide and sodium perchlorate, preelectrolyzed until free from sodium ion and recrystallized five times from water. Tetrabutylammonium iodide was "polarography grade" from Fluka AG. The methanol was from May and Baker, reagent grade, and used as received. The DMF was purified as in earlier work.[2]

Polarography. The half-wave potentials at a dropping mercury electrode were recorded in DMF solution using the same conditions as in earlier work.[2] A three-electrode apparatus similar to the Heath Model EUW 401 was used.

Electrolyses. An H-type cell with a cation exchange membrane (Amfion ® type C-311), clamped between two flat-flange joints, was used. The supporting electrolyte was a 0.05–0.5 M solution of tetramethylammonium chloride or lithium chloride in methanol. The cathode was a 20 cm^2 mercury layer and the anode a bundle of graphite rods. A silver chloride coated silver wire in a glass capillary containing supporting electrolyte served as the reference electrode. The catholyte was agitated by a stirrer which did not touch the mercury surface. The cell was immersed in ice water and the catholyte was kept deaerated with nitrogen. Current was supplied by a 200 V/1 A potentiostat built in this laboratory. The instantaneous current as well as its time integral were registered on a two-channel recorder. This arrangement, recommended by Fry,[16] made possible some important kinetic observations which have been commented on. An oscilloscope was connected across the cell to monitor the stability of the system. According to our experience some potentiostats (including commercial ones) cause oscillation in the 10–20 kHz region with some cells. The oscillation can reach an amplitude close to the full voltage swing of the potentiostat and has the consequence that the electrolysis does not take place at constant electrode potential. The remedy is to change the transfer function of the potentiostat by suitable damping.

After preelectrolysis, a weighed amount (2–5 mmol) of sulfone was added to the cathode compartment. The potentials of electrolysis are given in Table 2. The initial current was about 100 mA, and the experiments were run to exhaustion, at least 2 h. The half-life for the cases showing first-order kinetics was about 20 min. After completion of each run, a known amount of sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate (Merck), about 50 mg (0.3 mmol), was dissolved in the catholyte to serve as calibration substance as well as internal shift reference in the following analysis. The mercury was separated off and the methanol solution evaporated *in vacuo*. A few ml of deuterium oxide (99.5 % grade) was added and the evaporation repeated. The residue was dissolved in the minimum amount (1–3 ml) of deuterium oxide. The ^{1}H NMR spectrum was recorded on a Varian A-60 or T-60 instrument. The absolute amounts of the sulfinate ions could be calculated from the corresponding integrals of the peaks. Tetramethylammonium ion, when present in the supporting electrolyte, gave a huge peak at δ 3.2, but this

Table 3. 60 MHz ^{1}H NMR data for some sulfinate ions.

Ion	Chemical shift in ppm (D_2O, ref. $(CH_3)_3SiCD_2CD_2COONa$)
Methanesulfinate	2.29 (s)
o-Toluenesulfinate	2.54 (3 H, s), 7.25–7.95 (4 H, m)
o-tert-Butylbenzenesulfinate	1.52 (9 H, s), 7.45–8.15 (4 H, m)

did not interfere with any sulfinate ion signal needed for quantitative analysis in the present work.

The chemical shifts for the various sulfinate ion signals are given in Table 3.

In the analysis of the catholyte after electrolysis of benzyl *o-tert*-butylphenyl sulfone, signals from *o-tert*-butylbenzenesulfinate ion were observed. In the lithium chloride run, no signals were found between 3.0 and 6.0 ppm, the expected region for the methylene protons of phenylmethanesulfinate ion. Aryl-sulfonyl cleavage had apparently not taken place. (The absence of *tert*-butylbenzene in the catholyte was also verified by GLC analysis.)

For comparison, some runs were also made in DMF containing tetraethylammonium perchlorate (0.1 M). A cell with a glass frit as separator between the cathode and anode compartments was used since some runs were made at elevated temperatures. The reference electrode was a silver iodide coated silver wire in 0.1 M tetrabutylammonium iodide (in DMF), contained in a glass capillary. Identification of the sulfinate ions was made through ^{1}H NMR analysis as above after evaporation of the solvent *in vacuo*.

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A Comparison of the 1,2- and 1,4-Addition of Some Grignard Reagents with Different Halogens and Methyl Cuprates to 2-Furanacrolein

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When i-PrMgX (X = Cl, Br, I), *t*-BuMgX (X = Cl, Br), and $PhCH_2MgX$ (X = Cl, Br, I) react with 2-furanacrolein, the corresponding 1,2- and 1,4-addition products are formed. A heavier halogen in the i-Pr and *t*-Bu reagents enhances the formation of the 1,4-adducts, while the effect is the reverse for $PhCH_2$ reagents. A higher concentration of the Grignard reagent gives a higher yield of the 1,2-adduct, while the ratio of reagent to substrate does not affect the product distribution.

The methyl substituted 1,4-adduct is obtained with MeMgI/CuI and with $LiMe_2Cu$.

The initial complex, formed when the Grignard reagent is brought into contact with the substrate, is briefly discussed.

The influence of the halogen of the Grignard reagent on the distribution between 1,2- and 1,4-addition products has largely been neglected, although some different results have been published. In the reactions between 2-butyl crotonate and methyl-, propyl-, isopropyl-, and *tert*-butylmagnesium halide,[1] it was found that a heavier halogen in the reagent increased the yield of the 1,2-adduct. The same trend was also found in the reaction of butylmagnesium halide with acetals of acrylic aldehydes.[2] When the present work was already finished, an article which deals with the effect of the halogen in the reaction between RMgX and coumarin was published.[3] In that work a heavier halogen in isopropyl-, *sec*-butyl-, and *tert*-butylmagnesium halide was found to favour the 1,4-addition. The results were discussed on the basis of a theoretical model.

The Grignard reagent has been found to consist of the species RMgX and R_2Mg in equilibrium with each other (the Schlenk equilibrium[4]). It has also been established that RMgX predominates at equilibrium, but because R_2Mg is more reactive, a part of the reaction product is always formed by reaction between R_2Mg and the substrate.[5] Moreover, when the substrate is unreactive, most of the reaction product is formed by attack from R_2Mg.[6]

From these results, the conclusion can be drawn that a reactive substrate is needed in order to favour reaction with the RMgX part of the reagent and that a study of the effect of the halogen on the product distribution should be performed on a reactive substrate. Because aldehydes react fast with Grignard reagents, a conjugated aldehyde was considered suitable for this investigation.

Recent works on the furfurylidenemalonic ester system have shown that *tert*-butylmagnesium chloride and benzylmagnesium chloride can add in the 1,6- and 1,8-manners.[7] It therefore seemed interesting to choose 2-furanacrolein as the conjugated aldehyde.

It has been found in earlier studies of the reaction between conjugated aldehydes and Grignard reagents that the reagent, with the exception of tertiary reagents [8] and especially benzylmagnesium chloride,[9,10] adds almost exclusively in the 1,2-manner. With benzylmagnesium chloride the yield of 1,2-addition

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product varied between 7.5 and 78 % and the yield of 1,4-addition product between 28 and 0 %.

Large quantities of unidentified polymeric products have often been reported in the reaction of Grignard reagents with conjugated aldehydes. In these studies the composition of the reaction mixture has been analysed by chemical means or by distillation. Such analyses can affect the sensitive substances in the product mixture and lead to larger quantities of secondarily formed substances. In the present work the analysis was performed by GLC, and the ratios between the reaction products were calculated by measuring the peak areas.

It has been established that alkylmagnesium chlorides are best described as dimers throughout the whole concentration range, while bromides and iodides consist of associated and unassociated species in an equilibrium which depends on the concentration.[11] It therefore seemed meaningful to vary the concentration of the Grignard reagent in order to find out if a change in the composition of the reagent is followed by a change in distribution of the 1,2- and 1,4-adducts.

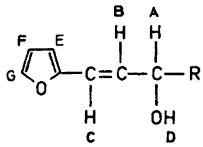
It is also known that different complexes are formed between reacted substrate molecules and unreacted reagent molecules,[12] and therefore the ratio of reagent to substrate was varied to find out if the appearance of these kinds of complexes affects the product distribution.

2-Furanacrolein has earlier been reacted with methylmagnesium iodide, ethylmagnesium bromide, and butylmagnesium chloride,[13] but only 1,2-addition was reported.

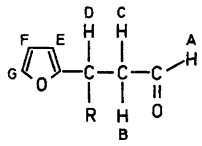
The methyl substituted 1,4-adduct was considered most easily obtainable with the aid of a copper reagent. The addition of copper(I) chloride to Grignard reagents has been shown to increase the yield of the conjugate addition product,[14] and the lithium diorganocuprates in particular are known to give high yields of the 1,4-adduct.[15]

RESULTS

When 2-furanacrolein reacts with Grignard reagents, a complex is immediately formed, which reacts further with more reagent. If less than one equivalent of the Grignard reagent is added to the aldehyde, the complex gives a yellow-brown precipitate. If the reaction is quenched at this stage, the aldehyde can be recovered quantitatively. The complex is probably formed from one molecule of the aldehyde by displacement of two ether molecules from the solvated Grignard reagent. If so, one ether molecule is displaced by the carbonyl oxygen, and the other by the furan oxygen. The second molecule of the Grignard reagent, which is needed to complete the reaction, is believed to react on the surface of the complex particle by a nucleophilic attack.



1a R = i-C_3H_7
b R = t-C_4H_9
c R = $PhCH_2$
d R = CH_3



2a R = i-C_3H_7
b R = t-C_4H_9
c R = $PhCH_2$
d R = CH_3

When isopropylmagnesium halide (RMgX; X = Cl, Br, and I) reacts with 2-furanacrolein, the corresponding 1,2-adduct 1-(2-furanyl)-4-methylpent-1-en-3-ol (*1a*) and 1,4-adduct 3-(2-furanyl)-4-methylpentanal (*2a*) are formed as the only products (GLC and NMR analysis). The products were separated by distillation and identified by spectroscopic means. Variation of the halogen and the concentration of the reagent resulted in the product distributions in Fig. 1. The chloride gives considerably more 1,2-

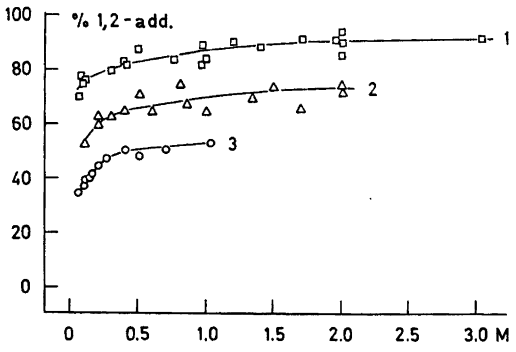


Fig. 1. Yield of 1,2-adduct *versus* the molarity of isopropylmagnesium halide (1,2- + 1,4-adduct = 100 %). 1, isopropylmagnesium chloride; 2, isopropylmagnesium bromide; 3, isopropylmagnesium iodide.

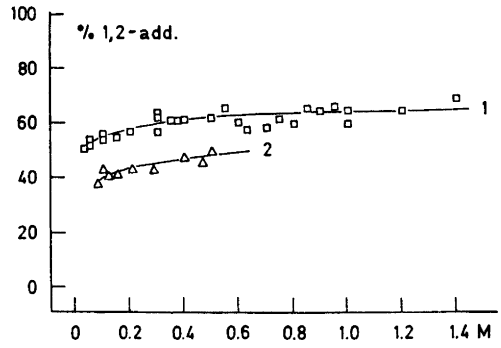


Fig. 2. Yield of 1,2-adduct *versus* the molarity of *tert*-butylmagnesium halide (1,2- + 1,4-adduct = 100 %). 1, *tert*-butylmagnesium chloride; 2, *tert*-butylmagnesium bromide.

addition product than the bromide and iodide, and the bromide in its turn more than the iodide.

When *tert*-butylmagnesium halide (RMgX; X = Cl and Br) reacts with 2-furanacrolein, the corresponding 1,2-adduct 1-(2-furanyl)-4,4-dimethylpent-1-en-3-ol (*1b*) and 1,4-adduct 3-(2-furanyl)-4,4-dimethylpentanal (*2b*) are the only products. These products were separated either by distillation or by preparative GLC, and identified as above. Variation of the halogen and the concentration of the Grignard reagent resulted in the distributions in Fig. 2. Also this chloride gives more of the 1,2-adduct.

Variation of the halogen and the concentration of the benzylmagnesium halide (RMgX; X = Cl, Br, and I) resulted in the product distributions in Fig. 3. The only reaction products formed are the 1,2-adduct 4-(2-furanyl)-1-phenylbut-3-en-2-ol (*1c*) and the 1,4-adduct 3-(2-furanyl)-4-phenylbutanal (*2c*). The chloride and the bromide give good yields of the 1,4-adduct, while the iodide almost exclusively gives the 1,2-adduct. The products were isolated by preparative GLC (the saturated aldehyde) and by steam distillation (the unsaturated alcohol).

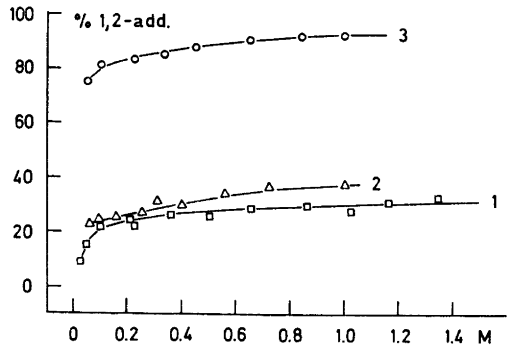


Fig. 3. Yield of 1,2-adduct *versus* the molarity of benzylmagnesium halide (1,2- + 1,4-adduct = 100 %). 1, benzylmagnesium chloride; 2, benzylmagnesium bromide; 3, benzylmagnesium iodide.

When methylmagnesium iodide reacts with 2-furanacrolein, the 1,2-adduct 4-(2-furanyl)but-3-en-2-ol (*1d*) is the only observed reaction product. A catalytic amount of copper(I) iodide added to the Grignard reagent in small portions during addition of the substrate failed to affect the fast reaction. When an equimolar amount of the copper salt was added to the Grignard reagent before the addition of the substrate, 16 % of the 1,4-adduct 3-(2-furanyl)butanal (*2d*) could be obtained. With lithium dimethylcuprate 85 % of the 1,4-adduct was formed. The separation of this product is simplified because no 1,2-adduct is formed in this reaction.

The reaction temperature (−15 to +25 °C) or the ratio of reagent to substrate (100:1 − 1.5:1) did not affect the observed product distribution in any of the Grignard reactions.

The first striking fact to be observed is the large proportion of 1,4-addition that can be achieved. Even with isopropyl reagent the conjugate addition product can become the main substance of the reaction mixture. Secondly, by combining the effect of the halogen with the effect of diluting the reagent, one obtains changes that can be of synthetical value, particulary in the case of benzylmagnesium halides.

The dilution of a Grignard reagent favours the 1,4-addition equally in the studied reactions, in spite of the dependence of the reagent association on the halogen of the reagent. This indicates a somewhat different molecularity in the transition state for the 1,2- and 1,4-reactions.

EXPERIMENTAL

The reactions between 2-furanacrolein and Grignard reagents were performed by adding 2-furanacrolein [16] dissolved in a minimal amount

of dry diethyl ether (1.0 g/10.0 ml) to a solution of a freshly prepared and titrated Grignard reagent. The reaction mixtures were worked up after about 5 min in the usual way using NH_4Cl-solution.

Analysis. After the reaction mixtures had been worked up, they were analysed by GLC using a 3 m (i.d. 1.7 mm) glass column packed with pretested 1 % XE-60 on 100–120 mesh Gas-Chrom Q supplied by Applied Science Laboratories Inc. The peaks were integrated by a Varian Integrator model 481. The mass spectra were taken on a combined GLC/MS instrument (LKB 9000). The preparative GLC separation was performed by using 5 % XE-60 on 60–80 mesh Chromosorb G in a 2 m (i.d. 4 mm) glass column. The fractionated distillation was done on a Todd distillation assembly. The NMR spectra were recorded on Bruker WH-270 and Perkin-Elmer R 12 spectrometers and the IR spectra on a Perkin-Elmer 257 spectrometer.

3-(2-Furanyl)-4-methylpentanal (2a), from the reaction between 2-furanacrolein and isopropylmagnesium bromide, was separated by fractionated distillation from a mixture containing 25 % of the aldehyde. The crude product (5.0 ml) was distilled, giving 0.5 ml of pure aldehyde, b.p. 66 °C/0.4 kPa. MS [IP 70 eV; *m/e* (% rel. int.)]: 166 (8, M), 137 (5, [M−CHO]), 123 (33, [M−C_3H_7]), 95 (100, C_6H_7O), 81 (25, C_5H_5O), 67 (91, C_4H_3O), 43 (37, C_3H_7), 41 (94, C_3H_5), 39 (73, C_3H_3). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.44 (1 H, dd, J_{AB} 2.4 Hz, J_{AC} 1.9 Hz, H_A), 7.29 (1 H, dd, J_{GF} 1.9 Hz, H_G), 6.26 (1 H, dd, J_{FE} 3.4 Hz, H_F), 6.01 (1 H, ddd, J_{EG} 0.7 Hz, J_{ED} 0.8 Hz, H_E), 3.14 (1 H, m, J 5.7 Hz, H_D), 2.74 (1 H, ddd, J_{BD} 9.3 Hz, J_{BC} 16.6 Hz, H_B), 2.63 (1 H, ddd, J_{CD} 5.2 Hz, H_C), 1.96 (1 H, m), 0.90 (3 H, d, J 6.7 Hz), 0.84 (3 H, d, J 6.7 Hz). IR (CCl_4): Aliphatic CH 2980–2880, aldehyde CH 2820 and 2720, aldehyde C=O 1735 cm^{-1}.

1-(2-Furanyl)-4-methylpent-1-en-3-ol (1a) was purified by distilling the crude product from the reaction of 2-furanacrolein with isopropylmagnesium chloride. By this simple method a 95 % pure product could be obtained, b.p. 87.5–88 °C/0.8 kPa. MS [IP 70 eV; *m/e* (% rel. int.)]: 166 (12, M), 149 (1, [M−OH]), 148 (2, [M−H_2O]), 123 (92, [M−C_3H_7]), 95 (34, C_6H_7O), 81 (36, C_5H_5O), 67 (50, C_4H_3O), 55 (100, C_4H_7), 43 (59, C_3H_7), 41 (97, C_3H_5), 39 (93, C_3H_3). ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.33 (1 H, dd, J_{GF} 1.9 Hz, H_G), 6.40 (1 H, dd, J_{CA} 1.0 Hz, H_C), 6.36 (1 H, dd, J_{FE} 3.5 Hz, H_F), 6.22 (1 H, dd, J_{EG} 1.0 Hz, H_E), 6.18 (1 H, dd, J_{BC} 16.0 Hz, H_B), 4.00 (1 H, m, J_{AB} 6.5 Hz, J 1.0 Hz, H_A), 1.81 (1 H, m), 1.57 (1 H, s, H_D), 0.97 (3 H, d, J 6.9 Hz), 0.95 (3 H, d, J 6.9 Hz). IR (CCl_4): Alcohol OH 3620 and 3490, aliphatic CH 2980–2880, olefinic C=C 1670 cm^{-1}.

3-(2-Furanyl)-4,4-dimethylpentanal (2b), from the reaction of 2-furanacrolein with *tert*-butylmagnesium chloride, was separated by fractionated distillation. The crude product (7.2 g), containing 35 % of the aldehyde, was distilled, giving 1.3 g (51 %) of 98 % pure aldehyde, b.p. 94 °C/1.3 kPa. MS [IP 70 eV; *m/e* (% rel. int.)]: 180 (18, M), 165 (4, [M−CH_3]), 151 (4, [M−CHO]), 124 (44, [M−C_4H_8]), 123 (13, [M−C_4H_9]), 95 (41, C_6H_7O), 81 (15, C_5H_5O), 57 (100, C_4H_9), 41 (24, C_3H_5). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.58 (1 H, dd, J_{AB} 2.5 Hz, J_{AC} 1.8 Hz, H_A), 7.30 (1 H, dd, J_{GF} 2.0 Hz, H_G), 6.28 (1 H, dd, J_{FE} 3.1 Hz, H_F), 6.04 (1 H, ddd, J_{EG} 1.0 Hz, J_{ED} 0.5 Hz, H_E), 3.09 (1 H, dd, H_D), 2.82 (1 H, ddd, J_{BD} 10.9 Hz, J_{BC} 16.5 Hz, H_B), 2.66 (1 H, ddd, J_{CD} 3.9 Hz, H_C), 0.93 (9 H, s). IR (CCl_4): Aliphatic CH 2960–2870, aldehyde CH 2820 and 2720, aldehyde C=O 1735 cm^{-1}.

1-(2-Furanyl)-4,4-dimethylpent-1-en-3-ol (1b). The residue from the distillation above was distilled over a short column. By this method 1.9 ml (40 %) of the unsaturated alcohol could be obtained. B.p. 127 °C/2.3 kPa. MS [IP 70 eV; *m/e* (% rel. int.)]: 180 (11, M), 165 (1, [M−CH_3]), 163 (1, [M−OH]), 123 (100, [M−C_4H_9]), 95 (16, C_6H_7O), 81 (9, C_5H_5O), 67 (14, C_4H_3O), 57 (23, C_4H_9). ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.33 (1 H, dd, J_{GF} 1.9 Hz, H_G), 6.41 (1 H, dd, J_{CA} 0.7 Hz, H_C), 6.36 (1 H, dd, J_{FE} 3.3 Hz, H_F), 6.24 (1 H, dd, J_{BC} 16.0 Hz, H_B), 6.22 (1 H, d, H_E), 3.89 (1 H, dd, J_{AB} 6.7 Hz, H_A), 1.26 (1 H, s, H_D) 0.97 (9 H, s). IR (CCl_4): Alcohol OH 3620, aliphatic CH 2960–2870, olefinic C=C 1670 cm^{-1}.

3-(2-Furanyl)-4-phenylbutanal (2c), from the reaction of benzylmagnesium chloride with 2-furanacrolein, was purified by preparative GLC. MS [IP 70 eV; *m/e* (% rel. int.)]: 214 (5, M), 185 (7, [M−CHO]), 123 (62, [M−C_7H_7]), 95 (100, C_6H_7O), 91 (64, C_7H_7), 67 (73, C_4H_3O). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.57 (1 H, dd, J_{AB} 2.0 Hz, J_{AC} 1.7 Hz, H_A), 7.3–7.0 (5 H, m), 7.28 (1 H, dd, J_{GF} 1.8 Hz, H_G), 6.20 (1 H, dd, J_{FE} 3.2 Hz, H_F), 5.90 (1 H, ddd, J_{EG} 1.0 Hz, J_{ED} 0.6 Hz, H_E), 3.56 (1 H, m, J 7.0 Hz, J 7.5 Hz, H_D), 3.03 (1 H, dd, J 13.7 Hz), 2.83 (1 H, dd), 2.65 (1 H, ddd, J_{BD} 7.8 Hz, J_{BC} 16.8 Hz, H_B), 2.58 (1 H, ddd, J_{CD} 6.1 Hz, H_C).

4-(2-Furanyl)-1-phenylbut-3-en-2-ol (1c), from the reaction of benzylmagnesium iodide with 2-furanacrolein, was purified by steam distillation. The crude product (5 g) was purified by the steam from 250 ml of water, giving 3,7 g of 94 % pure aldehyde. MS [IP 70 eV; *m/e* (% rel. int.)]: 214 (4, M), 196 (12, [M−H_2O]), 123 (100, [M−C_7H_7]), 95 (18, C_6H_7O), 91 (25, C_7H_7), 81 (20, C_5H_5O), 77 (18, C_6H_5), 67 (23, C_4H_3O), 55 (44, C_3H_3O). ^{1}H NMR (60 MHz, CCl_4): δ 7 (5 H, m), 7.05 (1 H, d, J_{GF} 2.0 Hz, H_G), 6.15 (1 H, d, H_C), 6.08 (1 H, dd, J_{FE} 3.2 Hz, H_F), 5.97 (1 H, dd, J_{BC} 15.3 Hz, H_B), 5.92 (1 H, d, H_E), 4.20 (1 H, dt, J_{AB} 4.0 Hz, H_A), 2.69 (2 H, d, J 6.2 Hz), 2.33 (1 H, s, H_D). IR (CCl_4): Alcohol OH 3620 and 3480, Ar CH 3100–3000, aliphatic CH 2960–2860, olefinic C=C 1665 cm^{-1}.

3-(2-Furanyl)butanal (2d), from the reaction of lithium dimethylcuprate with 2-furanacrolein. The reagent was prepared under N_2-atmosphere from methyllithium (59.6 mmol) and copper(I) iodide (5.2 g, 30 mmol) in absolute diethyl ether (distilled from sodium benzophenone ketyl) at 0 °C. After 15 min the reagent showed a negative Gilman Test I.[17] The reagent was cooled to about −35 °C in an acetone bath cooled with dry ice and 1.83 g (15 mmol) of 2-furanacrolein in ether was added *via* a hypodermic syringe. The reaction was worked up after 20 min. The GLC yield of the 1,4-adduct was 85 %. The crude product was distilled over a short column giving *ca* 1 ml of pure product; B.p. 85 °C/2.7 kPa. MS [IP 70 eV; *m/e* (% rel. int.)]: 138 (29, M), 123 (10, [M−CH_3]), 109 (12, [M−CHO]), 95 (100, C_6H_7O), 81 (15, C_5H_5O), 68 (40, C_4H_4O), 67 (38, C_4H_3O), 41 (48, C_3H_5), 39 (44, C_3H_3). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.72 (1 H, t, J_{AB} 2.0 Hz, J_{AC} 2.0 Hz, H_A), 7.29 (1 H, dd, J_{GF} 2.0 Hz, H_G), 6.26 (1 H, dd, J_{FE} 3.4 Hz, H_F), 6.00 (1 H, ddd, J_{EG} 1.0 Hz, J_{ED} 1.0 Hz, H_E), 3.41 (1 H, m, J 6.5 Hz, H_D), 2.76 (1 H, ddd, J_{BD} 6.5 Hz, J_{BC} 16.8 Hz, H_B), 2.56 (1 H, ddd, J_{CD} 7.2 Hz, H_C), 1.30 (3 H, d).

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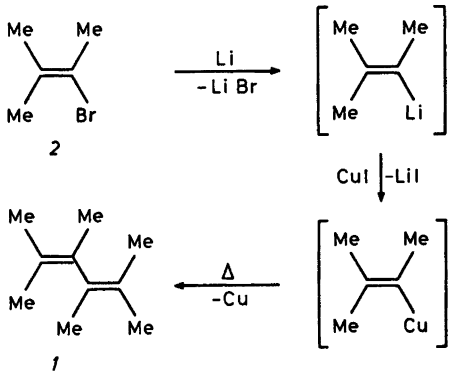
Synthesis and Molecular Structure of 2,3,4,5-Tetramethyl-2,4-hexadiene

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2,3,4,5-Tetramethyl-2,4-hexadiene (*1*) has been prepared in 23 % yield from 2-bromo-3-methyl-2-butene in a two-step synthesis. The molecular structure of *1* has been determined. The molecule was found to have *gauche* conformation.

2,3,4,5-Tetramethyl-2,4-hexadiene (*1*) was first isolated in 1955 by Braude and Evans [1] as a by-product (< 7 %) in the preparation of 1,2-dimethyl-1-propenyllithium from 2-bromo-3-methyl-2-butene (*2*). Later two synthetic pathways to this diene have been published. Criegee *et al.*[2] isolated *1* in 18 % overall yield in a five-step synthesis starting from 2-butyne and dimethylmaleic anhydride, while Forbes *et al.*[3] prepared the compound from 2-methyl-2-butene as starting material. For a number of reasons we found the last procedures unsatisfactory, and we therefore turned our attention to the original observation of Braude and Evans.



Scheme 1.

It has been shown recently that coupling of vinylic lithium [4] and Grignard [5] derivatives by means of copper(I) salts give the corresponding dienes in good yields, and this procedure was attempted for the preparation of diene *1*. The bromide *2* was converted into the corresponding lithium or Grignard derivative and either copper(I) chloride, copper(I) iodide or tetrakis[iodo(tributylphosphine)copper(I)] was used as coupling reagent. In all cases pure *1* was isolated, but the yields varied with the reagents used, being highest (23 %) from the reaction of the lithium derivative and cuprous iodide (Scheme 1).

The molecular structure of 2,3,4,5-tetramethyl-2,4-hexadiene poses interesting problems. Conjugated dienes show a strong preference for assuming an *s-trans* conformation, but because of the steric requirements of the methyl groups an *s-trans* conformation is energetically unfavourable for *1*. The determination of the preferred conformation of *1* is therefore of great interest. The obtained results are expected to contribute to elucidating the torsional potential energy of $C_{sp^2}-C_{sp^2}$ bonds.

EXPERIMENTAL

Preparation of 2,3,4,5-tetramethyl-2,4-hexadiene (1). Under a nitrogen atmosphere a solution of 75.0 g (0.50 mol) of 3-bromo-2-methyl-2-butene (*2*) [6] in 150 ml of dry ether was added to 7.8 g (1.13 mol) of finely cut lithium (0.9 % sodium) in 600 ml of dry ether with vigorous stirring and cooling (ice/water). After 20 h of stirring at 40 °C unreacted lithium was filtered off and the filtrate was added dropwise during

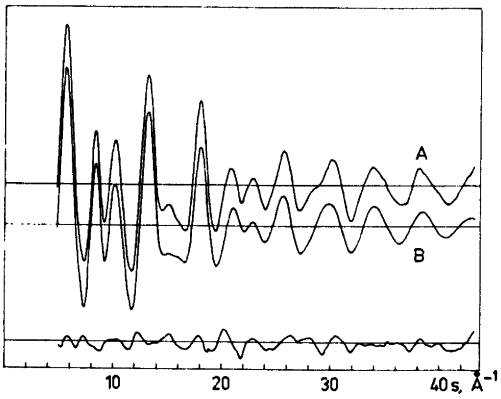


Fig. 1. Experimental (A) and theoretical (B) molecular intensity functions of *1* and the difference between the two.

1½ h to a suspension of 124.0 g (0.65 mol) of anhydrous cuprous iodide in 800 ml of dry ether kept at −60 °C (CO_2/acetone). After addition was complete the greyish mixture was stirred for 1 h at this temperature, then allowed to attain room temperature overnight, and finally heated with reflux for 2 h. 300 ml of 0.3 N HCl was added, the inorganic salts were removed by filtration, and the product was extracted with ether and dried ($MgSO_4$). Evaporation of the ether and fractionation of the residue through a spinning-band column gave 8.0 g (23 %) of *1* b.p. 73.5 °C (60 mmHg); n_D^{24} 1.4500 (lit.[3] b.p. 143–144 °C; n_D^{24} 1.4486); gas chromatography showed a purity of better than 98.5 %. The spectroscopic data are in accordance with those published.[2]

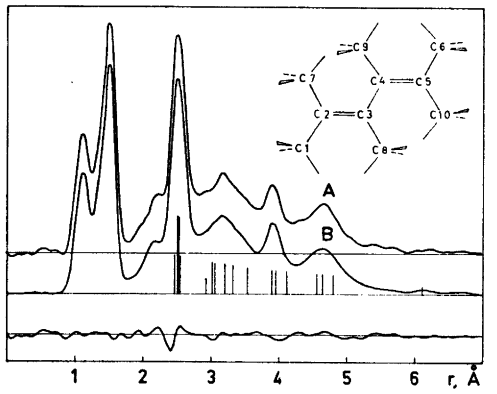


Fig. 2. Experimental (A) and theoretical (B) radial distribution functions of *1*, and the differences between the two. Artificial damping constant k = 0.0012.

Electron diffraction experiment. The electron diffraction data of *1* were collected with the Oslo Apparatus.[7] Two sets of photographic plates were recorded at nozzle-to-plate distances of 480.71 and 200.71 mm, respectively. Both sets were recorded at room temperature and at an electron wavelength of 0.064628 Å. From the two sets of data 5, respectively 4, plates were selected for further use. The data were treated in the usual way using the modification function $s/|f_C'|^2$.[8] Two average intensity curves were computed, one from each set of the data. The curves covered the s intervals 1.50 – 19.25 and 7.00 – 43.25 Å^{-1} with Δs values of 0.125 and 0.250 Å^{-1}, respectively. Finally a composite intensity curve was computed (see Fig. 1).

The experimental radial distribution (RD) curve [8] calculated by Fourier inversion of the composite intensity curve is shown in Fig. 2. The molecular structure of the title compound was determined by least squares refinements of the molecular intensities.

STRUCTURAL ANALYSIS

The molecular model that was used in the least squares refinements, was based on the following assumptions:

1. coplanar bonds around each of the CC double bonds;

2. local C_{3v} symmetry at each of the methyl groups;

3. the symmetry axis of each of the methyl groups coinciding with the adjoining CC bond;

4. one of the hydrogen atoms of each methyl group coplanar with the nearest CC double bond [$\omega(=C-C-H)=0°$].

The first two assumptions are reasonable. The latter two are probably not justified to the same extent, as the methyl groups in *cis*-2-butene [9] were found to be tilted away from the double bond (4.9°) and also rotated (9.0°) about the adjacent C – C bonds in such a way as to increase the distance between the two crowded hydrogen atoms. In the present case it was not possible to determine the tilt and rotation parameters of the methyl groups because of the larger complexity of this molecule. As the error limits of the tilt and rotational parameters determined for *cis*-2-butene were quite large and of the same order of magnitude as the observed deviations from the conditions inherent in assumptions 3 and 4, it was decided to use these in the present case. They will, however, hardly affect the determination of the carbon skeletal structure.

With the assumptions given above the geometry of *1* is determined by ten parameters. These are the four different bond distances (=C–C=, C=C, C–CH_3 and C–H), five bond angles ($\angle C_1-C_2=C_3$, $\angle C_2=C_3-C_8$, $\angle C_2=C_3-C_4$, $\angle C_3=C_2-C_7$, $\angle C-C-H$, see Fig. 2 for the numbering of atoms) and the dihedral angle of the central CC bond, $\omega(C_3-C_4)$.

By studying the experimental RD function it was fairly easy to rule out a molecular model of *1* with a planar carbon skeleton. The internuclear distances of a molecular model corresponding to *s-gauche* conformation fitted, however, quite well to the various peaks of the experimental RD-curve.

It was not possible to vary all CCC valence angles simultaneously in the least squares refinements. One valence angle was therefore kept constant during one run, but was varied systematically over the expected range. The value corresponding to the lowest squared error sum was then used for this angle parameter. As the angles $\angle C_2=C_3-C_8$ and $\angle C_3=C_2-C_7$ were found to be very similar, they were set equal in the latter part of this investigation.

Only a few of the vibrational amplitudes (u) could be refined together with the geometrical parameters. The other u values were either kept at the calculated values or adjusted somewhat based on the shape of the peaks in the RD-curve.

The RD-curve corresponding to the best molecular model determined by least squares refinements of the intensities showed obvious shrinkage effects when the dependent distances were based on an r_a structure. Especially the peak at 3.9 Å (C_3C_6 and C_7C_8) was clearly displaced to larger r-values in the theoretical RD-curve compared to the experimental one. These effects did, however, vanish when an r_α structure was studied, as will be seen from Fig. 2, which shows the experimental RD-curve together with the corresponding theoretical curve based on the best r_α structure. The mean vibrational amplitudes and the correction coefficients necessary to determine an r_α structure were calculated as described by Stølevik *et al.*,[11] using Allinger's force constants.[12]

The structural parameters determined for *1* are presented in Table 1. Table 2 gives the non-bonded carbon carbon distances together with the vibrational amplitudes that were used in the least squares analyses (u^{ED}) as well as those calculated (u^{calc}). Fig. 2 shows the numbering of the atoms in *1*, which is used in the present study. For the sake of convenience the model is shown with *s-trans* conformation.

DISCUSSION

The most important parameter in the 2,3,4,5-tetramethyl-2,4-hexadiene molecular structure is the C_3-C_4 dihedral angle, which determines the overall comformation of the molecule. It is well known that conjugated CC double bonds have a strong preference for assuming *s-trans* conformations wherever this is possible. In the present case an *s-trans* conformation is expected to be energetically unfavourable because of serious sterical interference between pairs of methyl groups. The C_3-C_4 dihedral angle is determined to be 60.0°, corresponding to *s-gauche*

Table 1. Geometrical parameters and vibrational amplitudes for 2,3,4,5-tetramethyl-2,4-hexadiene as determined from least squares refinements. The numbers in parentheses are standard deviation values from the least squares analyses. The bond distances are given as r_a values.

Distances	r_a (Å)	u (Å)	Angles	(°)
=C–C=	1.487 [a]	0.050	$\angle C_1-C_2=C_3$	125.2 [a]
–C=C–	1.349(2)	0.041(3)	$\angle C_2=C_3-C_8$	123.6(2)
=C–C–	1.515(1)	0.056(1)	$\angle C_2=C_3-C_4$	125.0(2)
C–H	1.117(2)	0.087(3)	$\angle C_3=C_2-C_7$	123.6 [b]
			$\angle C-C-H$	110.5(1)
			$\omega(C_3-C_4)$	60.0(<1)

[a] Determined by the combined trial and error/least squares method described in the text. [b] Assumed equal to $\angle C_2=C_3-C_8$.

Table 2. Non-bonded carbon carbon distances and the corresponding mean amplitudes of vibration.

	r_a (Å)	u^{ED} (Å)	u^{calc} (Å)
C_3C_9	2.459		0.0878
C_3C_5	2.493		0.0749
C_1C_7	2.503	0.0795[a](67)	0.0893
C_8C_7	2.543		0.0763
C_1C_3	2.516		0.0753
C_8C_9	2.922	0.1850	0.1873
C_3C_{10}	3.053	0.1550	0.1553
C_7C_{10}	3.204	0.2100	0.2115
C_1C_8	3.010	0.1530	0.1538
C_3C_5	3.202	0.1200	0.1403
C_9C_{10}	3.316	0.2000	0.2607
C_5C_9	3.529	0.1250	0.1263
C_3C_6	3.890	0.0788	0.0788
C_7C_8	3.947	0.0795	0.0795
C_7C_9	4.115	0.1860	0.1860
C_6C_7	4.553	0.2597	0.2770
C_2C_6	4.639	0.1325	0.1389
C_1C_9	4.791	0.1270	0.1366
C_1C_6	6.105	0.1302	0.1302

[a] Assumed equal.

conformation. The standard deviation of this parameter is found to be unusually small.

Similar sterical interferences between pairs of methyl groups as in *1* are encountered in the *trans, trans* and *cis, trans* isomers of 3,4-dimethyl-2,4-hexadienes.[10] These molecules were also found to have *s-gauche* conformations, but the dihedral angles were slightly larger (66.7 and 65.7°) than in *1*. The results obtained for the C_3-C_4 dihedral angle indicate that a three-fold barrier of rotation probably makes an important contribution to the torsional energy of a $C_{sp^2}-C_{sp^2}$ single bond.

The central CC single bond is found to be longer than the $C_{sp^2}-C_{sp^2}$ bond in butadiene (1.487 *vs.* 1.467 Å).[13] This effect probably reflects small differences in hybridization of the carbon atoms in the two molecules. This explanation is in accordance with the observation that the two other σ bonds directing from each of the carbon atoms under discussion makes a larger angle in *1* (123.6°) than in butadiene (119.5°).

The other bond lengths determined for *1* agree well with results obtained for structurally related molecules [14] and will not be discussed further.

The determined bond angles show that some of the strain in the molecule is relieved through enlarged valence angles at the double bonds. In *cis*-2-butene the CCC valence angle is found to be 125.4°. In the present case the C=C–C valence angles at the *cis* methyl groups are found to be 123.6°. In *1* the sterical strain is, however, substantial also on the other side of the double bond, and it will be seen that the CCC valence angles are slightly larger on this side (125°). The differences between the various valence angles at the double bonds are fairly small and the angles can hardly be claimed to be significantly different, even if the standard deviations of those angles that are varied in the least squares refinements are unusually small.

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Intermediates in the Barnol Biosynthesis in *Penicillium baarnense*

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By using appropriate ^{14}C-labelled phenolic substances as precursors in feeding experiments the following steps in barnol biosynthesis have been established:
acetate + malonate → 2,4-dihydroxy-6-ethyl-5-methylbenzaldehyde → 1,3-dihydroxy-4,6-dimethyl-2-ethylbenzene → barnol
P. baarnense can also reduce orcylaldehyde and 2,4-dihydroxy-5,6-dimethylbenzaldehyde to dimethylresorcinol and trimethylresorcinol, respectively.

Barnol (*9*) is the main secondary product when *Penicillium baarnense* is grown on Raulin-Thom medium whereas orsellinic acid and penicillic acid are the major metabolites when Czapek-Dox medium is used as substrate.

In order to investigate this phenomenon we found it important to know the detailed pathways of the formation of penicillic acid and barnol, respectively. We have recently been able to show how penicillic acid is formed in *Penicillium cyclopium*.[3] Some of the intermediates demonstrated in *P. cyclopium* have been found in *P. baarnense*.[4] One of them, namely 1,4-dihydroxy-2-methoxy-6-methylbenzene was biosynthesized from ^{14}C-labelled acetate and its conversion to penicillic acid in *P. baarnense* was confirmed.

Mosbach and Ljungkrantz [1,2] performed some radioactive feeding experiments that demonstrated the polyketide character of barnol and revealed interesting features of the origin of the three alkyl groups. One of the methyl groups attached to the aromatic nucleus is derived from the C-1 metabolism and is presumably inserted as *S*-adenosylmethionine. The other nuclear methyl group is the result of reduction of a former malonate carboxyl group. Finally, the ethyl group is formed by a *C*-methylation of an original acetate methyl group.

The potentiality of the organism to form orsellinic acid as well as barnol, but on different media, may indicate a biogenetic relationship between the two compounds. Theoretically, orsellinic acid could be a precursor of barnol or alternatively the primary polyketide chain [5] could be identical in the two cases. The former possibility can be tested by feeding ^{14}C-labelled orsellinic acid to the organism. When doing so no radioactivity is incorporated into barnol. Being well aware of the weakness of drawing conclusions of negative incorporation experiments we prefer to point to the successful feeding experiments that will be described for excluding orsellinic acid as a precursor of barnol.

Several other investigations indicate that biological *C*-methylation of an aromatic structure is a rare phenomenon and that the methyl group is preferably introduced at a prearomatic level. 5-Methylorsellinic acid [5] is then a possible precursor of barnol if the nuclear methyl group is substituted onto the open polyketomethylene chain, prior to cyclization and if the ethyl group is subsequently formed by a methylation reaction after cyclization. ^{14}C-Carboxyl labelled 5-methylorsellinic acid was synthesized and fed to the organism but no incorporation was obtained into barnol or any other phenolic compound. However, when ^{14}C-formyl labelled 5-methylorcylaldehyde was used as substrate two radioactive phenolic metabolites were isolated. By recrystallizations to constant specific radioactivity with authentic compounds

Table 1. Incorporation of ^{14}C-labelled precursors.

Radioactive substrate			
Compound	Spec. radioactivity dpm/mmol × 10^{-7}	Product	Incorporation of radioactivity %
Orcylaldehyde (*1*)	12.7	4,5-dimethylresorcinol (*2*)	6.1
2,4-Dihydroxy-5,6-dimethylbenzaldehyde (*3*)	1.3 1.3	4,5,6-trimethylresorcinol (*4*) 4,5,6-trimethylpyrogallol (*7*)	7.8 4.4
2,4-Dihydroxy-5,6-dimethylbenzoic acid (*5*)	4.7	4,5,6-trimethylpyrogallol (*7*)	0
4,5,6-Trimethylresorcinol (*4*)	5.0 5.0 [a]	4,5,6-trimethylpyrogallol (*7*) 4,5,6-trimethylpyrogallol (*7*)	0 10 [b]
2,4-Dihydroxy-6-ethyl-5-methylbenzaldehyde (*8*)	18.4	barnol (*9*)	24.0

[a] Cell-free experiment. [b] Value from radioscanning of TLC.

these phenols were identified as 4,5,6-trimethylresorcinol (*4*) and 4,5,6-trimethylpyrogallol (*7*), respectively. In the same way ^{14}C-formyl labelled orcylaldehyde gave rise to labelled 4,5-dimethylresorcinol (*2*). The results indicate the following. The methionine derived nuclear methyl group is introduced into the structure before the polyketide chain is ring-closed. The aldehyde group is reduced to a methyl group. The reduction of the original carboxyl group may thus pass the aldehyde level. The failure of metabolizing exogenous 5-methylorsellinic acid to trimethyl resorcinol or trimethylpyrogallol may depend on the inability of the organism to activate the aromatic carboxyl group with the possible formation of a thiol ester which may be the site for the initial reduction. The methylation of the acetate methyl group with the formation of the ethyl group of barnol apparently also takes place prior to cyclization of the open polyketide chain, as judged from the formation of the lower homolog and not barnol when 2,4-dihydroxy-5,6-dimethylbenzaldehyde (*3*) is given to the organism. Furthermore, when the organism is fed with ^{14}C-formyl labelled 2,4-dihydroxy-6-ethyl-5-methylbenzaldehyde (*6*) an appreciable conversion of the aldehyde into barnol is observed. Finally, the experiments indicate that in the formations of barnol and trimethylpyrogallol the methylation reactions and the reduction of the carboxyl group precede the hydroxylation of the aromatic structure. In an attempt to show the hydroxylation in a single step reaction ^{14}C-labelled 4,5,6-trimethylresorcinol was fed to the organism but no radioactive 4,5,6-trimethylpyrogallol could be isolated. To avoid permeability problems a corresponding experiment was performed with a homogenate of the cells whereby a significant formation of the pyrogallol derivative was obtained giving support to the suggestion that hydroxylation is the last step in the formation of barnol. The results from the various feeding experiments are shown in Table 1. Fig. 1 summarizes the reactions performed by the organism and Fig. 2 proposes the metabolic sequence of barnol formation as supported by the experimental evidences.

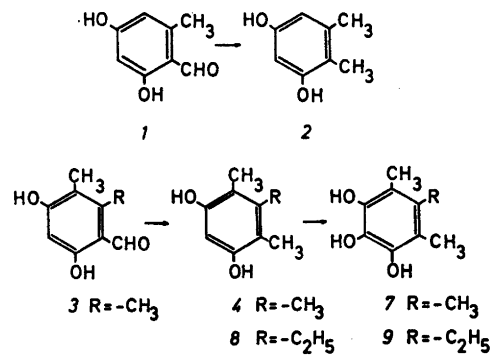


Fig. 1. Chemical transformations performed by *P. baarnense.*

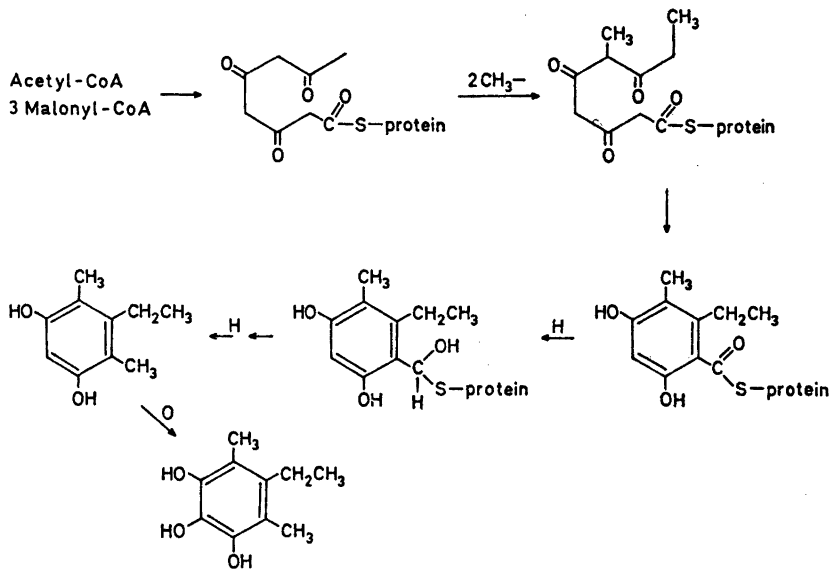


Fig. 2. Pathway suggested for the biosynthesis of barnol.

EXPERIMENTAL

Culture conditions. Penicillium baarnense v. Beyma, CBS 315.59 was grown in 500 ml conical flasks on a rotary shaker (2.5 cm stroke, 240 rpm) at 28 °C. Each flask contained 150 ml of Raulin-Thom medium with the following composition: $(NH_4)_2HPO_4$, 0.6 g; $(NH_4)_2SO_4$, 0.25 g; K_2CO_3, 0.6; $MgCO_3$, 0.4 g; $FeSO_4 . 7H_2O$, 0.07 g; $ZnSO_4 . 7H_2O$, 0.07 g; tartaric acid, 4.0 g; ammonium tartrate, 4.0 g; glucose, 75 g; yeast extract, 1.5 g; distilled water, 1500 ml.

Preparation of radioactive precursors

Orcylaldehyde (1). The aldehyde was prepared as described by Adams and Levine.[5] M.p. 184 °C (lit.[5] 178–180 °C), yield 85 %. Orcylaldehyde-^{14}C-formyl (12.7×10^7 dpm/mmol) was obtained by adding $Na^{14}CN$ (0.5 mCi) to the reacting $Zn(CN)_2$.

4,5-Dimethylresorcinol (2). Clemmensen reduction of *1* yielded *2* which was purified by recrystallization from water, m.p. 137–138 °C (lit.[6] 137–138 °C), yield 90 %.

2,4-Dihydroxy-5,6-dimethylbenzaldehyde (3). A Gattermann reaction on *2* gave the formyl derivative. The ^{14}C-labelled product was obtained by adding $Na^{14}CN$ (0.5 mCi) to the reaction mixture, m.p. 199–201 °C (lit.[7] 196–197 °C), yield 90 %, specific radioactivity 1.3×10^7 dpm/mmol.

4,5,6-Trimethylresorcinol (4). Compound *3* was reduced in a Clemmensen reaction. The product was extracted into ether, washed with aqueous $NaHCO_3$, water, and finally filtered through Florisil. Yield 85 %, m.p. 165–166 °C (from cyclohexane) (lit.[8] 163–165 °C). The ^{14}C-labelled substance was prepared in a similar manner.

2,4-Dihydroxy-5,6-dimethylbenzoic acid (^{14}COOH) *(5).* Acetylation of *3* (yield 90 %) and subsequent $KMnO_4$-oxidation (yield 65 %) gave the acetylated *5* m.p. 138–141 °C. Hydrolysis of the acetylated compound in 10 % aqueous KOH gave *5*.

2,6-Dihydroxy-3,4,5-trimethylbenzaldehyde (6). A Gattermann reaction was performed with $Zn(CN)_2$ and *4*. Compound *6* was purified by filtration of an ether solution through Florisil and subsequent sublimation at 130 °C/0.1 mmHg, m.p. 138–140 °C (lit.[8] 136 °C), yield 54 %.

4,5,6-Trimethylpyrogallol. (7). Compound *7* was obtained in a Dakin oxidation of *6* by adding 125 ml 3 % H_2O_2 for one hour to 180 mg (1 mmol) *6*, 0.36 ml (1 mmol) $[(CH_3)_4N]OH$ and 2 ml 50 % aqueous ethanol. The temperature was kept at 40–50 °C and the reaction performed under nitrogen. After 3 h 15 ml saturated aqueous $(NH_4)_2SO_4$ was added and *7* repeatedly extracted with ether. The extract was washed in order with aqueous $NaHCO_3$, $Na_2S_2O_4$, and water. The solid residue remaining after evaporation of the solvent was sublimed at 140 °C/1 mmHg and recrystallized from CCl_4. Yield 67 %, m.p. 166–169 °C (lit.[6] 165–166 °C). Anal. $C_9H_{12}O_3$: C, H.

2,4-Dihydroxy-6-ethyl-5-methylbenzaldehyde (8). 14.2 g 3,5-dimethoxyacetophenone was re-

duced in a Clemmensen reduction using a two phase solvent system (benzene-water). The product, a colorless oil (10.6 g, yield 81 %), was demethylated by refluxing with HI and red P in glacial acetic acid. The obtained 5-ethylresorcinol was formylated in a Gattermann reaction. The aldehyde was sublimed at 100 °C/0.1 mmHg and recrystallized from benzene, m.p. 99 – 100 °C (lit.[9] 99 – 100 °C), yield 98 %. 5-Ethyl-4-methylresorcinol was prepared in quantitative yield by a Clemmensen reduction of the aldehyde. ^{14}C-labelled *8* was synthesized as described for *1*. Purification of *8* was made by sublimation at 150 °C/0.1 mmHg and recrystallization from benzene, m.p. 158 – 159 °C (lit.[6] 157 – 158 °C), specific radioactivity 18.4×10^7 dpm/mmol.

^{14}C-Incorporation experiments

In the feeding experiments generally 2 – 9 mg of the synthesized radioactive precursors dissolved in a small volume of water were added to the organism grown for 3 to 4 days. After another day of incubation the phenolic products were extracted with ether from the acidified culture filtrate. The metabolic conversion of the added precursors and the nature of the formed radioactive phenolic compounds were checked by analyzing a small portion of the ether extract by TLC (benzene:dioxane:acetic acid (95:25:4), silica gel). After washing the ether solution with aqueous $NaHCO_3$ and water it was evaporated to dryness. The residue was usually sublimed before recrystallization to constant specific radioactivity with non-labelled carrier substance.

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Reductive Coupling of Anthracene with 1,2- and 1,3-Dihalides

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The reductive coupling of electrolytically generated anthracene anion radical with some 1,2- and 1,3-dihalides has been investigated. Coupling of 1,3-dihalides in the 1- or 2-position resulted in ring closure to cyclopentano anthracene derivatives.

Anion radicals, generated chemically [1] or electrochemically, [2,3] are useful reagents for reductive eliminations. Anion radicals may, however, couple with simple [4–6] alkyl halides or dihalides;[7,8] below is described the reaction between electrolytically generated anthracene (*1*) anion radical and the following 1,2- and 1,3-dihalides: 1,2-dichloroethane (*2*), 1,3-dibromopropane (*3*), and 1-bromo-3-chloropropane (*4*).

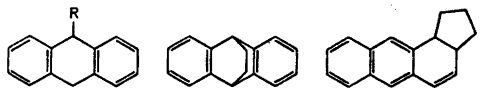
RESULTS

Electrochemical reduction of *1* in *N*,*N*-dimethylformamide (DMF) containing tetrabutylammonium iodide (TBAI) in the presence of *2* gave a mixture of 9-(2′-chloroethyl)-9,10-dihydroanthracene (*5*) and 9,10-dihydro-9,10-ethanoanthracene (*6*). The ratio of *5* to *6* depended on the relative concentrations of *1* and *2*, the higher the *2*/*1* ratio the higher the *5*/*6* ratio. The structure of *5* was proposed on the basis of the ^{1}H NMR and mass spectra (see Experimental). Besides *5* and *6* minor amounts of 9,10-dihydroanthracene were sometimes isolated.

In the presence of *3* the electrochemical reduction of *1* gave a mixture of 2,3,3a,11b-tetrahydrocyclopenta[*a*]-anthracene (*7*) and 9-(3′-bromopropyl)-9,10-dihydroanthracene (*8*). The proposal for the structure of *7* rested on the ^{1}H and ^{13}C NMR spectra, the UV and mass spectra. The UV spectrum excluded a 9,10-dihydroanthracene, the allylic coupling to the ethylenic protons in the ^{1}H NMR spectrum showing the position of the cyclopentano ring.

A reduction of *1* in the presence of *4* gave a mixture of *7* and 9-(3′-chloropropyl)-9,10-dihydroanthracene (*9*).

Table 1 shows the yields of the products, the *n*-values and the differences (ΔE) in the voltammetric peak potentials (E_p) between *1* and the dihalides.



5, R = CH_2CH_2Cl *6*, *7*,
8, R = $CH_2CH_2CH_2Br$
9, R = $CH_2CH_2CH_2Cl$

DISCUSSION

Cyclic voltammetry of *1* in the presence of *2*, *3*, or *4* showed that the peak current increased on addition of any one of the dihalides; the increase was largest for *3* and smallest for *2* and thus the larger the smaller ΔE_p (Table 1) was. This may be interpreted [10] as a reduction of the dihalide by electron transfer in solution from $1^{-\cdot}$. The catalytic reduction of the dihalides [2] explains the high *n*-values in the preparative reactions.

The reactions 1 to 5 have previously [2,6] been proposed for the reaction between electrochemically generated anion radicals ($A^{-\cdot}$) and alkyl halides; here the dihalides are represented by YBX.

$$A + e^- \rightleftharpoons A^{-\cdot} \quad (1)$$

$$A^{-\cdot} + YBX \rightleftharpoons A + YBX^{-\cdot} \quad (2)$$

Table 1. Product distribution in the electrochemical reduction in DMF/0.1 M TBAI of anthracene (*1*) in the presence of 1,2-dichloroethane (*2*), 1,3-dibromopropane (*3*), or 1-bromo-3-chloropropane (*4*).

1/mmol	Dihalide (mmol)	*n*	ΔE_p/V	Yield of products (%)	
10	*2*(50)	8 – 10	> 0.8	*5*(19)	*6*(57)
10	*2*(250)	10 – 12	> 0.8	*5*(38)	*6*(46.5)
10	*2*(375)	10 – 12	> 0.8	*5*(50)	*6*(27)
2.5	*3*(10)	7 – 8	0.33	*7*(55)	*8*(37)
2.5	*4*(10)	5 – 6	0.45	*7*(41)	*9*(34)

$$YBX^{-\cdot} \rightarrow YB^{\cdot} + X^{-} \quad (3)$$

$$A^{-\cdot} + YB^{\cdot} \begin{cases} \rightleftharpoons A + YB^{-} \rightarrow B + Y^{-} & (4) \\ \rightarrow YBA^{-} & (5) \end{cases}$$

Other mechanisms for the coupling reaction might be possible; the reactions 6 to 10 will also be discussed.

$$A + YB^{\cdot} \rightleftharpoons YBA^{\cdot} \xrightarrow{e^{-}} YBA^{-} \quad (6)$$

$$A + YB^{-} \rightleftharpoons YBA^{-} \quad (7)$$

$$A^{-\cdot} + YBX \rightleftharpoons YBA^{\cdot} + X^{-} \xrightarrow{e^{-}} YBA^{-} \quad (8)$$

$$AH^{-} + YBX \rightleftharpoons YBAH + X^{-} \quad (9)$$

$$A^{2-} + YBX \rightleftharpoons YBA^{-} + X^{-} \quad (10)$$

A nucleophilic substitution (eqn. 10) of anthracene dianion on YBX is unlikely for two reasons. The concentration of A^{2-}, formed by disproportionation of 2 $A^{-\cdot}$, is very low as $E_{\frac{1}{2}}^{1} - E_{\frac{1}{2}}^{2} = 0.7$ V, where $E_{\frac{1}{2}}^{1}$ and $E_{\frac{1}{2}}^{2}$ are the potentials of the first and second electron transfer, respectively; furthermore, the rate of electron transfer from A^{2-} to YBX would be rapid, and the electron transfer would result in the formation of $A^{-\cdot}$ and $YB^{\cdot}$ after loss of X^{-}, which ought to give a coupling analogous to eqn. 5.

A nucleophilic substitution (eqn. 9) of AH^{-} (dihydroanthracene anion) on YBX is difficult to exclude, but it cannot be the only reaction as it can neither explain the catalytic reduction of YBX indicated by the voltammetric results nor the formation of *6* or *7*. *6* and *7* are believed to be formed by an intramolecular nucleophilic substitution with YBA^{-} [the anion of 9-(2′-chloroethyl)-9,10-dihydroanthracene, respectively 2-(or 1-)-(3′-halopropyl)-1,2-didihydroanthracene].

The mechanism shown in eqn. 8 is also considered unlikely, as the S_N-reactivity of an anion radical of an aromatic hydrocarbon is low.[4,5]

A nucleophilic addition of YB^{-} to A (eqn. 7) would require a very fast addition reaction as YB^{-}, if formed at all, would lose Y^{-} with the formation of an alkene or a cyclopropane in a rapid reaction; the formation of such products probably accounts for the high *n*-value. Furthermore, eqn. 7 could in principle be a reversal of eqn. 4 followed by eqn. 5.

A coupling mechanism according to eqn. 5 and/or eqn. 6 seems thus operating. When ΔE_p is large, and the rate of eqn. 2 thus lower,[10] the colour of $1^{-\cdot}$ is visible in the neighbourhood of the electrode, whereas this is not so when ΔE_p is small. In the latter case the coupling takes place close to the electrode where the concentrations of A and $A^{-\cdot}$ are of comparable magnitude; under these conditions eqn. 6 cannot compete successfully with eqn. 5. At a greater distance from the electrode eqn. 6 might compete better with eqn. 5, but whether a significant part of the coupling proceeds *via* eqn. 6 is not clear. Other investigations [11,12] favour eqn. 5 as the coupling mechanism.

The electron uptake after the coupling required in eqn. 6 might occur *via* the electrode or *via* an anion radical $A^{-\cdot}$; if eqn. 6 occurs only far from the electrode, $A^{-\cdot}$ would be the electron source, but the concentration of $A^{-\cdot}$ would be low. $YBA^{\cdot}$ might abstract a hydrogen atom from solvent rather than accept an electron, but in that case the formation of *6* and *7* is difficult to explain.

Whether the coupling leading to *7* takes place preferentially in the 1- or 2-position is not clear as in both cases ring closure to *7* would result. In a similar coupling between $1^{-\cdot}$ and

t-butyl bromide the attack in the 2-position predominates, but here steric factors may play a greater role than in the present case.

The preparation of compounds *5* to *9* is a special example of a reaction between electrolytically generated anion radicals and YBX-compounds, where Y represents a functional group. The coupling of anion radicals with YBX-compounds, where Y represents other functional groups than halogen, is being investigated.

EXPERIMENTAL

General procedure for reductions. Anthracene (0.5 – 2 g) was dissolved in dry DMF (150 ml) containing TBAI (7.5 g) and the dihalide (1 – 5 ml) and reduced at a mercury pool electrode (area 25 cm^2) at −1.95 V *vs.* aq. SCE. After completion of the reduction the catholyte was diluted to 1 l with water and the products extracted with an organic solvent from which DMF and basic impurities were removed by washing with dilute acid and water.

Reduction of anthracene and 1,2-dichloroethane. The products were isolated by extraction with benzene. After evaporation of the washed and dried benzene layer the residue was separated on a column of alumina with 5 % ethyl acetate in light petroleum as eluent; isolated were *5* and *6*, yields are given in Table 1. If the DMF contained traces of proton donors some 9,10-dihydroanthracene was formed and lower yields of *5* and *6* obtained.

9-(2'-Chloroethyl)-9,10-dihydroanthracene, *5*, m.p. 154 – 156 °C. ^{1}H NMR ($CDCl_3$): δ 1.92 (*q*, *J* 6.5 Hz, 2 H, 1'-CH_2), 3.42 (t, *J* 6.5 Hz, 2 H, CH_2-Cl), 3.74 (d, *J* 20 Hz, 1 H, 10-CH), 4.02 (d, *J* 20 Hz, 1 H, 10-CH), 4.18 (t, *J* 7 Hz, 1 H, 9-CH), 7.07 – 7.40 (m, 8 H, arom. H). Mass spectrum, *m/e* (%): 244 (1), 243 (7), 242 (21), 241 (20), 240 (48), 207 (2), 205 (6), 203 (10), 202 (11), 193 (46), 191 (100), 189 (18), 178(20), 165 (25), 63 (22).

9,10-Dihydro-9,10-ethanoanthracene, *6*, m.p. 144 – 145 °C (142 – 143 °C).[13] ^{1}H NMR ($CDCl_3$):[14] δ 1.57 – 1.77 (m, 4 H, $(CH_2)_2$); 4.15 – 4.35 (m, 2 H, 9, 10-CH); 6.85 – 7.40 (m, 8 H, arom. H). The methylene protons in the ethylene bridge and the 9 and 10 protons formed an $A^1A^2A^3A^4B^1B^2$-system, which was analyzed employing LAOCN-3.[9] The best fit was obtained with the following parameters: $\delta A^1 = \delta A^2 = \delta A^3 = \delta A^4 = 1.67_5$, $\delta B^1 = \delta B^2 = 4.25$; $J_{A^1B^1} = J_{A^2B^2} = J_{A^1B^2} = 2.7$ Hz, $J_{A^1A^2} = J_{A^3A^4} = -14.0$ Hz, $J_{A^1A^3} = J_{A^1A^4} = 4.0$ Hz, $J_{A^1A^4} = J_{A^2A^3} = 12.0$ Hz. Other couplings = 0. Mass spetrum, *m/e* (%): 207 (1), 206 (9), 179 (22), 178(100), 176 (10), 165 (3), 152 (14), 151 (13), 88 (22).

Reduction of 1 *plus 1,3-dibromopropane (3).* The products were extracted with ether: the organic solvent was washed, dried and evaporated leaving a residue, which was separated into its components on a column of silica with carbon tetrachloride as eluent. *7* and *8* were isolated, *7* was eluted before *8*.

2,3,3a,11b-Tetrahydrocyclopentano[a]anthracene, *7*, m.p. 77 – 78 °C (light petroleum). ^{1}H NMR ($CDCl_3$): δ 1.35 – 2.25 (m, 6 H, $(CH_2)_3$), 2.75 – 3.33 (m, 2 H, H-3a, H-11b), 5.70 (double d, J_1 10 Hz, J_2 3 Hz, 1 H, H-4), 6.47 (double d, J_1 10 Hz, J_2 2 Hz, 1 H, H-5), 7.07 – 7.82 (m, 6 H, arom. H). ^{13}C NMR ($CDCl_3$): δ 23.78, 33.60, 33.35, 39.19, 42.63, 124.80, 125.19, 125.38, 125.94, 126.15, 127.09, 127.54, 130.66, 132.74, 133.16, 133.72, 137.34. Off-resonance decoupling of sp^3-carbons showed two methine and three methylene groups. Mass spectrum, *m/e* (%): 220 (100), 192 (76), 191 (90), 179 (69), 178 (78). IR (KBr) cm^{-1}: 2900 (w), 1480 (w), 1435 (w), 945 (w), 880 (s), 742 (s). UV (96 % ethanol) λ_{max}: 299 (log ε = 4.04), 288 (log ε = 4.11), 278 (log ε = 4.02), 263 (log ε = 4.32), 252 (log ε = 4.52), 246 nm (log ε = 4.53). Peak potential E_p (HME, DMF/0.1 M TBAI, v = 400 mV/s) = −2.42 V (aq. SCE).

9-(3'-Bromopropyl)-9,10-dihydroanthracene, *8*, liquid. ^{1}H NMR ($CDCl_3$): δ 1.6 – 1.95 (m, 4 H 1'-CH_2, 2'-CH_2), 3.05 – 3.3 (m, 2 H, −CH_2Br), 3.7 – 4.0 (m, 1 H, 9-CH), 3.79 (d, 19 Hz, 1 H, 10-C), 4.03 (d, 19 Hz, 1 H, 10-C), 6.95 – 7.35 (m, 8 H, arom. H). MS, m/e (%): 302 (0.1), 301 (0.2), 300 (0.4), 299 (0.4), 298 (0.5), 297 (0.3), 221 (0.1), 220 (0.4), 219 (0.5), 192 (1.8), 191 (5), 180 (19), 179 (100), 178 (37). IR (CCl_4) cm^{-1}: 3050 (m); 2910 (s), 1475 (s), 1445 (s), 1250 (m), 1235 (m), 953 (w).

Reduction of 1 *plus 1-bromo-3-chloropropane (4).* The reaction was performed as described above for *1*+*3*. *9* was isolated as well as *7*.

9-(3'-Chloropropyl)-9,10-dihydroanthracene, *9*, m.p. 41 °C (light petroleum). ^{1}H NMR ($CDCl_3$): δ 1.6 – 1.85 (m, 4 H, 1'-CH_2, 2'-CH_2), 3.25 – 3.5 (m, 2 H, 3'-CH_2Cl), 3.75 – 4.1 (m, 1 H, 9-CH), 3.83 (d, *J* 18.5 Hz, 1 H, 10-CH), 4.08 (d, *J* 18.5 Hz, 1 H, 10-CH), 7.10 – 7.30 (m, 8 H, arom. H). MS, *m/e* (%): 258 (0.1), 256 (0.4), 220 (1.1), 219 (1.6), 193 (9), 191 (10), 180 (17), 179 (100), 178 (32), 77 (13), 75 (20).

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Conformational Analysis of *N,N'*-Dimethyl-*N,N'*-diacyl-1,2-diaminoethanes and -1,3-diaminopropanes and Their Thio Analogues. A ^{1}H NMR and Molecular Mechanics Study Including Dipole-dipole Interactions

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The signals due to the *Z-Z*, *E-Z* and *E-E* forms of ten representative title compounds have been identified in the ^{1}H NMR spectra by solvent effects and coupling constants. In general, the *Z-Z* form shows a much stronger preponderance than expected on purely statistical grounds. For two simple *N,N'*-ethylenebisamides this is shown by molecular mechanics calculations to be due to a combination of steric and dipole interaction effects, leading to a general stabilization of the *gauche* relative to the *trans* form and to a stability order *Z-Z* > *E-Z* > *E-E*.

When studying the NMR spectra of some *N,N'*-dialkyl-*N,N'*-diacylethylenediamines, Siddall [1] observed that the *N*-alkyl proton shifts of the *Z* parts were different in the *Z-Z* and *E-Z* conformers, and similarly for the *E* parts in the *E-E* and *E-Z* forms. Since such differences are unlikely in the staggered *trans* conformation (Fig. 1a), he proposed an intramolecular association between the two amide groups, which should place them in closer proximity.

The investigation described here was initiated by the observation that the NMR spectrum of *N,N'*-dimethyl-*N,N'*-bis(phenylthioacetyl)-ethylenediamine (*8*) did not show the expected nearly statistical population ratio of 1:2:1 between the *E-E*, *E-Z*, and *Z-Z* conformers, but that the population ratio of the three conformers was about 1:3:6 in deuteriochloroform solution. In *N*-methyl-*N*-ethylthioacetamide the *E* and *Z* rotamers have nearly equal populations in $CDCl_3$ and C_6D_6 solutions,[2] and similar relations are expected for *8* in the absence of a specific interaction between the thioamide groups, *i.e.* the *E-E*, *E-Z*, and *Z-Z* conformer ratio should be close to 1:2:1.

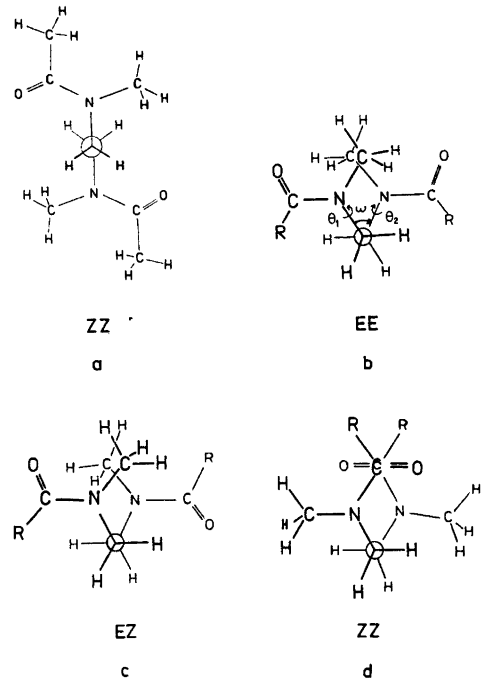
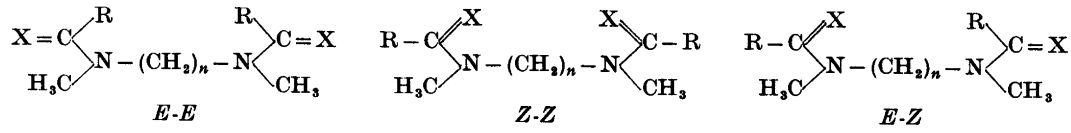


Fig. 1. The *trans Z-Z* conformation (a) and minimum energy conformations for the *E-E* (*b*), *E-Z* (c) and *Z-Z* (d) forms.

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E-E *Z-Z* *E-Z*

	R	X	*n*		R	X	*n*
1	H	O	2	*6*	CH_3	S	3
2	H	S	2	*7*	$PhCH_2$	O	2
3	CH_3	O	2	*8*	$PhCH_2$	S	2
4	CH_3	S	2	*9*	$PhCH_2$	S	3
5	CH_3	O	3	*10*	Ph	O	2

Scheme 1.

In order to identify the rotamers and to try to rationalize the observed conformer distribution, a number of analogous amides and thioamides derived from *N,N'*-dimethylethylenediamine and *N,N'*-dimethyl-propane-1,3-diamine (Scheme 1) have been prepared for an NMR study. Compounds *1* and *3* have been subjected to molecular mechanics calculations in order to find the minimum energy conformations and their energy differences and also to find the interactions responsible for the stabilization of the preferred conformations.

Diamides of the types studied here have been of interest as models for polyamides. Thus *3* and *10* were found to give rotamer ratios deviating rather much from the statistical one in phenol-water solution,[3] but this observation was only rationalized with reference to the rotamer ratio in methyl *N*-acetylsarcosinate.[4] For the latter compound, the assignment of NMR resonances is questionable, being based only on analogy with *N,N*-dimethylformamide. The same compounds have been studied in benzene solution, where a *Z-Z* to *E-Z* ratio of near unity was observed, but no *E-E* form was reported.[5] Compound *3* has also been studied by the LIS technique,[6] but the assignments for the uncomplexed diamide are at variance with those reached in the present study.

EXPERIMENTAL

Preparative part. N,N'-*Dimethyl*-N,N'-*diformylethylenediamine* (*1*) was prepared by refluxing *N,N'*-dimethylethylenediamine with an excess of ethyl formate for 4 h, followed by evaporation, recrystallization from benzene and vacuum sublimation. Colourless prisms in 76 % yield, m.p. 80 – 82 °C (lit.[7] 81 – 82 °C).

N,N'-*Dimethyl*-N,N'-*bis(thioformyl)ethylenediamine* (*2*) was prepared by refluxing *1* with excess P_4S_{10} in toluene for 2 h with stirring, followed by evaporation of the toluene solution and recrystallization of the residue from ethanol. Colourless prisms in 14 % yield, m.p. 135 – 140 °C. Found: C 40.7; H 6.76; N 15.5; S 35.9. Calc. for $C_6H_{12}N_2S_2$: C 40.9; H 6.86; N 15.9; S 36.4.

N,N'-*Dimethyl*-N,N'-*diacetylethylenediamine* (*3*) was obtained on reaction of *N,N'*-dimethylethylenediamine with excess acetic anhydride followed by fractional distillation. The product, b.p. 1 mmHg, 125 – 133 °C, crystallized from light petroleum (b.p. 80 – 100 °C) in 89 % yield as colourless prisms, m.p. 92.5 – 94 °C (lit. 90 – 92 °C,[8] 94.5 – 95 °C [3]). Anal. $C_6H_{16}N_2O_2$: C, H, N, O.

N,N'-*Dimethyl*-N,N'-*bis(thioacetyl)ethylenediamine* (*4*) was prepared essentially as *2* with *3* as starting material, but dioxane was used as solvent and the crude product was purified by extraction with chloroform, evaporation and crystallization of the residue first from toluene – light petroleum and then from toluene to give a 35 % yield of colourless prisms, m.p. 129 – 131 °C. Anal. $C_8H_{16}N_2S_2$: C, H, N, S.

N,N'-*Dimethyl*-N,N'-*diacetylpropane-1,3-diamine* (*5*) was prepared essentially as *3* starting from *N,N'*-dimethylpropane-1,3-diamine. The product was obtained as hygroscopic prisms, m.p. 63 °C, b.p. 2 mmHg, 190 – 194 °C, n_D^{20} 1.4845, in 61 % yield. Found: C 57.5; H 9.68; N 14.8; O 17.9. Calc. for $C_9H_{18}N_2O_2$: C 58.0; H 9.74; N 15.0; O 17.2.

N,N'-*Dimethyl*-N,N'-*bis(thioacetyl)propane-1,3-diamine* (*6*) was obtained as *4*, starting from *5*, in 20 % yield as colourless prisms, m.p. 128 – 130 °C. Anal. $C_9H_{18}N_2S_2$: C, H, N, S.

N,N'-*Dimethyl*-N,N'-*bis(phenylacetyl)ethylenediamine* (*7*) was prepared by Schotten-Baumann acylation of *N,N'*-dimethylethylenediamine with phenylacetyl chloride. The product could only be induced to crystallize after

column chromatography on activated silica. 2-Butanone eluted impurities, and *7* was eluted by a 1:1 (*v/v*) mixture of acetone and 2-butanone. It was obtained as colourless prisms, m.p. 79.5 – 82.5 °C, in 44 % yield after two recrystallizations from cyclohexane and one from toluene. Anal. $C_{20}H_{24}N_2O_2$: C, H, N, O.

N,N'-Dimethyl-N,N'-bis(phenylthioacetyl)-ethylenediamine (*8*) was prepared by reaction between methyl phenyldithioacetate [9] and *N,N'*-dimethylethylenediamine in the molar proportion 2:1 without solvent. The product was recrystallized four times from toluene to give a 60 % yield of colourless prisms, m.p. 145 – 148 °C. Anal. $C_{20}H_{24}N_2S_2$: C, H, N, S.

N,N'-Dimethyl-N,N'-bis(phenylthioacetyl)-propane-1,3-diamine (*9*) was prepared as *8*. Colourless prisms, m.p. 92 – 96 °C, were obtained in 25 % yield after two recrystallizations from toluene – light petroleum and two from toluene. Anal. $C_{21}H_{26}N_2S_2$: C, H, N, S.

N,N'-Dimethyl-N,N'-dibenzoylethylenediamine (*10*) was prepared by reaction of *N,N'*-dimethylethylenediamine with benzoyl chloride in dichloromethane in the presence of triethylamine. After 24 h the separated solid was triturated with hot water and recrystallized from ethanol to give a 66 % yield of colourless prisms, m.p. 174 – 175.5 °C (lit. [10] 177 – 178 °C). Anal. $C_{18}H_{20}N_2O_2$: C, H, N, O.

NMR measurements. These were performed with a JEOL model MH-100 NMR spectrometer, operating in the field sweep, synchro-track mode with the internal lock on TMS. The chemical shifts were read with a frequency counter, which measures the frequency difference between the lock signal and the observing frequency.

The populations of the observed conformers were determined by fitting calculated Lorentzians to the experimental *N*-methyl signals, taking coupling effects into account when appropriate.

The ASIS study was performed by measuring the spectra of compounds *1* – *10* as 0.1 M solutions in deuteriochloroform and in *o*-dichlorobenzene (ODC) and also in 3:1, 1:1, and 1:3 mixtures (*v/v*) of these solvents. To avoid exchange broadening, compound *3* was studied at + 25 °C, *5* at – 6 °C, and *10* at + 6 °C, whereas all other compounds were studied at ambient temperature (+ 35 °C).

The ASIS plots were constructed by the method of least squares, based on eqn. (1), where

$$\nu_x = aP(CDCl_3) + b \qquad (1)$$

ν_x is the frequency of the signal under study and $P(CDCl_3)$ is the volume percentage of this solvent. The ASIS are given by *a* in Table 3.

Molecular mechanics calculations. Geometries and conformational energies were calculated using Allinger's molecular mechanics method.[11,12] The 1973 force field [12] was employed in the

Table 1. Force field parameters. [a]

van der Waals constants

$E_v = -2.25\varepsilon(r^*/r)^6 + 8.28 \times 10^5 \varepsilon \exp(-r/0.0736r^*)$

Atom	r^*/Å	ε/kJ mol^{-1}
O	1.650	0.192
N	1.700	0.163

Stretching constants

$E_s = 301.0k_s(l-l_0)^2\ [1 + C_s(l-l_0)]\ \ C_s = -2.00$

Bond	l_0/Å	k_s/mdyn Å^{-1}
C=O	1.215	10.80
$C_{CO}-N(sp^2)$	1.367	5.00
$C(sp^3)-N(sp^2)$	1.449	3.40

Bending constants

$E_b = 0.091688k_b(\theta-\theta_0)^2[1 + C_f(\theta-\theta_0)]$
$C_f = -0.006$

Angle	θ_0/deg.	k_b/mdyn Å rad^{-2}
H – C = O	120.6	0.25
C – C = O	122.8	0.57
N – C = O	124.0	0.50
$H-C_{CO}-N$	110.0	0.40
$C-C_{CO}-N$	112.7	0.40
$C-N-C_{CO}$	121.0	0.70
C – N – C	115.0	0.50
H – C – N	109.5	0.42
C – C – N	109.5	0.42
Out-of-plane $N(sp^2)$	0.0	0.05
Out of plane C_{CO}	0.0	0.80

Stretch-bend constants

$E_{sb} = 10.50703k_{sb}(\theta-\theta_0)(l_1-l_0) + (l_2-l_0)$

Atoms	k_{sb}/mdyn rad^{-1}
X – F – Y	0.120
X – F – H	0.040

F, X, Y are C, N or O

Torsional constants

$E_t = V_1/2(1+\cos\omega) + V_2/2(1-\cos 2\omega) + V_3/2(1+\cos 3\omega)$

Angle	V_2	V_3/kJ mol^{-1}
H – C – C – N		2.22
N – C – C – N		2.22
X – C – N – Y		4.18
$X-C_{CO}-N-C$	25.1	
O = C – N – C	16.7	
$H-C-C_{CO}-N$		0.0
H – C – C = O		– 3.1

X = H or $C(sp^3)$; Y = C_{CO} or $C(sp^2)$

Bond moments

	μ/D
C=O	2.91
$C_{CO}-N$	– 1.16

[a] C – H parameters are taken from Ref. 12.

Table 2. ^{1}H Chemical shifts [a] of the different conformers of compounds *1 – 10*.

Compound No.	R	X	*n*	N–CH$_2$ *E-E*	N–CH$_2$ *E-Z(E)*	N–CH$_2$ *E-Z(Z)*	N–CH$_2$ *Z-Z*	N–CH$_3$ *E-E*	N–CH$_3$ *E-Z(E)*	N–CH$_3$ *E-Z(Z)*	N–CH$_3$ *Z-Z*	R *E-E*	R *E-Z(E)*	R *E-Z(Z)*	R *Z-Z*
1	H	O	2	3.40	3.46 [b]		3.55	2.90	2.92	2.94	3.00	–	– [c]	–	–
2 [d]	H	S	2	3.29	3.59 [b]		3.86	2.95	3.07	2.81	3.00	–	– [c]	–	–
3	CH_3	O	2	3.46	3.47 [b]		3.52	– [e]	2.95	3.03	3.03	2.00	2.08	2.10	2.03
4	CH_3	S	2	3.81	4.09 [b]		4.32	3.45	3.49	3.26	3.35	2.67	2.70	2.63	2.60
5	CH_3	O	3	– [e]	3.44	3.29	3.40	2.94	2.92	3.02	3.01	2.18	2.08	2.10	2.08
6	CH_3	S	3	– [e]	3.66	3.95	4.08	3.45	3.45	3.28	3.28	2.69	2.69	2.62	2.62
7	$PhCH_2$	O	2	– [e]	3.39 [b]		3.54	– [e]	2.94	2.91	3.00	– [e]	3.66	3.66	3.60
8	$PhCH_2$	S	2	– [e]	3.87 [b]		4.23	– [e]	3.45	2.97	3.20	– [e]	4.30	4.23	4.30
9	$PhCH_2$	S	3	– [e]	3.55	3.76	4.06	3.19	3.40	2.93	3.16	– [e]	4.33	4.22	4.27
10	Ph	O	2	3.39	3.62 [b]		3.93	2.77	3.24	2.73	3.11	–	–	–	–

[a] In ppm downfield from TMS. Solvent $CDCl_3$ unless stated otherwise. [b] Centre of A_2B_2 multiplet. [c] Not identified due to overlap. [d] In ODC for solubility reasons. [e] Not observed.

calculations of interactions involving only carbon and hydrogen. Parameters describing the amide skeleton were taken from earlier work [13] (carbonyl group parameters) or obtained by fitting to electron diffraction geometries of simple amides.[14–16] The amide skeleton force field is summarized in Table 1.

A problem in the parametrization is the choice of intrinsic torsional terms describing the variation in energy for rotation around the N(sp^3) – C(sp^3) and C(sp^3) – C(sp^2) bonds. The three-fold torsional terms chosen (Table 1) give 4.69 and 6.11 kJ/mol, respectively, for the barrier to rotation around these bonds in *N*-methylacetamide. In the calculated lowest energy conformation one C–H bond in the methyl group attached to the carbonyl group eclipses this group. The *N*-methyl group is staggered with respect to the C–N amide bond, in agreement with the electron diffraction structure.[15] There are no experimental data on the rotation barrier for the carbonyl-methyl group in amides but the barrier height has been estimated to be *ca.* 4 kJ/mol.[14,17] The only experimental value for the *N*-methyl rotational barrier comes from a recent measurement of ^{13}C relaxation times (T_1) in *N,N*-dimethylformamide.[18] From rate constants at –57.6 °C, $\Delta G^{\ddagger} = 7.5 - 8.4$ kJ/mol can be calculated. Since the uncertainties in these values are rather large, we have not made any attempt to reproduce them. In earlier work on amides and peptides V_3 terms for the *N*-methyl torsion varying from 2.1 – 6.3 kJ/mol have been used.[19] The twofold torsional terms for the C–N amide bond given in Table 1 were chosen to give a calculated barrier to rotation around this bond of *ca.* 82 kJ/mol.[20] The numbers used are only rough estimates since they are of minor importance in this work, where deviations from planarity of the amide skeleton are insignificant.

Dipole interaction energies were calculated from the expression (2).[21]

$$E_{DIP} = K\mu_A\mu_B (\cos X - 3 \cos \alpha_A \cos \alpha_B)/R^3D \quad (2)$$

where μ_A and μ_B are bond moments, R is the distance between the midpoints of the bond dipoles and X, α_A and α_B are angles describing their relative orientation. D is the dielectric constant and K is an energy conversion factor.

The bond moments used for the C=O and C–N dipoles (Table 1) reproduce the experimental magnitude (3.71 *D*) [22] and orientation (39.6° with respect to the N–C bond) [22] of the dipole moment in formamide.

RESULTS

The ^{1}H NMR spectral assignments of two representative compounds (*1* and *4*) are discussed in detail below, and data for the

Table 3. Aromatic solvent induced shifts [a] for the different conformers of compounds *1* and *3*–*10*.

Compound No.	N–CH_2				N–CH_3				R			
	E-E	*E-Z(E)*	*E-Z(Z)*	*Z-Z*	*E-E*	*E-Z(E)*	*E-Z(Z)*	*Z-Z*	*E-E*	*E-Z(E)*	*E-Z(Z)*	*Z-Z*
1	0.36	–	–	0.22	0.24	0.16	0.38	0.29	–	–	–	–
3	0.30	–	–	0.14	–	0.13	0.31	0.25	–	0.10	0.23	0.17
4	0.31	–	–	0.13	0.21	0.15	0.35	0.30	0.20	0.09	0.24	0.20
5	–	0.20	0.17	0.20	0.18	0.17	0.36	0.32	0.26	0.22	0.21	0.21
6	–	0.30	0.18	0.17	0.20	0.16	0.37	0.36	0.21	0.15	0.19	0.19
7	–	–	–	0.12	–	0.12	0.20	0.21	–	0.02	0.13	0.13
8	–	–	–	0.13	–	0.14	0.27	0.25	–	0.01	0.15	0.13
9	–	0.19	0.19	0.13	0.15	0.14	0.24	0.29	–	0.05	0.10	0.10
10	0.19	–	–	0.17	0.22	0.07	0.28	0.17	–	–	–	–

[a] *a* in eqn. 1.

remaining compounds are collected in Tables 2 and 3.

N,N'-*Dimethyl*-N,N'-*diformylethylenediamine* (*1*) in deuteriochloroform solution shows four narrow doublets in the *N*-methyl region, *A* (δ 2.90, *J* 0.5 Hz, p* 0.10), *B* (δ 2.92, *J* 0.5 Hz, p 0.22), *C* (δ 2.94, *J* 0.3 Hz, p 0.22) and *D* (δ 3.00, *J* 0.3 Hz, p 0.46). The ethylene part consists of two singlets, *E* (δ 3.40, p 0.10) and *F* (δ 3.54, p 0.46) and an A_2B_2 spectrum centered at δ 3.45 (p 2×0.22). The formyl proton region is not well resolved, and no assignments have been made here.

By assignment of the *N*-methyl signals in *N,N*-dimethylformamide using the NOE method, Anet and Bourn [23] have demonstrated that the formyl proton is more strongly coupled to the *Z* than to the *E* methyl proton. Hatton and Richards [24] have shown that the *E* methyl signal in *N,N*-dimethylamides is shifted more rapidly upfield on dilution with an aromatic solvent than the *Z* methyl signal, and this effect is ascribed to a time-average localization of the solvating aromatic molecules away from the negative end of the amide electric dipole (ASIS),[15,26] The ASIS values for signals *A* to *F* are 0.24, 0.16, 0.38, 0.29, 0.36 and 0.22, respectively, and $\Delta\nu_{AB}$ in the A_2B_2 spectrum is strongly increased with increasing ODC concentration.

The *A* and *B* signals are assigned to methyl protons in *E* forms ** by their larger couplings to the formyl proton and by their small ASIS values, and the populations together with the ASIS values for the methylene proton signals unequivocally assign *A* to the *E-E* form, *B* to the *E* part and *C* to the *Z* part in the *E-Z* form, and *D* to the *Z-Z* form. Signal *E* is assigned to the CH_2 protons in the *E-E* form and *F* to the same protons in the *Z-Z* form. Thus the *E-E*:*E-Z*:*Z-Z* ratio for this compound in deuteriochloroform is 0.10:0.44:0.46, which shows a smaller deviation from the statistical distribution than most of the other molecules.

N,N'-*Dimethyl*-N,N'-*bis(thioacetyl)ethylenediamine* (*4*). The spectrum of this compound in ODC is shown in Fig. 2. Signals *D* and *H* appear as quartets ($J = 0.5$ Hz) in expanded spectra, and either of them changes into a sharp singlet on selective irradiation of the other one.

With increasing temperature, all signals broaden, and above 150 °C *A* to *C* merge into one (N–CH_2), *D* to *G* into another (N–CH_3) and *H* to *K* into a third one (C–CH_3). The ASIS values for signals *A* and *C* to *G* are 0.13, 0.31, 0.15, 0.21, 0.30 and 0.35. These data together with the signal intensities clearly assign *A*, *F* and *J* to the *Z-Z* form, *D* and *H* to the *E* part and *G* and *K* to the *Z* part of the *E-Z* form, and the small signals *C*, *E* and *I* to the *E-E* form. The *B* multiplet is ascribed to the methylene protons of the *E-Z* form. The assignment of *D* and *H* to a *trans* methyl pair and *G* and *J* to a *cis* pair is further strengthened by the coupling, which is larger in the former case. Thus $^5J_{H-H,trans}$ in *N,N*-dimethylthioacetamide is 0.7 Hz and $^5J_{H-H,cis}$ is O Hz.[27]

* p stands for fractional population.

** Note that the *N*-methyl group is *Z* to the carbonyl group in the *E* form.

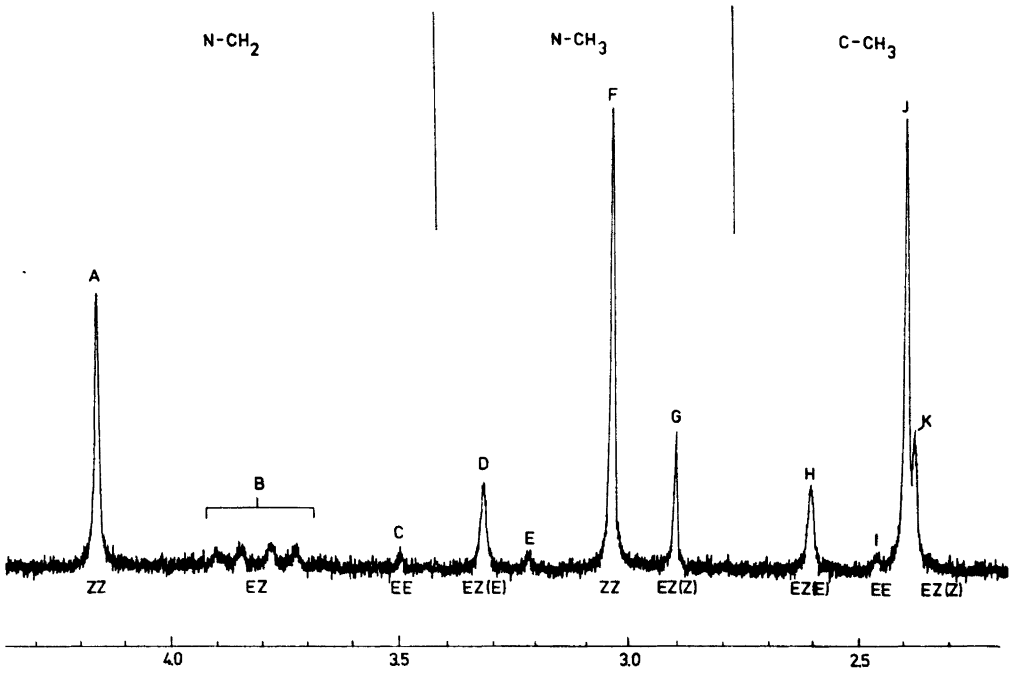


Fig. 2. ^{1}H NMR spectrum of *4* in ODC.

The *E-E : E-Z : Z-Z* ratio in this compound in deuteriochloroform solution is thus 0.02:0.30:0.68, *i.e.* it deviates more from the statistical distribution than in *1*.

DISCUSSION

The preponderance of the *Z-Z* over the other forms was first ascribed to a dipole-dipole attraction in a *gauche* conformation, since only in the *Z-Z* form could the amide/thioamide electric dipoles attain a favourable antiparallel arrangement (Fig. 1d). In the molecular mechanics calculations performed on compounds *1* and *3*, the minimum energy conformations were found by allowing the molecule to relax from all conceivable *gauche* and *anti* conformations. The dielectric constant D in expression (2) cannot be given the value appropriate for the bulk solvent. Rather it should be a composite of this constant and the (unknown) effective constant of the substrate. For the latter, some guidance may be obtained from the value of $D=4$, which Momany *et al.*[38] recommend for a polypeptide crystal. We have performed the calculations with three values for D (1.0, 4.0 and 8.0). The value for ODC is 9.93 and for deuteriochloroform 4.70.

The lowest-energy *E-E*, *E-Z*, and *Z-Z* conformations are shown in Fig. 1, and data for all energy minima are found in Table 5. The

Table 4. Populations of the different conformers.

Compound	Solvent	Ratio *E-E*	*E-Z*	*Z-Z*
1	$CDCl_3$	0.10	0.44	0.46
1	ODC	0.17	0.46	0.37
2	ODC	0.05	0.59	0.36
3	ODC	0.08	0.40	0.52
4	$CDCl_3$	0.02	0.30	0.68
4	ODC	0.05	0.44	0.51
4	DMSO-d_6	0.16	0.48	0.36
5	$CDCl_3$	0.09	0.55	0.36
6	$CDCl_3$	0.10	0.48	0.42
7	$CDCl_3$	—[a]	0.43	0.57
8	$CDCl_3$	0.10	0.29	0.61
9	$CDCl_3$	0.12	0.42	0.46
10	$CDCl_3$	0.08	0.18	0.74

[a] Not observed, possibly due to overlap in all solvents.

Table 5. Calculated minimum energy conformations.[a]

	ω^{0} [b]	θ^{0}_{1} [c]	θ^{0}_{2} [c]	μD	Energy/ kJ mol^{-1}
Compound *1*					
E-E	61	81	80	4.6	47.4
E-Z	57	83	75	2.4	44.0
Z-Z	−63	84	84	3.0	20.0
Compound *3*					
E-E (Fig. 1b)	62	90	90	5.2	56.9
E-Z (Fig. 1c)	57	91	78	2.9	49.1
Z-Z (Fig. 1d)	−64	86	86	2.2	20.0

[a] Data for $D = 1$. [b] Dihedral angle of ethane bond. [c] Dihedral angle of $N-CH_2$ bond. The angle is zero when the $N-C(=O)$ bond eclipses the ethane bond.

dependence of the energies of the most stable conformer on the dielectric constant is shown in Fig. 3. Three conformations with rather close-lying energies (within 1.2 kJ/mol) are found for the *E-Z* forms of both *1* and *3*, and the entropies of mixing at 307 K are included in the calculated energies. The importance of the value of the dielectric constant in empirical strain energy calculations on polar molecules has been demonstrated by Kumbar and Sankar.[29] These authors propose values in the range of 20 to 30 as a suitable compromise between the dielectric constants of the substrates (tryptamine and serotonin) and of water ($D = 78.5$) to give agreement between calculated and experimental conformations.

Inspection of Figs. 1 and 3 and Table 5 clearly shows that the *Z-Z* conformer has the lowest energy, and that it is relatively more stabilized in *3* than in *1*, all in agreement with the experimental results. It is evident that the anticipated dipole-dipole attraction in the *gauche Z-Z* form is of importance, though it decreases strongly with increasing dielectric constant (see, *e.g.*, *4*, Table 4).

The *gauche* form, however, is favoured not only by the dipole-dipole effect but also by a steric effect. The *trans* form has the higher steric energy for all arrangements of the amide groups, mainly due to interactions between the $N-CH_2$ group in one half of the molecule and the *N*-methyl and acyl groups in the other. The difference in steric strain between the *Z-Z* form and the other forms is less in *1* than in *3*, which may explain the observed population ratios. In particular, the *E-E* form in *1* is less disfavoured by steric effects than in *3*, which is also reflected in its relatively high population in the former compound.

It must be pointed out, however, that these calculations are rather approximate, partly because of the uncertainty in the effective dielectric constant and partly because it has not been possible to take the solvation energy into account. This latter contribution may, *e.g.*, be responsible for the discrepancy between

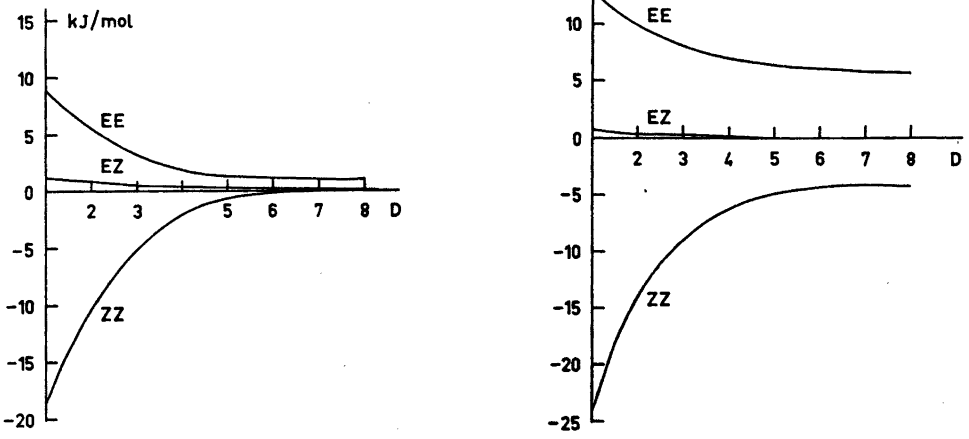


Fig. 3. Calculated minimum energy *versus* effective dielectric constant for *1* (a) and *3* (b).

the calculated differences in energy between the *Z-Z* form and the other forms of *3* on the one hand and the observed populations on the other hand. The calculated *Z-Z* : *E-Z* : *E-E* ratio at 305 K is 0.96:0.04:0 for $D=4$ and 0.86:0.12:0.02 for $D=8$. The experimental ratio in ODC is 0.52:0.40:0.08. In the *Z-Z* form (Fig. 1d) the *C*- and *N*-methyl groups in one amide part screen the carbonyl group in the other, thus hindering its solvation. In the low-energy *E-Z* and *E-E* forms, and in all forms of *1*, the carbonyl groups are less hindered.

The exchange between these three forms can only occur by rotation around the (thio)acyl-nitrogen bond, and it is therefore hindered by normal (thio)amide rotational barriers. By dissolving *4* and *8* and recording the NMR spectra in deuteriochloroform at low temperature, it has been possible to show that these compounds crystallize exclusively in the *Z-Z* form, and by monitoring the appearance of the *E-Z* form at -7 °C, it was shown that the free energy barrier to the *Z-Z* to *E-Z* exchange in *8* is 87.9 kJ/mol, in good agreement with the value of 90.4 kJ/mol found for *N,N*-dimethyl-thioacetamide in ODC.[30]

The conformer populations in the diamino-propane derivatives *5*, *6* and *9* follow a similar pattern. These compounds have a greater flexibility and have not been analyzed in detail. However, it is evident from models that when the propane chain has an energetically acceptable *gauche* (+)−*gauche* (−) conformation, the (thio)amide groups are only slightly more distant than in the *gauche* forms of the diamino-ethane derivatives and in suitable positions to interact in similar ways as in these.

To conclude, we find that the intramolecular association suggested by Siddall [1] is really working. The association is caused by a combination of dipole attraction effects and steric effects and it leads to a stabilization of the *Z-Z* form and a destabilization of the *E* − *E* form relative to the *E-Z* form. The steric part of these effects increases with increasing size of the (thio)acyl group, and the dipole part decreases with increasing polarity of the solvent.

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Hydroxylaminolysis of Anilides. V. H^+ Catalyzed Hydroxylaminolysis of *p*-Cyanoformanilide and *p*-Chloroformanilide. Differences in Substituent Effects Exerted by Substituents with Mesomeric and Inductive Action

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The kinetics of the hydroxylaminolysis of *p*-cyanoformanilide and *p*-chloroformanilide have been studied at 25 °C in the range $3.62 \leqslant -\log [H^+] \leqslant 9.79$. The concentration of hydroxylamine was varied from 0.2 M to 3.0 M ($\mu = 3.0$ by addition of KCl). The kinetics fit the mechanism suggested for the hydroxylaminolysis of *p*-nitroformanilide. With this mechanism, kinetic equations have now been derived for the dependence of k_{obs} on the concentration of the hydroxylamine system and on the pH. The new equations are closely related to those derived for other anilides. The rate constants for the previously studied formanilides are compared and the influence of substituents with and without mesomeric properties are discussed.

It has long been known [1] that nucleophiles showing α-effect, such as hydroxylamine and hydrazine, are unusually active towards amides and esters. Their reactivity far exceeds that expected from their base strength. This has been attributed to the presence of an $-OH$ or $-NH-$ group in the α-position to the amine nitrogen. It was early suggested [2] that the abnormal reactivity of hydroxylamine is due to hydrogen bonding between the α-group and the carbonyl oxygen. Such a bond should decrease the electron density at the carbonyl carbon and, possibly, hold the nucleophile in a favourable position for attack. Apart from the enhanced rates, until recently nothing unusual had been noted about hydroxylamine as a nucleophile. The relative rates of attack by hydroxylamine on different substrates were comparable with those shown by HO^- and by ordinary amines. In a recent paper from this Laboratory,[3] however, it was noted that the introduction of a *p*-nitro group into an anilide ring, which increases the rate of nucleophilic attack on the carbonyl carbon when the nucleophile is HO^- or an amine, decreases the rate when hydroxylamine was the attacking species. As an explanation for this phenomenon it was pointed out that *p*-nitro substitution lowers the electron density not only at the carbonyl carbon, which makes the nucleophilic attack easier, but also at the oxygen, which reduces the possibility of hydrogen bonding to the α-group. In order to obtain more information about the mechanism of the nucleophilic attack, two more anilides with electron withdrawing substituents have now been studied: *p*-cyanoformanilide, in which the substituent has the same type of electron withdrawing properties as the *p*-nitro group, and *p*-chloroformanilide, in which the substituent lacks $-E$ effect.

MATERIALS AND METHODS

p-*Chloroformanilide* (m.p. 93 – 94 °C, lit. 92 °C)[4] and p-*cyanoformanilide* (m.p. 188 – 189 °C, lit. 189 °C)[5] were prepared according to Huffman.[6] All other chemicals used were Merck reagent grade chemicals.

The hydroxylaminolysis reaction was studied in the range $4.80 \leqslant -\log [H^+] \leqslant 9.79$ for *p*-cyanoformanilide and $3.62 \leqslant -\log [H^+] \leqslant 9.79$ for *p*-chloroformanilide. [H+] denotes the

Table 1. Observed pseudo-first order rate constants, $k_{obs}/min^{-1} \times 10^{-4}$, for the formation of *p*-aminobenzonitrile from *p*-cyanoformanilide at different concentrations of the hydroxylamine system. The percentages denote the fractions of free hydroxylamine base.

C/M	3.00 %	5.00 %	10.0 %	20.0 %	40.0 %	60.0 %	80.0 %
0.20	0.98	0.84	0.91	0.90	0.99	1.07	0.74
0.60	2.75	2.79	3.09	3.55	4.32	4.98	4.85
1.00	4.67	4.73	5.65	7.05	10.7	13.2	14.0
1.40	6.30	6.82	8.35	10.8	20.1	25.6	28.5
1.80	8.23	8.76	11.5	16.5	30.4	37.9	45.7
2.20	10.7	11.7	15.1	23.2	41.3	57.7	67.8
2.60	12.1	14.2	19.0	29.9	56.5	77.6	91.0
3.00	14.5	16.7	22.7	38.4	73.1	96.4	122

C/M	90.0 %	C/M	99.00 %	99.90 %	99.97 %
0.20	1.25	0.98	–	9.15	–
0.60	4.80	0.99	9.58	–	9.56
0.98	11.9	1.73	–	26.4	–
1.37	24.9	1.78	31.0	–	–
1.74	41.3	1.79	–	–	30.2
2.09	56.2	2.54	–	–	62.3
2.46	78.4	2.58	64.5	–	–
2.94	107	2.59	–	63.8	–

concentration of H^+. The concentration of the hydroxylamine system was varied between 0.20 and 3.00 M. The kinetic experiments and assays were performed as in a previous work,[7] with the following modification: To increase the solubility of the *p*-cyanoformanilide, the reaction mixtures contained 1 % dimethyl sulfoxide. This addition allowed a reasonable amount of the anilide to dissolve but was too small to appreciably influence the correlation between the base percentages and the $[H^+]$ values. Therefore, these values were taken to be the same as for the water medium.

The molar absorption coefficients for the diazotized and coupled anilines were $\varepsilon = 45\,800$ at $\lambda = 540$ nm for *p*-aminobenzonitrile and $\varepsilon = 44\,400$ at $\lambda = 555$ nm for *p*-chloroaniline. The initial concentration of *p*-cyanoformanilide was $(0.3-0.9) \times 10^{-3}$ M and *p*-chloroformanilide $(0.25-2.8) \times 10^{-3}$ M.

Table 2. Observed pseudo-first order rate constants, $k_{obs}/min^{-1} \times 10^{-4}$, for the formation of *p*-chloroaniline from *p*-chloroformanilide at different concentrations of the hydroxylamine system. The percentages denote the fractions of free hydroxylamine base. * = corrected for acid hydrolysis.

C/M	0.20 %	1.00 %	5.00 %	10.0 %	20.0 %	40.0 %	60.0 %	80.0 %
0.20	1.04*	1.26	0.96	1.13	1.44	1.58	1.56	1.10
0.60	2.54*	2.75	3.96	4.94	8.92	9.29	10.1	8.60
1.00	4.33	5.37	8.18	10.7	17.7	23.8	28.8	24.2
1.40	6.05	7.72	13.1	18.2	31.8	43.7	62.3	48.1
1.80	8.28	10.8	18.8	27.0	48.9	68.8	103	82.9
2.20	9.91	13.4	24.2	39.1	69.0	102	153	121
2.60	12.0	16.3	30.6	49.1	90.5	141	210	173
3.00	12.9	19.3	37.2	60.4	120	183	286	225

C/M	90.0 %	C/M	95.0 %	C/M	99.00 %	99.90 %	99.97 %
0.20	0.94	0.20	0.67	–	–	–	–
0.60	7.05	0.60	6.22	–	–	–	–
0.98	20.6	1.00	18.3	1.00	15.6	14.8	14.9
1.35	41.0	1.38	34.9	1.78	48.7	–	46.4
1.70	64.8	1.79	58.6	1.79	–	43.7	–
2.14	100	2.17	84.9	2.55	102	–	–
2.53	144	2.56	122	2.57	–	–	87.6
2.79	168	2.95	157	2.60	–	91.8	–

Table 3. Values of p' and q. The q values are obtained from plots of $k_{obs}/[N]$ *vs.* C. The p' values are those that give the best fit to the experimental plots of k_{obs} *vs.* C, when introduced into eqn. (3).

% Base	$-\log [H^+]$ M	*p*-Cyano-formanilide p' M^{-1}	q	*p*-Chloro-formanilide p' M^{-1}	q
0.20	3.62	–	–	0.20	0.90
1.00	4.32	–	–	0.025	0.20
3.00	4.81	0.15	0.75	–	–
5.00	5.04	0.12	0.40	0.022	0.045
10.0	5.37	0.10	0.20	0.022	0.021
20.0	5.72	0.11	0.08	0.022	0.015
40.0	6.14	0.115	0.05	0.0205	0.003
60.0	6.50	0.12	0.01	0.022	–
80.0	6.92	0.105	–	0.013	–
90.0	7.27	0.087	–	0.010	–
95.0	7.60	–	–	0.0078	–
99.0	8.27	0.055	–	0.0063	–
99.90	9.27	0.055	–	0.0055	–
99.97	9.79	0.055	–	0.0055	–

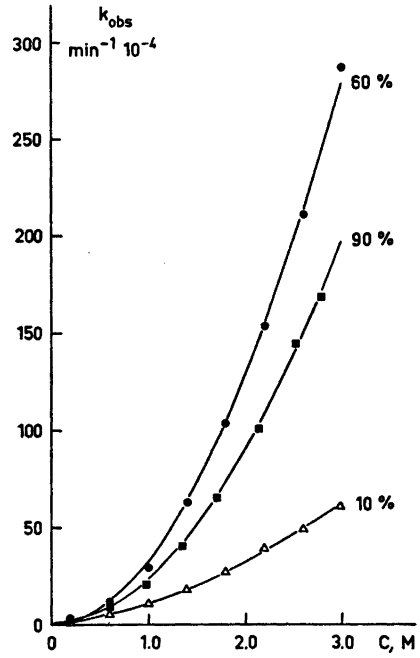


Fig. 1. *p*-Chloroformanilide. Plots of k_{obs} *vs.* C with 10, 60 and 90 % of the hydroxylamine system present as base. The points are experimental. The curves are calculated by means of eqn. (3) using $k_1 = 2.5 \times 10^{-1}$ min^{-1} M^{-1} and the values of p' and q listed in Table 3.

The k_{obs} values were determined from plots of log [remaining anilide] *vs.* time. Depending on the reaction rate, the hydroxylaminolysis was followed until 10 – 60 % of the *p*-cyanoformanilide and 4 – 90 % of the *p*-chloroformanilide had reacted. The rate constants were determined graphically or by means of least squares regression.

RESULTS

The determined pseudo-first order rate constants, k_{obs}, for the formation of *p*-aminobenzonitrile and *p*-chloroaniline from the corresponding anilides are listed in Tables 1 and 2. The tables show the variation of k_{obs} with the concentration of the hydroxylamine system (C) at various base percentages. (In the following, hydroxylamine base will be referred to as N, and the hydroxylammonium ion as HN). Table 3 lists the values of $-\log [H^+]$ corresponding to the different base percentages.

At low base percentages the dependency of k_{obs} on C is slightly more than first order in C as may be calculated from Tables 1 and 2. When the fraction of base is increased, the order of the dependency also increases, and eventually becomes purely second order. This may be seen in Fig. 1, which shows the plots of k_{obs} *vs.* C at 10, 60 and 90 % base for *p*-chloroformanilide.

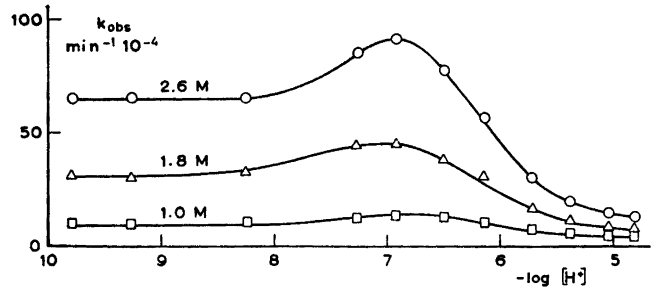


Fig. 2. *p*-Cyanoformanilide. Plots of k_{obs} *vs.* $-\log [H^+]$ at three different concentrations of the hydroxylamine system. The points are experimental. The curves are not calculated.

The magnitude of the k_{obs} values at a constant C is also pH dependent. At low base percentages the rates are low; they increase with the pH to reach a maximum at about 60 – 80 % base and then decrease to a limiting value. In Figs. 2 and 3, k_{obs} is plotted *vs.* $-\log [H^+]$ for 3 different values of C. In both figures the plots are bell shaped. In the *p*-chloroformanilide plot the maximum at $C = 2.6$ M is rather pointed and the limiting values at high pH are comparatively low. The *p*-cyanoformanilide plot, on the other hand, is smooth with high limiting values, resembling the corresponding plot for *p*-nitroformanilide.[3] It also reaches the maximum at a somewhat higher pH than *p*-chloroformanilide.

Figs. 4 and 5 show plots of $k_{obs}/[N]$ *vs.* C for *p*-cyano- and *p*-chloroformanilide. They are commented upon in the discussion part of this paper.

Although the investigations cover a pH range of up to 6 units, the only contribution to the reaction rates from acid [8] or alkaline [4] hydrolysis was in the *p*-chloroformanilide experiments at 0.2 % base for $C = 0.2$ and 0.6 M. In these runs, about 6 and 3 %, respectively, of the breakdown of the *p*-chloroformanilide can be ascribed to acid hydrolysis. The k_{obs} values given in Table 2 for these points are the corrected ones. In all other cases, the rate of breakdown due to acid or alkaline hydrolysis was calculated to be less than 1 % of the k_{obs} values and no correction was made.

DISCUSSION

A previous paper [9] presented a mechanism explaining the kinetics of the hydroxylaminolysis of different anilides. This mechanism is the same as the one shown in Scheme 1, except for the part within the dotted line, which has been added later. [3] From the mechanism, eqn. (1) was derived for the dependence of k_{obs} on C at constant pH:

$$k_{obs} = k_1[N]pC/(1+pC) \quad (1)$$

In this equation, k_1 is the same as in Scheme 1 and p is a pH dependent parameter.[7]

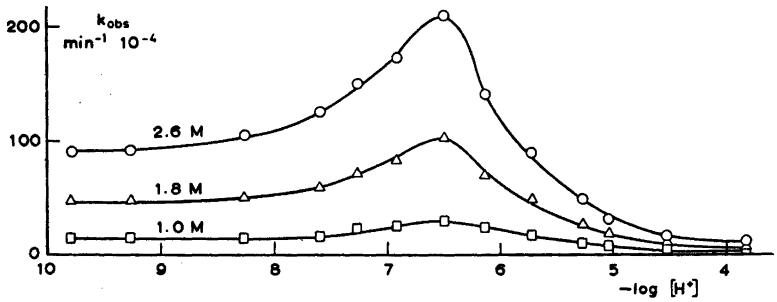


Fig. 3. *p*-Chloroformanilide. Plots of k_{obs} *vs.* $-\log [H^+]$ at three different concentrations of the hydroxylamine system. The points are experimental. The curves are not calculated.

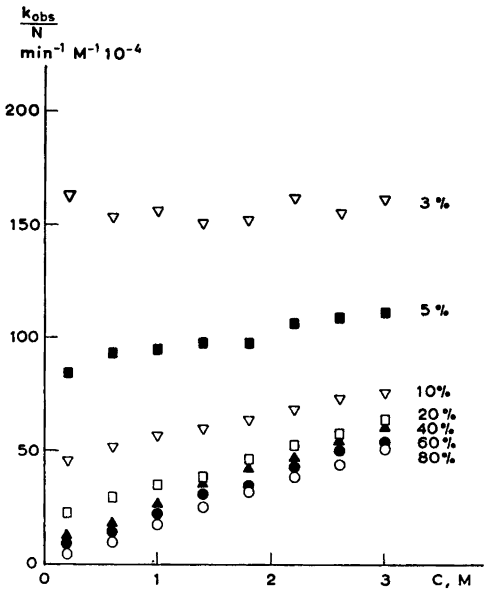


Fig. 4. *p*-Cyanoformanilide. Plot of $k_{obs}/[N]$ *vs.* C at seven different base percentages.

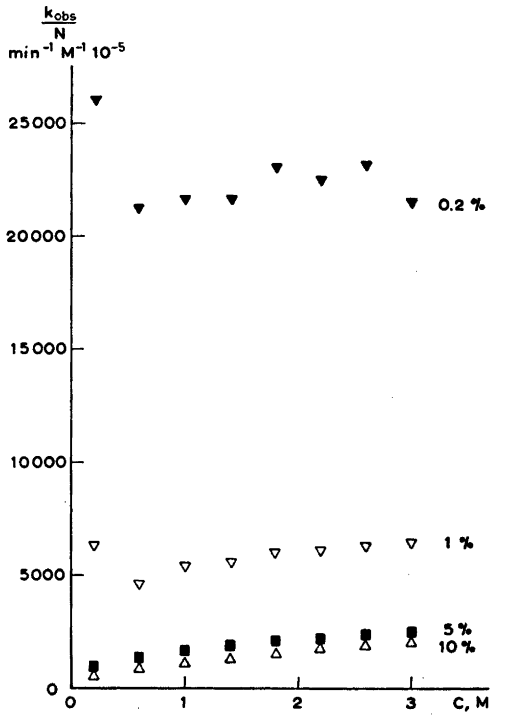
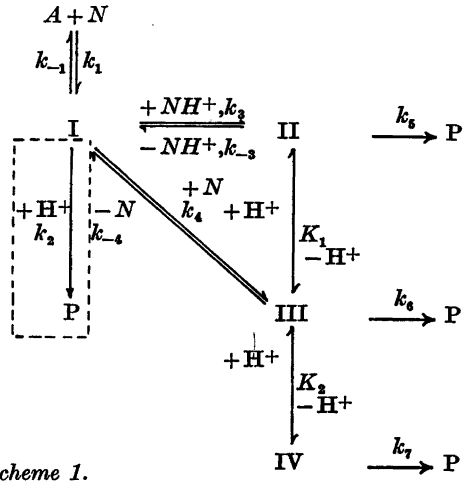


Fig. 5. *p*-Chloroformanilide. Plot of $k_{obs}/[N]$ *vs.* C at four different base percentages.

According to the plots of k_{obs} *vs.* C and k_{obs} *vs.* $-\log [H^+]$, the hydroxylaminolysis reactions of the anilides studied in the present investigation do not differ perceptibly from those of the anilides studied before. The order of dependency of k_{obs} on C rises from first to second with increasing base percentage and the pH-profiles are bell-shaped. Despite this, it was soon apparent that eqn. (1) did not give an acceptable fit to the experimental values.



Scheme 1.

Preliminary calculations showed that in the lower pH region the kinetics comprised a term first order in $[N]$ and first order in $[H^+]$. In a previous paper [7] the presence of a reaction path was discussed where the breakdown of the initial hydroxylamine-anilide intermediate is catalyzed by H^+ (k_2 in Scheme 1).

To see if this path could quantitatively account for the kinetic deviations from the previously adopted mechanism, an equation corresponding to eqn. (1) was derived from Scheme 1 by the method described earlier [9] (eqn. 2).

$$k_{obs} = k_1[N](p'C+q)/(1+p'C) \qquad (2)$$

N,C and k_1 are the same as in eqn. (1), p' is a pH dependent parameter, defined by eqn. (3) and q is defined by eqn. (4).

$$p' = \frac{(K_I + K_{IV}[H^+]/K_a)(1 + K_{II}[H^+] + K_V[H^+]^2)}{(1 + [H^+]/K_a)(1 + (K_{II} + K_{III})[H^+] + (K_V + K_{VI})[H^+]^2)(1 + k_2[H^+]/k_{-1})} \quad (3)$$

$$q = \frac{k_2[H^+]/k_{-1}}{1 + k_2[H^+]/k_{-1}} \quad (4)$$

Eqn. (3) is closely related to the equation describing the pH dependence of p,[7] and the constants K_I to K_{VI} denote the same ratios of the separate microconstants in Scheme 1 as in Ref. 7. The constants k_2 and k_1 also refer to Scheme 1.

Eqn. (2) may be rewritten as in eqn. (2a).

$$k_{obs}/[N] = k_1(p'C + q)/(1 + p'C) \quad (2a)$$

There are two important features of this equation. Firstly, when $C = 0$, $k_{obs}/[N] = k_1 q$. Since q is pH-dependent, plots of $k_{obs}/[N]$ *vs.* C will have pH-dependent intercepts. Secondly, at very high $[H^+]$, $q = 1$ which renders the equation independent of C; $k_{obs}/[N] = k_1$. Thus, if Scheme 1 is valid, k_1 may be evaluated from a plot of $k_{obs}/[N]$ *vs.* C, when the plot is parallel to the abscissa, and when k_1 is known q may be calculated from the intercept of the rest of the plots. This was done for *p*-cyano- and *p*-chloroformanilide (see Figs. 4 and 5). The result is listed in Table 4. When q is near to unity, the ratio $(p'C + q)/(1 + p'C)$ changes only little with C. Because of this, the k_1 values estimated as mentioned above may be too low. To compensate for this, the k_1 values have been rounded upwards, and are given in Table 4 with only 1 (2) significant figure (*cf.* Figs. 4 and 5).

Using the values of k_1 and q estimated from the plots of $k_{obs}/[N]$ *vs.* C and p' values found by iterative calculations, eqn. (2) gave good fits to all the plots of k_{obs} *vs.* C. The best fitting values of p' and q are listed in Table 3.

Since the values of k_1 have been rounded upwards, the values of q listed in Table 3 are all somewhat too small. In Fig. 1, showing the plots of k_{obs} *vs.* C at 10, 60 and 90 % base for *p*-chloroformanilide, the dots are experimental and the lines are calculated by means of eqn. (2) using the parameter values in Tables 3 and 4. The fit is representative for all the curves from the two substances. Thus, the kinetics of the hydroxylaminolysis reaction of *p*-cyano- and *p*-chloroformanilide may be completely explained by means of the equation derived from the mechanism shown in Scheme 1.

Substituent effects on formation of the intermediate I

Table 4 lists the k_1 values for the four formanilides studied. These values have been calculated by different methods and therefore the errors in the values may be of different relative size. In view of the later findings the method used for formanilide and *p*-nitroformanilide may have yielded k_1 values that are somewhat low but not so low as to influence the relative magnitudes. The effect of the substituents on k_1 will therefore be discussed only qualitatively.

In the case of the *p*-substituted formanilides, all of the substituents are electron-withdrawing. As seen, the *p*-chloro-group, which in this case has inductive effects, increases k_1 by a similar factor to that found in alkaline hydrolysis.[4] The *p*-cyano and *p*-nitro groups, on the other hand, which have both $-E$ and $-I$ effects reduce k_1. The different effects of the three substituents support the suggestion that the

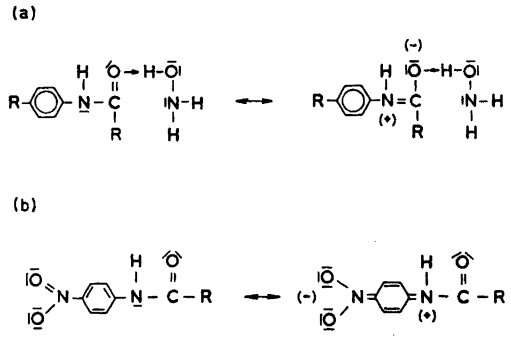
Table 4. Values of k_1, k_2/k_{-1} and k_4/k_{-1} for four formanilides. Both the constants and the ratios are calculated by means of methods, which are commented upon in the text.

	k_1/min^{-1} M^{-1} × 10^{-2}	$k_2(k_{-1})^{-1}/10^4$	$k_4(k_{-1})^{-1}/10^{-2}$
Formanilide	8.0	2	1.9
p-Chloroformanilide	25	0.5	0.55
p-Cyanoformanilide	2	8	5.5
p-Nitroformanilide	0.9	10	10

lowering of k_1 in the *p*-nitro- and *p*-cyano-substituted anilides is due to the mesomeric action of these groups.

In a previous paper[3] on *p*-nitrosubstituted anilides, two possible explanations for this phenomenon were presented.

(1) In the nucleophilic attack a hydrogen bond is formed between the hydroxylamine $-OH$ and the carbonyl oxygen. This bond may be stabilized by the mesomeric structure shown in Scheme 2a, except in anilides where the stabilization is hindered by competing mesomeric structures such as that shown in Scheme 2b.



Scheme 2.

The k_1 values of *p*-cyano- and *p*-chloroformanilide are compatible with this explanation.

(2) A hydrogen bond is formed between the hydroxylamine $-OH$ and the anilide nitrogen. The electron density at this nitrogen is lowered by all electron withdrawing substituents, which will result in a weaker hydrogen bond. Furthermore, *p*-substituents with $-E$ effects, such as the *p*-nitro group, change the geometry at the nitrogen, which may hinder the hydrogen bonded hydroxylamine in the attack on the carbonyl carbon. In view of the fact that introduction of a *p*-chloro group increases k_1, this explanation is no longer plausible.

H+ catalyzed breakdown of I

According to the plots of $k_{obs}/[N]$ *vs.* C, the H+ catalyzed breakdown of intermediate I is detectable (as an intercept) with all the formanilides studied, but in none of the acetanilides. The numerical value of k_2/k_{-1} may be evaluated by means of eqn. (4). This has been done for all the formanilides, and the resulting ratios are listed in Table 4.

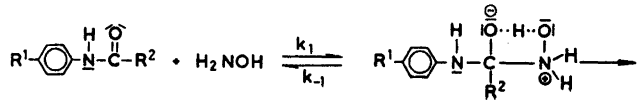
When calculating the ratios, the q values given in Table 3 were used for *p*-cyano- and *p*-chloroformanilide; for the other two q was obtained by dividing the estimated intercepts by k_1. Due to the error in the intercepts, all of the k_2/k_{-1} values should be considered rather rough estimations. They are all of about the same magnitude; possibly the formanilide and *p*-chloroformanilide values can be considered significantly lower than the others. Thus, the significance of the term k_2/k_{-1} in the overall kinetics as described by eqns. (2)–(4) is diminished by the introduction of the acyl $-CH_3$ group, and possibly increased by substituents with $-E$ effects.

This may be due to effects on k_2 or k_{-1} or both. The constant k_{-1} denotes the rate constant for the (spontaneous) breakdown of the anilide-hydroxylamine intermediate to starting materials (see Scheme 3). However, once the intermediate is formed, a $-CH_3$ group in R^2 should strengthen rather than weaken the intermolecular C–N bond.

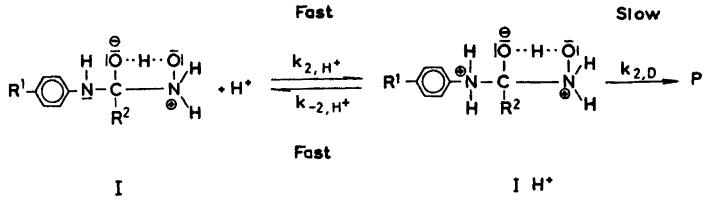
The substituent effect on the ratio k_2/k_{-1} must then be due to changes in k_2. In other words, the reaction $I + H^+ \xrightarrow{k_2} P$ is slower when R^2 is an electron donating group. This is contrary to what would be expected if k_2 denotes the protonation of the anilide nitrogen but in accord with the breakdown of a protonated intermediate IH^+. Thus, it is suggested that the path within the dotted line in Scheme 1 should be depicted as in Scheme 4.

Hydroxylamine catalyzed breakdown of I

In principle, constants K_I to K_{VI} in eqn. (3), denoting different ratios of the microconstants in Scheme 1, may be evaluated from plots of



Scheme 3.



Scheme 4.

p' *vs.* [H+]. In this case, however, the errors in p' are considered too great for the evaluation to give reliable results. The only constant that may be evaluated without computerized mathematical treatment is K_I, which represents the ratio k_4/k_{-1}. This is possible since at low [H+] eqn. (4) reduces to $p' = K_I$.

For all other anilides studied, the calculated values of K_I were very close to the p values used at high base percentages, where the reaction rates had reached the limiting values (*cf.* Figs. 2 and 3). Therefore the limiting values of the experimental p' at high base percentages for p-chloro- and p-cyanoformanilide may be used to discuss the substituent effect on K_I. These values are listed in Table 4 together with the calculated K_I from the formanilides studied earlier.

As seen, there is again a difference between the p-chloro-substituted and the p-cyano- and p-nitro-substituted anilides. In p-cyanoformanilide K_1 is higher than in formanilide and half way between the values in formanilide and p-nitroformanilide; the p-chloroformanilide K_I, on the other hand, is lower. It was earlier suggested [7] that the *HN* or *N* which is added in the k_3 and k_4 step is hydrogen bonded to the anilide nitrogen and to a hydrogen in the original nucleophile to form a cyclic intermediate. It was also suggested [3] that the increase in K_I following p-nitro substitution is due to lowered electron density at the carbonyl carbon, which in turn makes the hydrogens of the original nucleophile more easily accessible for such bonding. In view of the opposite effects exerted by electron withdrawing substituents with and without mesomeric action, it seems more likely that the increasing effect of the p-nitro- and p-cyano substituents is instead due to a change in the geometry at the nitrogen (see Scheme 2b) which facilitates the formation of the cyclic intermediate. The decreasing effect of the p-chloro substituent should then depend on the decrease in electron density at the nitrogen, which is not compensated for by a change in the geometry and which makes the nitrogen less accessible for hydrogen bonding.

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Aliphatic Hydroximates: Structure and Interconversion of Stereoisomers

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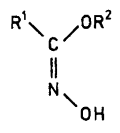
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Aliphatic alkyl hydroximates, produced by reaction of alkyl imidates with hydroxylamine, or by alkylation of alkyl hydroxamates, exist as *Z*- and *E*-isomers. Structural assignment rests on analogies in physical properties, as compared with those of the methyl *E*- and *Z*-acetohydroximates; the structure of the latter is unambiguously established by X-ray crystallography.

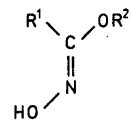
Consistently, the more stable isomers possess the *E*-configuration. In most cases they are partly convertible into the *Z*-isomers upon protonation. Intramolecular, bifunctional catalysis supposedly accounts for the lability of the *Z*-isomers of β-D-glucopyranosyl phenylacetohydroximates. The reversal of the relative stability, when compared with thiohydroximates, is commented upon.

The crystals of methyl *Z*-acetohydroximate are hexagonal, space group $P6_3/m$ with $a = 10.913(3)$, $c = 6.837(3)$ Å, $Z = 6$. The structure was solved by direct phasing technique using three-dimensional X-ray diffraction data and was refined by full-matrix least-squares methods. The final *R*-value is 0.063. The crystal structure is composed of layers of molecules, which form cyclic, hydrogen-bonded trimers. The conformation of the $CH_3-O-C=N-O-H$ moiety is *ap Z ap*.

An unambiguous structure determination of the ethyl hydroximates (*1Z* and *1E*), brought about by X-ray crystallography,[1,2] provided a secure foundation for assignment of structure to several pairs of analogous isomers of alkyl benzohydroximates, including a number of *O*-alkyl and *O*-acyl derivatives, on the basis of dipole moment measurements,[3] the propensity to undergo Beckmann rearrangement,[4,5]



Z



E

1, $R^1 = Ph$; $R^2 = Et$
2, $R^1 = Me$; $R^2 = Et$
3, $R^1 = R^2 = Me$
4, $R^1 = PhCH_2$; $R^2 = Me$
5, $R^1 = PhCH_2$; R^2 = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl

melting point consistencies,[3,5] stereomutation processes,[3,5] acidity properties,[3] and, to a limited extent, ^1H NMR spectroscopy.[5,12]

For the limited group of known aliphatic alkyl hydroximates, of particular interest to us in connexion with other studies, the extant data are less consistent. Thus, the ethyl acetohydroximate, prepared by Houben and Schmidt,[6] was subsequently shown [7] to produce an *O*-phosphate on treatment with PCl_5, rather than a carbamate, favouring its formulation as the *E*-isomer (*2E*). Repetition of the synthesis by Millen and Waters,[8] however, gave an 8:1 mixture of isomers, the more abundant of which was assigned the *Z*-configuration (*2Z*), on the basis of a questionable ^1H NMR analogy to the oxime series; on storage, stereomutation of the alleged *2E* to *2Z* was observed.[8] Conversely, a patent claim, comprising *O*-carbamoyl derivatives of aliphatic alkyl hydroximates,[9] contains a tentative assignment of *E*-configuration to the predominant Houben-synthesis hydroximates, partly convertible, upon protona-

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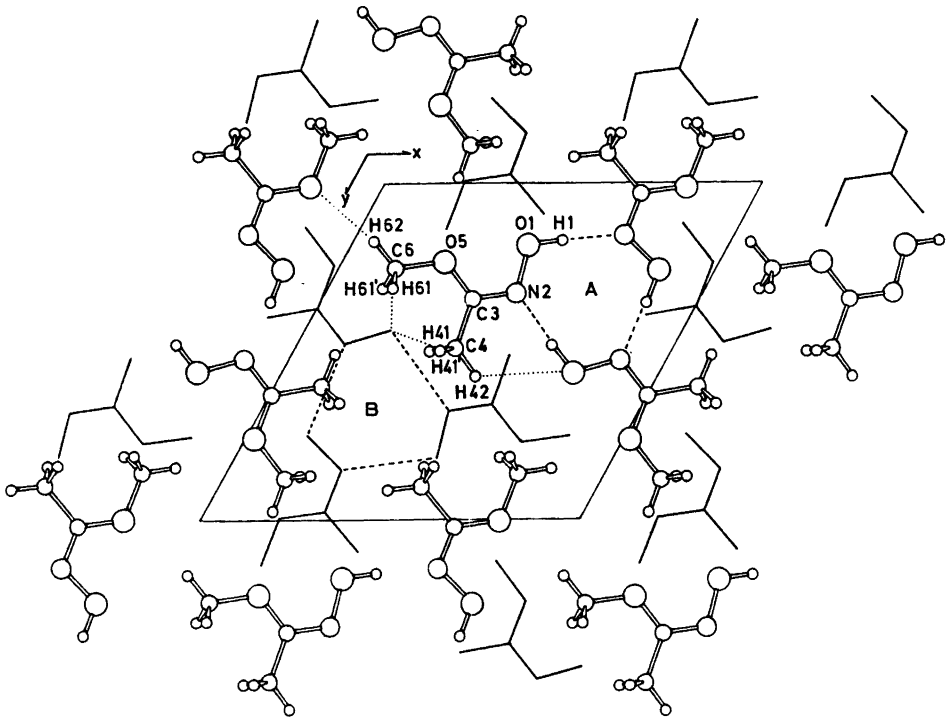


Fig. 1. The structure of methyl *Z*-acetohydroximate as viewed along an axis deviating 10° from the direction of the c axis to allow all the hydrogen atoms of the methyl groups to be seen. Dashed lines indicate hydrogen bonds, dotted lines indicate contacts between methyl hydrogen atoms and neighbouring oxygen atoms. Symbols A and B represent trimers from two layers, separated by 3.418 Å.

tion, into the *Z*-isomers. We report our endeavours to clear up these inconsistencies.

RESULTS

Conversion of methyl and ethyl acetimidate into the corresponding hydroximates, in yields of 25 and 53 %, respectively, was performed as described;[9] methyl phenylacetohydroximate was produced, in 42 % yield, by the same procedure. Chromatographic analysis and NMR spectroscopy served to ascertain the stereochemical homogeneity of all three products. On treatment with anhydrous HCl in ether solution, they all underwent stereomutation, and pure specimens of less stabile, higher melting isomers of the two acetohydroximates were isolated. In the phenyl-substituted series, however, the degree of conversion was much lower and separation of the stereoisomers failed. In acetonitrile solution, reconversion to the more stable isomers occurred, with half-lives at 24 °C corresponding to a free energy of activation of about 110 kJ/mol for ethyl and methyl acetohydroximate. In view of the partly conflicting character of the existing data, unambiguous structural assignment became mandatory. An X-ray determination of the higher melting isomer of methyl acetohydroximate proved it to constitute the *Z*-isomer (*3Z*).

X-Ray structure determination. The crystal is built up of layers of molecules, arranged in mirror planes (Fig. 1). In each layer the molecules form cyclic, hydrogen-bonded trimers, with the following dimensions of the hydrogen bond: O···N = 2.787(4) Å, H···N = 1.79 Å and ∠O−H···N = 177°. The molecular packing of the compound is very similar to that of acetoxime,[10] crystallizing in the same space group with similar lengths of the axes. In the

Table 1. Bond lengths (Å) and angles (°).

O1–N2	1.410(4)	O1–N2–C3	112.4(2)
N2–C3	1.274(5)	N2–C3–C4	118.3(3)
C3–C4	1.480(5)	N2–C3–O5	119.8(3)
C3–O5	1.336(3)	C4–C3–O5	121.9(3)
O5–C6	1.441(5)	C3–O5–C6	119.5(3)

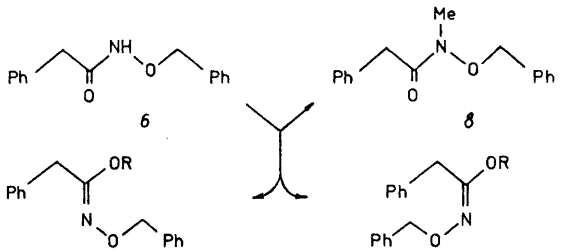
crystal structure of acetoxime only van der Waals forces connect the cyclic, hydrogen-bonded trimers, whereas, in the present structure of methyl *Z*-acetohydroximate, all the hydrogen atoms of the methyl groups are directed toward oxygen atoms of neighbouring molecules, *cf.* Fig. 1. These contacts, with C···H distances of 2.42–2.94 Å, may conceivably define the conformations of the methyl groups and hence contribute to the observed, smaller separation of the layers of trimers (3.418 Å) than in the structure of acetoxime (3.51 Å). The melting point, 126–127 °C, of methyl *Z*-acetohydroximate, when compared with those of the *E*-isomer (28–29 °C) and acetoxime (61 °C), also suggest that strong *inter*-molecular forces are operating in the crystal structure of the former compound.

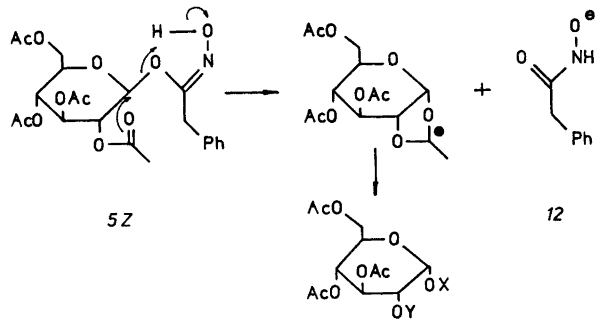
Bond lengths and angles are presented in Table 1, the former agreeing with the corresponding bonds in ethyl *E*- and *Z*-benzohydroximate;[2] some differences were found, however, between corresponding valency angles. In the planar methyl *Z*-acetohydroximate molecule the $O-C_{alkyl}$-bond is situated *antiperiplanar* to the C=N bond. In both isomers of ethyl benzohydroximate these bonds were *synperiplanar*.[2]

Chemical properties. Comparison of melting points, NMR data, and product compositions were utilized in assigning structures to the *2Z/2E* and *4Z/4E* pairs (*vide infra*).

O-Benzyl methyl phenylacetohydroximate, desired as a model for other studies, was produced by methylation of benzyl phenylacetohydroxamate (*6*). The product composition proved highly dependent on the conditions employed; observations, previously made in the aromatic series,[11] were roughly paralleled. Thus, treatment of *6* with diazomethane gave *7Z*, *7E*, and *8*, in the ratio 87:3:10, whereas the silver salt of *6*, on reaction with methyl iodide, yielded the same products, but in the ratio 18:56:26. Assignments (Table 2) were based on ^{1}H NMR characteristics and on the production of *7E* by *O*-benzylation of *4E*. Though stable in benzene solution at 23 °C, the neat *Z*-isomer *7Z* was thermally converted into *7E*, with a half-life of 45 min at 110 °C. In benzene, containing anhydrous HCl, the conversion into *7E* was complete after 2 h at 23 °C. On hydrogenolysis at 20 °C, *7E*, unexceptionally, afforded *4E*, whereas *4Z*, deriving from *7Z*, stereomutated to *4E* in the reaction mixture at a rate only slightly lower than that of the hydrogenolysis reaction.

Extension of the alkylation of *6* to reaction with tetra-*O*-acetyl-α-D-glucopyranosyl bromide furnished, in 75 % yield, an 84:16 mixture of the *O*-benzyl hydroximates *9Z* and *9E* (Table 2), convertible into homogeneous *9E* at 110 °C, with a half-life of 6.3 h, or on standing at 23 °C in HCl-containing benzene, the latter reaction being accompanied, however, by side reactions. Whereas controlled hydrogenolytic debenzylation of *9E* afforded *5E*, *9Z* invariably suffered more deep-seated cleavage, yielding, *inter alia*, 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (*10*),





2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (*11*), and phenylacetohydroxamic acid (*12*), rather than the desired hydroximate *5Z*.

DISCUSSION

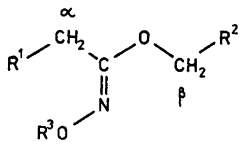
Structural assignments for the products listed in Table 2 rest primarily on (i) consistencies in melting point relations between the pairs of *E*- and *Z*-isomers, (ii) analogies in their NMR spectra, and (iii) the relative stability of the isomers, with reference, in each case, to the authentic (*3Z*/*3E*)-pair.

Within the series of aromatic alkyl hydroximates, the *E*-isomers have melting points higher than those of the *Z*-isomers, a 'rule' which has occassionally been invoked for stereochemical assignments.[5] Apparently, in the present series, the opposite holds true (*cf.* Table 2).

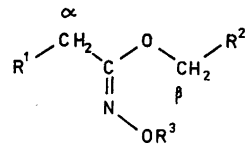
Table 2. ^{1}H NMR shifts of aliphatic hydroximates and some *O*-substituted derivatives.

Compound	M.p. °C	$-CH_2-C-$	$-CH-O-C-$	$-CH_2-O-H$
3Z[a]	126–127[b]	2.00[c]	3.85	
		(1.46)[c]	(3.12)	
3E	21–23[d]	2.00	3.67	
		(1.89)	(3.45)	
2Z	82–84[e]	2.00	4.14	
		(1.56)	(3.63)	
2E	23–24[f]	2.00	3.99	
		(1.90)	(3.90)	
4Z[g]		3.65	3.75	
		(2.93)	(3.27)	
4E	46–47	3.76	3.63	
		(3.74)	(3.36)	
5E	139–140	3.75	5.42	
		(3.10)	(5.47)	
7Z	oil	3.59	3.70	5.05
		(3.28)	(3.35)	(5.04)
7E	oil	3.70	3.62	4.97
		(3.67)	(3.40)	(4.99)
9Z	117–118	3.56	5.39	5.05
		(3.43)	(5.50)	(4.97)
9E	78–79	3.71	5.53	5.05
		(3.53)	(5.58)	(4.97)

[a] X-Ray analysis; present work. [b] Reported:[9] m.p. 123–124 °C. [c] Without parentheses: δ-values in $CDCl_3$; in parentheses: δ-values in C_6H_6. [d] Reported:[9] m.p. 28–29 °C. [e] Reported:[9] 84–85 °C. [f] Reported m.p. 25–26 °C;[6] 22–23 °C.[8] [g] Not isolated in pure form.



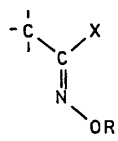
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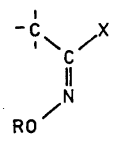
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In the ^{1}H NMR spectra of the aliphatic hydroximates a diagnostically useful feature appears to be the stronger deshielding of the α-protons in the *E*-isomers *13*, when measured in deuteriochloroform and/or benzene. Within the class of methyl and ethyl hydroximates, the more deshielded character, in $CDCl_3$-solution, of the β-protons in the *Z*-isomers *14*, paralleled in the aromatic series,[5,12] constitutes another useful structural character (Table 2). Similarly, the ^{13}C spectra are of diagnostic value. Both the α-C and β-C atoms of the *Z*-isomers *14* consistently resonate at lower field than the corresponding nuclei of the *E*-series (*13*). The observed, lower-field absorption of the sp^2-C in the *E*-isomers apparently constitutes an additional feature of diagnostic value (Table 3).

The factors dictating the ease of interconversion and stability of stereometric azomethines are multifarious and frequently subtle. Whereas oximes, *e.g.* (*15*, X = substituted C; R = H or alkyl), appear resistant to uncatalyzed, thermal stereomutation, aromatic *Z*-amidoximes (*15*, X = substituted N; R = H, or CH_2Ph)[13] share with both aromatic [12] and aliphatic alkyl *Z*-hydroximates (*15*, X = substituted O; R = H, alkyl, or benzoyl), the latter reported herein,



15



16

the propensity to undergo irreversible thermal transformation into the *E*-isomers (*16*). Within the analogous classes of aromatic [14] and aliphatic [15] thiohydroximates (*15*, or *16*, X = substituted S; R = H or CH_3), however, the opposite conversion, *i.e.* that of *16* into *15*, appears to be the favoured process, even when the more stable *Z*-isomers here possess the largest dipole moments.[14]

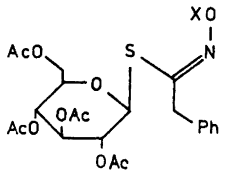
Like in aromatic *O*-alkyl benzohydroximates,[16] amidoximes,[13] and thiohydroximates,[14] the stereomutation of aliphatic hydroximates is considerably facilitated by *N*-protonation, supposedly ascribable, in all cases, to a diminished activation energy for rotation around the C–N-bonds in the protonated species. In the present series, the *Z*/*E*-composition of the protonated equilibrium systems, is highly substituent-dependent, varying from *ca.* 1:3, in the case of *2Z*/*2E* and *3Z*/*3E*, to about 1:9 for *4Z*/*4E*.

The observed, spontaneous fission of the bond between the anomeric carbon and the exocyclic *O*-atom in *5Z*, subsequent to its release from *9Z* on hydrogenolysis, is unparalleled in the *5E*-isomer and deserves comment. Bifunctional, intramolecular general acid and nucleophilic catalysis, as indicated in the formula scheme above, provides a satisfactory explanation for the facility of the reaction and the character of the observed products, *10*, *11* and *12*, and bears resemblance to the mechanism invoked for the facile hydrolysis of 2-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[17] A control experiment, excluding the participation of cyclic ions, in which the deacetylated analogue of *5Z* was generated upon hydrogenolysis in methanol, yielded, as expected, virtually homogeneous

Table 3. ^{13}C NMR shifts of aliphatic hydroximates and some *O*-substituted derivatives (in $CHCl_3$).

Compound	C_α[a]	C_β[a]	C(sp^2)
3Z	14.0	55.9	155.8
3E	12.7	53.7	163.5
2Z	15.1	64.4	154.9
2E	14.1	62.1	163.0
7Z	35.1	56.6	155.3
7E	33.6	54.0	163.5

[a] For α- and β-designations, see *13* and *14*.



17: X = H
18: X = SO_3^-

methyl α-D-glucopyranoside. In 2-propanol, the corresponding reaction proceeded so much slower that stereomutation of *5Z* into *5E* became the predominant reaction. The observed lability of *5Z* is in obvious contrast to the stability of tetra-*O*-acetyl-β-D-glucopyranosyl phenylacetothiohydroximate (*17*), convertible, *via* tetra-*O*-acetyl benzylglucosinolate (*18*), into benzylglucosinolate,[18] possessing *Z*-configuration, as inferred from its natural relationship to the allylglucosinolate ion, for which X-ray structural evidence exists.[19]

EXPERIMENTAL

Melting points are uncorrected. NMR-spectra were recorded on instruments of the types: Varian-HA-100 (^{1}H) and Bruker WH-90 (^{13}C), mass spectra on a Perkin-Elmer model 270 instrument, and rotations on a Perkin-Elmer 141 polarimeter. Analytical TLC was performed on coated silica gel PF_{254} plates (Merck), with detection in UV, or on spraying with 2 % $FeCl_3$ in 0.1 N hydrochloric acid.

X-Ray structure determination. The crystals used for the X-ray analysis were obtained by diffusion at room temperature of pentane into a solution of the compound in chloroform. Colourless, diamond-shaped crystals were formed.

Crystal data. Methyl *Z*-acetohydroximate, $C_3H_7NO_2$, M = 89.10. Space group $P6_3/m$ (No. 176), $a = 10.913(3)$, $c = 6.837(3)$ Å, $Z = 6$, $D_m = 1.26$ g cm^{-3}, $D_x = 1.26$ g cm^{-3}, $\mu(\mathrm{MoK}\alpha) = 0.75$ cm^{-1}. The unit cell parameters were refined by least-squares techniques from 30 diffractometer-measured θ angles. The crystal density was measured by flotation in a mixture of chlorobenzene and bromobenzene.

Data collection. Three-dimensional diffraction data were measured on a Nonius three-circle automatic diffractometer using the same technique as previously described.[20] All data were obtained from a single crystal with approximate dimensions 0.40 × 0.40 × 0.60 mm. The crystal was sealed in a glass capillary and mounted with [001] along the ϕ axis of the goniostat. Out of the 1332 reflections, ($hk\bar{l}$) and ($\bar{h}k\bar{l}$), measured in the range $2.5 \leq \theta \leq 25°$, 756 had $I_{net} \geq 3.0\sigma(I)$, where $\sigma(I)$ is the standard deviation from counting statistics. These reflections were regarded as observed, whereas the remaining were regarded as unobserved and excluded from the refinement procedure. Lorentz and polarization corrections were applied, but no absorption corrections were made.

Structure determination. The structure was solved by direct methods using the automatic phasing programme *MULTAN*.[21] An *E* map, based on 60 *E*-values with $|E| \geq 2.29$, revealed the positions of the six non-hydrogen atoms. Refinement of the positional and thermal (first isotropic and then anisotropic) parameters of these atoms, using the full-matrix least-squares method, led to an *R*-value of 0.095. The quantity minimized was $\sum w(|F_o| - |F_c|)^2$, where the weights were initially taken as unity. The difference Fourier map showed maxima in positions expected for all the hydrogen atoms of the structure. Introduction of the hydrogen atoms into calculated positions in the refinement as fixed parameters and with isotropic temperature factors set equal to those of the atoms to which they are bonded, led to a final *R*-value of 0.063 for all observed reflections. The weighting scheme used in the last cycles of refinement was of the form $w = 1/(1 + [(|F_o| - 7.5)/7.5]^2)$. Tables 4 and 5 list the final positional and thermal parameters for the non-hydrogen atoms, and hydrogen atoms, respectively. The final list of structure factors is available from one of the authors (I.K.L.) on request. All atoms within the molecule show large thermal motion in the *z* direction, *cf.* Table 4. As a test of the planarity of the molecule the atoms were allowed to move out of the mirror plane by least-squares refinement of the structure in the acentric space group $P6_3$. But the shifts of the atoms out of the plane were not significant, and the vibration of the atoms in the *z* direction were still large. Accordingly, the parameters from the refinement in the centrosymmetric space in group $P6_3/m$ were chosen as the final parameters. The programmes used in the crystallographic analysis were the same as those previously employed.[20] The X-ray atomic scattering factors used for hydrogen were those of Stewart, Davidson and Simpson,[22] and for oxygen, nitrogen and carbon those of Cromer and Mann.[23]

Methyl and ethyl E-*acetohydroximate* (3E) *and* (2E). The two esters were prepared from methyl and ethyl acetimidate hydrochloride and hydroxylamine hydrochloride, essentially as described in the patent literature.[9] NMR: *cf.* Tables 2 and 3.

Methyl E-*phenylacetohydroximate* (4E). Application of the same conditions to the reaction between methyl phenylacetimidate and hydroxylamine gave, after recrystallization from light petroleum, the expected ester (42 %), m.p. 42 – 45 °C. An analytical specimen was produced by repeated sublimation at 10^{-5} mmHg (bath

Table 4. Final positional and thermal parameters ($\times 10^3$) for the non-hydrogen atoms. The temperature factors are defined by: exp $[-2\pi^2 (U_{11}h^2a^{*2} + \cdots + 2U_{12}hka^*b^* + \cdots)]$.

Atom	x/a	y/b	z/c	U11	U22	U33	U12	U13	U23
O1	.4360(2)	.1809(2)	.2500	4.0(1)	4.3(1)	13.1(3)	2.6(1)	.0	.0
N2	.4756(3)	.3254(3)	.2500	3.5(2)	3.7(1)	9.1(3)	1.5(1)	.0	.0
C3	.3690(3)	.3435(3)	.2500	3.5(2)	3.9(2)	6.0(3)	1.7(1)	.0	.0
C4	.3948(4)	.4901(3)	.2500	5.4(2)	4.2(2)	11.3(3)	2.4(2)	.0	.0
O5	.2384(2)	.2315(2)	.2500	3.0(1)	4.0(1)	11.4(2)	1.7(1)	.0	.0
C6	.1183(3)	.2527(4)	.2500	4.1(2)	6.7(2)	11.0(4)	3.2(2)	.0	.0

40 °C), m.p. 46 – 47 °C. Anal. $C_9H_{11}NO_2$: C, H, N. ^{1}H NMR: *cf.* Table 2.

Methyl and ethyl Z-acetohydroximate (3Z) and (2Z). Partial stereomutation of the two *E*-esters was achieved by means of ethereal hydrogen chloride, essentially as described.[9] Selective extraction of unchanged *E*-esters with light petroleum facilitated the purification of the *Z*-isomers. *3Z:* Yield 19 % (recovered 28 % of the *E*-isomer), m.p. 126 – 127 °C (from $CHCl_3$: hexane), specimen for X-ray analysis. ^{1}H and ^{13}C NMR: *cf.* Tables 2 and 3. *2Z*: Yield 21 % (recovered 52 % of the *E*-isomer), m.p. 82 – 84 °C (84 – 85 °C [9]). ^{1}H and ^{13}C NMR: *cf.* Tables 2 and 3.

O-Benzyl phenylacetohydroxamate (6). Ethyl phenylacetate was converted into phenylacetohydroxamic acid (yield 63 %, m.p. 140 – 142 °C) by the method described for the preparation of benzohydroxamic acid.[24] *O*-Benzylation of the hydroxamic acid was performed, following the directions given for the synthesis of *O*-alkylated benzohydroxamic acids.[25]

O-Benzyl phenylacetohydroxamate (*6*) was obtained in 79 % yield, m.p. 77 – 79 °C (Lit.[26] 78 – 80 °C): ^{1}H NMR: δ 3.47 (2 H, br.s), 4.83 (2 H, s), 7.25 (5 H, s), 7.41 (5 H, s), 8.32 (1 H, br.s).

Methylation of O-benzyl phenylacetohydroxamate (6). (a) *With diazomethane.* A solution of *6* (4 mmol) in ether (4 ml) and methanol (1 ml) was treated, for 3 days at 22 °C, with a large excess of ethereal diazomethane. Estimated by ^{1}H NMR, the reaction mixture contained the *Z-O*-methyl (*7Z*), *E-O*-methyl (*7E*), and *N*-methyl (*8*) derivatives in the ratio 87:3:10. Preparative TLC, with benzene:ethyl acetate (9:1) as an eluent, afforded the major constituent (yield 69 %), R_F 0.65, consisting of methyl *Z-O*-benzyl phenylacetohydroximate (*7Z*), as a colourless oil, short-way distilled (10^{-5} mmHg, 80 °C) before analysis, n_D^{25} 1.5628. Anal. $C_{16}H_{17}NO_2$: C, H, N. ^{1}H and ^{13}C NMR: *cf.* Tables 2 and 3. MS [IP 70 eV; *m/e* (% rel.int.)]: 256 (25), 255 (90, M), 239 (10), 238 (50), 164 (10), 132 (49), 117 (10), 107 (15), 106 (10), 105 (40), 104 (23), 92 (60), 91 (100), 90 (15), 89 (20), 79 (10), 78 (10), 77 (41), 65 (74), 63 (10), 51 (29), 39 (24), 32 (25), and 28 (90).

With methyl iodide. The silver salt of (*6*) (4 mmol), prepared as described for a similar salt,[5] was suspended in ether (2 ml) and stirred for 40 h at 22 °C in the dark with methyl iodide (8 mmol). The oily reaction product (1.1 g), estimated, by ^{1}H NMR, to contain *7Z*, *7E*, and *8* in the ratio 18:56:26, was separated into three fractions on silica gel plates, with benzene ethyl acetate (9:1) as the solvent. The fastest moving band (R_F 0.9) (49 %) contained methyl *E-O*-benzyl phenylacetohydroximate (*7E*), purified by short-way distillation (10^{-5} mmHg, 75 °C), n_D^{25} 1.5512. Anal. $C_{16}H_{17}NO_2$: C, H, N. ^{1}H and ^{13}C NMR: *cf.* Tables 2 and 3. The MS was indistinguishable from that of *7Z*. The second band (R_F 0.7) contained *7Z* (14 %), identical with the material described above; from the slowest moving band (R_F 0.5) was obtained a 16 % yield of benzyl *N*-methylphenylacetohydroxamate (*8*), distilled at 10^{-5} mmHg (75 °C) before analysis. n_D^{25} 1.5630. Anal. $C_{16}H_{17}NO_2$: C, H, N. ^{1}H NMR: 3.20 (3H, s), 3.69 (2H, s), 4.75 (2H, s), 7.23 (5H, s), and 7.35 (5H, s). MS [IP 70eV; *m/e* (% rel.int.)]: 255 (14, M), 210 (15), 209 (90), 208 (36), 181 (20), 105 (10), 92 (85), 91 (100), 90 (15), 89 (20), 77 (30), 65 (85), 63 (15), 51 (20), 39 (30), 32 (30), and 28 (80).

O-Benzylation of 4E. A methanol solution (40 ml), containing sodium methoxide (30 mmol), methyl *E*-phenylacetohydroximate (*4E*) (30 mmol), and benzyl bromide (30 mmol), was refluxed for 2 h. After evaporation to dryness and extraction with chloroform, the residual oil was fractionally distilled at 11 mmHg to

Table 5. Positional and thermal parameters used for the hydrogen atoms.

Atom	x/a	y/b	z/c	B
H1	.519	.166	.250	3.7
H41	.345	.500	.131	4.5
H42	.491	.575	.250	4.5
H61	.127	.312	.131	3.8
H62	.020	.171	.250	3.8

give benzyl methyl ether (b.p. 57 – 59 °C) (1.1 g) and a higher-boiling fraction (b.p. 140 – 190 °C), consisting, according to ^{1}H NMR analysis, of about equal amounts of starting material (*4E*) and the *O*-benzyl derivative (*7E*). The latter was separated from other constituents by column chromatography on silica gel (200 g), with chloroform as the eluent. A 36 % yield was obtained of a colourless oil, identified, by n_D^{25} and ^{1}H NMR, as *7E*.

Hydrogenolysis of 7E *and* 7Z. Methyl *E*-*O*-benzyl phenylacetohydroximate (*7E*) (1.1 mmol) was dissolved in ethyl acetate (10 ml). 5 % Palladium on charcoal (250 mg) was added, and the mixture was shaken with hydrogen (3 at) at 22 °C for 2 h. After filtration and evaporation, an oil remained which, according to TLC and ^{1}H NMR, consisted of homogeneous *4E*. Subjecting the isomeric *Z*-*O*-benzyl methyl ester (*7Z*) to the same reaction conditions resulted in the production, after only 1 h, of a *ca.* 78:22 *E*/*Z*-ratio of the debenzylated species, with only traces present of the starting material. Prolonged reaction times gave more complicated mixtures. Performing the reaction in tetrahydrofuran and ether, or changing the catalyst to palladium oxide or palladium on barium sulfate, resulted in no improvement. On rapid and cautious TLC (benzene:ethyl acetate, 4:1), a colourless oil (12 %) was obtained and identified as methyl *Z*-phenylacetohydroximate (*4Z*) on the basis of ^{1}H NMR data (*cf.* Table 2). Attempts at further purification invariably resulted in its stereomutation to *4E*.

Tetra-O-*acetyl*-*β*-D-*glucopyranosyl*-Z-O-*benzyl phenylacetohydroximate* (9Z). A suspension of the silver salt of *O*-benzyl phenylacetohydroxamic acid (*6*) (15.5 mmol), drierite (5 g), and tetra-*O*-acetyl-α-D-glucopyranosyl bromide (14 mmol) in acetonitrile (40 ml) was stirred, in the dark, for 64 h at 22 °C. After filtration and evaporation, the reaction mixture was separated by preparative TLC on silica gel plates (benzene:ethyl acetate, 4:1) into a major (R_F 0.5), and a minor (R_F 0.6) product. The former was recrystallized from ethyl acetate: light petroleum to give a colourless product, m.p. 116 – 118 °C. An analytical specimen was produced by additional recrystallizations from the same solvent and aqueous methanol, m.p. 117 – 118 °C, $[\alpha]_D^{25}$ – 3.9° (*c* 1.0, chloroform). Anal. $C_{39}H_{33}NO_{11}$: C, H, N. By ^{1}H NMR spectroscopy, the product was assigned the *Z*-configuration *9Z*, *cf.* Table 2. The minor product, undoubtedly representing the *9E*-isomer, could be more conveniently isolated after treating the original glucoside-mixture (6.7 g) in benzene solution (40 ml) with HCl-saturated benzene (1 ml) for 20 h at 22 °C. After column chromatography [silica gel (200 g), chloroform:ethyl acetate (4:1)], the *E*-glucoside (*9E*, 3.2 g), m.p. 71 – 73 °C was obtained in virtually homogeneous form. An analytical specimen, m.p. 78 – 78.5 °C, was produced by two recrystallizations from ethyl acetate:light petroleum, $[\alpha]_D^{20}$ – 88.4 °C (*c* 1.0, chloroform). Anal. $C_{29}H_{33}NO_{11}$: C, H, N. ^{1}H NMR: *cf.* Table 2.

Hydrogenolysis of 9E *and* 9Z. Subjected to hydrogenolysis, as described above, the *O*-benzylated *E*-glucoside *9E* afforded, after 18 h, a mixture of compounds, which was separated by preparative TLC on silica gel plates, with chloroform:ethyl acetate (2:1) as a solvent, into starting material (R_F 0.7, 12 %), the expected, debenzylated glucoside tetraacetate (*5E*, R_F 0.4, 50 %), an oily material (R_F 0.2, 30 %), exhibiting an ^{1}H NMR spectrum in keeping with its identity as 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (*11*), admixed with traces of the 1,3,4,6-isomer (*10*),[27] and, finally, phenylacetamide (R_F 0.1, 18 %). Benzyl alcohol was not detected, and it was ascertained that the major product (R_F 0.4), when subjected to the hydrogenolysis conditions, did, in fact, undergo fission into phenylacetamide, *10* and *11*. Repeated recrystallizations of the major product from ethyl acetate:light petroleum gave an analytical specimen of *5E*, m.p. 139 – 140 °C, $[\alpha]_D^{20}$ – 91° (*c* 1.0, chloroform). Anal. $C_{22}H_{27}NO_{11}$: C, H, N. For ^{1}H NMR data, *cf.* Table 2. When the *Z*-isomer *9Z* was similarly treated, preparative chromatography of the reaction mixture, after 5 h, when no starting material was left, afforded the tetraacetates *11* and *10*, in addition to phenylacetohydroxamic acid (*12*), contaminated with traces of phenylacetamide, the latter shown to be slowly formed from *12* under the conditions employed. All attempts to conduct the reaction so that *5Z* could be isolated proved of no avail.

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Short Communications

Electrochemical Reductive Acetylation of Anthracene

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Electrochemical reductive acetylation of heteroaromatic compounds,[1] ketones,[2-4] nitro compounds,[5] and disulfides [6] has been described: in most of these examples the acetylation takes place at a hetero atom. Below is reported on an electrochemical reductive acetylation which involves formation of a carbon-carbon bond.

Results and discussion. Anthracene (*1*) in *N,N*-dimethylformamide (DMF) containing tetrabutylammonium iodide (TBAI) as supporting electrolyte is reduced more easily than acetic anhydride (*2*). Cyclic voltammograms of *1* in the absence and presence of *2* showed that the peak height of the first one-electron wave (formation of the anion radical) increased to approximately the height of a two-electron wave on addition of *2*; the peak potential of *1* was not affected by the addition of *2*.

Preparative reduction of *1* in the presence of excess of *2* gave a major product in good (66 – 75 %) yield together with small amounts of some side products. The major product was suggested to be the enol acetate of 9-acetyl-9,10-dihydroanthracene (*3*) from the mass spectrum, ^{1}H NMR and IR spectra. The MS indicated a molecular weight of 264, the ^{1}H NMR had singlets at δ 2.06 (3 H), 2.27 (3 H) and 3.84 (2 H), and the IR a carbonyl band at 1745 cm^{-1}. The compound was further characterized by acid hydrolysis to 9-acetyl-9,10-dihydroanthracene (*4*).

One of the minor products which was obtained in a small and variable yield (0 – 2 %) was suggested to be a mixture of the *cis* and *trans* forms of the diacetate of 9,10-diacetyl-9,10-dihydroanthracene (*5a*) and (*5b*) from the ^{1}H NMR spectrum and the mass spectrum. The reaction may be formulated as shown in Scheme 1.

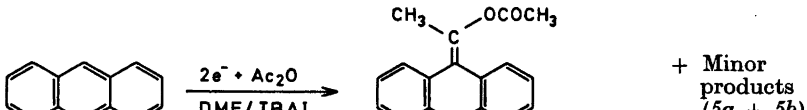
With lithium chloride as supporting electrolyte no *3* was formed but some *4* and 9,10-dihydroanthracene (*6*).

The reaction mechanism has not yet been established. A nucleophilic attack on acetic anhydride might occur, but attack by acetyl radicals formed by electron transfer from $1^{\cdot-}$ cannot be excluded. The nucleophile might be the anthracene anion radical, its dianion, or the monoanion of dihydroanthracene; the substrate for a radical attack might be anthracene or its anion radical.

The reductive acetylation is not limited to anthracene and similar aromatic hydrocarbons; an activated olefin, such as ethyl cinnamate (*7*), reacts similarly. *7* is thus reductively acetylated in good yield mainly to ethyl 3-phenyl-4-oxopentanoate which after reaction with hydrazine forms 4,5-dihydro-6-methyl-5-phenylpyridazin-3-(2*H*)-one.[7]

Experimental. Reductive acetylation of anthracene (1). 1 (2 g) was reduced at a mercury cathode in 150 ml of dry DMF/0.1 M TBAI containing 10 ml of acetic anhydride at – 2.0 V *vs.* aq. SCE. The acetic anhydride was, prior to the addition, purified by standing over activated alumina and 10 g of alumina were suspended in the catholyte. When the reduction was completed the catholyte was diluted with water and extracted three times with diethyl ether; the pooled ether extracts were washed with an aqueous sodium bicarbonate solution, dried with molecular sieves A4 and the solvent evaporated leaving 2.5 g of crude product. The crude product was separated on a column of silica using methylene chloride as eluent. *3* was eluted before *4a+b*.

3, Enolacetate of 9-acetyl-9,10-dihydroanthracene. Isolated was 1.92 – 2.20 g (66 – 75 %), m.p. 108 – 109 °C (ethanol). ^{1}H NMR



1 *3*

Scheme 1.

spectrum ($CDCl_3$): δ 2.06 (s, 3 H); 2.27 (s, 3 H); 3.84 (s, 2 H); 7.1 – 7.35 (m, 6 H); 7.40 – 7.65 (m, 2 H). IR spectrum (KBr) cm^{-1} (intensity): 2920 (w), 1745 (s), 1655 (m), 1445 (m), 1365 (ms), 1220 (s), 1155 (s), 1008 (m), 948 (m), 781 (s), 738 (s). Mass spectrum (*m/e* (%)): 264 (4), 222 (64), 179 (64), 178 (60), 43 (100).

4a+b, Enolacetate of 9,10-diacetyl-9,10-dihydroanthracene, 0 – 2 % yield, m.p. 152 – 158 °C (light petroleum), probably a mixture of the *cis* and *trans* forms. ^1H NMR spectrum ($CDCl_3$): δ 2.10 (s 6 H), 2.3 (two closely spaced singlets, 6 H), 7.1 – 7.6 (m 8 H). Mass spectrum (*m/e* (%)): 348 (1.5), 306 (10), 264 (28), 263 (38), 246 (17), 221 (16), 203 (17), 178 (22), 73 (68), 43 (100).

9-Acetyl-9,10-dihydroanthracene, (4). 3 was hydrolyzed in aqueous ethanolic 1 N HCl by boiling for 3 h under nitrogen. After cooling the mixture was diluted with water and extracted with ether, which was washed with an aqueous sodium bicarbonate solution and dried. Evaporation of the ether left a residue which was purified on a column of alumina using 5/95 ethyl acetate/light petroleum as eluent. *4* (liquid),[8,9] ^1H NMR (60 HMz, $CDCl_3$): δ 1.96 (3 H, s), 3.89 (1 H, d, *J* 18.5 Hz), 4.14 (1 H, d, *J* 18.5 Hz), 4.93 (1 H, broad s) 7.1 – 7.5 (8 H, m). Besides the couplings recorded, small unresolved long-range couplings between the 9 and 10 protons are found. *4* semicarbazone, m.p. 222 – 224 °C (224 – 226 °C).[9]

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The Reaction of 2-Methylphenylmagnesium Bromide with Ethyl 3-Coumarincarboxylate. Addition of Three Reagent Molecules Followed by a Ring Opening

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The reaction of 2-methylphenylmagnesium bromide with ethyl 3-coumarincarboxylate (*1*) has recently been studied in this laboratory.[1] The reaction product proved to be ethyl 4-(2-methylphenyl)-3,4-dihydrocoumarin-3-carboxylate (*2*), formed by a fast conjugate addition.

It has earlier been established that phenylmagnesium bromide to some extent can react further with the primarily formed 1,4-adduct giving slowly 3-(2-hydroxyphenyl)3-phenylpropiophenone on saponification.[2]

When 2-methoxyphenylmagnesium iodide was allowed to react with ethyl 3-coumarincarboxylate, several higher boiling products appeared if the reaction time was extended.[3]

Because 2-methylphenylmagnesium bromide can be considered to have a reactivity between that of phenylmagnesium bromide and 2-methoxyphenylmagnesium iodide it seemed interesting to look more closely at the reaction between 2-methylphenylmagnesium bromide and ethyl 3-coumarincarboxylate.

When the ratio of reagent to substrate was raised to 5 in the reaction of 2-methylphenylmagnesium bromide with ethyl 3-coumarincarboxylate and the reaction time was prolonged to 48 h, mainly one new product (GLC yield *ca.* 25 %) appeared. This compound, which was isolated by column chromatography, was formed rather slowly. By spectroscopic methods it was shown to be 1,3-di(2-methylphenyl)-3-[2′-(2-methylbenzoyloxy)phenyl]propan-1-one (*3*).

This compound is obviously formed by addition of three molecules of the Grignard reagent followed by a ring opening reaction during and after hydrolysis of the primarily formed magnesium complexes (Scheme 1). The ring opening reaction can be considered as an example of the reverse aldol condensation, where the equilibrium lies far towards the free ester and ketone.

The missing reactivity of the magnesium complex, which is formed by the first 1,4-addition, is explained by the lack of a well-defined carbonyl group. The actual reagent in

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spectrum ($CDCl_3$): δ 2.06 (s, 3 H); 2.27 (s, 3 H); 3.84 (s, 2 H); 7.1 – 7.35 (m, 6 H); 7.40 – 7.65 (m, 2 H). IR spectrum (KBr) cm^{-1} (intensity): 2920 (w), 1745 (s), 1655 (m), 1445 (m), 1365 (ms), 1220 (s), 1155 (s), 1008 (m), 948 (m), 781 (s), 738 (s). Mass spectrum (*m/e* (%)): 264 (4), 222 (64), 179 (64), 178 (60), 43 (100).

4a+b, Enolacetate of 9,10-diacetyl-9,10-dihydroanthracene, 0 – 2 % yield, m.p. 152 – 158 °C (light petroleum), probably a mixture of the *cis* and *trans* forms. ^1H NMR spectrum ($CDCl_3$): δ 2.10 (s 6 H), 2.3 (two closely spaced singlets, 6 H), 7.1 – 7.6 (m 8 H). Mass spectrum (*m/e* (%)): 348 (1.5), 306 (10), 264 (28), 263 (38), 246 (17), 221 (16), 203 (17), 178 (22), 73 (68), 43 (100).

9-Acetyl-9,10-dihydroanthracene, (4). 3 was hydrolyzed in aqueous ethanolic 1 N HCl by boiling for 3 h under nitrogen. After cooling the mixture was diluted with water and extracted with ether, which was washed with an aqueous sodium bicarbonate solution and dried. Evaporation of the ether left a residue which was purified on a column of alumina using 5/95 ethyl acetate/light petroleum as eluent. *4* (liquid),[8,9] ^1H NMR (60 HMz, $CDCl_3$): δ 1.96 (3 H, s), 3.89 (1 H, d, *J* 18.5 Hz), 4.14 (1 H, d, *J* 18.5 Hz), 4.93 (1 H, broad s) 7.1 – 7.5 (8 H, m). Besides the couplings recorded, small unresolved long-range couplings between the 9 and 10 protons are found. *4* semicarbazone, m.p. 222 – 224 °C (224 – 226 °C).[9]

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The Reaction of 2-Methylphenylmagnesium Bromide with Ethyl 3-Coumarincarboxylate. Addition of Three Reagent Molecules Followed by a Ring Opening

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The reaction of 2-methylphenylmagnesium bromide with ethyl 3-coumarincarboxylate (*1*) has recently been studied in this laboratory.[1] The reaction product proved to be ethyl 4-(2-methylphenyl)-3,4-dihydrocoumarin-3-carboxylate (*2*), formed by a fast conjugate addition.

It has earlier been established that phenylmagnesium bromide to some extent can react further with the primarily formed 1,4-adduct giving slowly 3-(2-hydroxyphenyl)3-phenylpropiophenone on saponification.[2]

When 2-methoxyphenylmagnesium iodide was allowed to react with ethyl 3-coumarincarboxylate, several higher boiling products appeared if the reaction time was extended.[3]

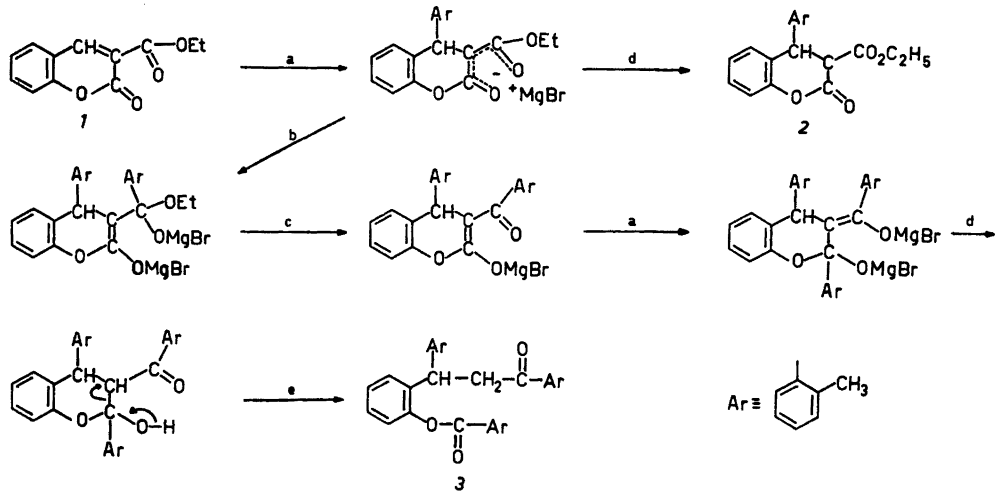
Because 2-methylphenylmagnesium bromide can be considered to have a reactivity between that of phenylmagnesium bromide and 2-methoxyphenylmagnesium iodide it seemed interesting to look more closely at the reaction between 2-methylphenylmagnesium bromide and ethyl 3-coumarincarboxylate.

When the ratio of reagent to substrate was raised to 5 in the reaction of 2-methylphenylmagnesium bromide with ethyl 3-coumarincarboxylate and the reaction time was prolonged to 48 h, mainly one new product (GLC yield *ca.* 25 %) appeared. This compound, which was isolated by column chromatography, was formed rather slowly. By spectroscopic methods it was shown to be 1,3-di(2-methylphenyl)-3-[2′-(2-methylbenzoyloxy)phenyl]propan-1-one (*3*).

This compound is obviously formed by addition of three molecules of the Grignard reagent followed by a ring opening reaction during and after hydrolysis of the primarily formed magnesium complexes (Scheme 1). The ring opening reaction can be considered as an example of the reverse aldol condensation, where the equilibrium lies far towards the free ester and ketone.

The missing reactivity of the magnesium complex, which is formed by the first 1,4-addition, is explained by the lack of a well-defined carbonyl group. The actual reagent in

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Scheme 1. a. 1,4-Addition; b. 1,2-addition; c. elimination of C_2H_5OMgBr; d. hydrolysis; e. ring opening.

the slow addition may be di(2-methylphenyl)-magnesium, which is present in equilibrium with the Grignard reagent and is more reactive.[4]

Experimental. Reaction of 2-methylphenyl-magnesium bromide with ethyl 3-coumarin-carboxylate.[5] A solution of ethyl 3-coumarin-carboxylate (5.79 g) in dry benzene (40 ml) was added to the Grignard reagent prepared from 2-bromotoluene (22.7 g), magnesium (3.2 g), and dry diethyl ether (110 ml). The reaction was worked up after 48 h. The yield of products was 10.4 g.

On treatment of the sirupy reaction product with ethanol, the 1,4-adduct (*2*) crystallized (4.4 g, from ethanol). The residue weighed 4.03 g and consisted mainly of one component (GLC analysis; column 1.5 m 1 % SE-30).

Chromatography. The column, Pharmacia SR-25/100, was packed with 428 ml alumina (Merck 1097). The height of the packing was 840 mm. A part of the residue (250 mg) was dissolved in 1.0 ml benzene and applicated at the lower end of the column. The speed of the liquid phase (benzene) was 21.6 ml/h regulated by a peristaltic pump, Desaga 131900. The eluate was divided into fractions of 5.4 ml. The fractions 55 – 75 contained the pure substance (*3*). Yield: 15.2 mg (from ethanol), m. p. 93 – 94 °C. MS [IP 70 eV; *m/e* (% rel. int.)]: 438 (1, M), 329 (2 [M – C_8H_7O]), 312 (15, [M – $C_8H_8O_2$]), 119 (100, C_8H_7O), 91 (26, C_7H_7), 65 (5, C_5H_5). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.98 (3 H, s), 2.02 (3 H, s), 2.53 (3 H, s), 3.49 (2 H, d, J 8.0 Hz), 5.00 (1 H, t), 6.7 – 8.2 (16 H, m). IR (CCl_4): 1750 (s), 1700 (s).

Hydrolysis. The substance (13 mg) was hydrolyzed with 5 drops of KOH in H_2O (1:1) and a small amount of ethanol. The mixture was boiled for 30 min and 5 drops of dimethyl sulfate was added during 30 min. After 2 h the mixture was dissolved in water and extracted with ether. The mass spectra (combined GC/MS; LKB 9000) of the two compounds in the ether extract revealed that the expected products had been formed, methyl 2-methyl-benzoate and 1,3-di(2-methylphenyl)-3-(2-methoxyphenyl)propan-1-one.

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Substituent Effects on Phosphorus Nucleophilicity in Phosphinoacetic Acid Derivatives

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Tertiary phosphines, although generally powerful nucleophiles, vary considerably in reactivity depending on the substituents on phosphorus. Davies and Lewis [1] and later Henderson and Buckler [2] have measured relative nucleophilicities toward EtI of a series of phosphines and the rate constants have, with limited success, been correlated with Taft σ^* values [2] or Kabachnik σ^{Ph} values.[3] The phosphines examined by Henderson and Buckler contained mostly unsubstituted radicals and, to our knowledge, no quantitative measurements of the influence of substituents in alkyl radicals on the nucleophilicity of phosphines have been published.

One of us has prepared some (cyanomethyl)-phosphines, $R_nP(CH_2CN)_{3-n}$, which show very low nucleophilic reactivities towards EtI.[4,5] This property is conventionally thought to reflect the strong $-I$ effect of the cyano group. In order to obtain more insight in the factors which influence phosphorus nucleophilicity we have measured the nucleophilic reactivity of some diphenylphosphinoacetic acid derivatives towards EtI in acetone (eqn. 1).

$$Ph_2PCH_2X + EtI \xrightarrow[\text{acetone, 35 °C}]{k} Ph_2EtP^+CH_2X\ I^- \quad (1)$$

X = COOH, COOEt, $CONH_2$, $CONMe_2$, CN

There is conflicting evidence in the literature concerning the reactivity of these phosphines towards alkyl halides. It is stated that Ph_2PCH_2COOH does not react with MeI, whereas Ph_2PCH_2COOEt does,[6] and that Ph_2PCH_2CN reacts with EtI.[4] We have found that all of the above mentioned phosphines react with EtI in acetone, and have determined the rate constants at 35 °C (Table 1). The rate constants do not correlate too well with the $\sum\sigma^*$ values (best least squares fit, $r = 0.93$, see Fig. 1), but the trend in nucleophilic reactivity, $Ph_2PCH_2CONMe_2 > Ph_2PEt \sim Ph_2PCH_2CONH_2 > Ph_3P \sim Ph_2PCH_2COOH \sim Ph_2PCH_2COOEt > Ph_2PCH_2CN$, is reasonable if mainly inductive effects are operating. A correlation with Kabachnik's σ^{Ph} substituent coefficients, which contrary to the σ^* values are derived from reactions of phosphorus compounds is not possible because of lack of data for the substituents of interest here.

Table 1. Rate constants for quaternization,[a] Taft σ^* values of substituents, and phosphorus lone-pair ionisation potentials of diphenylphosphinoacetic acid and related phosphines.

Compound	$k \times 10^5$/ l mol^{-1} s^{-1}	$\sum\sigma^*$ [c]	I.P. [e]
Ph_2PCH_2COOH	4.1	2.25	8.22
Ph_2PCH_2COOEt	3.9	1.91	8.16
$Ph_2PCH_2CONH_2$	10.7	1.61 [d]	8.18
$Ph_2PCH_2CONMe_2$	17.7		8.05
Ph_2PCH_2CN	0.40	2.91	8.44
Ph_2PMe		1.20	8.05
Ph_2PEt	11.2 [b]	1.10	7.98
Ph_3P	3.2 (3.8 [b])	1.80	7.88 (7.88 [f])

[a] With EtI in acetone, 35 °C. [b] Ref. 2. [c] σ^* = 0.60 for Ph, 0.00 for Me, −0.10 for Et, 1.05 for CH_2COOH, 0.71 for CH_2COOEt,[9] and 1.71 for CH_2CN.[10] [d] $\sigma^* = 0.41$ for CH_2CONH_2 calculated from the σ^* given [c] and pK_A-values for $CH_2(COOH)_2$, $EtOCOCH_2COCH$, H_2NCOCH_2COOH, and $NCCH_2COOH$ (2.78, 3.35, 3.64 and 2.47, resp.).[11] [e] Vertical ionisation potentials, eV. [f] Ref. 12.

Phosphorus lone-pair ionisation potentials (IP) have been suggested [7] as an alternative measure of the relative nucleophilicity of phosphines. We have measured the vertical IP's of the diphenylphosphinoacetic acid derivatives and of three related phosphines by He(I) photoelectron spectroscopy (Table 1) and correlated the rate constants for quaternization with these IP values (Fig. 2). The best least squares fit ($r = 0.91$) excepting triphenyl phosphine which

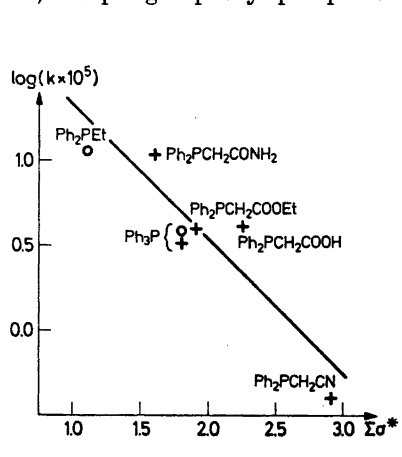


Fig. 1. Plot of rate constant *versus* Taft σ^* values for the reaction $R_3P + EtI \rightarrow R_3EtP^+ I^-$, solvent acetone, 35 °C. + = This study, O = Henderson and Buckler.[2]

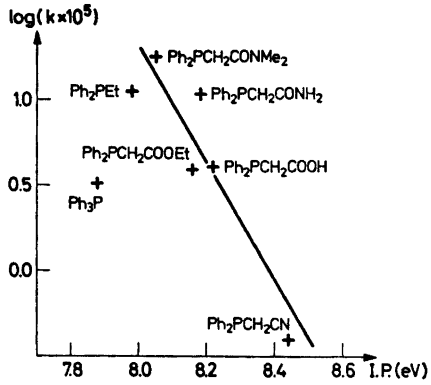


Fig. 2. Plot of rate constant *versus* lone-pair ionisation potential for the reaction $R_3P + EtI \rightarrow R_3EtP^+ I^-$, solvent acetone, 35 °C.

does not belong to the series Ph_2PCH_2X is shown in the figure.

While it is obvious that the deviations from linearity do exceed the experimental error it is also evident that IP's as a measure of nucleophilic reactivities of phosphines are not less reliable than the σ^* values. On the other hand a perfect linear correlation between IP's and free energies of activation can hardly be expected. IP's according to Koopmans theorem represent one-electron orbital energies. The latter quantities are related to activation energies through the general perturbation equation for reactions between soft acids and bases.[8] Even accepting the validity of both relations for the present case the correlation implies two rather crude approximations. IP's are recorded in the gas phase and may change to a varying degree on transfer of the compounds to acetone solution as a result of conformational changes and/or solvation effects. The perturbation treatment neglects the entropy contribution to the free energy of activation and is thus valid only to the extent that this contribution remains constant through the series compared.

In conclusion, we have shown that rate of quaternization does depend on the *measured* quantity, lone-pair ionisation potential, for the phosphines studied. However, the rather weak correlation indicates that additional factors influence the reaction rates and further data are needed in order to separate these. The present study further shows that a cyanomethyl group is the most potent of the radicals studied with respect to decreasing the nucleophilic reactivity of a tertiary phosphine, and that it excerts its effect largely by increasing the lone-pair ionisation potential of phosphorus.

Experimental. He(I) photoelectron spectra were obtained on a Perkin-Elmer PS 18 spectrometer. Calibrations were performed by measuring the 2P3/2 peaks of Ar and Xe present along with the samples. The phosphines are serious contaminants in the analyzer and therefore the calibration peaks were recorded immediately before and after the phosphine first I.P. in order to minimize spectrum drift. The I.P.'s were reproducible within ±0.03 eV. NMR spectra were run on a Bruker HX-90 E spectrometer. The δ values are given as positive for lowfield shifts and refer to internal TMS for δ_H and external 85 % H_3PO_4 for δ_P. The kinetic measurements were performed as described earlier [4] using the conductivity method of Henderson and Buckler.[2] The solvent was acetone (Merck, "zur Analyse") and dissolved oxygen was removed by boiling and cooling under nitrogen giving a conductivity of 0.1–0.2 μS. The solvent was not dried because the earlier described method [4] and other attempts (Al_2O_3, neutral, molecular sieves) caused substantial condensation to diacetone alcohol. Liquid phosphines were handled under nitrogen, and small amounts of hydroquinone were added to all solutions of phosphines in order to minimize oxidation. Solutions of phosphines were checked (^1H NMR) after the kinetic measurements and contained only a few percent phosphine oxides for the most oxidable phosphines. Ph_3P (Fluka) was purified by recrystallization from EtOH, m.p. 79.5–80.5 °C. The following compounds were prepared according to literature methods: Ph_2PMe;[13] Ph_2PCH_2COOH,[6] m.p. 123.5–124.5 °C ($MeOH-H_2O$), lit.[6] 120–121 °C. NMR [$(CD_3)_2CO$]: δ_P −18.4; δ_H 3.17 (CH_2, $^2J_{PH}$ 0.3 Hz); Ph_2PCH_2COOEt,[6] b.p. 135–137 °C/0.15 mmHg, lit.[6] 183–185 °C/5 mmHg. NMR ($CDCl_3$): δ_P −16.8; δ_H 3.11 (P-CH_2, $^2J_{PH}$ 0.5 Hz); $Ph_2PCH_2CONH_2$,[14] m.p. 182.5–184°C (CH_3CN), lit.[13] 173–174 °C. NMR [$(CD_3)_2SO$]: δ_P −17.5; δ_H 3.06 (CH_2, $^2J_{PH}$ 1.1 Hz); Ph_2PCH_2CN;[4] $Ph_3P^+ I^-$;[2] $Ph_2EtP^+CH_2CN\ I^-$.[4] Ph_2PEt was prepared in the same way as Ph_2PMe [13] (60 %).

N,N-*Dimethyl diphenylphosphinoacetamide* was prepared in the same way as Ph_2PMe.[13] Ph_3P (13.1 g, 0.05 mol) was added to a stirred solution of Na (2.3 g, 0.10 mol) in liq. NH_3 (100 ml) at −78 °C. After 15 min NH_4Cl (2.7 g, 0.05 mol) was added and then $ClCH_2CONMe_2$ [15] (6.1 g, 0.05 mol). The reaction mixture was left overnight for NH_3 to evaporate and then treated with H_2O (50 ml) and CH_2Cl_2 (100 ml). The CH_2Cl_2 solution was dried ($CaCl_2$) and evaporated to give crude $Ph_2PCH_2CONMe_2$ (13 g). Recrystallization from dry ether gave the pure compound, m.p. 99.5–100.5 °C (8.9 g, 65 %). Anal. $C_{16}H_{18}NOP$: C, H, N. NMR ($CDCl_3$): δ_P −19.0; δ_H 3.16 (CH_2, $^2J_{PH}$ 0.5 Hz); δ_H 2.90, 2.96 (CH_3). The compound is stable towards air in the solid state.

(Carboxymethyl)diphenylethylphosphonium iodide. Ph_2PCH_2COOH (2 mmol) and EtI (6 mmol) were refluxed in acetone (3 ml) for 24 h. The solution was evaporated to dryness and the residue recrystallized from H_2O. After drying at 80 °C/0.1 mmHg for 2 h the m.p. was

152.5–153.5 °C (dec.). Anal. $C_{16}H_{18}IO_2P$: C, H, I. NMR [$(CD_3)_2SO$]: δ_P 25.6; δ_H 4.72 (CH_2COOH, $^2J_{PH}$ 13.7 Hz).

(Ethoxycarbonylmethyl)diphenylethylphosphonium iodide. The compound was prepared as above and had m.p. 129.5–130 °C (from 2-PrOH). Anal. $C_{18}H_{22}IO_2P$: C, H, I. NMR [$(CD_3)_2SO$]: δ_P 26.0; δ_H 4.88 (CH_2COOEt, $^2J_{PH}$ 14.2 Hz).

(Carbamidomethyl)diphenylethylphosphonium iodide. The compound was prepared as above, m.p. 165–167 °C (from H_2O). Anal. $C_{16}H_{19}INOP$: C, H, N, I. NMR [$(CD_3)_2SO$]: δ_P 26.4; δ_H 4.57 (CH_2CONH_2, $^2J_{PH}$ 14.2 Hz).

(N,N-Dimethylcarbamidomethyl)diphenylethylphosphonium iodide. The compound was prepared as above, m.p. 235–237 °C (dec.; from EtOH). Anal. $C_{18}H_{23}INOP$: C, H, N, I. NMR [$(CD_3)_2SO$]: δ_P 26.3; δ_H 4.97 (CH_2CONMe_2, $^2J_{PH}$ 13.3 Hz).

Acknowledgements. We thank Mrs. Eva Asmussen for obtaining the kinetic data and the Danish Scientific Research Council for the purchase of the photoelectron spectrometer.

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Tropylium Ions. Part VIII.[1] General Base Catalysis in the Reaction of Phenyltropylium Ions with Water

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There are divided opinions about the occurrence [2–4] and importance [5] of general base catalysis in the reaction of carbonium ions with water and consequently also of general acid catalysis in the reverse dissociation reaction. Direct observations have earlier been reported for reactions of triarylmethyl cations [2–5] and for the hydration of *N*-substituted iminolactones.[6–7] In this paper we wish to report our observations about general base catalysis in the reaction of a representative of another cation system, phenyltropylium cations.

When dissolved in aqueous B–BH+ buffer solution, phenyltropylium cations undergo the reactions

$$Tr^+ + 2H_2O \rightleftharpoons TrOH + H_3O^+ \quad (1)$$

$$Tr^+ + OH^- \rightleftharpoons TrOH \quad (2)$$

and if general base catalysis by B contributes, the one-step reaction

$$Tr^+ + H_2O + B \rightleftharpoons TrOH + BH^+ \quad (3)$$

The observed pseudo first-order rate constant can be written as

$$k_{obs} = k_{H_2O} + k_{OH^-}[OH^-] + k_B[B] \quad (4)$$

where k_B is the second-order rate constant for catalysis by the base B. At constant buffer ratio (more exactly, at constant pH) the slope of the plot of k_{obs} *vs.* [B] gives k_B, and the intercept $k^{B=0}$ is $k_{H_2O} + k_{OH^-}[OH^-]$. If the intercepts $k^{B=0}$ measured at different buffer ratios are plotted as a function of $[OH^-]$, the values of k_{H_2O} and k_{OH^-} at zero concentration of buffer are obtained.

Data for the reaction of *p*-dimethylaminophenyltropylium cation with water in Dabco (1,4-diazabicyclo[2.2.2]octane)–DabcoH+ and Et_3N–Et_3NH^+ buffers are collected in Table 1. The rates were measured by stopped-flow technique at 25 °C and at constant ionic strength, regulated with potassium perchlorate.[8] Examination of the data gives the values of 1.4 $dm^3\ mol^{-1}\ s^{-1}$ and 2.3 $dm^3\ mol^{-1}\ s^{-1}$ for catalysis by Dabco and Et_3N, respectively, a value $0.8 \times 10^{-2}\ s^{-1}$ for k_{H_2O} and a value $1.5 \times 10^3\ dm^3\ mol^{-1}\ s^{-1}$ for k_{OH^-} at ionic strength 0.02 mol dm^{-3}, and a value $1.1 \times 10^{-2}\ s^{-1}$ for k_{H_2O} and a value $1.3 \times 10^3\ dm^3\ mol^{-1}\ s^{-1}$ for k_{OH^-} at ionic strength 0.1 mol dm^{-3}. Slightly

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(Ethoxycarbonylmethyl)diphenylethylphosphonium iodide. The compound was prepared as above and had m.p. 129.5–130 °C (from 2-PrOH). Anal. $C_{18}H_{22}IO_2P$: C, H, I. NMR [$(CD_3)_2SO$]: δ_P 26.0; δ_H 4.88 (CH_2COOEt, $^2J_{PH}$ 14.2 Hz).

(Carbamidomethyl)diphenylethylphosphonium iodide. The compound was prepared as above, m.p. 165–167 °C (from H_2O). Anal. $C_{16}H_{19}INOP$: C, H, N, I. NMR [$(CD_3)_2SO$]: δ_P 26.4; δ_H 4.57 (CH_2CONH_2, $^2J_{PH}$ 14.2 Hz).

(N,N-Dimethylcarbamidomethyl)diphenylethylphosphonium iodide. The compound was prepared as above, m.p. 235–237 °C (dec.; from EtOH). Anal. $C_{18}H_{23}INOP$: C, H, N, I. NMR [$(CD_3)_2SO$]: δ_P 26.3; δ_H 4.97 (CH_2CONMe_2, $^2J_{PH}$ 13.3 Hz).

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Tropylium Ions. Part VIII.[1] General Base Catalysis in the Reaction of Phenyltropylium Ions with Water

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There are divided opinions about the occurrence [2–4] and importance [5] of general base catalysis in the reaction of carbonium ions with water and consequently also of general acid catalysis in the reverse dissociation reaction. Direct observations have earlier been reported for reactions of triarylmethyl cations [2–5] and for the hydration of *N*-substituted iminolactones.[6–7] In this paper we wish to report our observations about general base catalysis in the reaction of a representative of another cation system, phenyltropylium cations.

When dissolved in aqueous B–BH+ buffer solution, phenyltropylium cations undergo the reactions

$$Tr^+ + 2H_2O \rightleftharpoons TrOH + H_3O^+ \quad (1)$$

$$Tr^+ + OH^- \rightleftharpoons TrOH \quad (2)$$

and if general base catalysis by B contributes, the one-step reaction

$$Tr^+ + H_2O + B \rightleftharpoons TrOH + BH^+ \quad (3)$$

The observed pseudo first-order rate constant can be written as

$$k_{obs} = k_{H_2O} + k_{OH^-}[OH^-] + k_B[B] \quad (4)$$

where k_B is the second-order rate constant for catalysis by the base B. At constant buffer ratio (more exactly, at constant pH) the slope of the plot of k_{obs} *vs.* [B] gives k_B, and the intercept $k^{B=0}$ is $k_{H_2O} + k_{OH^-}[OH^-]$. If the intercepts $k^{B=0}$ measured at different buffer ratios are plotted as a function of $[OH^-]$, the values of k_{H_2O} and k_{OH^-} at zero concentration of buffer are obtained.

Data for the reaction of *p*-dimethylaminophenyltropylium cation with water in Dabco (1,4-diazabicyclo[2.2.2]octane)–DabcoH+ and Et_3N–Et_3NH^+ buffers are collected in Table 1. The rates were measured by stopped-flow technique at 25 °C and at constant ionic strength, regulated with potassium perchlorate.[8] Examination of the data gives the values of 1.4 $dm^3\ mol^{-1}\ s^{-1}$ and 2.3 $dm^3\ mol^{-1}\ s^{-1}$ for catalysis by Dabco and Et_3N, respectively, a value $0.8 \times 10^{-2}\ s^{-1}$ for k_{H_2O} and a value $1.5 \times 10^3\ dm^3\ mol^{-1}\ s^{-1}$ for k_{OH^-} at ionic strength 0.02 mol dm^{-3}, and a value $1.1 \times 10^{-2}\ s^{-1}$ for k_{H_2O} and a value $1.3 \times 10^3\ dm^3\ mol^{-1}\ s^{-1}$ for k_{OH^-} at ionic strength 0.1 mol dm^{-3}. Slightly

Table 1. Reaction of *p*-dimethylaminophenyltropylium fluoroborate in aqueous buffered solutions at 25 °C.

pH	$[OH^-]$/ mol dm^{-3} 10^{-4}	$k^{B=0}$/ s^{-1}	k_{Dabco}/ dm^3 mol^{-1} s^{-1}	k_{Et_3N}/ dm^3 mol^{-1} s^{-1}
Ionic strength 0.020 mol dm^{-3}				
9.33	0.214	0.0405	1.28	
9.36	0.229	0.0414	1.30	
9.49	0.309	0.0545	1.32	
9.65	0.447	0.0748	1.68	
Ionic strength 0.100 mol dm^{-3}				
9.80	0.631	0.0925		2.13
10.22	1.660	0.226		2.25
10.60	3.981	0.525		2.46

higher values, 2.0×10^{-2} s^{-1} and 1.7×10^3 dm^3 mol^{-1} s^{-1}, have earlier been reported for the two last-mentioned rate constants measured in borate and triethylamine buffers at ionic strength 0.01 mol dm^{-3} at 23 °C.[9] The slight increase in the values of k_B with increasing concentration of *B* may be an indication that a second amine molecule facilitates the reaction of a water molecule.

Nucleophilic catalysis is unlikely. At 23 °C the rate constant for the reaction of the *p*-dimethylaminophenyltropylium cation with diethylamine is 4.2×10^4 dm^3 mol^{-1} s^{-1} and with ethylamine 2.8×10^3 dm^3 mol^{-1} s^{-1}.[10] These values are much higher than the value 2.3 dm^3 mol^{-1} s^{-1} found for triethylamine and imply the unimportance of nucleophilic catalysis. We therefore believe that the reaction of phenyltropylium cations with water involves general base catalysis.

Ritchie has suggested that the value of the rate constant ratio k_{H_2O}/k_{Dabco} may be a useful criterion for distinguishing between general base and nucleophilic catalysis.[4] The ratio varies from 0.06 mol dm^{-3} to 0.20 mol dm^{-3} for triarylmethyl cations and is 0.13 mol dm^{-3} for *N,O*-trimethylenephthalimidium cation.[2,4,7] For acetate esters, which are observed to be subject to nucleophilic catalysis, the ratio is much smaller, from 1.2×10^{-4} mol dm^{-3} to 1.5×10^{-7} mol dm^{-3}.[11] For *p*-dimethylaminophenyltropylium ion the ratio is 1.0×10^{-2} mol dm^{-3}.

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Liquid Scintillation Counting of Polar Molecules: Effects of pH on Water Association, Adsorption to Glass and Elution from Paper

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When weak β-emission from some polar molecules in aqueous solution was measured by liquid scintillation counting, unstable count rates were found.[1] The decrease of count rates with time may lead to great problems, especially in double labelling experiments. This "progressive" quenching was thought to be due to association of water to the polar groups of the radioactive molecules.[1] In preliminary experiments we found that liquid scintillation counting of adenosine monophosphate (5′-AMP) was accompanied by such "progressive" quenching. We also reported that the addition of acid could abolish the reduction of count rates with time.[2] We supposed that this effect of acid addition was caused by repression of dissociation of ionizing groups, decreasing the possibility of progressive association of water to the molecule. Changing of pH in order to diminish the polarity of a molecule could be a simple way to prevent "progressive" quenching for other molecules too, if the principle was generally applicable.

We therefore wanted to investigate the "progressive" quenching of various molecules in aqueous solution and study how pH-changes in both acid and basic direction influenced the count rates. For this purpose one small molecule (histamine) and one macromolecule (polylysine) with basic dissociable groups and a similar pair of molecules with acidic dissociable groups (5′-AMP and RNA) were examined. The molecules were chosen to assure clearly defined ionizable groups. In some experiments DNA was used instead of RNA to minimize the uncertainty of base hydrolysis of the acid macromolecules. [^{3}H]Histamine, enzymically prepared from [^{3}H]histidine ([3-^{3}H]-L-histidine, New England Nuclear Corporation, Boston, Mass.) with subsequent chromatographic purification,[3] was a generous gift from Dr. Sten Jacobsen, Department of Pharmacology, University of Oslo. [^{14}C]-5′-AMP ([8-^{14}C]-adenosine-5′-monophosphate), [^{3}H]-5′-AMP ([2-^{3}H]-adenosine-5′monophosphate) and [^{3}H]polylysine (Sigma Chemical Company, m.w. 70 000–200 000, mean m.w. 139 000) were obtained from the Radiochemical Centre, Amersham, U.K. ^{14}C-Labelled rat liver total RNA was obtained as described previously.[1] ^{3}H-Labelled DNA was extracted from *E. coli* by the method of Marmur [4] after labelling with [^{3}H]thymidine.

PPO (5 g), bis-MSB (0.5 g), naphthalene (80 g), 2-methoxyethanol (400 ml) and toluene (600 ml) were used as scintillation liquid. The spectrometer, a Packard Tri-Carb model 3365 fitted with bi-alkali photomultiplier tubes, was operated at 10 °C. The channels were adjusted so that chemical quenching reduced the tritium and the ^{14}C counts to the same extent. No adjustments of amplification or discriminator settings were done during the count period.

Count rates in aqueous solution were assayed by adding 50 μl of an aqueous solution of the radioactive material to 15 ml precooled scintillation liquid. After shaking, 450 μl of water, or a 0.5 M solution of HCl, NaOH or NaCl was added, and the counting started immediately. The normalized count rates reported are fractions of the initial count rates obtained. Initially, buffers of pH values slightly above or below the pK values of the radioactive molecules were used. However, when the scintillation liquid was added, the pH changed so much that only 0.5 M HCl or 0.5 M NaOH could assure pH values to the acidic or basic side of the pK values.

In some experiments known amounts of the radioactive material were applied and dried on 3×4 cm^2 of Whatman No. 1 paper. The paper was cut to pieces and shaken in 0.5 ml of one of the above solutions for 5 min at room temperature. Scintillation liquid (15 ml) was then added, and the shaking repeated. After cooling in the dark, the vials were shaken once more for 1 min before counting. These counts given in per cent of counts obtained from the respective radioactive compounds added directly to the scintillation liquid, were taken as an expression of the elution from the paper. This was checked by recounting of the solution after removal of the paper.

The results of the stability assays are depicted in Fig. 1. In the presence of H_2O or NaCl the count rates of 5′-AMP, RNA and polylysine declined markedly with time, whereas histamine count rates were only slightly reduced. Amino groups are not strongly polar. Therefore one might expect small effects on count rates in amine-containing compounds. The molecules containing phosphate groups showed greater instability in count rates than the others. Preliminary experiments using different polar groups seem to show a decreasing tendency towards instability in the following order: Phosphate groups, sulfate groups, carboxyl groups and amino groups. The drop in count rates was more pronounced for the macromolecules than for the small ones. This is probably a reflection of the secondary structure of the macromolecules in aqueous environ-

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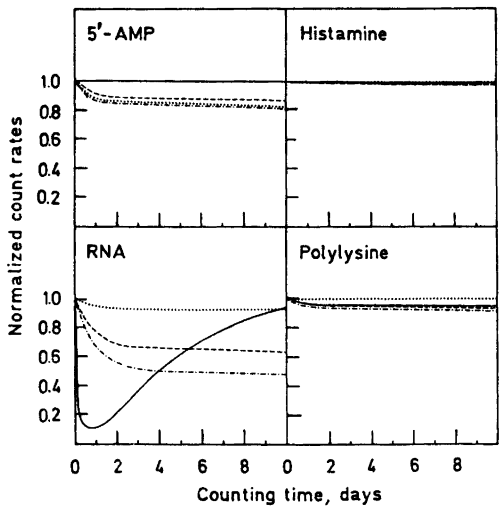


Fig. 1. Normalized count rates of AMP, histamine, RNA, and polylysine in different solutions as functions of time. Each substance was dissolved in 50 µl water and added to 15 ml scintillation liquid. Water (- - -), 0.5 M NaCl (— · —), 0.5 M HCl (—), or 0.5 M NaOH (···; 450 µl) was added, and the counting started.

ment where the polar groups, turning outwards, constitute a larger part of the molecular surface. The external standard count rates remained the same during the whole observation period, excluding the possibility of general quenching of the scintillation liquid. Usually, the falling count rates could not be attributed to adsorption by the vial walls, because the decline proceeded at the same rate both in glass and in polyethylene vials, and because count rates close to the originals could be obtained after transfer of the contents from one vial to another. In this case the mechanical shaking probably reversed the "progressive" quenching.[1] Occasionally, however, the count rates of AMP or histamine fell below the usual (data not shown). This was shown to be caused by adsorption to the vial walls, and was experienced more often when using new glass vials.

Addition of HCl to the AMP-vials stabilized the count rates while NaOH did not. Addition of NaOH to the polylysine vials stabilized the recordings while HCl was ineffective. These results were in accordance with the general repression-of-dissociation-hypothesis. The results obtained with NaOH for the histamine-vials did fit too, but the slight improvement also obtained with HCl in these vials is incompatible with the hypothesis. RNA was precipitated by acid and count rates dropped markedly. This was followed by a slow redissolution (if hydrolysis can be excluded) of the precipitate in the rather hydrophobic milieu. The final result — after 9 days — in RNA-vials with acid was thus in agreement with the hypothesis, but of little practical importance due to the precipitation. The addition of NaOH to RNA-vials stabilized the count rates which is in disagreement with the hypothesis. Base hydrolysis of the macromolecule could be excluded as identical results were achieved with DNA. Thus it seems probable that count rates may be stabilized by repression of the dissociation of the radioactive molecules in some instances, but the principle does not appear to be general.

Table 1. Per cent elution of AMP and histamine from Whatman No. 1 paper.[a]

Eluent	AMP	Histamine
H_2O	70 ± 2	64 ± 1
NaCl, 0.5 M	70 ± 2	76 ± 2
HCl, 0.5 M	87 ± 2	72 ± 2
NaOH, 0.5 M	55 ± 2	95 ± 2

[a] Mean of 6 parallels ± SEM in per cent of radioactivity applied.

Other advantages were obtained by the addition of acid to AMP-vials and base to histamine-vials as the adsorption to vials walls (see above) was abolished. However, we never observed adsorption in the presence of the salt solution either, so this might be attributable to the ionic strength of the solution. Elution from paper (Table 1), tried only for smaller molecules, as they are the ones usually chromatographed, was enhanced when the dissociation of polar groups was repressed. Thus the elution of AMP was increased to 87 % by HCl and of histamine to 95 % by NaOH. The lesser polarity of histamine may explain the differences between AMP and histamine.

When difficulties are encountered in liquid scintillation counting with water association to polar groups, adsorption to glass vials or incomplete elution, repression of dissociation of polar groups seems to be worth considering, when problems due to precipitation can be omitted. We have found comparable results with other biological molecules such as orotic acid, cystine and cyclic AMP.

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The Structure of Pinopalustrin and its Relations to Other Lignans

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Long ago a new phenol was isolated in the Research Center of Hercules Inc., Wilmington, Delaware. It was obtained from benzene extracts of exhumed stumps of *Pinus palustris* Miller, "long leaf pine". After removal of the resin acids, a residue remained which was dissolved in 2-propanol, whereupon the phenol gradually crystallized with solvent of crystallization. Evaporation of the solvent afforded an amorphous product, which was called pinopalustrin and which analysed for $C_{18}H_{16}O_5(OMe)_2$. It showed strong UV absorption at 230 and 280 nm.

The compound was first investigated at Hercules and later in Stockholm and a preliminary account of this work was given on the occasion of the "10de Nordiska Kemistmötet", Stockholm, August 17–21, 1959, by one of us (B.C.).[1]

Pinopalustrin gave a dimorphous dimethyl ether, $C_{18}H_{14}O_3(OMe)_4$, (Me_2SO_4/NaOH), MS: *m/e* 402 (M^+), which was soluble in hot alkali (lactone) and which on acetylation gave an acetate, $C_{18}H_{12}O_2(OMe)_4OAc$, m.p. 102–103 °C. Ethylation, (Et_2SO_4/NaOH), gave a non-crystalline diethyl ether, $C_{18}H_{14}O_3(OMe)_2(OEt)_2$.

Permanganate oxidation of the dimethyl ether gave veratric acid (55 %) and the diethyl derivative gave *O*-ethylvanillic acid (68 %). The dimethyl ether reacted with phenylmagnesium bromide to give a product which furnished benzophenone with periodate. Reduction with $LiAlH_4$ in ether gave a triol, $C_{18}H_{18}O_3(OMe)_4$, which consumed 1 mol of periodate.

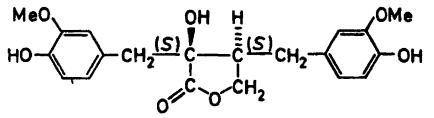
From these results and the mass spectrum of the dimethyl ether it became evident that pinopalustrin was a lignan and structure *1* (without stereochemistry) was proposed for it.[1] This work was not published, pending the elucidation of the configuration.

Our observations lay fallow for many years. We noticed, however, that the physical properties of pinopalustrin, of its derivatives and of the triol were closely related to those reported for the aglucone nortrachelogenin and the triol obtained from nortrachelogenin dimethyl ether.[2,3] Professor S. Nishibe kindly informed us that nortrachelogenin dimethyl ether was identical with pinopalustrin dimethyl ether (mixed melting point, superimposable IR spectra).

Table 1. Comparison of physical constants.

Compound	M.p./°C	$[\alpha]_D$/deg.
Pinopalustrin (*1*)	Amorphous	–
Nortrachelogenin (*2*)	Amorphous	−17 (EtOH)
Wikstromol (*3*)	Amorphous	+72 ($CHCl_3$)
Dimethyl ether of *1*	96–97, 127–128	−39 ($CHCl_3$)
Dimethyl ether of *2*	97–98.5	−46 (EtOH)
Dimethyl ether of *3*	96–97	+35 ($CHCl_3$)
Triol from dimethyl ether of *1*	133–134	−25 ($CHCl_3$)
Triol from dimethyl ether of *2*	132–133	−23 ($CHCl_3$)
Triol from dimethyl ether of *3*	130–131	+9 (EtOH)
Triol from gmelinol [5]	132	−3 (EtOH)
Same triol from olivil [2]	132–133	−22 ($CHCl_3$)
Carinol dimethyl ether [6]	130–131	−3 (EtOH)

Quite recently Tandon and Rastogi [4] isolated another lignan, wikstromol. The structure and configuration given are those of an enantiomer of pinopalustrin.



1

The pertinent data for pinopalustrin, nortrachelogenin and wikstromol are given in Table 1. It follows that the structure and absolute configuration of pinopalustrin are as in *1*.

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The Crystal Structure of Cudranone, 2,6,3′-Trihydroxy-4-methoxy-2′-(3-methyl-2-butenyl)-benzophenone: A New Antimicrobial Agent from *Cudrania cochinchinensis*

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Preliminary antimicrobial screening of the ethanolic extract of the Chinese plant *Cudrania cochinchinensis*, var. *gerontogea*, showed distinct activity against a wide range of microorganisms. The novel compound cudranone (2,6,3′-trihydroxy-4-methoxy-2′-(3-methyl-2-butenyl)-benzophenone), which was isolated [1] from the chloroform solubles of the ethanolic extract, was identified as one of the antimicrobial constituents. In order to confirm the structure assignment and also obtain conformational data on this novel antimicrobial agent, an X-ray crystallographic structure determination has been carried out.

A yellow prismatic crystal of approximate dimensions $0.19 \times 0.38 \times 0.48$ mm was selected for the crystallographic work. A computer-controlled Syntex P$\bar{1}$ four-circle diffractometer with graphite-monochromatized Mo$K\alpha$ radiation was utilized in the preliminary experiments and the collection of intensity data. Cell dimensions and their standard deviations were determined by a least-squares treatment of the angular coordinates of fifteen reflections. The crystal data are: 2,6,3′-trihydroxy-4-methoxy-2′-(3-methyl-2-butenyl)-benzophenone, $C_{19}H_{20}O_5$, $M = 328.35$ amu, space group Cc, $a = 9.037(2)$ Å, $b = 17.977(8)$ Å, $c = 10.402(3)$ Å, $\beta = 93.39°$ (2), $V = 1687.0(10)$ Å^3, $Z = 4$, $D_{calc} = 1.293$ g cm^{-3}, $F(000) = 696$.

Three-dimensional intensity data were recorded using the $\omega - 2\theta$ scanning mode with scan speed variable from 3 to 12° min^{-1} depending on the intensity of the reflection, and background counting time was equal to $0.7 \times$ scan time. The temperature was kept constant at 24 ± 2 °C. The variations in the intensity of three check reflections which were remeasured regularly, were random. The estimated standard deviations were taken as the square root of the total counts with a 2 % addition of the

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations for nonhydrogen atoms. The temperature factor is given by $\exp\{-2\pi^2[U_{11}(a^*h)^2 + U_{22}(b^*k)^2 + U_{33}(c^*l)^2 + 2U_{12}(a^*b^*hk) + 2U_{13}(a^*c^*hl) + 2U_{23}(b^*c^*kl)]\}$.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C1	.2205(0)	.1600(2)	.4482(0)	.0385(19)	.0327(17)	.0310(17)	−.0027(14)	−.0074(15)	.0016(14)
C2	.1758(5)	.0862(2)	.4222(5)	.0469(21)	.0329(18)	.0406(20)	−.0080(16)	−.0039(17)	.0021(16)
C3	.2303(6)	.0475(2)	.3205(5)	.0607(25)	.0345(19)	.0561(25)	−.0070(18)	.0012(21)	−.0128(18)
C4	.3294(6)	.0807(2)	.2432(5)	.0645(27)	.0495(24)	.0541(25)	−.0021(21)	.0075(22)	−.0152(20)
C5	.3747(6)	.1532(2)	.2654(5)	.0474(23)	.0545(24)	.0421(20)	−.0104(19)	.0065(17)	−.0056(19)
C6	.3186(5)	.1925(2)	.3675(4)	.0354(19)	.0322(17)	.0344(19)	−.0023(15)	−.0079(15)	.0034(15)
C7	.3695(5)	.2711(2)	.3887(4)	.0368(19)	.0385(19)	.0328(19)	−.0087(15)	−.0043(16)	.0029(15)
C8	.3173(5)	.3314(2)	.3057(4)	.0342(19)	.0356(18)	.0344(17)	−.0054(14)	−.0007(14)	.0023(15)
C9	.3853(5)	.4031(2)	.3189(5)	.0346(20)	.0351(19)	.0437(21)	−.0073(15)	−.0039(17)	−.0000(16)
C10	.3449(6)	.4612(2)	.2387(5)	.0519(22)	.0307(18)	.0551(23)	−.0073(16)	−.0008(19)	.0068(16)
C11	.2308(6)	.4511(2)	.1440(5)	.0464(21)	.0382(21)	.0464(22)	.0021(17)	.0013(18)	.0106(16)
C12	.1604(6)	.3837(2)	.1278(5)	.0450(21)	.0473(22)	.0370(20)	.0015(17)	−.0065(16)	.0020(17)
C13	.2020(5)	.3244(2)	.2077(4)	.0341(18)	.0368(18)	.0339(18)	−.0052(15)	.0006(15)	−.0015(15)
C14	.1544(5)	.2015(2)	.5587(5)	.0454(21)	.0331(17)	.0408(20)	−.0036(16)	−.0020(17)	−.0047(15)
C15	.2230(6)	.1789(2)	.6869(5)	.0486(21)	.0425(20)	.0400(21)	−.0057(18)	.0016(17)	−.0028(17)
C16	.2806(6)	.2214(2)	.7819(5)	.0397(21)	.0599(24)	.0455(21)	−.0024(18)	.0002(17)	−.0110(19)
C17	.2928(6)	.3046(2)	.7764(6)	.0571(27)	.0584(26)	.0778(32)	−.0024(21)	−.0032(23)	−.0338(24)
C18	.3419(7)	.1872(3)	.9059(5)	.0706(30)	.0953(35)	.0432(24)	−.0071(28)	−.0101(22)	−.0091(24)
O19	.0771(5)	.0564(2)	.5017(4)	.0749(19)	.0439(15)	.0619(19)	−.0303(14)	.0155(16)	−.0058(13)
O20	.4609(5)	.2827(1)	.4813(4)	.0577(17)	.0444(15)	.0507(16)	−.0168(13)	−.0249(14)	.0099(13)
O21	.4923(5)	.4149(1)	.4131(4)	.0510(17)	.0366(14)	.0612(18)	−.0163(12)	−.0226(14)	.0038(12)
O22	.1989(5)	.5117(2)	.0701(4)	.0750(22)	.0461(16)	.0680(20)	−.0013(15)	−.0148(17)	.0216(15)
C23	.0859(6)	.5040(3)	−.0319(5)	.0728(32)	.0723(31)	.0574(29)	.0185(26)	−.0093(26)	.0247(24)
O24	.1302(5)	.2591(1)	.1954(4)	.0520(16)	.0463(15)	.0459(14)	−.0169(13)	−.0205(12)	.0007(12)

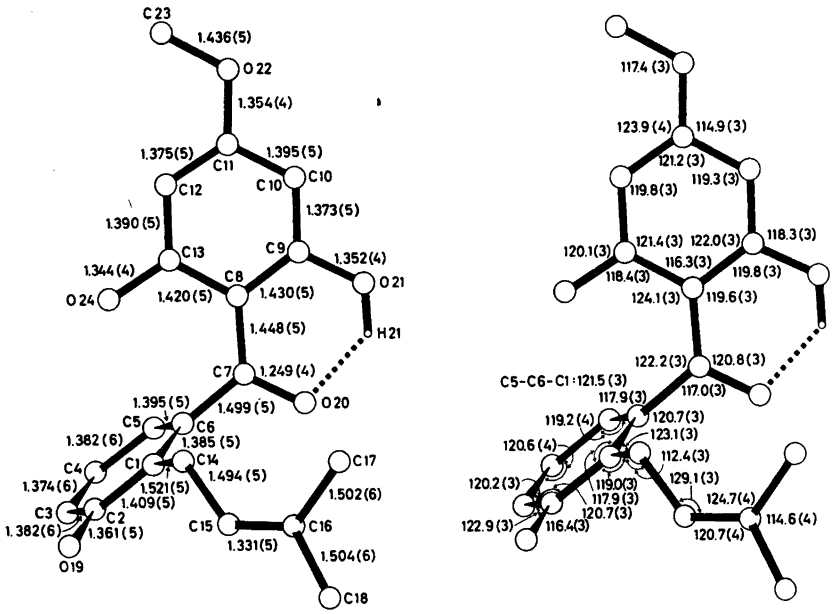


Fig. 1. Bond lengths (Å) and bond angles (°) with estimated standard deviations. The molecular conformation as seen down in the plane of the C8 – C13 ring is indicated.

net intensity for experimental uncertainties. Of the 1495 symmetry-independent reflections measured ($2\theta_{max} = 50°$), the 1408 which had intensities larger than twice their standard deviations were regarded as observed. The intensities were corrected for Lorentz and polarization effects.[2]

The phase problem was solved by the MULTAN [3] program package. The structure model with anisotropic thermal parameters for the nonhydrogen atoms was refined to a conventional R of 0.063. The computer programs used as well as programs subsequently employed, are part of a local (Oslo) assembly of programs.[4] Atomic scattering factors used were those of Doyle and Turner [5] for oxygen and carbon, and of Stewart *et al.*[6] for hydrogen. The hydrogen atoms were placed in calculated positions and included in the structure factor calculation with estimated isotropic thermal parameters. The distances indicated two *inter-* and one *intra*-molecular hydrogen bonds and the three hydrogens bonded to oxygens were placed accordingly. Full-matrix least-squares refinement of all nonhydrogen atomic parameters converged to a weighted R_w of 0.049 and an R of 0.041. Atomic parameters for nonhydrogen atoms are given in Table 1, and those used for hydrogen atoms may be obtained from the authors upon request, as may a list of observed and calculated structure factors.

Bond lengths and bond angles are listed in Fig. 1 where also the numbering of the atoms is indicated. The keto-group (C7 = O20) is nearly coplanar with the ring C8 – C13 (see Table 2), the dihedral angle O20 – C7 – C8 – C9 is only 8.4°(5). This approximate coplanarity of the keto-group with one of the phenyl rings, which is not found in other benzophenones,[7–11] is probably caused by the formation of an *intra*-molecular hydrogen bond from O21 to O20 (O – O distance: 2.501(3) Å). The strong conjugation between the keto-group and the

Table 2. Deviations from least-squares planes (Å × 10^3). The deviations for those atoms used to define the plane are given in italicized figures.

Atom	Plane A	Atom	Plane B
C1	*7*	C6	−277
C2	*−1*	C7	−57
C3	*−4*	C8	*−7*
C4	*4*	C9	*10*
C5	*2*	C10	*−8*
C6	*−7*	C11	*2*
C7	−14	C12	*0*
C8	−1195	C13	*2*
C14	−31	O20	72
C15	1297	O21	51
C16	1976	O22	−12
O19	−14	C23	−50
O20	1056	O24	48

C8 – C13 ring is reflected in a shortening of the C7 – C8 bond and a lengthening of the C8 – C9 and C8 – C13 bonds.

The angle between the planes through the phenyl rings is 77.1°, and the dihedral angle C1 – C6 – C7 – O20 is 74.0°(4) (see Fig. 1). This rotation of a phenyl ring relative to the keto-group and to the other phenyl ring is much larger than the rotations found in benzophenone,[7] 3,3′-dibromobenzophenone [9] and 4,4′-dimethoxybenzophenone,[8] where the angles between the phenyl rings and the plane defined by the atoms corresponding to C6, C7, C8 and O20 are about 25 – 35°, and the angles between the planes of the phenyl rings are about 55°. This large rotation must be a result of the *intra*-molecular crowding caused by the position of the large 3-methyl-2-butenyl group *syn* to O20 and also by the position of the hydroxyl group (O24) *syn* to the C1 – C6 ring. A similar situation is found in the molecules of *syn*- and *anti*-4-bromobenzophenone oxime *O*-picryl ethers [10] where the phenyl rings *syn* to the picryl groups are rotated 60 – 70°, relative to the plane through the oxime group, whereas the phenyl rings *anti* to the picryl groups are rotated only 18 – 23°.

The *inter*-molecular distances indicate two hydrogen bonds: one from O19 to O21 in position: $-0.5+x$, $-0.5+y$, z distance: 2.797(4) Å; and one from O24 to O20 in position: $-0.5+x$, $0.5-y$, $-0.5+z$, distance: 2.732(3) Å.

Acknowledgement. The authors thank Professor K. Seff for the use of the X-ray diffraction equipment at the Chemistry Department, University of Hawaii.

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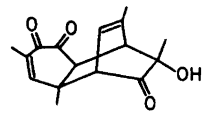
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On the Crystal Structure of 5,8-Etheno-3,4a,7,9-tetramethyl-4a,5,6,7,8,8a-hexahydrochromene-7,8a-diol-2,6-dione, Formed by Periodate Oxidation of 2,4-Dimethylphenol

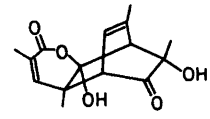
BENGT KARLSSON,[a] BENGT O. LINDGREN,[b] ANNE-MARIE PILOTTI [a] and ANNE-CHARLOTTE SÖDERHOLM [a]

[a] Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden and [b] Swedish Forest Products Research Laboratory, S-114 86 Stockholm, Sweden

A previous study [1] has shown that the action of sodium periodate on 2,4-dimethylphenol yields three products: 2,4-dimethyl-*p*-quinol, the Diels-Alder dimerization product [2] of 2,4-dimethyl-*o*-quinol, and the Diels-Alder adduct [3] (*1*) of the *o*-quinol and 3,5-dimethyl-1,2-benzoquinone. We have now obtained from 2,4-dimethylphenol, in small yield, another periodate oxidation product, $C_{15}H_{18}O_5$, with m.p. 172.5 – 174.5 °C. Crystallographic examination shows that it is 5,8-etheno-3,4a,7,9-tetramethyl-4a,5,6,7,8,8a-hexahydrochromene-7,8a-diol-2,6-dione (*2*), evidently formed by periodate oxidation of the adduct (*1*).



1



2

Experimental. The oxidation of 2,4-dimethylphenol with periodate was carried out and the oxidation mixture was worked up essentially as described in the previous paper.[1] From the acidification of 0.5 M aqueous NaOH solution with glacial acetic acid, a precipitate of a colourless compound, m.p. 172.5 – 174.5 °C, was obtained. Its mass spectrum showed a molecular ion at *m/e* 278, calc. for $C_{15}H_{18}O_5$, 278.

X-Ray experimental and structure elucidation. Cell dimensions were determined, by least-squares refinement, from the angular positions of 25 well-centered reflexions on a Philips PW 1100 diffractometer. Crystal data are: $a = 16.513(4)$, $b = 6.987(2)$, $c = 12.146(4)$ Å, $\beta = 102.91(2)°$, $V = 1366$ Å^3, space group $P2_1/n$, $Z = 4$, $\varrho_{X-ray} = 1.35$ g cm^{-3}, $\mu(CuK\alpha) = 8.53$ cm^{-1}.

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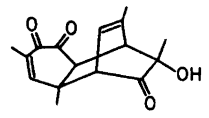
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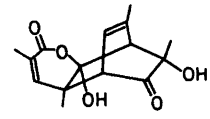
BENGT KARLSSON,[a] BENGT O. LINDGREN,[b] ANNE-MARIE PILOTTI [a] and ANNE-CHARLOTTE SÖDERHOLM [a]

[a] Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden and [b] Swedish Forest Products Research Laboratory, S-114 86 Stockholm, Sweden

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X-Ray experimental and structure elucidation. Cell dimensions were determined, by least-squares refinement, from the angular positions of 25 well-centered reflexions on a Philips PW 1100 diffractometer. Crystal data are: $a = 16.513(4)$, $b = 6.987(2)$, $c = 12.146(4)$ Å, $\beta = 102.91(2)°$, $V = 1366$ Å^3, space group $P2_1/n$, $Z = 4$, $\varrho_{X-ray} = 1.35$ g cm^{-3}, $\mu(CuK\alpha) = 8.53$ cm^{-1}.

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1909 unique reflexion intensities recorded out to $\theta = 58°$, however, only 502 fulfilled the criterion that the net intensity, I, be greater than $2.5\sigma(I)$, where $\sigma(I)$ is based on counter statistics. Attempts to solve the structure by diverse direct method approaches failed, probably due to the difficulties of obtaining reliable triple-product sign relationships between different parts of the reflexion sphere. At this stage another crystal was used to collect a second data set (II). Of the 2570 unique reflexions collected out to $\theta = 70°$, 1049 were considered significantly above background on the criterion $\sigma(I) < 0.3I$. All 20 non-hydrogen atoms were found from an E map computed with the best phase set obtained from the MULTAN [4] direct phase determination procedure from 187 normalized $|E|$ values $\geqslant 1.8$ (excluding the $8|E|$ values greater than 4.3) and 1125 triple-product sign relationships.

With the exception of H(O16), which could not be positioned, the hydrogen atoms were located partly from chemical reasoning and partly from a difference Fourier synthesis. The positions, as well as assigned isotropic temperature factors, were included but not refined in the full-matrix least-squares treatment.

However, since the refinement of the structural parameters did not converge properly for set II, presumably as an effect of slight twinning in the crystal, set I was employed for the final refinement. Assuming anisotropic thermal parameters for the non-hydrogens, only half a molecule at a time could be refined with this set, due to the lack of data. The final value of R is 0.060. The Freeman [5] scattering factors for C and O and those of Stewart *et al.*[6] for H were used throughout the refinement, as well as the weighting scheme of Hughes.[7] The final positional parameters for the non-hydrogen atoms are listed in Table 1, and those for the

Table 1. Positional parameters ($\times 10^4$) of the non-hydrogen atoms. Estimated standard deviations are given in parentheses.

ATOM	X	Y	Z
O(1)	3427(5)	7396(11)	5387(6)
C(2)	3930(7)	7464(22)	6436(11)
C(3)	4268(7)	5592(18)	7003(9)
C(4)	4068(8)	4044(17)	6445(9)
C(4A)	3522(8)	3737(17)	5321(10)
C(5)	2753(7)	2576(18)	5505(8)
C(6)	2194(8)	2181(18)	4343(11)
C(7)	1871(7)	4049(18)	3611(9)
C(8)	2237(8)	5669(18)	4406(10)
C(8A)	3191(7)	5695(19)	4727(8)
C(9)	1977(7)	5541(18)	5498(10)
C(10)	2213(7)	3866(17)	6049(9)
O(11)	4056(6)	9004(13)	6859(7)
C(12)	4796(8)	5836(25)	8150(10)
C(13)	3986(8)	2526(19)	4554(9)
O(14)	1943(6)	661(14)	3967(7)
C(15)	930(8)	4129(19)	3305(10)
O(16)	2161(5)	4014(13)	2606(5)
O(17)	3529(5)	5955(13)	3763(6)
C(18)	1442(8)	7025(18)	5889(9)

Table 2. Positional parameters ($\times 10^3$) of the hydrogen atoms.

ATOM	X	Y	Z
H(C4)	435	280	682
H(C5)	273	106	587
H(C8)	203	697	401
H(C10)	204	345	678
H1(C12)	495	444	876
H2(C12)	454	674	862
H3(C12)	536	633	813
H1(C13)	413	157	497
H2(C13)	354	215	383
H3(C13)	448	320	426
H1(C15)	80	560	289
H2(C15)	69	310	290
H3(C15)	84	401	402
H(O17)	309	672	320
H1(C18)	149	679	677
H2(C18)	89	704	521
H3(C18)	167	841	579

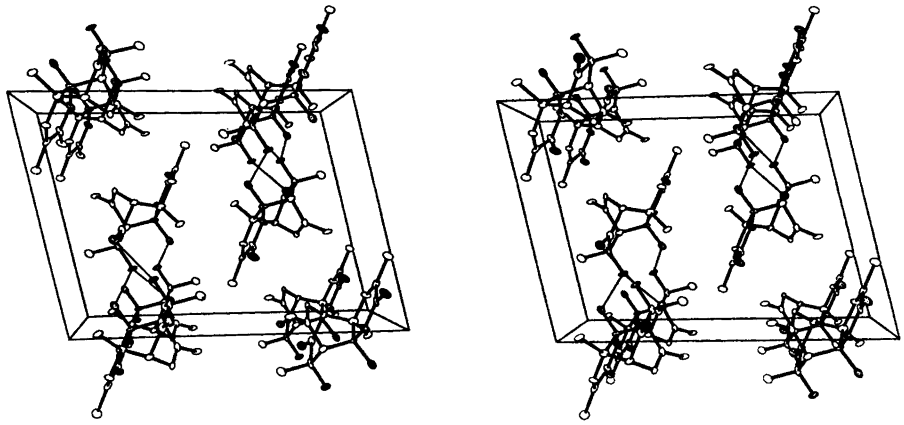


Fig. 1. A stereoscopic view of the molecular packing. Hydrogen atoms are omitted. Oxygens are marked with shaded ellipsoids. Intermolecular O – O contacts shorter than 3.0 Å are indicated as weak lines.

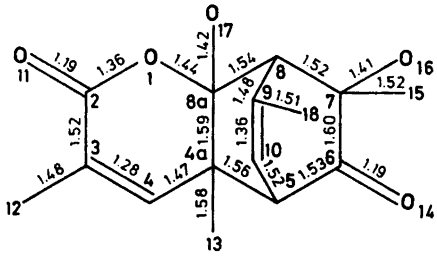


Fig. 2. Bond distances involving the non-hydrogen atoms.

hydrogens in Table 2. A list of the observed and calculated structure factors is available from the authors on request.

Results and discussion. The molecular geometry and packing pattern for this new type of periodate oxidation product from 2,4-dimethylphenol is shown in Fig. 1. Bond distances involving non-hydrogen atoms, as well as the numbering system used, are given in Fig. 2. The standard deviations of the bond distances are estimated to be in the range 0.012 – 0.018 Å; an effect of the lack of data. The derived C(3) – C(4) double bond length is 1.28 Å, which is more than 3σ shorter than what is generally observed. The C(sp^2) – C(sp^3) bond C(6) – C(7), on the other hand, is too long, perhaps indicating that the estimate of the esd's is too small. The C(4a) – C(8a) bond is lengthened, which is also observed in related compound,[2,3,8–10] although the elongation measured in this investigation is not significant. The mean C – H bond length is 1.04 Å; the C – H bonds are distributed in the range 0.84 – 1.22 Å.

The O(1) – C(2) – C(3) – C(4) – C(4a) – C(8a) ring is planar within ±0.018 Å, having the atoms C(4a) and C(8a) farthest out of the plane. The magnitudes of the torsion angles around this ring are less than 3.7°. The other three six-membered rings in the molecule are all boat-shaped.

Four short intermolecular O – O contacts are found between molecules related by the 2_1 axis at $\frac{1}{4},y,\frac{1}{4}$ and its equivalents. These contacts are marked in Fig. 1 with weak lines. The atoms involved, their symmetry codes and the distances between them are:

$O(14)^{i} - O(16)^{ii} = 2.90$ Å (i $\equiv x,y,z$
ii $\equiv \frac{1}{2}-x,y-\frac{1}{2},\frac{1}{2}-z$)
$O(16)^{i} - O(17)^{ii} = 2.79$ Å.

Only three other intermolecular contacts shorter than 3.5 Å are observed, neglecting the hydrogens:

$C(5)^{i} - O(11)^{iii} = 3.46$ Å (iii $\equiv x,y-1,z$)
$O(11)^{i} - C(15)^{iv} = 3.45$ Å (iv $\equiv x+\frac{1}{2},\frac{3}{2}-y,z+\frac{1}{2}$)
$O(14)^{i} - O(17)^{ii} = 3.24$ Å

This investigation has received financial support from the Swedish Natural Science Research Council and from the Tri-Centennial Fund of the Bank of Sweden. The authors are indebted to Professor Peder Kierkegaard for his stimulating interest in this investigation. We also wish to thank Dr. Sven Westman for correcting the English of this article.

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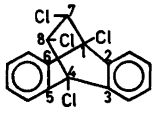
Chlorinated Polycyclic Compounds. I. Reactions of 9,10-Dichloroanthracene with *cis*- and *trans*-1,2-Dichloroethylene

TAPIO MIETTINEN

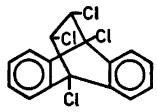
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In addition to the normal Diels-Alder reaction product, *syn*- and *anti*-1,8-dichlorodibenzobicyclo[3,2,1]octadien-4-one were formed in the reactions of 9,10-dichloroanthracene with *cis*- and *trans*-1,2-dichloroethylene, respectively. Synthetic routes to several 1,8-dichloro- and 1,5,8-trichlorosubstituted derivatives of dibenzobicyclo[3,2,1]octadiene are described.

Although it is known that substituents at the 9- and 10-positions of anthracene considerably reduce its reactivity towards dienophiles,[1] the reaction between 9,10-dichloroanthracene and *trans*-1,2-dichloroethylene gave good yields of *trans*-1,4,7,8-tetrachlorodibenzobicyclo[2,2,2]-octadiene (*1*), when the starting materials were heated at 210 – 250 °C for 24 – 96 h. When

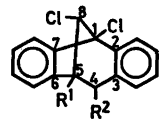


1

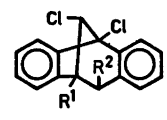


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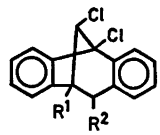
synthetic grade dichloroethylene was used without drying the normal Diels-Alder product was invariably accompanied by a carbonyl compound to which structure *11a* was assigned. In addition to *1* and *11a* some minor components were formed, of which only the rearranged tetrachloride *9c* was identified. Similarly, the reaction of 9,10-dichloroanthracene with *cis*-1,2-dichloroethylene gave *2*, *12a* and *7c*. The yield of the normal product was somewhat lower than with the *trans* isomer and more insoluble material, resulting from polycondensation reactions of dichloroethylene, was formed. Although careful drying of the starting materials considerably reduced the amount of the ketones *11a* and *12a*, they could not be entirely eliminated, the rest being evidently due to moisture in air present in the pressure vessel.



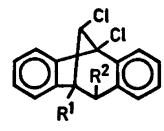
3 R^1 = H
7 R^1 = Cl



4 R^1 = H
8 R^1 = Cl

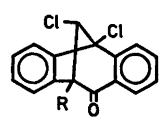


5 R^1 = H
9 R^1 = Cl

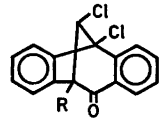


6 R^1 = H
10 R^1 = Cl

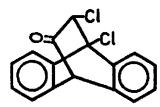
in compounds *3-10*:
a R^2 = OH
b R^2 = OAc
c R^2 = Cl



11a R = H
b R = Cl



12a R = H
b R = Cl



13

Reduction of ketone *11a* with sodium borohydride gave only the *endo* alcohol *3a* (*exo* hydride attack) whereas ketone *12a* gave

Table 1. Dibenzobicyclo[3,2,1]octadiene derivatives.

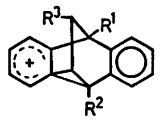
Compound	No.	M.p./ °C	ν_{max}/ cm^{-1}	Chemical shift/δ						$J_{4,5}$/ Hz	$J_{5,8}$/ Hz
				endo-4-H	*exo*-4-H	*syn*-8-H	*anti*-8-H	5-H	Other		
1-*anti*-8-Dichloro-4-oxo	*11a*	143	1692			5.04		4.30			
1-*syn*-8-Dichloro-4-oxo	*12a*	117	1695				5.13	4.18			4.8
1,5-*anti*-8-Trichloro-4-oxo	*11b*	168	1700			5.13					
1,5-*syn*-8-Trichloro-4-oxo	*12b*	162	1703				5.22				
1-*anti*-8-Dichloro-*endo*-4-hydroxy	*3a*	190	3230		5.10	4.70		4.03	1.67 (OH)	5.6	
1-*anti*-8-Dichloro-*exo*-4-hydroxy	*4a*	161	3140	4.71		5.03		3.76	2.00	1.8	
1-*syn*-8-Dichloro-*endo*-4-hydroxy	*5a*	142	3250		5.07		4.87	3.70	1.22	5.3	5.3
1-*syn*-8-Dichloro-*exo*-4-hydroxy	*6a*	130	3310	4.43			4.90	3.71	2.87	1.4	5.0
1,5-*anti*-8-Trichloro-*endo*-4-hydroxy	*7a*	125	3400		5.07	4.77			2.10		
1,5-*anti*-8-Trichloro-*exo*-4-hydroxy	*8a*	139	3300	4.76		5.20			3.10		
1,5-*syn*-8-Trichloro-*endo*-4-hydroxy	*9a*	121	3350		5.12		4.94		2.07		
1,5-*syn*-8-Trichloro-*exo*-4-hydroxy	*10a*	130	3460	4.45			4.96		2.80		
1-*anti*-8-Dichloro-*endo*-4-acetoxy	*3b*	155	1733		6.18	4.68		4.12	2.06 (OAc)	5.6	
1-*anti*-8-Dichloro-*exo*-4-acetoxy	*4b*	168	1735	5.87		5.03		3.78	2.13	2.0	
1-*syn*-8-Dichloro-*endo*-4-acetoxy	*5b*	125	1724		6.28		4.85	3.97	2.06	5.5	5.5
1-*syn*-8-Dichloro-*exo*-4-acetoxy	*6b*	155	1728	5.60			4.79	3.75	2.13		4.7
1,5-*anti*-8-Trichloro-*endo*-4-acetoxy	*7b*	231	1750		6.52	4.83			2.13		
1,5-*anti*-8-Trichloro-*exo*-4-acetoxy	*8b*	161	1735	6.25		5.23			2.15		
1,5-*syn*-8-Trichloro-*endo*-4-acetoxy	*9b*	176	1730		6.58		4.93		2.12		
1,5-*syn*-8-Trichloro-*exo*-4-acetoxy	*10b*	141	1732	6.03			4.88		2.20		
1-*endo*-4-*anti*-8-Trichloro	*3c*	132			5.50	4.61		4.00		5.2	
1-*exo*-4-*anti*-8-Trichloro	*4c*	129		5.10		5.20		3.98		2.0	
1-*endo*-4-*syn*-8-Trichloro	*5c*	94			5.67		4.85	3.76		4.8	5.5
1-*exo*-4-*syn*-8-Trichloro	*6c*	145		4.92			4.81	3.88			5.0
1-*endo*-4,5-*anti*-8-Tetrachloro	*7c*	113			5.53	4.69					
1-*exo*-4,5-*anti*-8-Tetrachloro	*8c*	157		5.35[a]		5.32[a]					
1-*endo*-4,5-*syn*-8-Tetrachloro	*9c*	146			5.64		5.00				
1-*exo*-4,5-*syn*-8-Tetrachloro	*10c*	159		5.10			4.93				
1,4,4-*anti*-8-Tetrachloro	*16*	151				5.19		4.41			
1,4,4-*syn*-8-Tetrachloro	*17*	129					4.84	4.19			4.7

[a] Assignment is tentative.

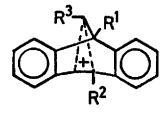
a mixture of both epimers with an *endo-exo* ratio of 30:70. The same behaviour has been observed in the reduction of 8-trichloromethyl derivatives of dibenzobicyclo[3,2,1]octadiene, where obstruction of the C-4 *exo* position by the bulky *syn*-8-trichloromethyl group causes complete *endo* specifity of hydride attack.[2] To obtain both epimeric alcohols, *3a* was treated with thionyl chloride to give a mixture of the trichlorides *3c* and *4c* which on acetolysis with acetic acid and silver acetate gave a mixture of the acetates *3b* and *4b*. Hydrolysis of the acetates with ethanolic potassium hydroxide gave the epimeric alcohols *3a* and *4a*.

The structure of *9c* was established by the following reaction sequence: Acetolysis of *9c* gave a mixture of two acetates which were hydrolyzed to the corresponding alcohols. On oxidation with Jones reagent both of these alcohols gave the same ketone *12b*. When this ketone was dehalogenated with zinc and ethanol, a quantitative yield of *12a* was obtained. The same reaction sequence applied to *7c* gave another ketone *11b* which furnished about 60 % of *11a* when treated with zinc and ethanol. The fact that in the latter reduction several other compounds are formed, may reflect the greater reactivity of the *anti*-8-chlorine compared with the *syn*-8-chlorine.[3-5]

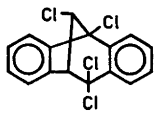
Because of the lack of the 5-proton, the stereostructure at C-4 in the 1,5,8-trichloro-substituted compounds *7 – 10* cannot be concluded directly from the ^{1}H NMR spectra, in contrast to the 1,8-dichlorosubstituted compounds, *3 – 6*, where the *endo* and *exo* epimers can easily be distinguished by the coupling constants between the *4*- and *5*-protons.[6] In the latter, the *endo-4*-proton absorbs at a higher field than the epimeric *exo*-4-proton by δ 0.33 – 0.75 which is in good agreement with the results obtained with compounds unsubstituted at the 1-position.[6] That this rule can be extended to the 1,5,8-trichloroderivatives, was confirmed by equilibration studies. In the dibenzobicyclo [3,2,1]octadiene series the *endo*-substituted derivatives have been shown to be thermodynamically more stable than the corresponding *exo* epimers and in most cases an *exo-endo*-conversion takes place on heating or by acid catalysis.[7,8] Thus reaction of *7a – 10a* with thionyl chloride gave epimeric mixtures which upon further heating slowly shifted towards the *endo* chloride. Solvolysis of the tetrachlorides *7c – 10c* with acetic acid and silver acetate gave acetate mixtures that did not change on further heating. Only when 5 % of sulfuric acid was added, equilibrium was reached. If stronger acid was used (30 % H_2SO_4 in HOAc) the only product was the dibenzobicyclo[2,2,2] octadiene derivative *13*.



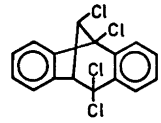
14a $R^1 = R^2 = H, R^3 = Cl$
b $R^1 = R^2 = R^3 = Cl$



15a $R^1 = R^2 = H, R^3 = D$
b $R^1 = R^2 = R^3 = Cl$



16



17

If *cis-* or *trans*-7,8-dichlorodibenzobicyclo [2,2,2]octadiene is solvolyzed with acetic acid and silver acetate, the *syn-* or *anti*-8-chlorodibenzobicyclo[3,2,1]octadienyl-4-acetates are formed, respectively.[7] This rearrangement is thought to require the intermediacy of carbonium ions of the type *14a*.[8] In the 1,4,7,8-tetrachloroderivatives it can be expected that the inductive effect of the 1- and 4-chlorine atoms would retard the rate of ionisation. In fact, *1* and *2* were found to be inert in the reaction conditions mentioned above and only when strong acid is used can the ionisation be forced to occur. Thus solvolysis of *1* or *2* in a mixture containing 60 % sulfuric acid in acetic acid and a slight excess of silver acetate gave the rearranged ketones *11a* or *12a* in high yield. As in the rearrangement of *7b – 10b* to *13* the normal reaction gives an unstable intermediate that is hydrolyzed to the ketone. Like most rearrangement reactions of the dibenzobicyclo[2,2,2]octadiene system.[7-9] these rearrangements are stereospecific: *1* gave only the *anti* compound and *2* only the *syn* compound.

When the ketones *11a* and *12a* are formed in the autoclave the carbonium ion intermediate *14b* can be attacked either by a hydroxyl ion derived from moisture present in the reaction

mixture or by a chloride ion. In the latter case the tetrachlorides *16* and *17* should be formed. The former possibility seems more likely because a ^{1}H NMR comparison of the mother liquors from the crystallization of *1* and *2* with the tetrachlorides *16* and *17* showed the absence of the latter. *16* and *17* were prepared from the corresponding ketones with phosphorus pentachloride. Even if *16* and *17* are hydrolyzed readily, they should remain when all water in the reaction vessel has been consumed.

The formation of *9c* and *7c*, although in a low yield, is of interest, because it offers a case where intervention of ionic species such as *15b* could be assumed to explain the bond migration from C-5 to C-4. However, chlorine substitution at C-5 makes the intermediate differ essentially from *15a*,[10] making this ion energetically still more unfavorable. Therefore, another reaction pathway consisting of direct chloride ion attack on the three-membered ring in *14b* with consequent bond migration to the adjacent carbon atom should also be considered.

EXPERIMENTAL

^{1}H NMR spectra were recorded with a JEOL JNM-PMX 60 spectrometer in CCl_4 solution and IR spectra with a Perkin-Elmer 125 spectrometer using KBr pellet technique. All mass spestra (70 eV) exhibited a typical isotope pattern consistent with the number of chlorine atoms present in the molecule. The parent peak based on ^{35}Cl and the base peak are given. *trans*-1,2-Dichloroethylene was synthetic grade reagent from Fluka or Merck, *cis*-1,2-dichloroethylene was obtained by fractional distillation of a commercial isomer mixture. Silica gel used for columns was H & W silica gel M.F.C. and for TLC Merck's Kieselgel 60 PF. Light petroleum refers to the fraction boiling 80 – 100 °C. Melting points are not corrected.

Reaction of 9,10-dichloroanthracene with trans-*1,2-dichloroethylene.* In a typical experiment 24.7 g (0.1 mol) of 9,10-dichloroanthracene[11] and 291 g (3.0 mol) of *trans*-1,2-dichloroethylene were heated for 24 h at 210 °C in a 1 l steel pressure vessel. After cooling, excess dichloroethylene was removed, 1200 ml of light petroleum was added to the residue and the mixture was stirred overnight. The solution was filtered and the filtrate was passed through a silica gel column (50 g) followed by 2000 ml of light petroleum. After removal of the solvent, the yellow oily residue crystallized on standing to give 22.6 g (66 %) of *1*, m.p. 153 °C (EtOH). δ 4.30 (2 H, s), 7.1 – 7.4 (4 H, m), 7.5 – 7.9 (4 H, m).

To obtain the ketone *11a*, the column was eluted with 1500 ml of benzene. The solvent was removed and the residue dissolved in 100 ml of acetone, 5 g of activated charcoal was added and the solution boiled for 20 min. The mixture was filtered and to remove any residual carbon the filtrate was passed through a small (5 g) silica gel column and the column was flushed with 50 ml of acetone. After removal of acetone the product was crystallized from ethanol to give 3.3 g (11 %) of *11a*, *m/e* 288(25), 253(100).

The same starting mixture as above heated for 96 h at 250 °C gave 51 g of material insoluble in light petroleum, 6.6 g (19 %) of *1* and 4.1 g (14 %) of *11a*. The mother liquors from the crystallization of both *1* and *11a* were combined and all volatile material was removed by steam distillation. From the spectrum of the residue it was estimated to contain about two thirds of higher molecular weight condensation products of dichloroethylene, the rest being a mixture of *1*, *11a* and some unknown compounds. The most important of the latter group was separated by preparative TLC (several elutions with light petroleum) and two recrystallizations from EtOH. 0.24 g (0.7 %) of pure *9c* was obtained, *m/e* 342(28), 307(100).

Reaction of 9,10-dichloroanthracene with cis-*1,2-dichloroethylene.* The reaction was carried out in the same manner as with *trans*-dichloroethylene with somewhat different separation of the products. A typical experiment gave 10 g of material insoluble in light petroleum. According to ^{1}H NMR the solution contained the two principal products in a ratio of 54:46 which corresponds to a yield of about 34 % of *2* and 29 % of *12a*. As the chromatographic separation was much more difficult than with the *trans* adduct, the product mixture, after removal of excess dichloroethylene, was dissolved in 200 ml of acetone and 10 g of activated charcoal was added. The solution was refluxed for 20 min and the charcoal and acetone removed. The residue was crystallized from EtOH to give 7.1 g (21 %) of *2*, m.p. 199 °C, δ 4.54 (2 H, s), 7.1 – 7.5 (4 H, m). 7.6 – 8.0 (4 H, m). The mother liquor was evaporated, steam distilled and fractionated on a silica gel column (100 g). Two main components were separated and crystallized from EtOH to give 3.8 g (11 %) of *2* and 6.2 g (21 %) of *12a*. MS of *12a m/e* 288(25), 217(100).

A run for 96 h at 250 °C gave 64 g of material insoluble in light petroleum, 5.3 g (15 %) of *2* and 7.5 g (26 %) of *12a*. 0.35 g (1.0 %) of *7c* was separated from the mother liquor using the same procedure as for *9c*. MS of *7c m/e* 342(28), 202(100).

Reduction of the ketones 11a *and* 12a. A solution of 2.89 g of the ketone (10 mmol) and 0.38 g of $NaBH_4$ (10 mmol) in 100 ml of EtOH was refluxed for 20 min. The solution was poured into cold water, acidified with HCl and extracted

twice with ether. The ethereal solution was dried and evaporated. ^{1}H NMR examination of the reduction product showed that *11a* gave almost pure *endo* alcohol. Crystallization from 80 % aqueous EtOH gave 2.18 g (76 %) of *3a*. The product from *12a* exhibited an *endo-exo* ratio of about 30:70. The epimers were separated by preparative TLC (repeated elutions with a 1:1 mixture of benzene and light petroleum) and purified by crystallization from 80 % aqueous EtOH. 0.40 g (14 %) of the *endo* alcohol *5a* and 1.42 g (49 %) of the *exo* alcohol *6a* were obtained.

Reactions of the alcohols 3a, 5a – 10a *with thionyl chloride.* Epimeric mixtures resulting from reduction of ketones *11a* and *12a* or hydrolysis of acetates *7b – 10b* were used as starting material. The alcohol (0.5 – 1.0 g) was refluxed with 10 ml of $SOCl_2$ for 20 h. Excess $SOCl_2$ was removed under reduced pressure, the residue dissolved in ether and the ether solution washed with saturated $NaHCO_3$ solution. After drying, ether was removed and the residue examined by ^{1}H NMR. Following mixtures were obtained (starting compounds, *endo-exo* ratio of resulting chloride mixture): *3a*; 60:40, *5a*, *6a* 28:72, *7a*, *8a* 45:55, *9a*, *10a* 65:35. Analytical samples were prepared by chromatographic separation of the epimers (elution with light petroleum) and crystallization from 80 % aqueous EtOH.

Acetolysis of the chlorides 3c – 10c. A mixture of 1 mmol of the chloride (0.31 g of trichloride or 0.34 g of tetrachloride), 0.20 g (1.2 mmol) of AgOAc and 10 ml of HOAc was refluxed for 20 h. Acetic acid was removed under reduced pressure, the residue dissolved in acetone, the solution filtered and evaporated. Following mixtures were obtained (starting compounds, *endo-exo* ratio of resulting acetate mixture): *3c*, *4c* 19:81, *5c*, *6c* 24:76, *7c*, *8c* 22:78, *9c*, *10c* 52:48. Analytical samples were prepared by chromatographic separation of the epimers (elution with a 1:1 mixture of benzene and light petroleum) and crystallization from EtOH.

When 0.05 g of the *exo* acetate *8b* was refluxed for 20 h with a mixture of 1 ml of H_2SO_4 and 9 ml of HOAc, complete epimerization to the *endo* acetate *7b* occurred. Similarly *10b* gave *9b*. 0.4 g of *10b* refluxed for 20 h with a mixture of 2 ml of H_2SO_4 and 8 ml of HOAc gave, after the usual work-up and crystallization from EtOH, 0.24 g (76 %) of *13*. The same product was obtained from *8b*, m.p. 131 °C, δ 4.13 (1 H, s), 4.79 (1 H, s), 7.1 – 7.5 (6 H, m), 7.6 – 7.9 (2 H, m). ν_{max} 1740 cm^{-1}.

Hydrolysis of the acetates 3b – 10b. The acetate (1 mmol; 0.33 g of a dichloro compound or 0.37 g of a trichloro compound) was stirred with 10 ml of 10 % KOH in EtOH for 80 min at room temperature. Water and HCl was added and the product was obtained by ether extraction and purified by crystallization from 80 % aqueous EtOH.

Oxidation of the alcohols 7a – 10a. The alcohol (0.1 – 0.5 g), dissolved in acetone, was treated with Jones reagent until the oxidation was complete (as shown by TLC). After the usual work-up the pure ketones were obtained by crystallization from EtOH. MS of *11b m/e* 322(15), 251(100), *12b m/e* 322(16), 251(100).

Dechlorination of the ketones 11b *and* 12b *with zinc and ethanol.* A mixture of 0.2 g of *12b*, 0.4 g of Zn-powder and 10 ml of EtOH was refluxed for 8 h. Ethanol was removed, the residue dissolved in acetone, the solution filtered and evaporated. According to ^{1}H NMR the product was almost pure *12a*. Two recrystallizations from EtOH gave 0.11 g (62 %) of pure ketone, m.p. 116 °C. It showed no melting point depression when mixed with a sample of *12a* obtained from the reaction of 9,10-dichloroanthracene and *cis*-1,2-dichloroethylene.

When the same procedure was used for the dechlorination of the *anti*-8-chloro ketone *11b* (0.2 g), a mixture of several compounds was obtained, the major component being the expected ketone *11a*. Separation by TLC (elution with a 1:1 mixture of benzene and light petroleum) and crystallization from EtOH gave 0.07 g (39 %) of pure product, identical with *11a* from the Diels-Alder reaction. The other products were not identified.

Acetolysis of the tetrachlorides 1 *and* 2. A mixture of 3.44 g (0.01 mol) of *1*, 3.50 g (0.021 mol) of AgOAc, 40 ml of H_2SO_4 and 60 ml of HOAc was refluxed for 20 h. The mixture was cooled and poured into cold water. The aqueous solution was extracted three times with ether, the ether solution dried and evaporated. The residue was crystallized from EtOH to give 2.3 g (81 %) of *11a*. Similarly 3.44 g of *2* gave 1.90 g (66 %) of *12a*.

Synthesis of the tetrachlorides 16 *and* 17. *11a* (0.5 g) was refluxed for 80 min with a mixture of 5 g of PCl_5 and 5 g $POCl_3$. The hot mixture was carefully poured into vigorously stirred ice water. When excess reagent was completely decomposed, the aqueous solution was extracted twice with ether, the ethereal solution dried and evaporated. The crude product was purified by TLC (elution with light petroleum) followed by crystallization from EtOH to give 0.49 g (82 %) of *16*. Similarly, the ketone *12a* gave 0.41 g (69 %) of *17*.

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Semi-empirical Parameters in π-Electron Systems. XIV. Parameters for the Methoxy and Ethyl Groups

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The parametrization of the methoxy and ethyl groups for the modified PPP method originally proposed by Roos and Skancke has been analysed. From the basic assumptions of the method it is found that the method should be able to distinguish between the methoxy group and the hydroxy group, which was treated in an earlier paper, while it cannot always account for the experimentally observed differences between methyl- and ethyl-substitution. Ionization potentials and electronic spectra of substituted benzenes are presented.

INTRODUCTION AND DETAILS OF THE CALCULATIONS

Some years ago Roos and Skancke [1] proposed a systematic way to parametrize the PPP method on the basis of an analysis of the ZDO approximation by Fischer-Hjalmars.[2] This work has been extended to include various heteroatoms and substituents in conjugated systems.[1–14] Later on Roos [15,16] generalized the method to metal complexes and Sundbom and Henriksson applied it to the interpretation of the electronic spectra of metal-organic complexes (copper and iron) with very good results.[17–19]

In Refs. 3 and 7 the methyl and hydroxy groups were parametrized. In some vanadium-porphyrins, which the present authors are studying now, the porphin ring has methoxy and ethyl groups as substituents. To include these groups in this method one can think of two different ways. Either one can try to build them with the existing parameters for $-CH_3$ and $-OH$, just adding the missing ones, or one can treat them as complete groups. The way to include these two new groups in the method is determined by the following experimental findings. The ionization spectra and the UV absorption spectra of substituted benzenes show a strong similarity between methyl- and ethyl-substitution and between hydroxy- and methoxy-substitution. The exchange of a hydrogen atom for a methyl group leads only to a small modification of the inductive and mesomeric effects of the original group. In our model this means that the original oxygen π-orbital in the hydroxy or the pseudo π-orbital in the methyl group is perturbed by this exchange. How this affects the original $-OH$, respectively $-CH_3$, parameters is discussed below. But first we want to make a remark about an alternative approach to our problem. One could try the hypothesis, that the methoxy group contributes to the π-system a real π-orbital on the oxygen atom *and* a pseudo π-orbital on the methyl group with a total of four electrons. That would imply a hyperconjugation effect across the $O-CH_3$ bond. Some preliminary tests on anisole along this line gave very unsatisfactory results and clearly demonstrated that this second approach is unrealistic.

Fischer-Hjalmars [2] showed, that in the formally orthogonalized atomic orbital basis the core parameter W_μ is local to the first order in the overlap while $\beta_{\mu\nu}$ and $\gamma_{\mu\nu}$ are local to the second order (we use the notations of Ref. 1). For that reason Roos and Skancke made W_μ dependent on the nearest neighbours while the other parameters were directly transferable. In the discussion of the various param-

eters we let χ stand for the π-orbital of the substituent in question. Going from $-OH$ to $-OCH_3$ and from $-CH_3$ to $-C_2H_5$ should then be accomplished by reoptimizing W_χ in the first place. The one-center integral $\gamma_{\chi\chi}$ has previously been estimated from atomic spectral data or from the assumed form of χ. In this case it is very difficult to say how χ is modified by changing a hydrogen atom for a methyl group in the substituent. We will return to this problem later on. Experiments with this parameter show, that the results are very insensitive to reasonable variations in the value of $\gamma_{\chi\chi}$. The two-center integrals $\gamma_{\chi C}$ and $\beta_{\chi C}$ have been obtained in the earlier papers by fitting to experimental data on a few standard molecules. In this case we do not have enough data to make a least squares fit for W_χ, $\gamma_{\chi C}$ and $\beta_{\chi C}$. But $\gamma_{\chi C}$ and $\beta_{\chi C}$ are local to the second order and should not vary by a change in the environment of χ, unless χ is strongly affected by this change. Again the results vary rather slowly with these two parameters. And at last $\Delta W_C(\chi)$, which is the correction to W_C due to χ as nearest neighbour to C, depends only on χ and not on the environment of χ. This analysis turns out to hold quite well for the hydroxy-methoxy case as our results will show in the next section.

For the ethyl group the situation is more complicated. Experimentally it is found, that ethylbenzene has a lower ionization potential (IP) and a smaller red shift of the ^1L_b band with respect to benzene than methylbenzene. Apparently the method cannot reproduce both these properties at the same time. We will discuss several points connected with this problem. Diminishing the absolute value of W_χ lowers the IP but increases the red-shift at the same time. The first observation we want to make is that, the method gives a too low transition energy for the ^1L_b band in benzene, 39.2×10^5 m^{-1} compared to the experimental value of 39.5×10^5 m^{-1}. Roos used methyl-substituted ethylenes to determine the methylparameters. The calculated energy of the ^1L_b band in toluene comes out right on the observed value. This means, however, that the calculated red shift of toluene (and also for the other methyl-substituted benzenes) is too small if we take the calculated spectra of benzene as standard. In this sense the value obtained for ethylbenzene is better though the absolute value of the transition energy is off compared to the experimental one. Secondly, looking at the suggested form of the π-orbital of the methyl group, one should expect the replacement of one of the hydrogens for a $-CH_3$ to affect to a greater extent the form of the orbital and thus the parameters of this group, than in the hydroxy-methoxy case, where the π-orbital is the oxygen $2p_z$ orbital. In the ethyl group it is not unreasonable to think that the π-orbital partly spreads into the CH_3 group. CNDO calculations [20] do show such a shift in the highest occupied π-orbital in ethylbenzene. The size of the shift depends on the conformation. It is only a few percent for the case with the ethyl group in the benzene plane, while it is quite large when the group is rotated out of the plane. This discussion suggests, that the π-orbital of the ethyl group is a little more diffuse and centered further away from the ring than the methyl π-orbital. That should lead to poorer overlap with the ring, which means a numerically smaller beta value for the bond and smaller repulsion integrals, both $\gamma_{\chi\chi}$ and $\gamma_{\chi C}$. The effect on ΔW (χ) is not so obvious from the change in χ. Variations along these lines were not able to lower the IP and the red shift at the same time. The last point we want to make is that the rotational barrier in ethylbenzene is very small. Experimentally it has been estimated to be around 5.4 kJ/mol.[21] Both *ab initio* [22] and CNDO [20] predict a barrier of about 8.4 kJ/mol and the conformation with the ethyl out of the benzene plane as the most stable one. In this conformation the strict symmetry separation of σ and π breaks down. It is not surprising then that the parametrization, which does not take into account σ-π-mixing, cannot completely reproduce the experimental results. A detailed study of the difference between methyl- and ethyl-substitution is really beyond the limits of the applicability of the π-electron theory.

GEOMETRY

The same geometries have been used as for the corresponding hydroxy- and methyl-substituted molecules. This is very reasonable as neither the hydroxy [7] nor the methyl param-

Table 1. Comparison of calculated and experimental ionization potentials (eV).

Molecule	Calc.	Symmetry (for C_{2v})	Observed Adiabatic	Vertical
Benzene [a]	9.25	a_2, b_1	9.25 [f]	9.40 [h]
Phenol [b]	8.45	b_1	8.46 [f]	8.75 [h]
	9.14	a_2	9.34 [f]	9.45 [h]
Anisole	8.20	b_1	8.20 [f]	8.54 [h]
	9.12	a_2	9.10 [f]	9.37 [h]
1,2-Dimethoxybenzene	7.77			
	8.53			
1,3-Dimethoxybenzene	7.88			
	8.34			
1,4-Dimethoxybenzene	7.61	b_1		7.90 [h]
	9.00	a_2		9.19 [h]
1,4-Bromohydroxybenzene [c]	8.43	b_1		8.52 [h]
1,4-Bromomethoxybenzene	8.22	b_1		8.49 [h]
	9.06	a_2		9.65 [h]
p-Methoxybenzaldehyde	8.55	b_1		8.87 [h]
	9.66	a_2		9.77 [h]
	9.98	n		
Benzaldehyde [d]	9.44	b_1	9.46 [f]	9.80 [h]
	9.71	a_2		10.00 [h]*
	10.10	n		
Toluene [e]	8.84	b_1	8.84 [f]	8.9 [h]
	9.19	a_2	9.15 [f]	9.13 [h]
Ethylbenzene	8.77	b_1	8.77 [g]	
	9.18	a_2		
1,2-Diethylbenzene	8.50			8.91 [i]
	8.92			
1,3-Diethylbenzene	8.54			8.99 [i]
	8.88			
1,4-Diethylbenzene	8.36	b_1		8.93
	9.11	a_2		

[a] Ref. 1. [b] Ref. 7. [c] Ref. 9. [d] Ref. 14. [e] Ref. 3. [f] Ref. 24. [g] Ref. 25. [h] Ref. 26. [i] Ref. 27, electron impact data.
* Estimated by us from the unresolved first band in Fig. 11, Ref. 26. The Refs. 24 and 26 refer to photoelectron spectroscopy data.

eters [3] are distance dependent. A change in the distance ring-substituent would only affect the coulomb integrals between the substituent and its non-nearest neighbours with very small influence on the final results.

RESULTS AND DISCUSSION

The parameter W_χ was optimized by fitting the first IP and the first transition in anisole and ethylbenzene to experimental data. The other parameters retained their values (from $-OH$ and $-CH_3$) in agreement with the analysis above.

The new parameters are

$W_{OCH_3} = -10.42$ eV; $W_{C_2H_5} = -11.18$ eV

As mentioned in the previous section the results for the ethyl group are not entirely satisfactory but we give them for completeness. In the tables we include also some molecules treated in earlier papers in this series to simplify the discussion.

Ionization potentials. The calculated and experimental ionization potentials (IP's) are presented in Table 1. When Roos and Skancke [1] initiated this method only adiabatic ionization potentials were available for the molecules of interest. In the following papers [3–14] this practice was continued for consistency. Experimentally the difference between the adiabatic and vertical IP's is of the order 0.2 to 0.4 eV. Our theoretical values are obtained as usual

Table 2. Calculated and observed electronic transitions in 10^5 m^{-1}.

Theoretical			Compilation by Petruska [23]			Observed					
ν_{max}	$-\Delta\nu$ [a]	f_{calc}	$-\Delta\nu_{0-0}$ [b]	f	solvent	ν_{max}	intensity	ν_{max}	intensity	ν_{max}	intensity
Benzene [c]											
39.17		0.0				39.4	[g]	39.4	$f=0.001$ [h]	39.5	[i]
50.62		0.0				49.8		49.5	0.10	50.6	[j]
56.14		1.19				56.3		54.4	0.69	55.9	[j]
Phenol [d]											
37.89	1.28	0.013	1.74(1.80)	−(0.017)	vap. (iso)	37.02	0.02 [g]	37.2	$\log\varepsilon=3.34$ [k]	36.7	$\log\varepsilon=3.18$ [l]
47.07	3.55	0.158	2.10			46.94	0.132	47.6	3.78	47.0	3.78
53.69	2.45	0.830	1.70			54.04	0.636			52.7	4.70
54.72	1.42	1.036				55.90	0.467				
Anisole											
37.38	1.79	0.019	1.85	0.020	hex	37.02	0.023 [g]	36.90	$a=2200$ [m]	36.90	[n]
45.72	4.90	0.212	3.40			46.62	0.175	45.5			
52.22	3.92	0.576				53.88	0.585				
54.40	1.74	0.922				55.65	0.371				
1,2-Dimethoxybenzene											
36.18	2.99	0.024	2.40	0.028	hex	36.43	$a=3920$ [n]				
43.04	7.58	0.130				44.44	11700				
50.53	5.61	0.946									
50.72	5.42	0.542									
1,3-Dimethoxybenzene											
36.40	2.77	0.015	2.50	0.024	hex	36.64	[n]				
44.18	6.44	0.081									
49.62	6.52	0.962									
49.82	6.32	0.511									
1,4-Dimethoxybenzene											
35.62	3.55	0.054	4.50	0.032	hex	34.60	$a=3010$ [n]				
43.38	7.24	0.337				44.35	8870				
52.31	3.83	0.650									
54.50	1.64	1.010									

Table 2. Continued.

1,2-Dihydroxybenzene [d]									
36.93	2.24	0.017	2.70	0.026	hex	36.05	[n]		
44.79	5.83	0.105	2.80						
52.00	4.14	1.061							
52.36	3.78	0.753							
1,3-Dihydroxybenzene [d]									
37.08	2.09	0.011	2.60	0.022	hex	36.36	[n]		
45.59	5.03	0.067	3.80						
51.53	4.61	1.088							
51.95	4.19	0.736							
1,4-Dihydroxybenzene [d]									
36.54	2.63	0.041	5.40	0.032	hex	34.07	$a=2810$ [n]		
44.97	5.65	0.275				44.54	5180		
53.35	2.79	0.828							
54.66	1.48	1.064							
p-Bromomethoxybenzene									
37.26	1.91	0.000				35.46	$a=1600$ [n]	35.46	$a=1750$ [m]
43.79	6.83	0.342				44.15	14200	44.00	12500
51.35	4.79	0.708							
54.46	1.68	0.935							
p-Bromohydroxybenzene [e]									
37.6	1.57	0.001				35.40	$a=1930$ [n]	35.52	$a=1750$ [m]
44.9	5.72	0.31				44.25	12100	44.64	10000
52.5	3.64	0.88							
54.6	1.54	1.06							
p-Methoxybenzaldehyde									
31.37		0.0							
35.91		0.017							
40.12		0.504							
48.23		0.422							
52.22		0.259							
54.28		0.719							
57.43		0.102							

Table 2. Continued.

Theoretical			Compilation by Petruska [23]			Observed					
ν_{max}	$-\Delta\nu$ [a]	f_{calc}	$-\Delta\nu_{0-0}$ [b]	f	solvent	ν_{max}	intensity	ν_{max}	intensity	ν_{max}	intensity
Toluene [f]											
38.84	0.33	0.011	0.61(0.61)	−(0.003)	vap. (iso)						
49.95	0.67	0.018	1.40								
55.65	0.49	1.212	1.40								
55.85	0.29	1.143									
Ethylbenzene											
38.73	0.44	0.012	0.55	0.003	iso						
49.68	0.94	0.032									
55.46	0.68	1.203									
55.69	0.45	1.119									
1,2-Diethylbenzene											
38.41	0.76	0.014	1.03	0.003	iso						
48.81	1.81	0.032									
54.86	1.28	1.209									
54.97	1.17	1.103									
1,3-Diethylbenzene											
38.51	0.66	0.012	1.06	0.003	iso						
49.08	1.54	0.017									
54.81	1.33	1.229									
54.99	1.15	1.114									
1,4-Diethylbenzene											
38.07	1.10	0.043	1.25	0.005	iso						
48.98	1.64	0.081									
55.15	0.99	1.203									
55.80	0.34	1.071									

[a] Frequency shift relative the calculated benzene band. [b] Frequency shift relative the observed benzene band. [c] Ref. 1. [d] Ref. 7. [e] Ref. 9. [f] Ref. 3. [g] Ref. 28, vapour. [h] Ref. 29, hexane. [i] Ref. 30, vapour. [j] Ref. 31, vapour. [k] Ref. 32, cyclohexane. [l] Ref. 29, heptane. [m] Ref. 33, cyclohexane. [n] Ref. 34, MeOH.

as minus the energy of the orbital in question according to Koopmans' theorem. For the comparison theory-experiment the adiabatic IP's or the vertical IP's lowered by the above-mentioned difference should be used.

For the parametrization we used for anisole the adiabatic photoelectron spectroscopy value given by Turner,[24] which is the same reference as was used for the hydroxy group.[7] For ethylbenzene we used the adiabatic spectroscopic value reported by Hammond *et al.*[25]

The degenerate e_{1g} level in benzene is split into b_1 and a_2 in C_{2v} (monosubstitution and *para*-disubstitution). The substitution orbital(s) is (are) of b_1 symmetry and can mix directly with the b_1 orbital of the e_{1g} level and also to a smaller extent with the deeper lying a_{2u} orbital, which also transforms as b_1 in C_{2v} symmetry. The a_2 orbital, which cannot mix directly with the substituent orbital(s), should be less affected by the substitution than the b_1 orbital. The table shows a very good agreement between calculated and experimental data for the first ionization potential b_1. The second ionization potential, which corresponds to the a_2 orbital, requires a comment. The $-CH_3$, $-C_2H_5$, $-OH$ and $-OCH_3$ groups have very small effect on the a_2 level as expected and the agreement between theory and experiment is very good for this band too. It is only in the case of bromosubstitution the method fails to show a fairly strong stabilization of the a_2 level experimentally observed. In all the other cases this level remains very near the e_{1g} level in benzene.

Electronic spectra. The calculated and observed spectra are collected in Table 2. In the cases where we have used Petruska's [23] compilation of the spectra of substituted benzenes for the comparison of theory and experiment, we have assumed that the shifts of ν_{max} are very similar to the shifts in the 0–0 bands given by Petruska. Most of the experimental spectra are taken in solution. Unfortunately this makes the comparison of the effects of the hydroxy and methoxy groups rather difficult as the former group but not the latter one is readily available for hydrogen bonding, solute-solute and when the solvent is an alcohol also solute-solvent. It is quite probable that the solvent shifts (including effects solute-solute) will be different for the two groups. The observed differences are very near the experimental accuracy too. For these reasons the evaluation of the results for the electronic transitions will not be as clear as in the case of the ionization potentials, where the experiments are in the vapour phase.

The calculated positions of the bands are very good. But the methoxy group is always predicted to give larger red shifts than the hydroxy group, while the experimental data do not show a definite trend on that point. The agreement on the variation of the intensities of the bands is very good between theory and experiment. The methoxy group should intensify the ^1L_b and ^1L_a bands and lower the intensity of the ^1B band compared to the hydroxy group.

CONCLUSIONS

The comparison of the hydroxy and methoxy groups and the methyl and ethyl groups provides a good illustration of the consistency in the basic approximations of the method. The reoptimization of W_χ to account for the change from $-OH$ to $-OCH_3$ or from $-CH_3$ to $-C_2H_5$ gave very good results for the prediction of the first IP's while the results were not so conclusive with respect to the electronic spectra. However, this might be related to the fundamental problem of relating theoretical spectra, which correspond really to the vapour phase, and experimental solution spectra. We feel this problem is specially serious for the case $-OH$ and $-OCH_3$. Experimentally $-CH_3$ and $-C_2H_5$ substitutions are very similar and apparently the method cannot account for the fine differences due to the reasons given at the end of the introduction.

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Tobacco Chemistry. 40. Syntheses of (12*R*,13*R*)- and (12*S*,13*S*)-8,12-Epoxy-14-labden-13-ol and (13*R*)- and (13*S*)-8,13-Epoxy-14-labden-12-one, Four Tobacco Diterpenoids

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Two of the title compounds, (12*R*,13*R*)- and (12*S*, 13*S*)-8,12-epoxy-14-labden-13-ol (*9*, *10*) were obtained as major products on peracid oxidation of (12*Z*)-abienol (*8*), a reaction also furnishing small amounts of (12*R*,13*R*)- and (12*S*,13*S*)-8,13-epoxy-14-labden-12-ol (*12*, *11*), which were converted to the other two title compounds (13*R*)- and (13*S*)-8,13-epoxy-14-labden-12-one (*6*, *5*) by oxidation using Jones' reagent. The stereostructures of *9* and *10* were determined by an X-ray analysis of (12*R*)-14,15-bisnor-8,12-epoxylabdane-13-one (*16*), prepared by oxidative degradation of the 8,12-epoxyalchohol (*9*), and by consideration of the reaction mechanism involved in the peracid oxidation.

^{13}C NMR data for the synthetic and some related compounds are given and the effects of steric factors on their ^{13}C shieldings are discussed.

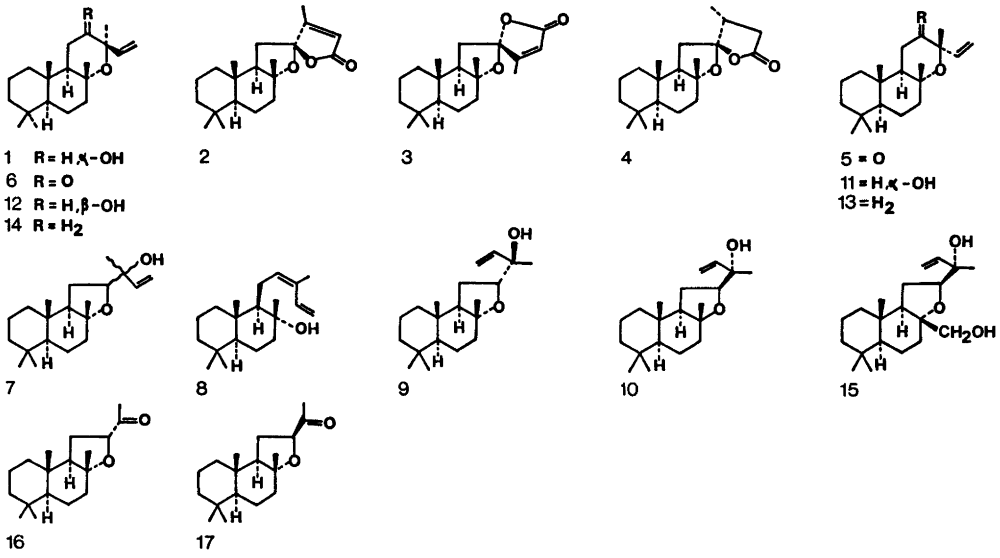
Recent phytochemical studies have disclosed that cultivars of *N. tabacum* L. generally produce diterpenoids of either the thunbergane or the labdane types.[1,2] The majority of the tobacco labdanoids known to date have been encountered in commercial Turkish and Greek tobaccos. They comprise (12*S*, 13*R*)-8,13-epoxy-14-labden-12-ol (*1*),[3] α- and β-levantenolide (*2*, *3*) [4,5] and α_2-levantanolide (*4*) [6] of Turkish tobacco, and (13*S*)- and (13*R*)-8,13-epoxy-14-labden-12-one (*5*, *6*) [7] and four stereoisomers of 8,12-epoxy-14-labden-13-ol (*7*) [8] of Greek tobacco. Since these compounds differ only with respect to their side-chain oxygenation and stereochemistry, it is reasonable to assume that they may be formed in tobacco by the non-stereospecific oxidation of an appropriate labdane precursor. (12*Z*)-abienol (*8*), which is present in the gummy exudate of green leaves of labdane producing tobacco cultivars, is an attractive possibility, particularly since studies have shown that it is lost during leaf processing.[2] The present communication describes the synthesis of four of the above tobacco labdanoids from (12*Z*)-abienol (*8*).

RESULTS

Treatment of (12*Z*)-abienol (*8*) with *m*-chloroperbenzoic acid in chloroform afforded two major products *9* (71 %) and *10* (25 %). These proved to be identical to two of the stereoisomers of (12ξ,13ξ)-8,12-epoxy-14-labden-13-ol (*7*), previously encountered in Greek tobacco and then designated "Ib" and "Id" respectively.[8]

Two alcohols, *11* and *12*, each comprising some 2 % of the reaction mixture, were also obtained. Both compounds gave mass spectra displaying diagnostically important peaks at *m/e* 306 (M), 291 (M − 15), 273 (M − 33), 236 (A), 235, 206 (B), 192 (C) and 177 (192 − 15), which are indicative of 8,13-epoxy-labdenes having a hydroxyl substituent at C-12 (Fig. 1).[7]

In agreement with this the ^1H NMR spectra of these compounds (*11*, *12*) displayed signal patterns typical of a vinyl group attached



to a non-protonated carbon, *i.e.* one-proton doublets of doublets at δ 5.20 ($J_{AB}=2.5$ and $J_{AX}=10$ Hz), 5.42 ($J_{AB}=2.5$ and $J_{BX}=17.5$ Hz) and 5.84 ($J_{AX}=10$ and $J_{BX}=17.5$ Hz) and at δ 5.23 ($J_{AB}=1.5$ and $J_{AX}=11$ Hz), 5.42 ($J_{AB}=1.5$ and $J_{BX}=18$ Hz) and 6.32 ($J_{AX}=11$ and $J_{BX}=18$ Hz), respectively. The presence of the hydroxyl group in *11* and *12* was confirmed by the IR spectra (*11*: 3580 cm^{-1} and *12*: 3630 and 3460 cm^{-1}) and by the ^1H NMR spectra, which contained a one-proton triplet at δ 3.69 ($J=3.5$ Hz) and a one-proton doublet of doublets at δ 3.50 ($J=5$ and 10 Hz), respectively. Furthermore, it was concluded from the magnitudes of these coupling constants that the secondary hydroxyl group at C-12 in *11* is axial (α-oriented), while that in *12* is equatorial (β-oriented). The ^{13}C NMR spectra of the two alcohols (*11* and *12*) containing signals due to one sp^2 methine, one sp^2 methylene, one sp^3 oxygen-carrying methine and two sp^3 oxygenated and non-protonated carbon atoms were in harmony with these assignments. (*cf.* Table 1). Oxidation using Jones' reagent converted the axial alcohol (*11*) to the ketone *5*, which was identical with (13*S*)-8,13-epoxy-14-labden-12-one previously found in Greek tobacco.[7] Since oxidation of the equatorial alcohol (*12*) afforded (13*R*)-8,13-epoxy-14-labden-12-one (*6*),[7] the alcohols obtained as minor products of the peracid oxidation of (12*Z*)-abienol (*8*) can be formulated as (12*S*, 13*S*)- and (12*R*,13*R*)-8,13-epoxy-14-labden-12-ol (*11* and *12*).

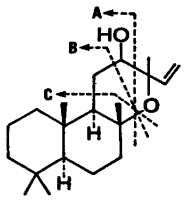


Fig. 1. Characteristic cleavage reactions in the mass spectra of 8,13-epoxy-14-labden-12-ols.

Configuration of the (12ξ,13ξ)-8,12-epoxy-14-labden-13-ols 9 *and* 10. Since the ^{13}C NMR spectrum of the 8,12-epoxy alcohol *9* exhibited C-12 and C-17 signals at 81.6 and 21.3 ppm, while the corresponding signals for the isomer *10* were located at 85.3 and 25.4 ppm, it was concluded that these compounds differ with respect to the orientation of the C-12 substituent. In the absence of information on the steric dependence of the effects of substituents on the shieldings of the carbon atoms of tetrahydrofuran rings, these data did not, however, allow configurational assignments. Nor was conclusive evidence obtained when lanthanide-induced shift (LIS) measurements were employed, although these were useful for the assignments of the ^{13}C NMR spectra of *9* and *10*.

Table 1. Carbon-13 chemical shifts and assignments for *5*, *6*, *8*–*14*, *16* and *17*.

Compound	Carbon 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
13	39.0	18.6	42.1	33.2	56.4	19.9	43.2	74.8	55.7	36.9	15.4	35.8	73.0	147.8	110.1	28.5	25.5	33.4	21.3	15.3
11	38.7	18.5	42.1	33.2	56.4	19.9	42.6	75.4	49.1	36.4	23.0	70.4	76.8	143.1	116.1	27.5	25.4	33.3	21.2	15.6
5	38.6	18.3	41.8	33.2	55.9	19.7	41.8	75.2	52.6	36.8	33.1	211.8	82.7	141.5	114.9	26.6	22.5	33.3	21.5	14.4
14	39.4	18.7	42.2	33.4	56.5	19.9	43.1	76.1	58.5	39.6	15.9	34.9	73.3	147.8	109.5	32.7	24.0	33.3	21.3	15.9
12	39.3	18.6	42.3[a]	33.3	56.3	19.9	42.1[a]	76.0[b]	57.8	36.8	25.2	77.6	76.2[b]	140.5	116.9	28.0	25.5	33.3	21.2	16.0
6	38.7	18.3	41.9[a]	33.2	56.0	19.7	42.1[a]	75.6	54.6	37.0	33.8	211.8	81.8	142.4	113.1	28.6	22.2	33.4	21.5	14.6
9	39.8[a]	18.4	42.4	33.1	57.3	20.6	39.6[a]	81.4	60.2	36.3	24.0	81.6	74.5	143.1	112.9	23.5	21.3	33.5	21.1	14.8
16	39.9[a]	18.3	42.4	33.1	57.2	20.7	39.8[a]	82.8	59.5	36.4	27.1	81.0	211.1			26.7	21.5	33.5	21.1	14.9
10	40.8	18.4	42.5	33.1	57.1	21.4	40.2	81.3	60.8	36.4	24.0	85.3	73.2	144.3	112.5	23.2	25.4	33.5	21.0	15.9
17	40.0	18.4	42.5	33.1	57.1	20.8	40.0	82.4	60.7	36.4	26.1	82.7	211.3			26.8	23.0	33.5	21.1	15.4
8	40.2	18.6	41.9	33.3	56.2	20.3	44.0	74.3	62.2	39.0	23.2	133.9	130.8	133.7	113.7	19.9	24.4	33.5	21.6	15.5

[a,b] Assignment may be reversed.

In order to clarify this question the two allylic alcohols *9* and *10* were converted by careful osmium tetroxide–sodium periodate oxidation to the corresponding nor-ketones *16* and *17*, respectively. Both these nor-compounds gave mass spectra containing diagnostic peaks at *m/e* 263 (M−15), 235 (M−43), 217 and 191, IR spectra having carbonyl bands at 1720 cm^{-1} and ^1H NMR spectra showing characteristic methyl singlets at δ 2.21 (*16*) and 2.25 (*17*).

Saturation of the 17-methyl singlet at δ 1.17 produced a 13 % nuclear Overhauser enhancement of the H-12 signal for the nor-ketone *16*, whereas the intensity of the H-12 signal for the other nor-ketone *17* was not enhanced when its C-17 signal at δ 1.07 was irradiated. This result is in accordance with 12*R*-configurations in the tetrahydrofurans *9* and *16* and 12*S*-configurations in the tetrahydrofurans *10* and *17*. The ^1H NMR spectra of *9* and *10* supported this view. Thus, while *9* gave rise to a one-proton triplet at δ 3.94 ($J = 5.5$ Hz), the spectrum of *10* contained a multiplet at δ 3.86 ($W_{1/2} =$ 16 Hz) reminiscent of the H-12 signal for (12*S*)-8,12-epoxylabdane-13,17-diol (*15*).[9]

Conclusive evidence for the absolute configurations at C-12 was obtained from an X-ray analysis of the nor-ketone *16*, which crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 19.811$ Å, $b = 10.904$ Å, $c = 7.574$ Å, $Z = 4$. The crystals were unstable in X-rays and in air at room temperature. The structure was solved by direct methods using data collected on two crystals with the computer-controlled Philips PW1100 diffractometer. Data from only one of the crystals ($30° \leq \theta \leq 70°$) were, however, used in the structure refinement ($R = 0.072$). Non-hydrogen atoms were refined with anisotropic thermal parameters but hydrogen atoms have so far not been included in the least-squares procedure. A stereoscopic view of the nor-ketone *16*, summarising the *X*-ray result to be discussed in detail elsewhere, is given in Fig. 2.

Thus, the 8,12-epoxy alcohols *9* and *10* have 12*R*- and 12*S*-configurations, respectively; the chiralities of the remaining unknown asymmetric centre, C-13, in these compounds followed from a consideration of the reaction mechanism involved in the peracid oxidation of (12*Z*)-abienol (*8*).

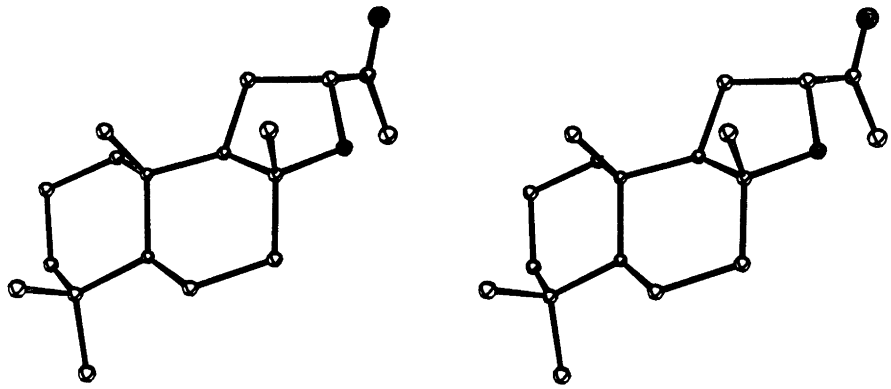
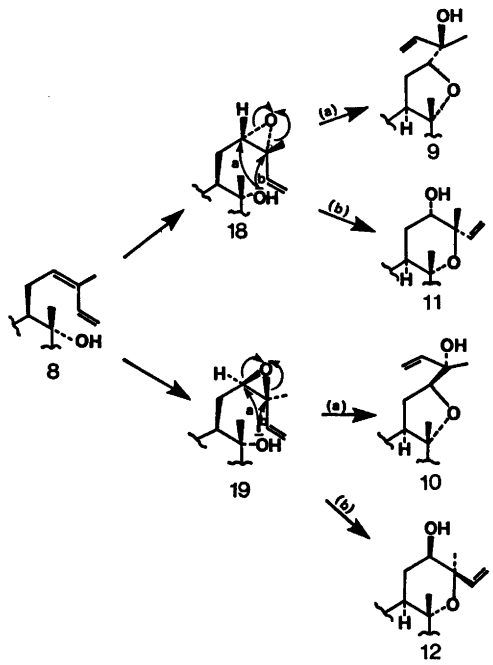


Fig. 2. A stereoscopic view of (12*R*)-14,15-bisnor-8,12-epoxylabdan-13-one (*16*).

Peracid oxidation of linalool is known to proceed *via* the initial generation of two diastereomeric 1,2-epoxides, which are easily converted by acid or heat to two pairs of diastereomeric 1,4- and 1,5-epoxides, the former of which is the main product.[10] It is evident from the structures and proportions of the products obtained that (12*Z*)-abienol (*8*) and peracid react in an analogous manner (*cf.* Scheme 1). Thus, the formation of the two minor oxidation products *11* and *12*, whose structures have been established as (12*S*,13*S*)- and (12*R*,13*R*)-8,13-epoxy-14-labden-12-ol, may be explained as being due to an attack of the 8α-hydroxyl group at the fully substituted C-13 in the intermediate epoxides (12*S*,13*R*)- and (12*R*,13*S*)-12,13-epoxy-14-labden-8-ol (*18*, *19*), respectively, or their equivalents. Similarly, an S_N2 type of oxide opening at the secondary epoxide carbon, C-12, by attack of the 8α-hydroxyl group in the epoxides *18* and *19* would give the diastereomeric (12*R*,13*R*)- and (12*S*,13*S*)-8,12-epoxy-14-labden-13-ols. Since it has been established from the results presented above that, of the 8,12-epoxy alcohols, *9* has the 12*R*- and *10* the 12*S*-configuration, it can be concluded that their chiralities at C-13 are *R* and *S*, respectively.



Scheme 1. Reaction mechanism for the peracid oxidation of (12*Z*)-abienol (*8*).

In conformity with previous findings,[10,11] the use of an acidic medium increased the proportions of the 1,5-epoxides (*11*, *12*). Thus, treatment of (12*Z*)-abienol (*8*) with *m*-chloroperbenzoic acid in acetic acid afforded a mixture of *9*, *10*, *11*, *12* in the ratio 53:15:19:13.

^{13}C NMR spectra. ^{13}C NMR spectroscopy was used in the present study to facilitate the structure determination of the various synthetic products. Since these comprise pairs of diastereoisomers, it was also of interest to evaluate the effects of steric factors on their ^{13}C shieldings.

Table 1 lists the ^{13}C shieldings of (12*Z*)-abienol (*8*) and its oxidation products (*5*, *6*, *9*–*12*, *16*, *17*), as well as those of manoyloxide

(*13*) and epimanoyloxide (*14*), which have been included for comparison. The assignments of the resonances due to C-1 to C-7, C-9, C-10, and C-18 to C-20 for these compounds rest primarily on a comparison with published data for other labdanoids.[12,13] The C-5 and C-9 signals appeared as broad and well-resolved doublets, respectively, in the single-frequency off-resonance decoupled spectra, an observation which corroborated their previous assignment. The identification of the remaining signals for the 8,13-ether bridged compounds (*5*, *6*, *11*, *12*) was guided by the substituent parameters previously obtained for podocarpanes having a 12-oxo or 12-hydroxyl group.[14] The use of shift reagents, $Yb(fod)_3$ and Yb (dpm_3), solved the assignments of the spectra of (12*Z*)-abienol (*8*) and the 8,12-ether bridged compounds *9* and *10*, while the shift analysis for the nor-compounds *16* and *17* was completed by spectral comparison with *9* and *10*.

Reversal of the configuration at C-13 in manoyloxide (*13*→*14*) causes deshielding of C-9 (2.8 ppm) and of C-16 (4.2 ppm), the latter then occupying an equatorial orientation. In contrast, C-17 undergoes shielding (−1.5 ppm), which indicates that the syndiaxial effect of the vinyl group is smaller than that of the methyl group.

Introduction of an axial hydroxyl group in manoyloxide (*13*→*11*) produces a shielding effect at C-9 (−6.6 ppm). As expected, the γ-effect on C-9 caused by the equatorial hydroxyl group in (12*R*,13*R*)-8,13-epoxy-14-labden-12-ol (*12*) is considerably smaller (−0.7 ppm), thus confirming the configurations at C-12 in *11* and *12*.

The methyl group at C-13 being γ-gauche to the equatorial hydroxyl group at C-12 in (12*R*,13*R*)-8,13-epoxy-14-labden-12-ol (*12*) suffers an expected upfield shift of 4.7 ppm. This result is of diagnostic importance since, having established the orientation of the hydroxyl group, the chemical shift value of the C-16 signal in this compound (*12*; 28.0 ppm), when compared with the corresponding values for manoyloxide (*13*; 28.5 ppm) and epimanoyloxide (*14*; 32.7 ppm) is compatible only with an α-orientation of the C-16 methyl group. The small shielding effect (−1.0 ppm) observed at C-16 on introduction of an axial hydroxyl group in manoyloxide (*13*→*11*) can be explained in terms of an γ-*anti* heteroatom effect.[15]

It is noteworthy that the shielding at C-14 and the deshielding at C-15 caused by the introduction of the hydroxyl group is larger in (12*R*,13*R*)- than in (12*S*,13*S*)-8,13-epoxy-14-labden-12-ol (*12*, *11*), an effect which in the case of the different C-14 shieldings may possibly be related to the fact that C-14 experiences γ-gauche interactions with two larger atoms at C-12 in *12* (OH and C-11), but with only one larger atom (OH) in *11*.

The introduction of a 12-oxo group in manoyloxide (*13*→*5*) and in epimanoyloxide (*14*→*6*) causes shielding at C-16. As previously observed in related cases,[12] the effect is larger at the equatorial than at the axial methyl group, −4.1 as against −1.9 ppm.

With the stereochemistry of the 8,12-epoxy alcohols *9* and *10* at hand, it can be concluded that the interaction between C-17 and the β-oriented substituent at C-12 in *10* gives rise to a deshielding of C-17 (25.4 ppm in *10* as against 21.3 ppm in *9*), which may be analogous to the syn-diaxial δ-effect previously observed for substituents attached to six-membered rings.[16–19] A similar effect, although less marked, is found in the spectra of the nor-compounds *16* and *17*, *i.e.* the C-17 signal occurs at 23.0 ppm in the spectrum of the compound having the acetyl-group β-oriented (*17*), while it appears at 21.5 ppm in the spectrum of the C-12 epimer (*16*).

EXPERIMENTAL

Melting points, rotations, infrared and mass spectra were recorded on Leitz Wetzlar, Perkin-Elmer 141, Perkin-Elmer 257 and LKB 2091 instruments respectively. ^1H NMR (100 MHz) and Fourier transform ^{13}C NMR (25.16 MHz) spectra were obtained in $CDCl_3$ solutions using TMS as internal standard on a Varian XL-100 spectrometer equipped with S-124 FT and disc accessories and controlled by a Varian 620/L (16K) computer. Gas chromatography (GC) was performed on a Varian 1700 instrument using a glass capillary column (0.35 mm × 25 m) coated with Emulphor for analytical work and a steel column (10 mm × 2 m) packed with Apiezon L (2.5 %) on Chromosorb for preparative work. GC integrations were carried out on an Autolab System 1 integrator.

Isolation of (12Z)-abienol (8). Commercial Canada balsam, Chroma-Gesellschaft, Stutt-

gart-Untertürkheim, was chromatographed over silica gel using a light petrol/acetone gradient to give (12*Z*)-abienol, indistinguishable from an authentic sample,[20] in approximately 10 % yield. The homogeneity of the isolated material was checked by ^1H and ^{13}C NMR spectroscopy. Relative shifts of the ^{13}C NMR signals for *8* on addition of Yb(fod)$_3$ were: C-1 12; C-2 7; C-3 6; C-4 8; C-5 17; C-6 21; C-7 54; C-8 100; C-9 47; C-10 22; C-11 32; C-12 18; C-13 10; C-14 6; C-15 2; C-16 0; C-17 51; C-18 4; C-19 6; C-20 16. The measurements were made within the linear LIS range (shift reagent substrate ratio 0 – 0.5) and were normalized by arbitrarily assigning the value 100 to the carbon signal exhibiting the largest shift.

Treatment of (12Z)-abienol (8) with m-chloroperbenzoic acid. A: A solution of 516 mg of (12*Z*)-abienol (*8*) in 20 ml of acetic acid was stirred with 360 mg of *m*-chloroperbenzoic acid. After 45 min at room temperature the reaction mixture was diluted with water and extracted with ether. The ether solution was washed with aqueous $NaHCO_3$, dried and evaporated. Chromatography over silica gel furnished 65 mg of (12*S*,13*S*)-8,13-epoxy-14-labden-12-ol (*11*) and 273 mg of a mixture consisting of three components. This was separated by preparative GC into 110 mg of (12*R*,13*R*)-8,12-epoxy-14-labden-13-ol (*9*), 31 mg of (12*S*,13*S*)-8,12-epoxy-14-labden-13-ol (*10*) and 69 mg of (12*R*,13*R*)-8,13-epoxy-14-labden-12-ol (*12*).

(12S,13S)-8,13-epoxy-14-labden-12-ol, (*11*) gum, $[\alpha]_D + 30°$ (*c* 1.2 in $CHCl_3$), (Found: M^+ 306. Calc. for $C_{20}H_{34}O_2$: mol. wt 306) IR band at 3580 cm^{-1}, ^1H NMR peaks at δ 0.80 (6 H, s), 0.86 (3 H, s) 1.29 (3 H, s), 1.33 (3 H, s), 3.69 (1 H, t, $J = 3.5$ Hz), 5.20 (1 H, dd, $J = 2.5$ and 10 Hz), 5.42 (1 H, dd, $J = 2.5$ and 17.5 Hz) and 5.84 (1 H, dd, $J = 10$ and 17.5 Hz) and MS peaks at *m/e* (%): 306 (M, 0.1), 291 (1), 288 (0.4), 273 (2), 236 (2), 235 (2), 221 (2), 217 (2), 206 (1), 192 (100), 191 (17), 177 (98), 149 (6), 137 (13), 136 (15), 123 (33), 95 (22), 81 (25), 69 (31), 55 (23), and 41 (24).

(12R,13R)-8,12-epoxy-14-labden-13-ol, (*9*) was identical in all respects ($[\alpha]_D$, IR, ^1H NMR, MS and GC retention time on a capillary column) with isomer "Ib" of Greek tobacco.[8] Relative shifts of the ^{13}C NMR signals on addition of Yb(dpm)$_3$ were: C-1 0; C-2 0; C-3 0; C-4 0; C-5 2; C-6 4; C-7 10; C-8 22; C-9 21; C-10 5; C-11 33; C-12 57; C-13 100; C-14 47; C-15 27; C-16 58; C-17 12; C-18 0; C-19 0; C-20 3.

(12S,13S)-8,12-epoxy-14-labden-13-ol, (*10*) gum, $[\alpha]_D$ −15° (*c.* 2.3 in $CHCl_3$) had IR bands at 3580, 3480 and 1645 cm^{-1}, ^1H NMR peaks at δ 0.83 (3 H, s), 0.87 (6 H, s), 1.19 (6 H, s), 3.86 (1 H, m, $W_{1/2} = 16$ Hz), 5.11 (1 H, dd, $J = 1.5$ and 10.5 Hz), 5.30 (1 H, dd, $J = 1.5$ and 17 Hz), and 5.98 (1 H, dd, $J = 10.5$ and 17 Hz). Relative shifts of the ^{13}C NMR signals on addition of Yb(dpm)$_3$ were: C-1 3; C-2 1; C-3 1; C-4 1; C-5 3; C-6 3; C-7 7; C-8 21; C-9 15; C-10 5; C-11 28; C-12 44; C-13 100; C-14 46; C-15 28; C-16 53; C-17 12; C-18 0; C-19 0; C-20 1. The mass spectrum and the GC retention time were indistinguishable from those of isomer "Id" of Greek tobacco.[8]

(12R,13R)-8,13-epoxy-14-labden-12-ol, (*12*) had m.p. 100 – 102 °C, $[\alpha]_D + 37°$ (*c.* 0.78, $CHCl_3$) (Found: M^+ 306. Calc. for $C_{20}H_{34}O_2$: mol. wt 306). IR bands at 3630 and 3460 cm^{-1}, ^1H NMR peaks at δ 0.77 (3 H, s), 0.79 (3 H, s), 0.86 (3 H, s), 1.34 (3 H, s), 1.45 (3 H, s), 3.50 (1 H, dd, $J = 5$ and 10 Hz), 5.23 (1 H, dd, $J =$ 1.5 and 11 Hz), 5.42 (1 H, dd, $J = 1.5$ and 18 Hz), and 6.32 (1 H, dd, $J = 11$ and 18 Hz) and MS peaks at *m/e* (%): 306 (M, 1), 291 (1), 288 (1), 273 (1), 236 (2), 235 (3), 221 (3), 217 (2), 206 (1), 192 (100), 191 (23), 177 (99), 149 (18), 137 (15), 136 (16), 123 (33), 95 (20), 81 (23), 69 (30), 55 (19) and 41 (21).

B: Treatment of 500 mg of (12*Z*)-abienol (*8*) with 400 mg of *m*-chloroperbenzoic acid in 20 ml $CHCl_3$ for 1 h at ambient temperature afforded a mixture from which 131 mg of (12*R*,13*R*)-8,12-epoxy-14-labden-13-ol (*9*) and 50 mg of (12*S*,13*S*)-8,12-epoxy-14-labden-13-ol (*10*) were isolated by preparative GC.

Preparation of (13S)-8,13-epoxy-14-labden-12-one (5). To a solution of 38 mg of *11* in 5 ml of acetone, 0.7 ml of Jones' reagent [14 mg CrO_3 in 0.7 ml of aqueous H_2SO_4 (20 %)] was added with stirring. After 10 min at room temperature the reaction mixture was worked up in the usual manner. Chromatography over silica gel gave 19 mg of (13*S*)-8,13-epoxy-14- labden-12-one (*5*), which was identical in all respects with an authenthic sample.[7]

Preparation of (13R)-8,13-epoxy-14-labden-12-one (6). Treatment of 20 mg of *12* with 0.4 ml of Jones' reagent in 5 ml of acetone afforded 15 mg of (13*R*)-8,13-epoxy-14-labden-12-one (*6*), indistinguishable from an authentic sample.[7]

Preparation of (12R)-14,15-bisnor-8,12-epoxylabdan-13-one (16). A solution of 70 mg of *9* in 20 ml of dioxane/H_2O (3:1) was stirred with 15 mg of osmium tetroxide at 0 °C for 10 min. After addition of 300 mg of sodium periodate the mixture was stirred at 0 °C for 60 h. Dilution with water, extraction with ether and chromatography over silica gel using a hexane/ether gradient afforded 39 mg of (12*R*)-14,15-bisnor-8,12-epoxylabdan-13-one (*16*), which had m.p.'s 77 – 79, 108 – 109 °C, IR band at 1720 cm^{-1}, ^1H NMR peaks at δ 0.84 (6 H, s), 0.88 (3 H, s), 1.17 (3 H, s), 2.21 (3 H, s) and 4.38 (1 H, dd, $J = 3.5$ and 9.5 Hz) and MS peaks at *m/e* (%): 263 (M – 15, 1), 235 (35), 217 (18), 191(100), 137 (38) 109 (28), 95 (21), 81 (21), 69 (33), 55 (14), 43 (24) and 41 (21).

Preparation of (12S)-14,15-bisnor-8,12-epoxylabdan-13-one (17). Treatment of 30 mg of *10* with 5 mg of osmium tetroxide and 100 mg of sodium periodate in 5 ml of dioxane/H_2O (3:1) at 0 °C for 6 h gave 13 mg of (12*S*)-14.15-bisnor-8,12-epoxylabdan-13-one (*17*), which had m.p.'s

82–84, 105–107 °C, IR band at 1720 cm^{-1}, ^1H NMR peaks at δ 0.83 (6 H, s) 0.88 (3 H, s), 1.07 (3 H, s), 2.25 (3 H, s) and 4.32 (1 H, t, $J = 8$ Hz), MS peaks at m/e (%): 278 (M, 1), 263 (3) 235 (42), 217 (20), 191 (100), 137 (45), 109 (26), 95 (18), 81 (20), 69 (43), 55 (15), 43 (18) and 41 (21).

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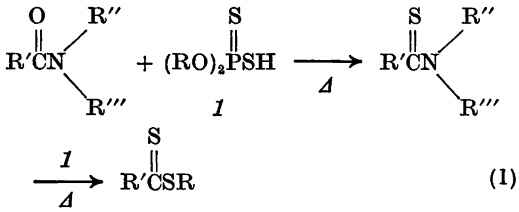
Studies on Organophosphorus Compounds. XVII.* About *O,O*-Diethyl Dithiophosphoric Acid as Thiation and Alkylation Agent. A New Route to Dithiocarboxylic Esters and (Ethylthio) Heterocycles

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By heating thioloesters with *O,O*-diethyl dithiophosphoric acid, *2*, at about 120 °C the corresponding dithioesters are produced in appreciable yields. Extended reaction time causes formation of ethyl dithiocarboxylic esters. Also common esters produce, after reaction with *2*, thiono- and/or dithiocarboxylic esters provided the reaction temperature is higher (140 °C). Potential hydroxyheterocycles (2- and 4-hydroxypyridine, saccharin) and *2* give the corresponding (ethylthio) heterocycles.

In a preceding paper thiation and alkylation properties of *O,O*-dialkyl dithiophosphoric acids, *1*, were established in reactions with carboxamides.[1] Thus thiocarboxamides were prepared from carboxamides and *1* after a relatively short reaction time while prolonged heating afforded the corresponding alkyl dithiocarboxylic esters.



(1)

Similarly Oae *et al.* have shown that ketones and aldehydes were transformed into the corresponding thiocarbonyl compounds or the trimers of these.[2] This paper will describe further applications of *O,O*-diethyl dithiophosphoric acid, *2* (*1*, R=Et), in reactions with carbonyl containing compounds such as carboxylic esters, thiolocarboxylic esters and some potential hydroxy heterocycles.

RESULTS AND DISCUSSION

Thiolocarboxylic esters react smoothly in neat *2* at 120 °C to give dithiocarboxylates in good yields (Table 1). Further heating resulted in formation of appreciable amounts of ethyl dithiocarboxylates. In one case this was dramatically demonstrated in product distribution by reaction of phenyl thiobenzoate with *2* during different reaction times (see Table 2). Most of the reaction mixtures were contaminated with ethyl dithiocarboxylates if total conversion of starting materials was desired.

In an attempt to prepare thionocarboxylic esters we also reacted carboxylic esters with *2*. To get any conversion of the starting materials higher reaction temperatures were needed than in the previous reactions. Thus reaction of ethyl benzoate afforded a mixture of the expected ethyl thionobenzoate (45 %) and ethyl dithiobenzoate (11 %), while the only product isolated from reaction of ethyl heptanoate was ethyl dithioheptanoate (66 %). Whether the formation of dithiocarboxylic esters results from alkylation of or rearrangement[3] of pri-

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Table 1. Synthesis of dithiocarboxylates [a] RCOSR′ + *2* → RCSSR′ (*3*)

R	R′	Reaction time/h	Reaction temp./°C	Yield of *3*	B.p./mmHg or m.p., °C Found	Lit.	H NMR ($CDCl_3$)
C_6H_5	CH_3	8	120	79	146/15	142/12[6]	2.7 (s, 3 H); 7.1–8.0 (m, 5 H)
C_6H_5	CH_2CH_3	7	120	92	150/12	97/0.5[7]	1.4 (t, 3 H); 3.4 (q, 2 H); 7.2–8.2 (m, 5 H)
C_6H_5	$CH_2C_6H_5$	6	120	81	156–57/0.2	159–60/0.3[7]	4.6 (s, 2 H); 7.1–8.2 (m, 10 H)
C_6H_5	C_6H_5	8	120	59	62	60–61[7]	7.2–7.7 (m, 8 H) 8.0–8.3 (m, 2 H)
CH_3	$CH_2C_6H_5$	5	125	66	85–88/0.4 [b]		2.8 (s, 3 H) 4.4 (s, 2 H) ~7.3 (m, 5 H)
CH_3	C_6H_5	9	120	85	63–65/0.5 [b]		2.8 (s, 3 H); ~7.4 (m, 5 H)
$CH_3CH_2CH_2$	CH_2CH_3	7	120	95	73–75/10	75/9[8]	1.0 (t, 3 H); 1.3 (t, 3 H); 1.8 (m, 2 H); 2.9 (t, 2 H);

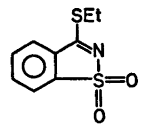
[a] 0.02 mol of thiolocarboxylate and 15 ml of *2* were used in each experiment.
[b] Elemental analyses were in accordance with the proposed structures.

marily formed thionocarboxylic esters is at present uncertain. Compared to improved tetraphosphorus decasulfide sulfurizations [4] our method seems to be advantageous in many cases (higher yields) except under conditions when *2* being a pronounced alkylation agent. Such alkylations were found to predominate in reactions of *2* with potential hydroxyheterocycles. Thus 3-ethylthiobenzoisothiazole-*S*-dioxide, *7*, was prepared from saccharin, 2-ethylthiopyridine, *8a*, from 2-pyridone, and 4-ethylthiopyridine, *8b*, from 4-pyridone. A similar alkylative sulfurization of carbostyril by *2* has been reported by Oae *et al.*[5]

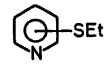
Table 2. Time dependent product distribution.

PhC(=O)SPh (*4*) —*2*→ PhC(=S)SPh (*5*) + PhC(=S)SEt (*6*)

Reaction time/h	Reaction temp./°C	Yield of *4*/%	*5*/%	*6*/%
8	120	34	58	8
10	120	12	18	67
12	120	–	10	86



7



8a: 2-SEt
8b: 4-SEt

Finally, it should be mentioned that *O,O,S*-triethyl dithiophosphate, *9*, was isolated in most of our reactions (with the hydroxypyridines and saccharin appreciable amounts of *9* were formed), which shows that *2* itself is alkylated under these reaction conditions.

$S=P(OEt)_2(SEt)$

9

EXPERIMENTAL

^{1}H NMR spectra were recorded at 60 MHz on a Varian A-60 spectrometer. TMS was used as internal reference standard. Chemical shift values are expressed in δ values. Mass spectra were recorded on a CEC 21-104 mass spectrometer operating at 70 eV using direct inlet. Silica gel 60 (Merck) was used for column chromatography. Commercial *O,O*-diethyl dithiophosphoric acid was kindly delivered by Cheminova A/S, DK-8260 Lemvig. Elemental analyses were performed by Novo Industry A/S, Copenhagen. M.p.'s and b.p's are uncorrected.

Reactions of *thiolocarboxylic esters* were performed as indicated in Table 1. The reaction mixtures were neutralized with K_2CO_3-solution, and H_2O (200 ml) and ether (100 ml) were added. The phases were separated and the H_2O-phase was further extracted with ether (2 × 100 ml). The combined ether phases were washed with H_2O (2 × 50 ml), dried ($MgSO_4$) and the ether evaporated. The dithiocarboxylic esters, *3*, were purified by column chromatography (eluted with light petroleum or 5 % v/v ether/light petroleum). The products were characterized by their b.p./m.p. and ^{1}H NMR spectra (Table 1).

Carboxylic esters. Ethyl benzoate (0.02 mol) was heated at 140 °C with 15 ml of *2* for 10 h. The reaction mixture was worked up as above affording 2.0 g of product consisting of a mixture of ethyl thionobenzoate (yield 45 %) and ethyl dithiobenzoate (yield 11 %). These two compounds could not be separated by column chromatography, but they were identified according to ^{1}H NMR: ^{1}H NMR ($CDCl_3$) of ethyl thionobenzoate: 1.5 (t, 3 H); 4.7 (q, 2 H); 7.2 – 8.2 (m, 5 H). ^{1}H NMR ($CDCl_3$) of ethyl dithiobenzoate: 1.4 (t, 3 H); 3.4 (q, 2 H); 7.2 – 8.2 (m, 5 H). These spectra were in accordance with spectra of separately prepared samples.

Ethyl heptanoate (0.02 mol) was heated at 140 °C with 15 ml of *2* for 9 h. The same working-up procedure as above afforded 2.5 g (66 %) of ethyl dithioheptanoate. B.p. 79 – 81 °C/0.2 mmHg (lit.[7] b.p. = 71 °C/0.1 mmHg). ^{1}H NMR ($CDCl_3$): 0.9 (t, 3 H): 1.2 – 2.0 (m, 8 H); 1.3 (t, 3 H); 3.0 (t, 2 H); 3.2 (q, 2 H).

Hydroxyheterocycles. Saccharin (0.02 mol) was heated at 130 °C with 15 ml of *2* for 3 h. The reaction mixture was then neutralized with K_2CO_3-solution, and H_2O (200 ml) and CH_2Cl_2 (100 ml) were added. The phases were separated and the H_2O-phase was further extracted with CH_2Cl_2 (2 × 100 ml). The combined CH_2Cl_2-phases were washed with H_2O (2 × 50 ml), dried ($MgSO_4$) and the CH_2Cl_2 evaporated. The residue was refluxed in ether (50 ml) and upon cooling 3-ethylthiobenzoisothiazole-*S*-dioxide, *7*, precipitated. 4.1 g (93 %) were filtered off. M.p. 187 °C. (lit.[9] m.p. 183 – 184 °C). ^{1}H NMR ($CDCl_3$): 1.5 (t, 3 H); 3.4 (q, 2 H); 7.6 – 7.8 (m, 4 H).

2-Pyridone (0.02 mol) was heated at 120 °C with 15 ml of *2* for 30 min. The same working-up procedure as for dithiocarboxylates afforded 1.0 g (36 %) of 2-ethylthiopyridine, *8a*. B.p. 90 – 92 °C/16 mmHg (lit.[10] b.p. 205 – 207 °C). ^{1}H NMR ($CDCl_3$): 1.3 (t, 3 H); 3.1 (q, 2 H); 6.8 – 7.5 (m, 3 H); 8.3 – 8.4 (m, 1 H).

4-Pyridone (0.02 mol) was heated at 120 °C with 15 ml of *2* for 15 min. The same working-up procedure as for dithiocarboxylates afforded 1.2 g (43 %) of 4-ethylthiopyridine, *8b*. B.p. 122 °C/23 mmHg (lit.[11] b.p. 123 – 126 °C/23 – 24 mmHg). ^{1}H NMR ($CDCl_3$): 1.4 (t, 3 H); 3.0 (q, 2 H); 7.0 – 7.1 (m, 2 H); 8.3 – 8.4 (m, 2 H).

A second compound (3 – 4 g) was isolated in all reactions of potential hydroxyheterocycles, which was identified as *O,O,S*-triethyl dithiophosphate, *9*, B.p. 120 – 121 °C/15 mmHg (lit.[12] b.p. 128 °C/20 mmHg). ^{1}H NMR ($CDCl_3$): 1.4 (t, 9 H); ~2.8 (m, 2 H); ~4.1 (m, 4 H). MS *m/e* (% rel. int.): 214 (M^+, 14), 186 [$(EtO)_2$-PSSH, 42], 158 ($M - 2C_2H_4$, 42), 153 (M – SEt, 12), 142 (17), 125 (31), 121 (78), 113 (28), 109 (29), 97 (100), 93 (71), 65 (95). A GLC chromatogram showed only one peak.

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Chemistry of *gem*-Dihalocyclopropanes. XI. Reactions of Dihalocarbenes with Allylic Alcohols

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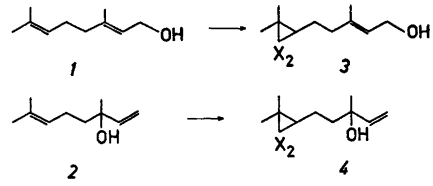
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Reactions of dichloro- or dibromocarbene, generated by the phase transfer method, with a number of allylic alcohols resulted in formation of the corresponding dihalocyclopropane derivatives in variable yields. The addition to linalool occurred exclusively at the trisubstituted double bond in excellent yields. In two examples insertion at the O—H bond was an important reaction. The results indicate no electronic interaction between the hydroxyl group and the carbene leading to stereochemical consequences.

Addition of dihalocarbene to allylic alcohols has been reported by several groups. Seyferth and Mai [1] studied particularly cyclic alcohols using phenyl bromodichloromethyl mercury as the source of dichlorocarbene; *trans* stereochemistry was observed in all cases, indicating the lack of any directing effect of the hydroxyl group. The reaction of bicyclo [6.4.0]dodec-1(8)-en-12-ol with dibromocarbene, generated from bromoform and potassium *t*-butoxide, has been reported,[2] but the cycloaddition product was not isolated. A number of open chain alcohols have also been studied. Allyl alcohol and dichlorocarbene, generated as above, gave products derived from reaction at the O—H bond only.[2,3] Alkyl-substituted allylic alcohols, however, reacted with dichlorocarbene,[1,4] dibromocarbene [5] and chlorofluorocarbene [6] by addition to the double bond. A preferred stereochemistry was not reported in any of the additions, but a high degree of regioselectivity was observed [4] in the addition of dichlorocarbene to (*E*)-3-methyl-2,6-heptadiene-1-ol, under phase transfer conditions.[7]

In the present paper we want to report on additions of dihalocarbenes to a number of allylic alcohols which provided examples of both regio- and stereoselectivity. The carbenes were generated by the phase transfer method and in some cases the yields were excellent.

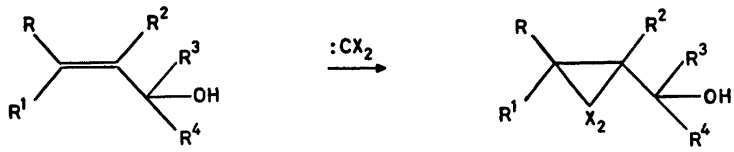
The work was initiated after we had observed a surprising difference in behaviour of geraniol (*1*) and linalool (*2*) towards dichlorocarbene; the former was considerably less reactive and a low yield of a high-boiling liquid consisting of at least six components was obtained. Attempts to separate them resulted in partial decomposition, but the main component was most probably compound *3*. On the other hand, linalool reacted rapidly and regioselectively with dichlorocarbene to yield 89 % of compound *4* (X = Cl); the corresponding dibromo derivative *4* (X = Br) was obtained in 93 % yield under conditions generating dibromocarbene (Scheme 1).



Scheme 1.

The two terpene isomers differ structurally in the allylic hydroxyl function, and apparently the primary allylic hydroxyl group not only competes with the double bonds for dihalocarbene, but it also seemed to have a retarding effect on the rate of addition. It was therefore of interest to study the behaviour of a variety of allylic alcohols under similar conditions.

Table 1. Reactions of allylic alcohols with dihalocarbenes.

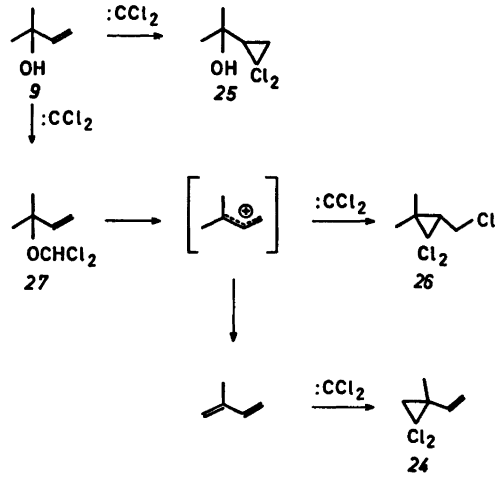


Starting material	Product		Yield %
5	*13*[a]	$R=R^1=R^3=R^4=H$; $R^2=CH_3$; X = Cl	70[b]
5	*14*	$R=R^1=R^3=R^4=H$; $R^2=CH_3$; X = Br	34[c]
6	*15*[d]	$R=R^1=R^3=H$; $R^2=R^4=CH_3$; H = Cl	75
6	*16*[e]	$R=R^1=R^3=H$; $R^2=R^4=CH_3$; X = Br	62
7	*17*[f]	$R^1=R^3=R^4=H$; $R=R^2=CH_3$; X = Br	45
8	*18*[g]	$R=R^1=R^3=CH_3$; $R^2=R^4=H$; X = Cl	90
8	*19*[h]	$R=R^1=R^3=CH_3$; $R^2=R^3=H$; X = Br	74
9	[i]	$R=R^1=R^2=H$; $R^3=R^4=CH_3$; X = Cl	
10	*20*	$R=R^1=H$; $R^2=R^3=R^4=CH_3$; X = Cl	69
11	*21*	$R=R^1=R^3=R^4=CH_3$; $R^2=H$; X = Cl	88
11	*22*	$R=R^1=R^3=R^4=CH_3$; $R^2=H$; X = Br	88
12	*28*	$R=R^1=R^3=CH_3$; $R^3=R^4=H$; X = Cl	81

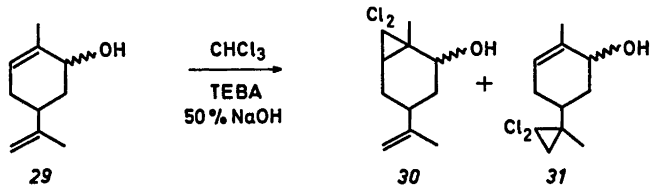
[a] A 4:1 ratio of *9* and *23* (2,2-dichloro-1-methyl-1-dichloromethoxymethylcyclopropane) was formed in 20 % yield. [b] A 3-fold excess of $CHCl_3$/NaOH and 72 h reaction time. [c] Recovered starting material 51 %. [d] Ratio of diastereomers 48:52. [e] Ratio of diastereomers 45:55. [f] A mixture of *E* and *Z* isomers in a ratio of 1:1; 3-fold excess of $CHBr_3$/NaOH. [g] Ratio of diastereomers 90:10 determined by GC. [h] Ratio of diastereomers 81:19 determined by NMR. [i] Six products besides carbon monoxide were formed. Compounds *24*, *25* and *26* (Scheme 2) were identified.

The allylic alcohols (*5* – *12*) used in this study were either commercial samples or prepared according to known procedures. Usually a two-fold excess of haloform, a three-fold excess of base and 16 h reaction time were used to ensure completion of the reactions at room temperature. As catalysts either benzyltriethylammonium chloride (TEBA) or tributylamine were employed; however, the latter was in all cases inferior and the results summarized in Table 1 are those from reactions with TEBA as catalyst.

Insertion at the O – H bond is an observable reaction in the case of the primary alcohol *5*, but methyl substitution at the double bond renders this the preferred site of attack by the carbene in contrast to allylic alcohol itself.[3] However, the products from the reaction of alcohol *9* with dichlorocarbene strongly suggest that also in this case insertion into the O – H bond occurred initially. Carbon monoxide escaped during the reaction and a mixture of six compounds was formed in low yield. These were separated by preparative GC, and on the basis of spectroscopic evidence and comparison with authentic material we were able to ascertain the structures of the three main components *24*, *25* and *26*, representing 81 % of the mixture. The structures of the three



Scheme 2.



29 30 31

Scheme 3.

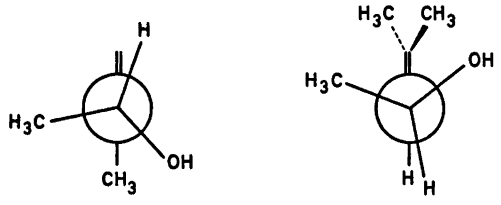
minor components are uncertain. The results are rationalized in Scheme 2. We suggest that fragmentation of a carbene intermediate derived from the insertion product *27* takes place; reactions of the resulting carbenium ion provides the necessary substrates for formation of the observed dichlorocarbene addition products. Other results which support the proposed mechanism have been reported.[8]

With the other alcohols of Table 1 addition to the double bond was the exclusive reaction. It appears that the hydroxyl function deactivates the double bond of allylic alcohols towards electrophilic reagents and that alkyl substituents are a prerequisite for a reasonable yield of cycloaddition products. This is further supported by the results from the reaction of dichlorocarbene with carvol (*29*); almost 50 % of the product derives from addition to the disubstituted exocyclic double bond (Scheme 3).

The formation of a stereoisomeric mixture of the cyclopropanes obtained from dibromocarbene addition to the alcohol *7* deserves further comment. The apparent lack of stereospecificity is not compatible with a dihalocarbene addition; however, recovered starting material *7* consisted of a mixture of *E* and *Z* isomers in a ratio of 2:1, not significantly different from that observed for the product. Clearly a fast base-catalyzed isomerization of the alcohol *7* had occurred prior to the carbene addition. There are only a few reported examples of base-induced isomerization of allylic alcohols [9] and none of them are under phase transfer conditions. Abstraction of a proton at either of the methyl groups would lead to an allylic carbanion that could isomerize. We cannot distinguish between the two mechanisms but it is interesting that the structurally isomeric alcohols 2-ethyl-2-propen-1-ol and 2-methyl-3-buten-1-ol were not detected in the product.

The double bonds of both compounds *6* and *8* are prochiral; *e.g.* addition of dihalocarbene could produce mixtures of diastereomers which indeed occurred in both cases. From the reaction of either dichloro- or dibromocarbene with *6* almost equal amounts of two diastereomers were obtained, in agreement with the observation of Santelli and Bertrand;[5] however, the corresponding reactions with *8* exhibited considerable stereoselectivity as shown by the 9:1 and 4:1 ratios of diastereomers obtained from addition of dichlorocarbene and dibromocarbene, respectively, in contrast to the lack of selectivity reported by the above authors [5] and others.[1,4] The question arises whether the selectivity originates in steric factors or a guiding effect of the hydroxyl group. The latter is clearly not important in the case of dichlorocarbene addition to cyclic allylic alcohols,[1] but in other carbenoid additions [10] and in epoxidation reactions [11] it has been observed.

On the basis of molecular models it seems plausible that the alcohol *8* should prefer the conformation depicted in Fig. 1. Assuming a ground state conformational control of products the preferred direction of dihalocarbene addition to the double bond of this conformer should indicate which effects are important in determining the stereochemistry. The structure of the most abundant dia-



6 8

Fig. 1. Assumed preferred conformations of compounds *6* and *8*.

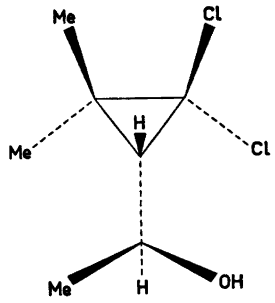


Fig. 2. Perspective drawing of the structure of 1-(2,2-dichloro-3,3-dimethylcyclopropyl)ethanol *18*.[12]

stereomer of *18* was determined [12] by X-ray crystallography (Fig. 2). In the crystal state the chlorine atoms and the hydroxyl group are on the same side of the plane comprising the methine carbon and bisecting the cyclopropane ring. This configuration indicates that addition of dichlorocarbene to the double bond has occurred preferably from the least sterically hindered side but also in close proximity to the hydroxyl group; hence, from this result no conclusion can be drawn about the factors determining the observed selectivity. However, from the alcohol *6* practically equal amounts of the diastereomers *15* were obtained and by considering in the same way preferred addition to the most plausible conformers (Fig. 1) it seems that the effect of the hydroxyl group is only steric.

All evidence so far available on the addition of dichloro- or dibromocarbene to allylic alcohols suggests that the hydroxyl group renders the double bond less reactive and further that the interaction is negligible between the hydroxyl oxygen and the approaching carbene in reference to stereochemical consequences. However, the difference in reactivity between geraniol (*1*) and linalool (*2*) towards dihalocarbene remains unexplained.

EXPERIMENTAL

General. Most of the spectral and gas chromatographic equipment used has been described previously.[13] ^{13}C NMR spectra were obtained on a JEOL FX 60. ^{1}H NMR were recorded at 60 MHz when not stated otherwise. Elemental analyses were carried out by Ilse Beetz Microanalytical Laboratory, 8640 Kronach, West Germany.

Allylic alcohols. Geraniol (*1*), linalool (*2*), 2-methyl-2-propen-1-ol (*5*), 2-methyl-3-buten-2-ol (*9*) and carvol (*29*) were obtained commercially. The following alcohols were prepared according to the literature: 3-methyl-3-buten-2-ol (*6*),[15] 2,3-dimethyl-3-buten-2-ol (*10*),[15] 2,4-dimethyl-3-penten-2-ol (*11*) [17] and 2,3-dimethyl-2-buten-1-ol (*12*);[14] in the case of compound *10* the yield was increased to 82 % by using methyllithium.

(E)-2-Methyl-2-buten-1-ol (7) was prepared in 95 % yield by the lithium aluminium hydride reduction of tiglaldehyde at −80 °C for 5 min and 0 °C for 15 min, b.p. 57 – 58 °C/23 mmHg, n_D^{23} 1.4420 (lit.[14] b.p. 74 °C/60 mmHg, n_D^{20} 1.4421). The IR and NMR spectra were in accordance with those published.[14,15]

5-(2,2-Dichloro-3,3-dimethylcyclopropyl)-3-methyl-1-penten-3-ol (4, X=Cl). A mixture of 30.8 g (0.20 mol) of linalool (*2*), 35.8 g (0.30 mol) of $CHCl_3$, 32.0 g (0.40 mol) of 50 % aq. NaOH, and 0.4 g triethylbenzylammonium chloride (TEBA) was stirred vigorously for 16 h at 20 °C. Water was added and the product extracted with CH_2Cl_2. The combined organic phases were washed with water and dried ($MgSO_4$). Evaporation of the solvent left a pale yellow residue which distilled at 98 °C/0.01 mmHg through a short path apparatus to yield 42.0 g (89 %) of *4*. Anal. $C_{11}H_{18}Cl_2O$: C, H. ^{1}H NMR (CCl_4): δ 1.17 (3 H, s), 1.27 (3 H, s), 1.35 (3 H, s), 1.0 – 1.7 (5 H, m), 1.70 (1 H, s), 4.7 – 6.1 (3 H, vinyl m). IR (film): 3400 (s), 1630 (w), 825 (s) cm^{-1}.

5-(2,2-Dibromo-3,3-dimethylcyclopropyl)-3-methyl-1-penten-3-ol (4, X=Br), m.p. 41 – 45 °C (from pentane). Anal. $C_{11}H_{18}Br_2O$: C, H. ^{1}H NMR (CCl_4): δ 1.19 (3 H, s), 1.28 (3 H, s), 1.38 (3 H, s), 1.1 – 1.7 (5 H, m), 1.66 (1 H, s) 4.8 – 6.1 (3 H, vinyl m). IR (film) 3400 (s), 750 (s) cm^{-1}.

2,2-Dichloro-1-methylcyclopropylmethanol (13) and 2,2-dichloro-1-methyl-1-dichloromethoxymethylcyclopropane (23). The product consisted of a mixture that was separated by prep. GC (10 % QF1, 155 °C):

13 (shorter retention time) b.p. 84 – 86 °C/9 mmHg, n_D^{21} 1.4907. Anal. $C_5H_8Cl_2O$: C, H. ^{1}H NMR (98 MHz, CCl_4): δ 1.23 (1 H, d, J 7 Hz), 1.39 (1 H, d, J 7 Hz), 1.45 (3 H, s), 1.63 (1 H, broad s), 3.61 (1 H, d, J 12 Hz), 3.73 (1 H, d, J 12 Hz). IR (film): 3320 (s), 1035 (s), 760 (s) cm^{-1}.

23 b.p. 88 °C/9 mmHg, n_D^{24} 1.4720. ^{1}H NMR (98 MHz, CCl_4): δ 1.32 (1 H, d, J 7.5 Hz), 1.45 (3 H, s), 1.51 (1 H, d, J 7.5 Hz), 4.14 (1 H, d, J 11.5 Hz), 4.40 (1 H, d, J 11.5 Hz), 8.04 (1 H, s). ^{13}C NMR (15.0 MHz, CCl_4): δ 18.5, 29.2, 30.9, 64.5, 67.2, 158.9. IR (film): 1690 (s), 1460 (s), 1165 (s), 1050 (s), 968 (m), 764 (s) cm^{-1}. No elemental analysis was obtained due to instability.

2,2-Dibromo-1-methylcyclopropylmethanol (14), b.p. 60 – 61 °C/0.9 mmHg, m.p. 66.5 °C (from pentane). Anal. $C_5H_8Br_2O$: C, H. ^{1}H NMR

(benzene): δ 1.08 (1 H, d, J 7.5 Hz), 1.24 (1 H, d, J 5 Hz), 1.32 (3 H, s), 2.57 (1 H, s), 3.52 (1 H, s). IR (film): 3390 (s), 1040 (s), 685 (s) cm^{-1}.

1-(2,2-Dichloro-1-methylcyclopropyl)ethanol (15), b.p. 86–90 °C/15 mmHg. Anal. $C_6H_{10}Cl_2O$: C, H. GC (10 % PEG 4000, 160 °C) revealed the presence of two diastereomers in a ratio of 48:52. They were separated by prep. GC (same column):

a. (shorter retention time) m.p. 39–40 °C (from pentane). ^1H NMR (CCl_4): δ 1.1–1.3 (8 H, overlapping s and d), 2.50 (1 H, broad s), 3.67 (1 H, q, J 6.5 Hz). IR (film): 3570 (w), 3400 (s), 750 (s) cm^{-1}.

b. ^1H NMR (CCl_4): δ 1.1–1.5 (8 H, overlapping s and d), 2.77 (1 H, broad s), 3.60 (1 H, q, J 6.5 Hz). IR (film): 3570 (w), 3350 (s), 750 (s) cm^{-1}.

1-(2,2-Dibromo-1-methylcyclopropyl)ethanol (16), b.p. 51–52 °C/0.1 mmHg, n_D^{21} 1.5422. Anal. $C_6H_{10}Br_2O$: C, H. ^1H NMR (CCl_4): δ 1.10–1.95 (8 H, complex m), 2.42 (1 H, s), 3.41–3.91 (1 H, m). IR (film): 3580 (m), 3410 (s), 1020 (s), 1010 (s), 690 (s) cm^{-1}. According to GC (10 % PEG 4000, 145 °C) the product consists of a mixture of diastereomers in a ratio of 45:55.

(E)- and (Z)-2,2-Dibromo-1,2-dimethylcyclopropylmethanol (17). Distillation of the reaction mixture gave two main fractions:

a. (10 %), b.p. 45 °C/16 mmHg, of a 2:1 mixture of (*E*)- and (*Z*)-*7*.

b. (45 %), b.p. 50–52 °C/0.05 mmHg, of a 1:1 mixture of (*E*) and (*Z*)-*17*. Found: C 28.45; H 3.95. Calc. for $C_6H_{18}Br_2O$: C 27.93; H 3.91. ^1H NMR (CCl_4): δ 1.15 (3 H, d, J 5 Hz), 1.18 (3 H, d, J 5.5 Hz), 1.25 (3 H, s), 1.33 (3 H, s), 1.25–1.80 (2 H, complex m), 3.10 (2 H, broad s), 3.55 (1 H, d, J 10.5 Hz), 3.62 (1 H, d, J 12 Hz), 3.66 (1 H, d, J 10.5 Hz), 3.72 (1 H, d, J 12 Hz). IR (film): 3575 (w), 3380 (m), 1025 (s), 796 (s), 767 (s) cm^{-1}.

1-(2,2-Dichloro-3,3-dimethylcyclopropyl) ethanol (18). The product was shown by GC (10 % PEG 4000, 150 °C) to consist of two diastereomers in a ratio of 90:10.

a. The major isomer was pure by recryst. from pentane, m.p. 71–72 °C (lit.[1] 73–73.5 °C).

b. The minor isomer was obtained pure by prep. GC (same column as above). ^1H NMR (CCl_4): δ 1.16 (1 H, d, J 9 Hz), 1.32 (3 H, s), 1.36 (3 H, d, J 6 Hz), 1.38 (3 H, s), 1.97 (1 H, s), 3.64 (1 H, d q, J 9, J 6 Hz). IR (film): 3580 (w), 3320 (s), 805 (s) cm^{-1}. The above reaction was followed by GC and shown to be complete after 3.5 h. In two separate experiments a 10 % excess of NaOH/$CHCl_3$ yielded 44 % and a 50 % excess gave 64 % of the adducts *18*; with 50 % excess but without the catalysts TEBA 19 % of *18* was isolated. In all these experiments starting material was recovered.

1-(2,2-Dibromo-3,3-dimethylcyclopropyl)ethanol (19). The product was distilled at bath temperature < 100 °C/0.01 mmHg (lit.[4] b.p. 80 °C/0.15 mmHg) as a 81:19 mixture of diastereomers *a* and *b*. The distillate crystallized and separation of the diastereomers was achieved by recrystallization and chromatography on silica gel.

a. M.p. 84–85 °C (from pentane). ^1H NMR (CCl_4): δ 1.23 (3 H, d, J 6 Hz), 1.25 (3 H, s), 1.37 (1 H, d, J 9 Hz), 1.38 (3 H, s), 2.42 (1 H, s), 3.61 (1 H, d q, J 9, J 6 Hz). IR (CCl_4): 3610 (s), 3470 (m), 1095 (s) cm^{-1}.

b. M.p. 63–65 °C (from pentane). ^1H NMR (CCl_4): δ 1.24 (1 H, d, J 9 Hz), 1.35 (3 H, s), 1.40 (3 H, d, J 6 Hz), 1.45 (3 H, s), 3.53 (1 H, d q, J 9, J 6 Hz). IR (CCl_4): 3620 (s), 3480 (m), 1100 (s) cm^{-1}.

2-(2,2-Dichloro-1-methylcyclopropyl)propan-2-ol (20), b.p. 78–81 °C/17 mmHg. Anal. $C_7H_{12}Cl_2O$: C, H. ^1H NMR (CCl_4): δ 1.00 (1 H, d, J 7 Hz), 1.27 (3 H, s), 1.28 (3 H, s), 1.50 (3, H, s), 1.89 (1 H, broad s), 2.10 (1 H, d, J 7 Hz). IR (film): 3580 (m), 3470 (m), 750 (s) cm^{-1}.

2-(2,2-Dichloro-3,3-dimethylcyclopropyl)-propan-2-ol (21), b.p. 66–67 °C/8 mmHg. Anal. $C_8H_{14}Cl_2O$: C, H. ^1H NMR (CCl_4): δ 1.12 (1 H, s), 1.26 (3 H, s), 1.37 (3 H, s), 1.47 (6 H, s), 1.75 (1 H, s). IR (film): 3580 (m), 3460 (m), 835 (s), 760 (s) cm^{-1}.

2-(2,2-Dibromo-3,3-dimethylcyclopropyl)-propan-2-ol (22), b.p. 53 °C/10^{-2} mmHg, m.p. 22–23 °C (from pentane). Anal. $C_8H_{14}Br_2O$: C, H. ^1H NMR: δ 1.26 (4 H, ss), 1.42 (3 H, s), 1.50 (6 H, s), 1.84 (1 H, s). IR (film): 3580 (s), 3490 (m), 955 (s), 715 (s) cm^{-1}.

Reaction of 2-methyl-3-buten-2-ol (9) with excess dichlorocarbene. A mixture of 8.6 g (0.10 mol) of *9*, 35.7 g (0.30 mol) of $CHCl_3$, 24.0 g (0.30 mol) of 50 % aq. NaOH and 0.2 g of TEBA was stirred vigorously for 16 h at room temperature. The reaction mixture was worked up in the usual way. A sample of the gas evolved during the reaction was collected over CCl_4 and characterized as CO (IR). The crude reaction mixture consisted of starting allyl alcohol (63 %) and 6 products, according to GC (20 % SE 30). The products were separated by prep. GC (same column): *a* (shortest retention time). 24 % of the product was shown to be 1,1-dichloro-2-methyl-2-vinylcyclopropane (*24*) by comparison with an authentic sample.[18] *b*. 19 %, 2-(2,2-dichlorocyclopropyl)propan-2-ol (*25*). Anal. $C_6H_{10}Cl_2O$: C, H. ^1H NMR (CCl_4): δ 1.23 (3 H, s), 1.48 (3 H, s), 1.5–1.8 (4 H, m). IR (film): 3590 (s), 3450 (s), 750 (s) cm^{-1}. *c*. 38 %, 1,1-dichloro-2-chloromethyl-3,3-dimethylcyclopropane (*26*). Anal. $C_6H_9Cl_3$: C, H. ^1H NMR (CCl_4): δ 1.28 (3 H, s), 1.42 (3 H, s), 1.3–1.8 (1 H, m), 3.2–4.0 (2 H, m). IR (film): 2970 (s), 850 (s), 720 (s) cm^{-1}. The three remaining compounds, *d*, *e*, and *f* comprising 19 % of the product were not obtained pure enough for identification.

2,2-Dichloro-1,3,3-trimethylcyclopropylmethanol (28), m.p. 75 °C (from hexane). Anal. $C_7H_{12}Cl_2O$: C, H. ^1H NMR (CCl_4): δ 1.24 (3 H, s), 1.30 (6 H, s), 2.61 (1 H, s), 3.72 (2 H, broad s). IR (KBr): 3250 (s), 1020 (s), 840 (s) cm^{-1}.

Reaction of carvol (29) with dichlorocarbene. A mixture of 7.6 g (50 mmol) of carvol, 6.0 g (50 mmol) of $CHCl_3$, 4.0 g (50 mmol) of 50 % aq. NaOH, and 0.2 g of TEBA was stirred vigorously for 16 h at room temperature. The reaction mixture was worked up in the usual way. Short path distillation gave 3.3 g (43 %) of carvol and 4.4 g (38 %) of a mixture of monoadducts, b.p. 102–106 °C/0.01 mmHg. ^1H NMR (CCl_4) (with approx. integrals): δ 1.0–2.2 (13 H, overlap. s and m), 3.7–4.1 (2 H, m) 4.66 (1 H, broad s), 5.45 (0.5 H, m). IR (film): 3340 (s), 1640 (m), 750 (m) cm^{-1}.

On the basis of the integrals of the NMR-resonances at δ 4.66 and 5.45 we concluded that the two monoadducts *30* and *31* are present in approximately equal amounts. Small amounts of diadducts m.p. 110–115 °C (pentane) could be isolated from the residue after distillation.

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Formation of Aromatic Compounds from Carbohydrates. V.* Reaction of D-Glucose and Methylamine in Slightly Acidic, Aqueous Solution

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The title reaction produced a humin-like precipitate. In the supernatant, D-fructose and compounds *1*–*18*, comprising furans, pyrroles, phenols, and enols, were identified. The bicyclic products, 1-methyl-5-[5-(hydroxymethyl)-2-furfuryl]pyrrole-2-carboxaldehyde (*13*), 5-(5-formyl-2-furfuryloxymethyl)-1-methylpyrrole-2-carboxaldehyde (*15*), 5,5′-(oxydimethylene)-bis-[1-methylpyrrole-2-carboxaldehyde] (*16*), 5-(hydroxymethyl)-1,1′-dimethyl-4,5′-methylenedipyrrole-2-carboxaldehyde (*17*), and 1-methyl-5-(3,4,5-trihydroxybenzyl)pyrrole-2-carboxaldehyde (*18*) seem to be new compounds. 2′,4′,5′-Trihydroxyacetophenone (*12*) has apparently not been identified previously as a degradation product of carbohydrates.

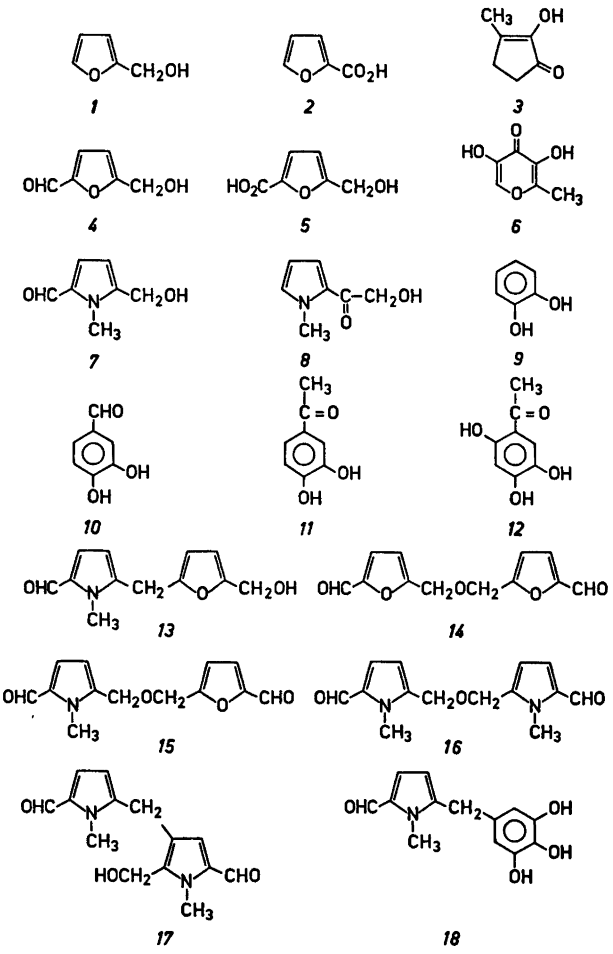
The previous papers [1–4] in this series focused on the degradation of aldoses or hexuronic acids to phenolic and enolic compounds, believed to be intermediates in the yellowing of cellulosic products during manufacture, storage and use. After accelerated ageing of bleached pulp, low-molecular carbohydrates and their degradation products may be extracted with ethanol.[5] Such extracts also contain amino acids,[6] which are known to accelerate and modify the degradation of carbohydrates under neutral or slightly acidic conditions.[7] In modern forestry, where the utilization of the whole tree increases the protein content of the pulp, a pronounced effect on the yellowing process may be expected. The so-called non-enzymic browning or Maillard reaction between carbohydrates and amino compounds is particularly important in food chemistry and may considerably affect the colour, aroma, and nutritional value of food.[7]

The present paper deals with the Maillard reaction in a simple model system, *viz.*, D-glucose and methylamine in aqueous acetic acid. The results were reported briefly at a recent meeting.[8] The same reaction has been studied previously,[9–11] and several products (particularly furans and pyrroles) were identified. These were separated mainly by gas chromatography or distillation. We preferred liquid chromatography for the separation to be able also to identify hydrophilic and less volatile products.

RESULTS

In previous investigations of the title reaction, a fairly dilute aqueous solution, of D-glucose, methylamine, and acetic acid in the approximate molar ratio 3:1:1 was refluxed for 2 h, cooled and then extracted with ethyl acetate.[9,10] Alternatively, a concentrated solution of equimolar amounts was refluxed for 1 h.[11] We followed essentially the former procedure, but exploratory, small-scale experiments using various reflux periods indicated that the maximum yield of extracted material was approached only after *ca.* 24 h. In subsequent large-scale experiments, the solution was accordingly refluxed for 24 h. During that time, the pH dropped from 5.1 to 4.2, and a dark-brown powder precipitated. The elemental composition of the dry material approximated

* Part IV. See Ref. 4.



to $C_{13.0}H_{14.2}NO_{4.6}$. On the basis of eqn. 1, the precipitate accounted for 3.7 % of the glucose and 5.7 % of the methylamine. Sugar analysis [12] of the supernatant showed glucose in 51 % of the original amount and a 7 % yield of D-fructose. The solution was extracted with ethyl acetate, followed by 2-butanone. The extracted material accounted for 2.5 – 3 % of the glucose and probably 4 – 5 % of the methylamine. Chromatography of the extracts on Sephadex LH-20 columns resulted in 9 main fractions, which were rechromatographed on silica gel columns (Table 1). In this way each of the compounds *1* – *18* was isolated, except for the mutual separation of the minor products *10* and *11*. No efforts were made to obtain optimum yields.

Compounds *1* – *6*, *9* – *12*, and *14* were identical (TLC, MS, and ^{1}H NMR) with authentic samples, while *7* and *8* were identified by means of previously published spectral data (MS, IR, and ^{1}H NMR).[10] The new compounds *13* and *15* – *18* may be regarded as anhydro-dimers of *7* or as anhydro-codimers of *7* with *1*, *4*, or pyrogallol (*19*). Their molecular formulas were established by high resolution mass spectrometry and their structures determined mainly by comparing their spectra (Table 2) with those of the respective monomers and the known anhydro-dimer *14*.[3,13]

Thus, the mass spectrum of each new compound showed the two arylcarbonium ions, expected from simple cleavage of the interannular chain, and/or closely related fragments.

Table 1. CC fractionation of extracts from the filtered title reaction mixture.

Fraction [a] No. (mg) [b]	Major (minor) components	Silica gel eluent (v/v)	Isolated compounds (mg)
Ethyl acetate extract (7.3 g)[b]			
1 (268)	*1*, *4* (*5*)	CH_2Cl_2–MeCN, 4:1	*4* (51)
2 (1257)	*4*, *7*, *8* (*1–3*, *6*)	CH_2Cl_2–MeCN, 4:1	*1* (9), *2* (8), *3* (5), *6* (6)
3 (876)	*7*, *8* (*15*)	CH_2Cl_2–Me_2CO, 8:1	*7* (183), *8* (162)
4 (141)	*14* (*15*)	CH_2Cl_2–MeCN, 4:1	*14* (3),[c] *15* (2)
5 (185)	*13* (*9*, *16*, *17*)	$(CH_2)_6$–Me_2CO, 2:1	*9* (2), *13* (18), *16* (7)
6 (135)	(*17*)	CH_2Cl_2–MeCN, 8:1	*17* (4)
7 (108)	(*10*, *11*)	$(CH_2)_6$–Me_2CO, 2:1	*10*+*11* (7)[d]
8 (462)	*18* (*12*)	CH_2Cl_2–MeCN, 4:1	*12* (9), *18* (14)
2-Butanone extract (2.5 g)[b]			
9 (242)	*4*, *7* (*5*, *6*, *16*)	CH_2Cl_2–EtOH, 9:1	*5* (11)

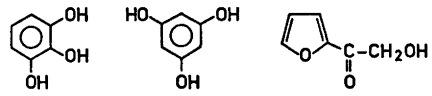
[a] Eluted from Sephadex LH-20. [b] After evaporation. [c] Identified only by TLC and MS. [d] Identical (TLC, MS, ^1H NMR) with an authentic 1:1 mixture.

Table 2. Spectral data.

Assignment [a]	*7* [b]	*13* [c]	*15*	*16*	*17*	*18*
MS [IP 70 eV; *m/e* (% rel. int.)]						
M, obs.		219.089	247.083	260.115	260.114	247.083
M, calc.		219.090	247.084	260.116	260.116	247.084
Strongest peaks above *m/e* 65	139 (100)	219 (100)	122 (100)	122 (100)	260 (100)	110 (100)
	122 (53)	94 (73)	94 (83)	94 (76)	151 (79)	247 (60)
	82 (30)	190 (51)	123 (48)	123 (71)	110 (61)	138 (54)
	67 (26)	160 (40)	67 (24)	67 (25)	123 (59)	81 (33)
	94 (22)	111 (33)	109 (22)	93 (20)	82 (58)	82 (32)
	138 (22)	117 (33)	81 (21)	260 (15)	94 (36)	108 (19)
	110 (20)	132 (33)	93 (18)	108 (13)	122 (31)	109 (17)
	124 (17)	93 (28)	82 (15)	82 (12)	150 (30)	218 (15)
	68 (17)	77 (22)	95 (15)		185 (27)	77 (14)
	80 (11)	108 (21)	247 (12)		93 (24)	122 (12)
IR [$CHCl_3$; $\tilde{\nu}_{max}$ (cm^{-1})]						
2-CHO	1660 (s)	1660 (s)	1660 (s)	1660 (s)	1660 (s)	1660 (s)
2′-CHO	–	–	1680 (s)	1660 (s)	1660 (s)	–
OH	3380 (s)	3420 (s)	–	–	3420 (m)	3380 (s)
^1H NMR [100.0 MHz, $(CD_3)_2CO$; δ]						
1-Me	3.92 (s)	3.89 (s)	3.92 (s)	3.91 (s)	3.88 (s)	3.86 (s)
2-CHO	9.48 (s)	9.46 (s)	9.55 (s)	9.55 (s)	9.45 (s)	9.43 (s)
3-H	6.84 (d)	6.87 (d)	6.90 (d)	6.90 (d)	6.88 (d)	6.85 (d)
4-H	6.16 (d)	6.06 (d)	6.28 (d)	6.26 (d)	5.98 (d)	5.97 (d)
5-CH_2	4.62 (s)	4.03 (s)	4.66 (s)	4.61 (s)	3.93 (s)	3.90 (s)
1′-Me	–	–	–	3.91 (s)	4.00 (s)	–
2′-CHO	–	–	9.62 (s)	9.55 (s)	9.48 (s)	–
3′-H	–	6.02 (d)	7.37 (d)	6.90 (d)	6.67 (s)	–
4′-H	–	6.18 (d)	6.66 (d)	6.26 (d)	–	6.22 (s)[d]
5′-CH_2	–	4.46 (s)	4.62 (s)	4.61 (s)	4.69 (s)	–
^1H NMR [100.0 MHz, $(CD_3)_2CO$; $\|J\|$ (Hz)]						
3-H, 4-H	4.1	4.0	4.1	3.9	4.1	4.0
3′-H, 4′-H	–	3.1	3.6	3.9	–	–

[a] Primed numbers refer to the right-hand ring in the formula. [b] *Cf.* Ref. 10. [c] *m/e* 122 (20 %). [d] 4′- and 6′-H.

Such ions derived from the *7* residue(s) appeared at *m/e* 122 and 94 (loss of CO). As expected,[14] this mode of cleavage was most pronounced for the ethers *15* and *16*. Each formyl group attached to a pyrrole or furan ring gave rise to a carbonyl band in the IR spectrum at 1660 or 1680 cm^{-1}, respectively. Broadening of certain ^1H NMR signals revealed weak but unambiguous allylic couplings to all methylene protons, except for those of the hydroxymethyl group in *17*. Although disregarded in Table 2, these couplings permitted complete analysis of the spectra by successive spin decoupling. The resulting assignments are listed in Table 2 and establish the proposed substitution patterns.



19 *20* *21*

When sprayed with *p*-anisaldehyde in sulfuric acid, all the furan derivatives displayed a blue colour, whereas all pyrroles turned yellow to brown. Accordingly, *13* and *15* showed a blue-green colour. Unlike phloroglucinol (*20*), compounds *18* and *19* produced a characteristic dark-grey colour when sprayed with aqueous iron(III) chloride or ammonium molydate,[15] and formed borate complexes, ruling out the isomer of *18* derived from *7* and *20*. The complex formation was demonstrated by electrophoresis experiments at pH 9.0 with and without borate.[16] The migration rates (mm/h) were as follows.

Compound	*7*	*18*	*19*	*20*
Without borate	0	0	5	0
With borate	0	44	68	0

DISCUSSION

The total yields of *1–18* were not determined but in each of fractions 1–9 (Table 1), the approximate proportions of the components were estimated by TLC. From the results and from the weights of the evaporated fractions, it was clear that *4*, *7*, and *8* were the main products, followed by *1*, *13*, *14*, and *18*. Compounds *1*, *4*, and *6–8* have previously been identified as title reaction products.[10] Compounds *9*, *10*, and *14* have been obtained from glucose or fructose,[3] and compound *2* from D-glucuronic acid,[1] under similar conditions but in the absence of methylamine. Compounds *3* and *11* are formed on degradation of glucose in alkali,[2] and compound *5* from solutions of *4* by autoxidation.[17] However, *12*, *13*, and *15–18* have apparently not been identified previously as degradation products of carbohydrates.

The dehydration of hexoses to *4*, 2-furyl hydroxymethyl ketone (*21*), and other products is well-known and its mechanism is fairly well understood.[18] The reaction is catalyzed by amino compounds, owing to the transient formation of enamines and/or immonium ions.[7] Cyclization of such intermediates is no doubt responsible for products *7* and *8*.[19] The ready conversion of *4* to *14*[13] indicates that the bicyclic products *13–17* are formed by acid-catalyzed S_N1 reactions between the relatively abundant compounds *1*, *4*, and *7*, the aryl-carbonium ion corresponding to *4* or *7* alkylating *1*, *4*, or *7* at oxygen or in the most reactive free ring position (Friedel-Crafts alkylation). It is not clear whether *18* is formed by similar alkylation of *19* or one of its precursors, since *19* should react in position 4 rather than 5. Although no free *19* was detected in the title reaction mixture, traces are formed in the absence of methylamine.[3]

Products *3*, *5*, *6* and *9* may have been formed by dehydration, accompanied by oxidation or reduction. Since all these products are minor, air oxidation and disproportionation reactions – of the Cannizzaro type, for example – are perhaps not ruled out. However, a fragmentation-recombination sequence seems more probable for *3* and *9*, because these are formed on degradation of either xylose or glucose in alkali.[2] In any case, as pointed out previously for *11* and related compounds,[2] such reactions are necessary to explain the formation of products *1*, *2*, and *10–12*. Experiments with pure *9* or hydroxyhydroquinone in acetate or formate buffer indicated that *10–12* are not formed by Friedel-Crafts acylation.

The elemental composition of the precipitate is close to that of soil humin [20] and indicates considerable aromatization. It is also in good agreement with the net reaction scheme (eqn. 1)

$$2.0\ C_6H_{12}O_6 + CH_3NH_2 \rightarrow C_{13.0}H_{14.2}NO_{4.6} + 7.4\ H_2O \qquad (1)$$

Disregarding the modest amounts of *1*, its derivative *13*, and some minor products, the title reaction is therefore essentially composed of isomerization and dehydration steps.

The above results indicate that the formation of pyrroles is partly responsible for the loss of lysine and other essential amino acids in food through the Maillard reaction. However, a comparable part of the lost amino nitrogen is found in the insoluble fraction. Being fairly stable, the isolated pyrroles are probably not intermediates in the formation of this fraction.[21] An exception is *18*, which is easily converted to coloured products. Minor products like *9–12*, *19*, or other derivatives of *9* [3] may also be reactive and important in colour formation.

EXPERIMENTAL

General

Melting points are corrected. Evaporations were performed at reduced pressure below 50 °C. Electrophoresis was carried out on Whatman No. 1 paper in 0.05 M phosphate or phosphate-borate buffer (0.05 M in each) at pH 9, 15 °C, and 1500 V. The spots were visualized with aq. *p*-anisidine hydrochloride and iron(III) chloride. TLC was performed on silica gel (Merck, HF_{254}). After inspection of the plates in UV light, ethanolic *p*-anisaldehyde-sulfuric acid, aq. iron(III) chloride, and aq. ammonium molybdate–acetic acid [15] were used as spray reagents. CC was carried out on Sephadex LH-20 or silica gel (Mallinckrodt, 100 mesh). Sugars were analyzed as their per(trimethylsilyl) ethers by GLC [12] on a Varian 1840 instrument, equipped with flame-ionization detectors and 1.8 m × 2 mm i.d. glass columns. These contained 3 % OV-1/Varaport 30 (100–120 mesh). The nitrogen flow rate was 30 ml/min and the temperature programmed from 100 to 250 °C at 6 °C/min. Low and high resolution mass spectra were recorded at 70 eV on Varian MAT CH 7 and AEI MS 902 instruments, respectively, the latter spectra at the Institute of Medical Biochemistry, University of Gothenburg. The samples were introduced directly into the ion source or through a Varian 1740 gas chromatograph, operated at 150 °C and 25 ml He/min and fitted with a 1.8 m × 2 mm i.d. glass column. This contained 3 % OV-225/Gas-Chrom Q (100-120 mesh). IR spectra were recorded on a Perkin-Elmer 337 instrument, using chloroform as solvent. ^1H NMR spectra were recorded at 100.0 MHz and *ca.* 30 °C on a Varian HA-100 D instrument, equipped with a Varian VFT-100 Fourier Transform System. Acetone-d_6 was used as solvent.

Materials

Solvents, reagents, and reference compounds other than those listed below were commercial samples of good grade. All solvents were freshly distilled before use.

5-(Hydroxymethyl)-2-furoic acid (5) was prepared by oxidation of *4* (94 mg) with freshly prepared silver(I) oxide in aq. alkali, *cf.* Ref. 22. The ethyl acetate extract of the acidified reaction mixture yielded *5* (55 mg, 52 %), which was recrystallized from chloroform-ethanol, m.p. 159–162 °C (lit.[23] 160–162 °C, decomp.).

3,5-Dihydroxy-2-methyl-4H-pyran-4-one (6) was a gift from Dr. P. E. Shaw, Winter Haven, Florida, U.S.A.

2′,4′,5′-Trihydroxyacetophenone (12), *cf.* Ref. 24. Perchloric acid (5 ml 70 %) was carefully added to a stirred solution of hydroxyhydroquinone (4.2 g, 33 mmol) in acetic anhydride (16 g, 160 mmol). The stirring was continued for 2 h on a steam bath. The mixture was cooled, neutralized with aq. sodium carbonate, and extracted with ethyl acetate. The concentrated extract was put on a silica gel column, which was eluted with dichloromethane-ethanol, 20:1 (v/v). Sublimation of the evaporation residue from the first fraction at *ca.* 0.5 mmHg yielded *12* (0.62 g, 11 %) as yellow crystals, m.p. 204–206 °C (lit.[25] 204–206 °C).

5,5′-(Oxydimethylene)di-2-furaldehyde (14) was obtained from D-fructose.[3]

Title reaction procedure

A solution of anhydrous D-glucose (520 g, 2.9 mol), aq. methylamine (72 g 40 %, 0.93 mol), and glacial acetic acid (59 g, 0.98 mol) in water (3.7 l) was refluxed for 24 h and then cooled to 20 °C. The dark-brown precipitate was collected, washed with water, and then dried to constant weight at 60 °C and 0.1 mmHg, yielding a hygroscopic amorphous powder (13.7 g). Found: C 60.5; H 5.5; N 5.1; O 28.6. Calc. for $C_{13.0}H_{14.2}NO_{4.6}$: C 60.5; H 5.6; N 5.4; O 28.5. Apart from a small portion used for sugar analysis,[12] the combined filtrate and washings were extracted with ethyl acetate (4 × 600 ml), followed by 2-butanone (4 × 600 ml). The extracts were fractionated as follows (Table 1).

The ethyl acetate extracts were combined, dried with sodium sulfate and evaporated to constant weight. The residue was put on a 95 × 5 cm Sephadex LH-20 column. This was eluted with water, yielding fractions 1–7. Subsequent elution with 95 % ethanol yielded fraction 8. The separation was monitored by TLC, using chloroform-glacial acetic acid, 9:1 (v/v), as eluent. The 2-butanone extracts were treated in the same way, but an 80 × 2 cm column was used, and one aq. fraction (9) was

collected. Fractions 1–7 were extracted with ethyl acetate and fraction 9 with 2-butanone. The extracts and fraction 8 were dried and evaporated as above. Each residue was put on a silica gel column. This was eluted by the solvent system listed in Table 1. The separation was monitored by TLC, using the same eluent. Evaporation of the purest fractions yielded *1–9*, *12–18*, and a mixture of *10* and *11*, generally as brownish syrups and each in sufficient amount and purity for proper characterization and identification.

Characterization of new compounds

Compound *15* was crystallized from hexane-acetone, m.p. 63–65 °C; compound *16* from hexane, m.p. 108–109 °C; and compound *17* from hexane-acetone, m.p. 121–122 °C. For spectral data, the reader is referred to Table 2.

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Decomposition of the *p*-Chlorophenyltriazene and *N*-Nitrosoacetamide of Glucosylamine. Formation of Cyclohexyl and Methyl Glucopyranosides

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N-Acetyl-2,3,4,5-tetra-*O*-acetyl-*N*-nitroso-β-D-glucopyranosylamine and 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*p*-chlorophenyltriazene, have been synthesized and their reactions in dichloromethane, methanol and cyclohexanol have been studied. In dichloromethane, penta-*O*-acetyl-β-D-glucopyranose and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine are formed. When the degradations are performed in methanol or cyclohexanol, the corresponding α- and β-glucosides are obtained with the former predominating. The best yields of glucosides are obtained from the triazene derivatives.

Decomposition of *N*-acetyl-2,3,4,6-tetra-*O*-acetyl-*N*-nitroso-β-D-glucopyranosylamine (*1*) in dichloromethane yields penta-*O*-acetyl-β-D-glucopyranose. When, however, the *N*-nitroso derivative is refluxed in methanol, a mixture of methyl tetra-*O*-acetyl-α- and β-D-glucopyranosides (3:1) is obtained.[1] This was suggested as an alternative way of preparing glycosides; especially α-linked glycosides. In the present work, the study has been extended to include the reaction of the *N*-nitroso derivatives in cyclohexanol and the reactions of the triazene derivative of glucosylamine in dichloromethane, methanol, and cyclohexanol.

RESULTS

The synthesis of *N*-acetyl-2,3,4,6-tetra-*O*-acetyl-*N*-nitroso-β-D-glucopyranosylamine (*1*) is described in Ref. 1.

When 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (*2*)[3] is reacted with *p*-chlorobenzenediazonium tetrafluoroborate in *N*,*N*-dimethylformamide, crystalline 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*p*-chlorophenyltriazene (*4*) is obtained. Unlike *1*, compound *4* is stable at room temperature.

When heated in various solvents, the *N*-nitroso and triazene derivatives decompose with evolution of molecular nitrogen. The resulting mixtures were analysed by GLC-MS. When authentic samples were not available, a part of each reaction mixture was separated by column chromatography and the components were identified.

Decomposition of *1* in dichloromethane gives mainly penta-*O*-acetyl-β-D-glucopyranose (*7*), as reported earlier[1] (Table 1). When refluxed in dichloromethane in the presence of an acidic catalyst (boron trifluoride), *4* yields *2* and not 2,3,4,6-tetra-*O*-acetyl-*N*-*p*-chlorophenyl-β-D-glucopyranosylamine (*8*) as might have been expected.

When *1* is refluxed in methanol/pyridine (1:1)[1] or cyclohexanol/pyridine (1:1) the methyl- and cyclohexyl glucopyranosides *9*, *10*, *11*, and *12*, respectively, are formed. The ratio between α- and β-linked glucopyranosides is about 3:1 in both reactions, but the cyclohexyl glucopyranosides are obtained in lower yields (Table 1). In each of the above solvents, *N*-acetyl-2,3,4-tri-*O*-acetyl-*N*-nitroso-α-L-arabinopyranosylamine reacts similarly to *1*, giving the α- and β-anomers in similar yields and ratios. Refluxing *4* in methanol and cyclohexanol, respectively, in the presence of boron trifluoride yields the corresponding glucopyranosides, *9*,

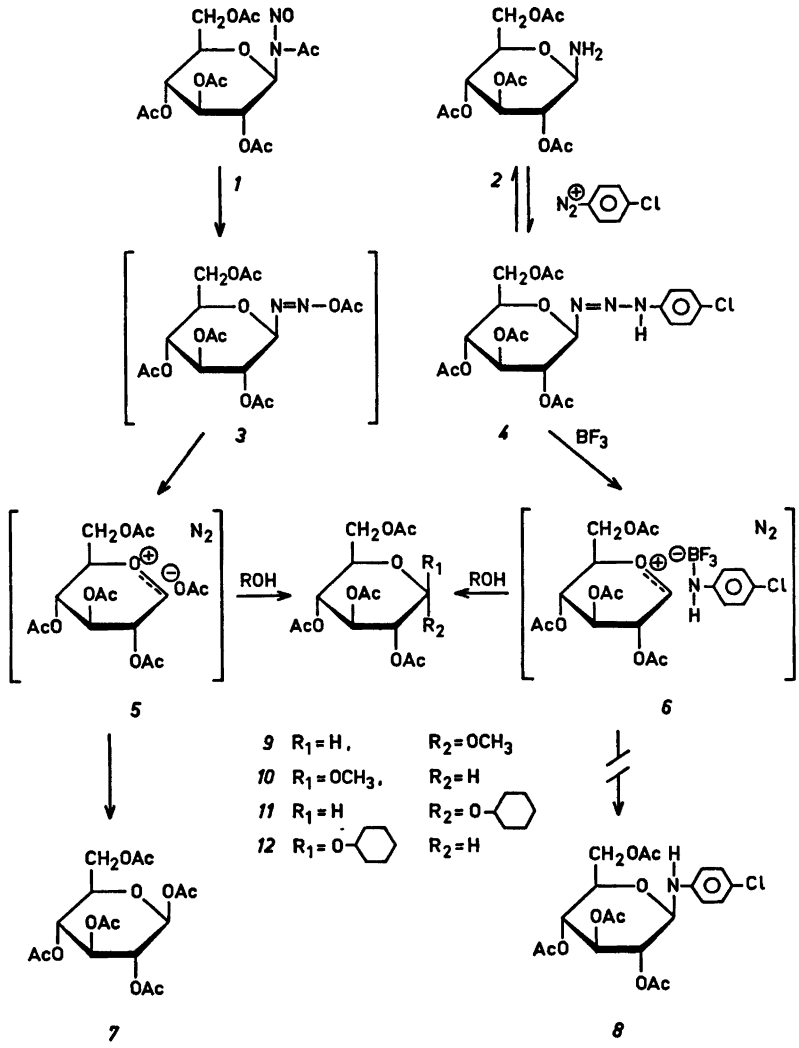


Table 1. The decomposition products and their yields [a] as estimated by GLC.

Decomposition of	Product (yield %)
1 in CH_2Cl_2/pyridine	*7*(65)
1 in CH_3OH/pyridine	*9*(75), *10*(25)
1 in $C_6H_{11}OH$/pyridine	*7*(29), *11*(31), *12*(11)
4 in $CH_2Cl_2BF_3$	*2*(15)
4 in CH_3OH/BF_3	*9*(85), *10*(15)
4 in $C_6H_{11}OH/BF_3$	*11*(60), *12*(15)

[a] The yields are calculated against *myo*-inositol, which is added as an internal standard.

10, *11* and *12*. The α-anomer is formed in higher yield in these reactions (Table 1). The yields of glucosides in most reactions were not quantitative. Other products, not accounted for, must have been formed.

DISCUSSION

Mechanisms for the decomposition of aliphatic *N*-nitrosamides and 1,3-di-substituted triazenes have been reviewed.[5] Thus, *N*-nitrosamides are believed to rearrange to a diazoester (*3*), which loses molecular nitrogen and an ion pair (*5*) is formed. When the reaction

is performed in an inert solvent, the ion pair collapses to yield the ester or possibly an alkene. When a large cation is involved in the ion pair, the reaction proceeds essentially with retention of configuration.[5] It has also been shown that neighbouring group participation is not important in these reactions.[5] In accordance with these findings, decomposition of *1* in inert solvents should give predominantly the β-anomer, which was also found.

Solvolysis of aliphatic *N*-nitrosoamides in protic solvents leads to products with retained or inverted configuration.[5]

If we assume that a solvent-separated ion pair is formed in the solvolysis reaction of *1*, this ion pair should react with the solvent giving both α- and β-linked glycosides. However, α-linked glycosides are formed preferentially.

The mechanism for the decomposition of triazenes (*4*) is similar to that for nitrosamide decomposition, but the reaction is catalyzed by acids.[5] As in the nitrosamide decomposition, loss of nitrogen leads presumably to the formation of the ion pair *6*. The ion pairs *5* and *6* have identical cations but different anions and should behave analogously on solvolysis. The triazene derivatives are more stable than the *N*-nitroso derivatives and give higher yields of glycosides in reactions with alcohols.

EXPERIMENTAL

General methods. Melting points are corrected. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. NMR spectra were recorded at 100 MHz and ca. 30 °C on a Varian HA-100 D spectrometer, IR spectra with a Perkin-Elmer 337 spectrometer. TLC was performed on Silica Gel F_{254} (Merck) and column chromatography on Silica Gel 60 (Merck). GLC was conducted at 190–220 °C with a Varian model 2700 instrument fitted with a glass column containing 3 % of OV-225 on Gas-Chrom Q (100–120 mesh). For quantitative evaluations of the results, obtained by GLC, an Autolab, minigrator was used. GLC-MS was performed with a Varian CH 7 mass spectrometer.

Decomposition reactions of 1. The reactions in dichloromethane/pyridine and methanol/pyridine are already described.[1] Decomposition of *1* in cyclohexanol/pyridine (1:1) was performed and the reaction mixture was processed and analysed as described for the reaction of *1* in methanol/pyridine. Cyclohexyl glucosides were obtained in 40 % yield and the ratio between α and β-anomers was 3:1 (Table 1) as analysed by GLC and the products identified by GLC-MS.

The detector responses and relative retention times of acetylated cyclohexyl α- and β-D-glucopyranosides were determined by reference to a constant amount of *myo*-inositol.

In a preparative experiment, *N*-acetyl-tetra-*O*-acetyl-β-D-glucopyranosylamine (3 g) was dissolved in pyridine (30 ml) at 0 °C, dinitrogen tetraoxide (3 g) in dichloromethane (25 ml) was then added. After 30 min, the mixture was purged with nitrogen for 1 h at 0 °C. Cyclohexanol (30 ml) was added and the reaction mixture was heated for 1 h at 50 °C. The solution was concentrated and the residue was fractionated on a Silica Gel column (3 × 50 cm), which was irrigated with light petroleum–ethyl acetate (1:1 v/v). The separation was followed polarimetrically and by TLC. Three main products, *7* (0.8), *11* (0.94 g) and *12* (0.33 g) were obtained. Compound *7* had m.p. 135 °C, $[\alpha]_{578}$ +4° (*c* 1, chloroform); lit.[8] m.p. 132–134 °C, $[\alpha]_D$ +4°. Compound *11* had m.p. 38–39 °C, $[\alpha]_{578}$ +115° (*c* 1.8, chloroform); lit.[9] m.p. 40 °C, $[\alpha]_D^{20}$ +112°. Compound *12* had m.p. 121 °C, $[\alpha]_{578}$ −21°, lit.[9] m.p. 121–122 °C, $[\alpha]_D^{20}$ −23.8°.

*Synthesis of 1-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-3-*p*-chlorophenyltriazene (5).* To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (*3*) (1 g) in *N*,*N*-dimethylformamide (5 ml) was added Na_2CO_3 (0.30 g) at 0 °C followed by a solution of *p*-chlorobenzenediazonium tetrafluoroborate (0.65 g) in *N*,*N*-dimethylformamide (10 ml).[4] After stirring at 0 °C for 5 min, chloroform (50 ml) was added and the reaction mixture was washed with ice-water (2 × 25 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated to dryness. After recrystallisation (dichloromethane, light petroleum) 1.2 g of *5* was obtained as yellowish crystals and had m.p. 189–191 °C, $[\alpha]_{578}$ +2.5 (*c* 1.5, chloroform); λ_{max} ($CHCl_3$) 5.7 (C=O) 6.8 (C=C) and 7.2 (O–$COCH_3$) μm. ^{1}H NMR ($CDCl_3$): δ 7.35 (s, 4 H, phenyl), 5.54–5.10 (m, 3 H, H-1, H-2, H-2, H-3), 5.15 (q, 1 H, $J_{4,3}$ 7.5, $J_{4,5}$ 9.5, H-4), 4.3 (q, 2 H, $J_{5,6'}$ 4.5, $J_{6,6'}$ 12.5, H-6), 4.1 (q, 2 H, $J_{5,6}$ 2.5, $J_{6,6'}$ 12.5, H-6), 3.9 (m, 1 H, H-5), 2.06, 2.04, 2.03, 2.01 (4 s, 4 OAc). *Anal.* Calc. for $C_{20}H_{24}O_9N_3Cl$: C 49.4; H 4.9; N 8.7; Cl 7.3. Found: C 49.4, H 5.2, N 8.2, Cl 7.0.

Decompositions reactions of 4. (a) To a stirred solution of *4* (0.34 g) and a drying agent ($MgSO_4$, 1 g) in dichloromethane was added 45 % boron trifluoride–ethyl ether complex (0.4 ml). The colour changed from yellow to dark brown within 3 min at room temperature and TLC revealed that no starting material was left. Another 50 ml of dichloromethane was added and the reaction mixture was extracted with an aqueous solution of sodium hydrogen carbonate (2 %, 500 ml) and water (2 × 100 ml).

The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness. Recrystallisation from dichloromethane/light petroleum yielded *2* (50 mg). The compound, 2,3,4,6-tetra-*O*-acetyl-*N*-*p*-chlorophenyl-β-D-glucopyranosylamine (*8*) could not be detected in the reaction mixture.

(b) The reaction was performed as described in (a), except that methanol (50 ml) instead of dichloromethane was added. Evolution of nitrogen was observed and after the reaction had gone to completion (20 min), pyridine (10 ml) was added together with an internal standard (*myo*-inositol). The reaction mixture was evaporated to dryness and acetylated. GLC revealed that the methyl glucosides [1] had been obtained in quantitative yield with an α/β ratio of 6/1 (Table 1).

(c) The reaction was performed as described in (b), using cyclohexanol (50 ml) instead of methanol. The reaction mixture was heated for 15 min at 95 °C. Cyclohexyl glucopyranosides were formed in 80 % yield and the α/β ratio was 4:1. The analyses were performed as described above.

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Alkali and Oxygen—Alkali Treatment of 4-Deoxy-2,3-hexodiulose and 3-Deoxy-*erythro*-pentose

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In oxygen-free solutions ($NaHCO_3$ and NaOH), the decomposition of 4-deoxy-2,3-hexodiulose, *1*, to 3-deoxypentulose and formic acid is more important than rearrangement reactions. These give rise to 3-deoxy-2-*C*-(hydroxymethyl)pentonic and 1,4-anhydro-3-deoxypentitol-2-carboxylic acids. In NaOH a substantial amount of 3,4-dideoxypentonic acid is formed from 3-deoxypentulose. During treatment of *1* under oxygen pressure the cleavage to 3,4-dihydroxybutanoic and glycolic acids is the predominant reaction. Large amounts of less polar, UV-absorbing solutes were formed, particularly in the absence of oxygen.

3-Deoxy-2-*C*-(hydroxymethyl)pentonic (isosaccharinic) acids are formed by endwise degradation of cellulose and other 1,4-linked polysaccharides in alkaline media.[1] Investigations carried out in recent years by improved chromatographic methods show, however, that under the conditions employed in technical processes large amounts of several other hydroxy acids are produced.[2] The isosaccharinic acids are formed by a benzilic acid rearrangement [3] of 4-deoxy-2,3-hexodiulose (*1*). We now report a detailed study of the hydroxy acids formed from this dicarbonyl compound. Since 3-deoxypentulose was found to be an intermediate, results with 3-deoxy-*erythro*-pentose (which is rapidly isomerized) are included.

RESULTS AND DISCUSSION

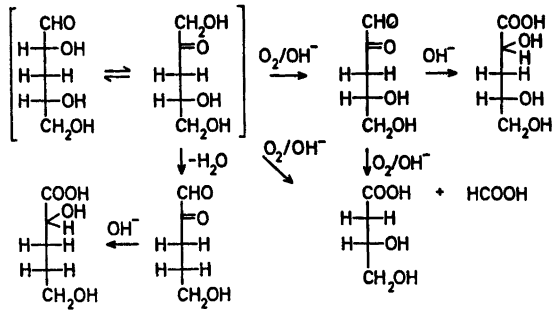
Degradation of 3-deoxy-erythro-*pentose.* The most abundant aliphatic carboxylic acid formed from 3-deoxy-*erythro*-pentose in aqueous NaOH at 75 °C in the absence of oxygen was 3,4-dideoxypentonic acid (yield 28 %). Except for 2,4-dihydroxybutanoic and 2-hydroxypropanoic acids (total yield 9 %) no appreciable amounts of other non-volatile carboxylic acids were produced.[4] Most of the sugar was converted to cyclic UV-absorbing compounds which were held firmly by the ion exchange resin (*cf.* Ref. 5).

Experiments in $NaHCO_3$ medium in the absence of oxygen showed that the yield of aliphatic hydroxy acids was even lower. Hence, the yield of 3,4-dideoxypentonic acid was only 10 % and the yields of 2,4-dihydroxybutanoic and 2-hydroxypropanoic acids less than 2 % under conditions which led to almost complete destruction of the sugar (5 h, 99 °C, 0.3 M $NaHCO_3$).

Table 1 shows that the formation of 3,4-dideoxypentonic acid was suppressed by the presence of oxygen and that 2,4-dihydroxy-

Table 1. Monocarboxylic acids obtained after degradation of 100 mg of 3-deoxy-D-*erythro*-pentose in 0.05 M sodium hydroxide at 90 °C under oxygen pressure (0.4 MPa) for 15 min.

Acid	Yield/mg
3-Deoxy-*threo*-pentonic	13.5
3-Deoxy-*erythro*-pentonic	2.3
3,4-Dideoxypentonic	6.6
3,4-Dihydroxybutanoic	25.6
2-Hydroxypropanoic	1.8
Glycolic	6.1
Formic	17.5
Total	73.4



Scheme 1.

butanoic acid was not formed. The formation of the two diastereomeric 3-deoxypentonic acids suggests that 3-deoxypentosulose is an important intermediate when oxygen is present. A benzilic acid rearrangement of this dicarbonyl compound explains the formation of these acids (Scheme 1). The observation that the *threo* form was the most abundant, is in agreement with the product composition observed after alkaline treatment of this dicarbonyl compound in the absence of oxygen.[6] The very large amounts of 3,4-dihydroxybutanoic and formic acids formed during O_2-NaOH-treatment of 3-deoxy-*erythro*-pentose suggest that the oxidative cleavage of its enediolate form and of 3-deoxypentosulose is important.

The higher yield of hydroxy acids obtained in the presence of oxygen shows that the reactions which give rise to nonpolar compounds are less important than in oxygen-free medium.

Degradation of 4-deoxy-2,3-hexodiulose (1). In the experiments with sodium hydroxide, *1* was consumed completely while about 1 % remained after the treatments in $NaHCO_3$ solution. No sugars were present after the treatments in NaOH while in $NaHCO_3$ in the absence of oxygen 3-deoxypentulose was the main product (Table 2). It has been postulated [7] that the corresponding 1,2-enediol is formed as an intermediate, after the loss of C-1 in *1* as formic acid, during alkali degradation of hexoses substituted at C-4. In agreement with this reaction scheme, formic acid was the most abundant acid produced in oxygen-free solution.

The instability of reducing sugars in strongly alkaline solution explains the absence of 3-deoxypentulose in the solutions obtained after the treatments in sodium hydroxide. The fact that formic acid was by far the most abundant acid in the absence of oxygen, together with other results referred to below, strongly indicates that 3-deoxypentulose is an important intermediate in this medium as well.

As already mentioned this intermediate gives a very low yield of aliphatic hydroxy acids, rather it yields a complex mixture of cyclic UV-absorbing solutes. Compounds of this type were eluted from the anion exchanger with water. Partition chromatography of this solution (without previous evaporation) in 92.4 % ethanol on the sulfate form of an anion exchanger showed that at least ten UV-absorbing compounds were present. A larger proportion was obtained in the fraction eluted with 5 M acetic acid. In the experiment in NaOH this fraction corresponded to 40 % of the starting material, while in $NaHCO_3$ medium the corresponding figure was 16 % (Table 2).

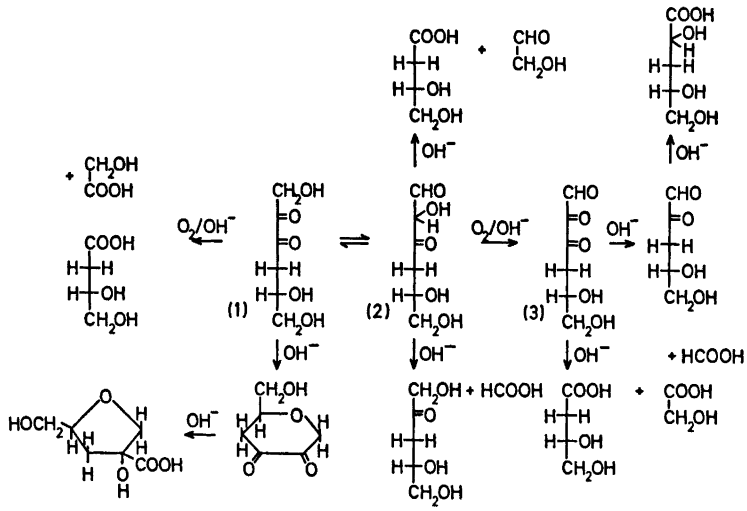
Only a minor amount of 3-deoxypentulose was present after the O_2-$NaHCO_3$-treatment. The low amount of formic acid produced in the experiments under oxygen pressure shows that reaction paths *via* 3-deoxypentulose are of little importance in the presence of oxygen.

Table 2 shows that the benzilic acid rearrangement of *1* to 3-deoxy-2-*C*-(hydroxymethyl) pentonic acids [8] is less important in bicarbonate than in sodium hydroxide solution. The same is true of the formation of 1,4-anhydro-3-deoxypentitol-2-carboxylic acid [8] which is an important reaction product in sodium hydroxide (Scheme 2). This acid has previously been isolated in appreciable amounts after alkali treatment of hydrocellulose and cellobiose.[4,9] Competing fragmentation reactions, of greater importance in the presence of oxygen, explain

Table 2. Products obtained after degradation at 90 °C of 100 mg of 4-deoxy-2,3-hexodiulose (*1*, purity 94 %).

	Reaction time 30 min				Reaction time 15 min[a]			
	0.05 M $NaHCO_3$		0.05 M NaOH		0.05 M $NaHCO_3$		0.05 M NaOH	
	mg	mmol mol reacted *1*	mg	mmol mol reacted *1*	mg	mmol mol reacted *1*	mg	mmol mol reacted *1*
Hexoses (incl. uloses)	1.5	14	0		1.3	12	0	
Tetroses (incl. ulose)	0.5	6	0		<0.1		0	
3-Deoxypentulose	25.3	325	0		1.5	19	0	
3-Deoxy-2-*C*-hydroxymethyl-*threo*-pentonic acid[b]	2.9	28	9.3	89	1.6	15	6.4	61
3-Deoxy-2-*C*-hydroxymethyl-*erythro*-pentonic acid[b]	0.4	3.8	2.7	26	<0.1	1.0	1.4	13
1,4-Anhydro-3-deoxypentitol-2-carboxylic acid	0.5	5.3	2.5	27	0.1		2.0	21
3-Deoxy-*threo*-pentonic acid[b]	0.2	2.2	0.6	6.9	0.5	5.7	7.9	91
3-Deoxy-*erythro*-pentonic acid[b]	0.1	1.2	0.3	3.4	0.3	3.4	2.0	23
3,4-Dideoxypentonic acid	<0.1		9.6	120	—		0.3	3.8
3,4-Dihydroxybutanoic acid	4.8	70	4.3	62	32.9	472	26.6	382
2,4-Dihydroxybutanoic acid	—		1.9	27	—		0.2	2.9
Glyceric acid	<0.1		<0.1		0.1	1.6	<0.1	
2-Hydroxypropanoic acid	<0.1		1.0	19	<0.1		1.8	34
Glycolic acid[c]	2.6	60	2.1	48	24.1	547	16.3	370
Formic acid[c]	12.8	480	16.6	620	1.4	53	4.8	180
C-(2,3-Dihydroxypropyl)tartronic acid	—		—		0.4	3.6	1.0	8.9
Identified compounds (total)	51.6	996	50.9	1048	64.2	1133	72.1	1191
Nonidentified compounds								
Eluted with water	11.2		4.9		17.8		2.0	
Eluted with 5 M acetic acid	15.9		39.8		16.5		25.7	

[a] Partial pressure of oxygen 0.4 MPa. [b] Relative amounts of the diastereomers determined by ion exchange chromatography. [c] Determined by ion exchange chromatography.



Scheme 2.

the lower yield of these acids in the experiments with oxygen present.

In the absence of oxygen, 3,4-dideoxypentonic acid was the second most abundant acid formed in sodium hydroxide. This supports the conclusion that 3-deoxypentulose is an important intermediate. Only a trace amount was formed in bicarbonate solution, explained by the greater stability of 3-deoxypentulose in this medium and by its ability to be easily converted to cyclic compounds.

The fact that only a trace amount of 3,4-dideoxypentonic acid was formed in bicarbonate medium in the absence of oxygen may seem puzzling since this acid was a major reaction product after treatment of hydrocellulose with 0.2 M $NaHCO_3$ at 120 °C.[5] Separate experiments with 3-deoxy-*erythro*-pentose in bicarbonate medium show, however, that the yield of 3,4-dideoxypentonic acid increases markedly with increasing reaction temperature and bicarbonate concentration.

The observation that 3,4-dideoxypentonic acid was virtually absent after the O_2-NaOH-treatment supports the conclusion that reaction paths *via* 3-deoxypentulose are of little importance in the presence of oxygen. The low recovery of strongly polar solutes in these experiments (64 and 72 %) indicates that less polar compounds are also formed *via* other reaction routes.

The diastereomeric 3-deoxypentonic acids formed in large amounts during O_2-NaOH-treatment of *1* are among the more abundant acids produced during a similar treatment of hydrocellulose.[10] The results obtained in the present work show that the reaction path *via* *1* (see Scheme 2) is very important although these acids can also be formed from hexosulose end groups.[11] A possible route for their formation is *via* oxidation of *2* (Scheme 2) to a compound containing three carbonyl groups (*3*). Elimination of formic acid followed by benzilic acid rearrangement of the 3-deoxypentosulose intermediate, yields the expected 3-deoxy-pentonic acids.[12]

As shown by Machell and Richards, 3,4-dihydroxybutanoic and glycolic acids are the predominant products obtained by treatment of *1* in hydrogen peroxide solution.[3] Similarly, these acids are among the most abundant acids produced during O_2-NaOH-treatment of hydrocellulose.[10] Table 2 shows that the same holds true for the treatment of *1* with oxygen in both alkaline media. Evidently, the oxidative cleavage of the bond between C-2 and C-3 in *1* is a rapid reaction and of greater importance than the reactions which give rise to formic acid and 3-deoxy-pentulose. 3,4-Dihydroxybutanoic and glycolic acids can also be formed from *3* in an analogous way to the formation of formic and 3-deoxy-pentonic acids.

In agreement with the results obtained with hydrocellulose the yields of these acids were much less in the absence of oxygen.[9] Hydrolytic cleavage of *2* (see Scheme 2) will give rise to 3,4-dihydroxybutanoic acid and glycolaldehyde, which is unstable in the alkaline solutions. Among the remaining reaction products were tetroses and hexoses formed by aldol condensation. As expected these sugars were present in detectable amounts only after the treatments in bicarbonate solution.

In the experiments carried out in the absence of oxygen no dicarboxylic acids of the types produced during alkali treatment of cellulose [13] and cellobiose [4] were recorded. On the other hand appreciable amounts of *C*-(2,3-dihydroxypropyl) tartronic acid were formed in the presence of oxygen. This acid is the major dicarboxylic acid formed during O_2-NaOH treatment of cellulose [14] and cellobiose.[15] Evidently one reaction path for the formation of this acid is *via 1*. Only trace amounts of other dicarboxylic acids were recorded.

EXPERIMENTAL

Preparation and characterization of 1. Maltose (20 g) was heated [3] under paraffin oil in 1000 ml of 0.05 M NaOH for 5 h at 25 °C. A cation exchanger (H^+) was added under stirring to decrease the pH to 7. The solution was then passed through 35 ml of an anion exchanger in the acetate form. The effluent was evaporated to a syrup at 35 °C. The syrup was chromatographed in 85 % (w/w) ethanol at 40 °C on an anion exchange resin in the sulfate form (Dowex 1-X8, 14 × 720 mm, 24 – 32 μm). The first 400 ml of eluate were evaporated. Rechromatography on the same column gave a broad peak (refractive index detector) with an adjusted retention volume of 1.5 bed volumes. This fraction was evaporated and rechromatographed in 92.4 % ethanol on Dowex 1-X8 (6 × 615 mm, 13 – 18 μm) in the sulfate form. The yield of the syrup was 1 %.

Oxidation with hydrogen peroxide gave 3,4-dihydroxybutanoic and glycolic acids.[3] Gas chromatography of the oxime-Me_3Si derivatives showed that besides the major product (*1*), 3 % of 3-deoxypentulose and 3 % of unknown compounds were present. The mass spectrum (AEI Organic MS-20, EI, 70 eV) of the major component confirmed the identity. [*m/e* 552, M^+, (0.1 % of base peak at *m/e* 73) 537 (0.4), 462 (0.9), 449 (2.4), 373 (4.4), 359 (3.2), 345 (0.8), 317 (1.2), 293 (0.5), 283 (2.3), 253 (5.3), 230 (3.0), 218 (5.0), 215 (2.5), 205 (9.0), 191 (11.0), 188 (7.0), 147 (36.0), 117 (6.5), 103 (16.0)]. Reduction to 3-deoxyhexitols confirmed that the purity was 94 %.

Procedure. In the experiments in the absence of oxygen paraffin oil was layered onto a boiled solution (90 ml) of NaOH or $NaHCO_3$. The solution was kept at 90 °C and the sample (60 mg) dissolved in boiled water (5 ml) was injected with an all-glass syringe into the aqueous solution which was stirred magnetically. The experiments under oxygen pressure (0.4 MPa) were made in a Teflon-lined autoclave under efficient magnetic stirring. Oxygen (10 ml/min determined at 22 °C and atmospheric pressure) was passed through the preheated solution and the sample was introduced as described above. After the reaction times given in the tables the reaction vessels were cooled with ice water.

Analyses. Formic acid was determined in an aliquot of the reaction solution.[10] The other products were analyzed after a group separation on an anion exchange resin in the acetate form. Sugars and other strongly polar non-electrolytes were washed out with water (5 bed volumes). Monocarboxylic acids and "less polar solutes" were eluted with 5 M acetic acid (15 bed volumes). Dicarboxylic acids were eluted with 0.5 M magnesium acetate (4 bed volumes).

The sugars were determined by partition chromatography in 85 % (w/w) ethanol on the sulfate form of an anion exchanger or on a cation exchanger in the lithium form.[16,17] The peak positions and colour responses were compared with those of authentic samples. The results were confirmed by gas chromatography of the oxime – Me_3Si derivatives.[18] The mass spectrum of the major sugar was consistent with that of 3-deoxypentulose. Partition chromatography of the *erythro* and *threo* forms of 3-deoxypentitol obtained after borohydride reduction confirmed the indentity. This method was also employed for confirmation of the identity of the tetroses.

The fraction eluted with 5 M acetic acid was rechromatographed in 0.08 M sodium acetate (acetic acid added to pH 5.9) and in 0.5 M acetic acid on anion exchange columns coupled to a three-channel analyzer.[19] The quantitative determinations of the monocarboxylic acids were made either from these chromatograms or by temperature programmed (80 – 240 °C) gas chromatography of the Me_3Si derivatives on two parallel columns.[20] Perseitol was used as internal standard. Analysis of a solution containing known amounts of gluconic, arabinonic and glyceric acids showed that the response of the detector (FID) was approximately proportional to the weight of the derivatives. This relationship was employed for the determination of the acids obtained in the present work. Final identification was obtained by gas chromatography-mass spectrometry.[18,21]

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Halogenation of Enamines. I. Synthesis of Haloketones from Enamines. α-Halogenated Pinacolones

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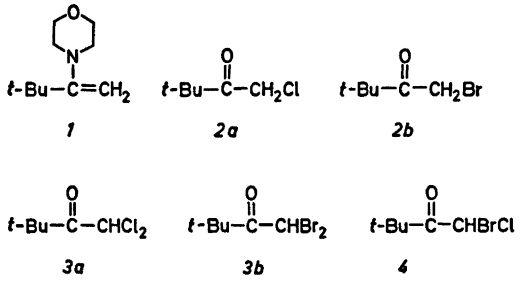
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The reactions of 2-(4-morpholinyl)-3,3-dimethyl-1-butene, *1*, with chlorine and bromine were studied under varying conditions for possible transformations to mono- and dihaloketones. A convenient synthesis of 1-chloro-3,3-dimethyl-2-butanes is described. The usefulness and limitations of the reaction of *1* with halogen for obtaining haloketones are briefly discussed.

The preparation of 1-monohalo- and 1,1-dihalo-3,3-dimethyl-2-butanone, *2a*, *2b*, *3a*, *3b* (Fig. 1) has been reported by several authors.[1–9] These methods include both the direct halogenation of the parent ketone [1–6] and indirect routes.[7–9] *2a* cannot, however, be prepared in good yields from pinacolone using simple procedures. Photochlorination in the liquid phase yielded 23 % of *2a*.[1] Vapour phase chlorination of pinacolone under various conditions has been reported as giving acceptable yields of *2a* (85–88 %).[2,3] However, these methods call for special equipment which is not ordinarily available. *2a* has been obtained indirectly by the action of butyllithium or *N*-piperidyllithium on the lithium 1,1-dichloro-3,3-dimethyl-2-butanolate in yields of 51 and 88 %, respectively.[7] The reaction of diazoketone with hydrogen halide is often used for the synthesis of halomethyl ketones [10] and a yield of 64 % of *2a* was obtained by this method, (see experimental part). The diazoketone method is not an attractive one since it involves the use of excessive amounts of diazomethane.

1-Bromo-1-chloro-3,3-dimethyl-2-butanone, *4*, does not seem to have been reported previously. It can be prepared from *2b* and sulfuryl chloride, (see experimental part).

Enamines can be used for the synthesis of α-haloketones [11–15] and the purpose of the present investigation was to study whether the enamine *1* can be a useful substrate for the preparation of the haloketones shown in Fig. 1. The aim was to determine suitable conditions for simple procedures using cheap and readily available chemicals and glassware. A method for obtaining *2a* was particularly sought since this ketone is rather difficult to obtain, (see above). The synthetic routes studied are shown in Scheme 1.



1 2a 2b

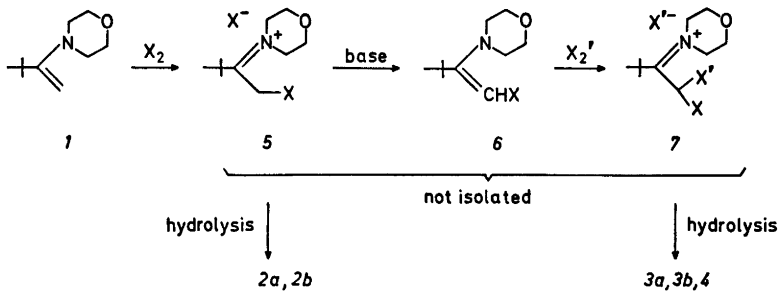
3a 3b 4

Fig. 1.

METHODS

The enamine *1* was obtained from pinacolone, morpholine and titanium(IV) chloride,[16] with modifications given in the experimental part.

The conversion of *1* to the haloenamines *6a* and *6b*, in 50 and 48 % yields, respectively, have been described by Duhamel *et al.*[17] They used the technique of bubbling chlorine diluted with nitrogen, *viz.* adding a solution of bromine to an ethereal solution of *1* at low temperature,



1 5 6 7

2a, 2b 3a, 3b, 4

Scheme 1. Order of halogenation: *a*, X = X′ = Cl; *b*, X = X′ = Br; *c*, X = Cl and X′ = Br; *d*, X = Br and X′ = Cl.

followed by addition of triethylamine. According to our experience, an inverse addition procedure gives better yields, *i.e.* a *rapid* addition of the enamine to a solution of the halogen. The reaction of enamines with halogen is very fast and a thorough and rapid mixing of the reactants is necessary in order to minimize competing side reactions, such as proton abstraction by *1* from the haloimmonium ions *5a* and *5b*.

The syntheses of bromomethyl isopropyl ketone and dibromomethyl isopropyl ketone were recently reported.[14,15] In these preparations (2-(4-morpholinyl)-3-methyl-1-butene was reacted with bromine in methylene chloride at low temperature followed by hydrolysis to afford the bromomethyl ketone, *viz.* reacted consecutively in methylene chloride with bromine, triethylamine and a second portion of bromine followed by hydrolysis to afford the dibromomethyl ketone. In order to investigate whether this technique could be used for the synthesis of the haloketones in Fig. 1 and to determine suitable conditions, the reactions in Scheme 1 were subjected to variations. Some of the experiments are reported in Tables 1 and 2 and some experimental findings are given in the text. Since we were interested in the direct conversion of *1* and, if possible, a one-pot procedure for obtaining *2a*, *2b*, *3a*, *3b* and *4*

Table 1. Synthesis of dihalopinacolones using triethylamine as base. Base treatment 1h, and second halogenation 1 h.

Order of halogenation [a]	Base treatment Temp./°C	Second halogenation Temp./°C	Yield of haloketones % 2a	2b	3a	3b	4	Others
a	−20 [b]	−20 [c]	42		48			−
a	0	0	5		65			2 [d]
a	RT	0	5		54			3 [e]
a	RT	RT	3		41			14 [e]
b	0 [b]	0		17		40		
b	0	0		11		47		
b	0	RT		18		48		
b	RT	0		15		63		
b	RT	RT		21		69		
c	RT	RT					18	57 [f]
c	RT	0					52	27 [f]
c [g]	RT	0					46	38 [f]
c [g]	RT	RT					29	46 [f]
c [h]	0	0					22	59 [f]
d [h]	0	0					10	33 [f]

[a] See Scheme 1. [b] 15 min. [c] 30 min. [d] Pinacolone. [e] 1,1,1-Trichloro-3,3-dimethyl-2-butanone. [f] The distribution of the different haloketones is not shown. [g] Propene was bubbled prior to the second halogenation. [h] Triethyl ammonium bromide was precipitated by trituration with carbon tetrachloride and removed by filtration prior to the second halogenation step.

Table 2. Synthesis of dihalopinacolones using ion-exchange resin Amberlyst A 21 as base.

Order of halogenation [a]	Base treatment Amount of resin g/mmol of *1* [b]	Time h	Yield of haloketones % *3a*	*4*	Others [c]
a	0.2 B	0.5	55		16
a	0.4 B	0.25	63		15
a	0.4 B	1	53		16
a	0.6 B	1	49		19
a	0.6 C	–	49		14
c	0.4 B	0.2		53	26
c	0.6 C	–		50	43
d	0.4 B	0.2		53	46
d	0.6 C	–		16	66

[a] See Scheme 1. [b] B, batch; C, column. [c] The distribution of the individual different haloketones is not shown.

without isolation of the intermediates, methods involving the isolation of these intermediates were not further studied.

Different techniques were tested for effecting the deprotonation of *5* to the corresponding haloenamine *6*. Triethylamine was tested for possible one-pot procedures. The weakly basic ion-exchange resin Amberlyst A 21, with tertiary amino groups as active groups was studied in batch and column operations.

The choice of solvent requires a comment. Chloroform and methylene chloride are useful solvents for the reactions in Scheme 1, since the immonium salts are soluble in these solvents. Methylene chloride was used for bromination reactions and chloroform for chlorinations. The first halogenation step must be performed at low temperature. The freezing point of chloroform imposes a lower limit for chlorinations. Methylene chloride cannot be used for chlorinations since preparing solutions of chlorine in this solvent sometimes leads to a vigorous chlorination of the solvent. This does not occur with chloroform if care is taken to keep the concentration of chlorine low (≤ 0.5 M).

The yields of the haloketones reported in Tables 1 and 2 were determined by gas chromatography using internal standard technique.

RESULTS

Monohaloketones. The preparation of the monohaloketones was best performed by adding *1* to a well-stirred solution of an equivalent amount of the halogen at low temperature, followed by hydrolysis at room temperature. In small scale runs at -65 °C, *2a* was obtained in 75–77 % yield (GC). The amount of *3a* was less than 1 %. Use of higher temperature gave mixtures of *2a* and *3a*. Synthetic scale (0.1 mol) preparation of *2a* gave 72 and 68 % as isolated yields in a duplicate run. The bromoketone *2b* was obtained in 78 % yield by bromination in methylene chloride at -78 °C.

Dihaloketones, triethylamine as base. Some experimental findings not shown in Table 1 ought to be mentioned. The first halogenation was performed at low temperature. In the second halogenation step, greater yields of *3a* were obtained if a solution of chlorine in carbon tetrachloride was added to the chloroenamine *6a*, because a more concentrated solution of chlorine can be prepared in this solvent. A rapid mixing of the reactants is obviously necessary for achieving a good conversion to *3a*. Use of more dilute chlorine in chloroform gave poorer yields. The results given in Table 1 were obtained where carbon tetrachloride was used in the second chlorination step. In the second bromination step a *ca.* 1 M solution of bromine in methylene chloride was used. A dilute solution gave poorer yields and neat bromine occasionally gave tarry products.

A slight excess of triethylamine was used. The amount of base was not varied, since it was found in the analogous preparation of dibromomethyl isopropyl ketone that the use of large excess of base did not improve the yield.[15]

One result shown in Table 1 requires a brief comment. While chlorination at ambient temperature gives some 1,1,1-trichloro-3,3-dimethyl-2-butanone, *8*, (identified by GC-MS, see experimental part) neither the corresponding tribromoketone nor pivalic acid (haloform cleavage hydrolysis) could be detected (GC) from bromination under similar conditions. All attempts at preparing *8* by independent routes failed and it was thus not possible to unambiguously establish the structure of the compound supposed to be *8* by chromatography using pure *8* as reference and for spiking experiments.

Attempts at preparing *4* by sequential introduction of different halogens into *1* have not been successful. The best yield recorded was 52 % and the amount of other haloketones present in the hydrolysate was 27 %. The possibility that the first halogenation had not gone to completion and that remaining halogen might have given unwanted side reactions in subsequent steps was studied by quenching the reaction mixture with propene prior to the addition of base. However, no increase in the yield was found by this technique. A modified technique was tried for reversing the order of halogenation, *viz.* bromination followed by chlorination, which made it necessary to remove bromide ions from the reaction mixture. The removal was performed by precipitation of the triethyl ammonium bromide formed by trituration with carbon tetrachloride. The filtrate was then added to a solution of chlorine. The yield of *4* by this procedure was only 10 % and of other haloketones 33 %. Use of the same technique, but with chlorination followed by bromination, yielded 22 % of *4* and the major part of 59 % was a mixture of other haloketones.

Dihaloketones, ion-exchange resin as base. Since the syntheses of dihalopinacolones were not promising when triethylamine was used, polymer-bound bases were tried. The weakly basic ion-exchange resin Amberlyst A 21 seemed to be a promising alternative to triethylamine in these syntheses. The high content of water, *ca.* 50 % in the commercial product must, however, be reduced (see experimental part.). As Table 2 shows, the use of ion-exchange resin as a heterogeneous base did not give better yields than the use of triethylamine. One exception is noted: When reactions were carried out with bromination prior to chlorination a better yield of *4* was obtained with ion-exchange resin than with triethylamine.

CONCLUSION

The monochloroketone *2a* is conveniently prepared from *1* and since it is not easily and safely prepared by routine procedures this method may be synthetically useful. The haloketones *2b*, *3a* and *3b* are more easily prepared by direct halogenation of pinacolone. *4* is obtained in good yield by chlorination of *2b*. The route to these ketones *via* enamine halogenation do not seem to offer any advantages.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian A-60A instrument. Mass spectra were obtained on an LKB 9000 Mass Spectrometer. GC-analyses were performed on a PYE M 64 Gas Chromatograph with flame ionization detector. The identities of the various ketones present in the reaction mixtures were established by analyses on two different GC-columns: 10 % APM (270 cm, 6 mm o.d.) and 12 % QF-1 (270 cm, 6 mm o.d.) on Chromosorb W AW-DMCS 110–120 mesh. 2,5-Dimethyl-3-hexanone was used as internal standard. Integrated peak areas were used for quantifications. A Houston Instrument integrating recorder was used. Pure samples of authentic ketones were prepared by independent routes and used as references and for calibration of FID-responses.

2-(4-Morpholinyl)-3,3-dimethyl-1-butene *1* was prepared according to White and Weingarten.[16] However, when prepared according to the original directions (room temperature) moderate yields were obtained, despite the extended reaction time of 150–200 h. Greater yields were obtained by refluxing the reaction mixture after the titanium(IV) chloride had been added. A yield of 55 % of *1* was obtained using pentane as solvent and allowing a reaction time of 24 h. When benzene was used as solvent yields of 65–70 % were obtained after 24 h, but the crude reaction product caused severe foaming on distillation. This was not encountered when pentane was used. B.p. 99–101 °C/35 mmHg.

1-Bromo-3,3-dimethyl-2-butanone, *2b*,[4] *1,1-dichloro-3,3-dimethyl-2-butanone*, *3a* [4] and *1,1-dibromo-3,3-dimethyl-2-butanone*, *3b* [6] were prepared by published methods for use as GC-references.

1-Bromo-1-chloro-3,3-dimethyl-2-butanone, *4*. 1.79 g (10 mmol) of *2b* and 0.81 ml of sulfuryl chloride were mixed and stirred at room temperature for 6 h. The temperature was then raised to 55–60 °C and the mixture stirred for

another 3 h. 5 ml of water was added to destroy remaining SO_2Cl_2 and the mixture was stirred at this temperature for 1 h and then allowed to cool to room temperature. 5 ml of methylene chloride was added to dissolve the precipitated ketone. The organic layer was washed three times with distilled water and then dried ($MgSO_4$). Evaporation of the solvent and recrystallisation of the crude product from hexane yielded 1.85 g (85 %) of *4*.

M.p. 60–61 °C. ^{1}H NMR (CCl_4) δ 1.32 (9 H, s) and 6.50 (1 H, s). MS (20 eV) Typical ClBr isotope cluster at *m/e* 212, 214, 216 (0.1 %) (molecule ion), 155, 157, 159 (1.0 %) (M–*t*-Bu) and 127, 129, 131 (0.2 %) (CHClBr). Base peak (100 %) at 57 and 85 (22.4 %) shows the presence of the pivaloyl group.

MS determination of 1,1,1-trichloro-3,3-dimethyl-2-butanone, 8. The presence of *8* in the reaction product was found by GC-MS analysis. No molecule ion was detected even at low ionization potential (14 eV). The base peak (100 %) appearing at *m/e* 57 and a peak at 85 suggest the presence of the pivaloyl group. A typical Cl_3 isotope cluster of low abundance (0.1 %) at 117, 119, 121, 123 is from the CCl_3 fragment and a similar cluster at 187, 189, 191, 193 is from the ($M-CH_3$) ion.

General procedure for halogenation of 1. Monohalogenation. To a cooled (–65 °C, $CHCl_3$ or –78 °C, CH_2Cl_2) solution of the halogen (0.5 M) was rapidly added, with vigorous stirring 1.69 g (10 mmol) of *1* in 20 ml of solvent. Stirring was continued for 5 min, then the cooling bath was replaced by a room temperature water bath, and 20 ml of water and 5 ml of 2 % $NaHSO_3$ were introduced. The mixture was stirred for 1 h to complete hydrolysis. The layers were separated and the aqueous layer extracted with 5 ml of CH_2Cl_2. The combined organic layers were transferred to a volumetric flask and the volume adjusted to 50 ml. To an aliquot was added a known amount of 2,5-dimethyl-3-hexanone and the sample analyzed by GC. The yields given are the averages of two analyses by GC.

Dihalogenation, triethylamine as base. The first halogenation was performed as for monohalogenation. After 5 min the cooling bath was replaced by an ice bath and 1.40 g of triethylamine in 5 ml of solvent was added in one portion with vigorous stirring. After the time given in Table 1 15 ml of 1 M solution of the halogen was added in one portion with vigorous stirring. After 1 h 20 ml of distilled water was introduced, followed by a careful addition of just enough 5 % $NaHSO_3$ solution to cause complete decolourisation of the halogen. The mixture was stirred for 1 h. The aqueous layer was extracted with 10 ml of CH_2Cl_2 and the combined organic layers were adjusted to 100 ml and analyzed as above.

Dihalogenation, Amberlyst A 21 ion-exchange resin as base. First halogenation as above, but 5 mmol of *1* was used.

A, Batch procedure. The resulting reaction mixture after the first halogenation was warmed to 0 °C and the amount of dried (see below) ion-exchange resin given in Table 2 was added. The mixture was stirred magnetically for the time given in Table 2. The resin was removed by filtration and washed three times with 3 ml of solvent. The filtrate was added in one amount to a well-stirred solution of the halogen (50 % excess) at –50 °C. The reaction mixture was stirred with no external cooling for 30 min and at room temperature (water bath) for 30 min. Hydrolysis and GC-analysis as above.

B, Column procedure. The reaction mixture from the first halogenation was slowly passed through a column (10 mm id) filled with 3.0 g of ion-exchange resin in the solvent used. This gave *ca.* 15 cm of bed height. The column was eluted with 20 ml of solvent. The second halogenation, hydrolysis and analysis were performed as for batch procedure.

Drying of Amberlyst A 21. Mere drying *in vacuo* over phosphorus(V) oxide was unsatisfactory, a more laborious drying procedure was necessary: The moist resin was extracted in a Soxhlet extractor for 24 h with ethanol refluxing from calcium oxide heated to 900 °C for 12 h in an oven prior to use. Extraction with ethanol was repeated once. The final drying was performed by extracting with ethanol boiling from a solution of sodium ethoxide in diethyl phthalate.[18] During this process good stirring was necessary to prevent bumping. The resin was free from ethanol by evaporation at 100 °C/10 mmHg for 6 h and at 1 mmHg for 10 h. The weight loss of the resin by this procedure was 45–48 %. The dried resin was stored in vacuum over phosphorus(V) oxide.

Synthesis of 1-chloro-3,3-dimethyl-2-butanone, 2a. From diazoketone. The reaction was performed in a magnetically stirred 3 l Erlenmeyer flask. Diazomethane (0.60 mol) was prepared in ethereal solution according to *Organic Syntheses* [19] and dried over solid KOH overnight. The dried diazomethane solution was used without purification. Pivaloyl chloride (Fluka, pract.) 24.1 g (0.20 mol) in 100 ml of dry ether, was added dropwise with stirring to the diazomethane solution at 0 °C over a period of 1.5 h followed by stirring at room temperature for 2 h. Dry hydrogen chloride was then bubbled through the solution. The yellow colour from the diazoketone disappeared in a few minutes. 200 ml of water was added to destroy unreacted pivaloyl chloride. The ethereal layer was washed three times with 100 ml of 5 % $NaHCO_3$ and finally with sat. NaCl. Drying ($MgSO_4$) and evaporation of ether yielded 17.2 g of *2a* (64 %). The purity of the crude product was 95 % (GC, ^{1}HNMR). Distillation yielded 14.1 g (52 %) of pure (> 99 %) *2a*.

B.p. 83.5–85 °C/35 mmHg. 2,4-Dinitrophenyl hydrazone m.p. 142.5–143 °C (lit.[3] 143–144 °C). ^{1}HNMR (CCl_4) δ 1.33 (9 H, s) and 4.40 (2 H, s).

From the enamine. To a well-stirred solution of 7.1 g of chlorine in 200 ml of chloroform at −65 °C was added in one portion 16.9 g of *1* (0.10 mol) in 40 ml of chloroform. After 5 min the cooling bath was replaced by a room temperature water bath, and 200 ml of water and 10 ml of 2 % $NaHSO_3$ was added. Stirring of the mixture was continued for 1 h. The aqueous layer was extracted with 50 ml of chloroform. The combined organic layers were washed twice with 50 ml of water and finally with sat. NaCl. The chloroform was removed by distillation with a short column. By this procedure residual water is removed by azeotropic distillation. The residue from this distillation was distilled under reduced pressure and yielded 9.46 g (72 %) of *2a*. A high boiling residue remained in the distillation flask. TLC-analysis showed a complex mixture of at least 11 products. The nature of these were not elucidated.

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The Basicity of 2,3,4,5,6-Pentafluorodiphenylamine. The Use of ^{19}F NMR Chemical Shifts in Protonation Studies

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The pK_{BH^+} of 2,3,4,5,6-pentafluorodiphenylamine, as determined by use of UV spectroscopy in aqueous methanesulfonic acid solutions, is -4.35 ± 0.13. The use of ^{19}F NMR spectroscopy for this purpose was less satisfactory because the chemical shifts appeared to be subject to solvent effects in highly concentrated acid solutions.

A variety of techniques (including both spectrometric and electrochemical methods) have been used to determine the extent of protonation of organic compounds.[1-3] In general, it appears that UV-VIS spectroscopy is the most reliable method for compounds that contain a suitable chromophore. Hence the extent of protonation of almost all aromatic compounds may be determined by this method; however, aliphatic compounds which often lack suitable chromophores may more easily be studied by the use of NMR spectroscopy.[4] In this paper we report on the determination of the basicity of 2,3,4,5,6-pentafluorodiphenylamine (*1*) by use of UV spectroscopy and describe some interesting results that were obtained when an attempt was made to use ^{19}F NMR spectroscopy for the same purpose.

EXPERIMENTAL

2,3,4,5,6-Pentafluorodiphenylamine. This compound was prepared as previously described.[5]

Methanesulfonic acid solutions. Commercially available methanesulfonic acid was dried over phosphorus pentoxide and vacuum distilled (b.p. 115–116 °C/0.07 kPa) under a nitrogen atmosphere.[6] Portions of this acid were then diluted with distilled water and standardized by titration against sodium hydroxide.

Determination of the pK_{BH^+} value of 2,3,4,5,6-pentafluorodiphenylamine by UV spectroscopy. 2,3,4,5,6-Pentafluorodiphenylamine (0.136 g) was dissolved in 25.0 ml of acetonitrile. A portion of this solution (16 μl) was transferred to a volumetric flask containing 5.0 ml of standardized methanesulfonic acid. Three ml of the acid solution was then transferred to a 1.00 cm cell which was placed in the thermostated (25.0 °C) cell compartment of a Unicam SP500 spectrophotometer. The maximum absorbance in the region of 260–268 nm was recorded and tabulated (Table 1). The wavelength of the

Table 1. Absorbance data for 0.0210 M 2,3,4,5,6-pentafluorodiphenylamine in aqueous solutions of methanesulfonic acid.

Methanesulfonic acid/wt. %	$-H_0$[a]	Absorbance	log I[b]
49.2	1.96	0.717	
51.1	2.02	0.712	
57.5	2.53	0.706	
60.1	2.72	0.695	
66.0	3.22	0.666	−1.07
69.4	3.54	0.616	−0.74
71.8	3.77	0.568	−0.53
74.5	4.04	0.489	−0.27
76.2	4.16	0.420	−0.08
78.2	4.32	0.341	0.14
80.0	4.53	0.274	0.33
81.8	4.81	0.204	0.57
84.0	5.18	0.147	0.85
89.0	6.13	0.085	
93.2	6.92	0.068	
96.5	7.52	0.068	
98.6	7.74	0.066	

[a] Bascombe, K. N. and Bell, R. P. *J. Chem. Soc.* (1959) 1096. [b] $I = [BH^+]/[B] = (0.717 - \text{Absorbance})/(\text{Absorbance} - 0.066)$.

Table 2. ^{19}F NMR chemical shift data for 2,3,4,5,6-pentafluorodiphenylamine in aqueous solutions of methanesulfonic acid.

Methanesulfonic acid/wt. %	$-H_0$[a]	δ *meta*–δ *ortho*/ ppm	δ *meta*–δ *para*/ ppm	log *I*
46.2	1.75	13.93	0	
50.0	2.00	13.77	0	
62.4	2.90	13.72	0	
64.6	3.10	13.73	0	
69.7	3.57	13.78	1.36	−0.77
74.3	4.00	13.26	2.94	−0.34
76.2	4.16	12.99	4.07	−0.12
78.2	4.32	12.77	4.88	0.03
80.1	4.52	12.53	5.86	0.22
81.1	4.66	12.42	6.46	0.34
82.9	4.98	12.20	7.21	0.52
84.2	5.20	12.11	7.62	0.63
85.0	5.34	12.00	8.08	0.79
87.5	5.84	11.87	8.51	
90.4	6.40	11.45	8.87	
93.7	7.04	11.68	9.17	
97.0	7.56	11.62	9.24	
101.0	7.92	11.54	9.34	
101.0	7.92	11.51	9.30	

[a] Bascombe, K. N. and Bell, R. P. *J. Chem. Soc.* (1959) 1096.

maximum found at 268 nm in 50 % methanesulfonic acid decreased with increasing acid concentration until it was 260 nm in 98 % methanesulfonic acid.

Attempted use of ^{19}F NMR for studying the basicity of 2,3,4,5,6-pentafluorodiphenylamine. A stock solution of *1* in dimethyl sulfoxide (DMSO) was prepared. Twenty μl of the solution was added to 1.0 ml of standardized methanesulfonic acid and the ^{19}F spectrum obtained using a BRUKER HX-90 NMR spectrometer. Since we were interested only in the relative differences in chemical shifts, no internal standard was added. Instead the chemical shifts of the *ortho*- and *para*-fluorines were measured relative to those of the *meta*-fluorines. The results presented in Table 2 were obtained.

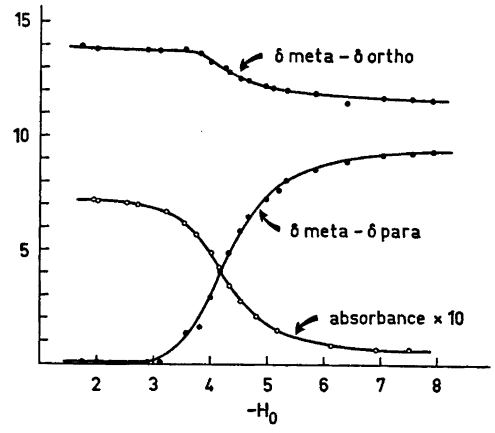


Fig. 1. Titration curves for the protonation of *1* in methanesulfonic acid.

RESULTS

The results obtained are summarized in Tables 1 and 2. That the observed changes correspond to a protonation reaction can be seen from the sigmoidal curves found in Fig. 1. The inflection points for all of these curves are at about −4.4 thus confirming that the changes in the ultraviolet spectrum and in the chemical shifts are likely due to the same cause.

DISCUSSION

Determination of the basicity of 1. The protonation of a weak organic base may be described in terms of eqns. (1) and (2) where log I = log ([BH$^+$]/[B]) and H_x is an acidity function

$$BH^+ \rightleftharpoons^{K_{BH^+}} B + H^+ \quad (1)$$

$$pK_{BH^+} = H_x + \log I \quad (2)$$

that is appropriate for the base under investigation.[3] The applicability of a particular acidity function is most easily evaluated by plotting log I *vs.* $-H_x$; the observance of a linear plot of near unit slope may be taken as an indication that a satisfactory function has been found. In the case of *1* the H_0 acidity function appears to be quite satisfactory (see Fig. 2). This is not surprising since the H_0 function was developed by use of aromatic amine indicators [7] and subsequent work has shown that substituted diphenylamines conform to this function in their protonation behaviour.[8] Furthermore, Yates and Shapiro [9] have shown that the function is also applicable to fluorinated anilines.

The pK_{BH^+} value obtained from a statistical analysis of the plot presented in Fig. 2 is -4.35 ± 0.13. Such a result is not unreasonable when comparison is made with the basicities of other diphenylamines.[8]

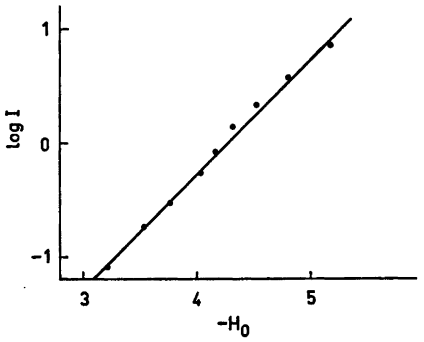


Fig. 2. Plot of log I *vs.* $-H_0$ based on ultraviolet measurements. Slope = 1.02 ± 0.03. Intercept = 4.43 ± 0.13.

Further evidence for the reliability of this result comes from a consideration of the differences in basicities between substituted anilines and the corresponding substituted diphenylamines. As the data presented in Table 3 indicate the diphenylamines are approximately 4 units less basic; *i.e.*, introduction of a phenyl group decreases the pK_{BH^+} values by about 4 acidity units. Since the pK_{BH^+} of pentafluoroaniline is known [9] to be -0.36 ± 0.02 it is consistent to find that the value for 2,3,4,5,6-pentafluorodiphenylamine is -4.35 ± 0.13. Consequently it appears as if this result may be accepted with considerable confidence.

Table 3. Comparison of pK_{BH^+} values of substituted anilines and the corresponding diphenylamines.

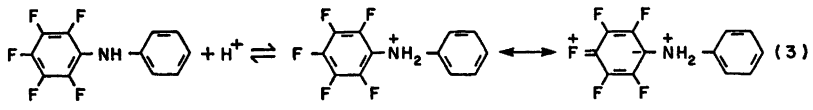
Substituent	Aniline	Diphenylamine	Difference
4-Methyl	5.08 [a]	1.20 [c]	3.88
4-Methoxy	5.34 [a]	1.36 [c]	3.98
3-Methoxy	4.23 [a]	0.40 [c]	3.83
None	4.63 [a]	0.78 [c]	3.85
3-Chloro	3.46 [a]	−0.45 [c]	3.91
4-Chloro	4.15 [c]	0.01 [c]	4.14
3-Nitro	2.50 [e]	−1.61 [c]	4.11
4-Nitro	1.02 [e]	−3.13 [c]	4.12
2-Nitro	−0.29 [e]	−4.12 [c]	3.83
Pentafluoro-	−0.36 [b]	−4.35 [d]	3.99
		Average	3.96 ± 0.12

[a] *Handbook of Chemistry and Physics*, Forty-ninth Ed., The Chemical Rubber Publishing Co., Cleveland 1969, p. D-87. [b] Yates, K. and Shapiro, S. A. *Can. J. Chem. 50* (1972) 581. [c] Dolman, D. and Stewart, R. *Can. J. Chem. 45* (1967) 903. [d] This work. [e] Bascombe, K. N. and Bell, R. P. *J. Chem. Soc.* (1959) 1096.

The use of ^{19}F NMR spectroscopy for basicity determinations. Several examples are known where both ^1H [4] and ^{13}C NMR spectroscopy [10] have been used for basicity measurements. In order to further expose the use of this potentially important technique the ^{19}F NMR spectra of *I* in various methanesulfonic acid solutions have been examined and an attempt has been made to use the chemical shifts for basicity measurements.

In order to minimize solvent effects on the chemical shifts no internal standard was used. Instead the chemical shifts of the *ortho*- and *para*-fluorines were compared with those for the *meta*-fluorines (Table 1). This method rests on the assumption that the chemical shifts of different fluorines will be affected differently by protonation and that these variations can be taken as a measure of the extent of protonation. Since all of the atoms in a simple molecule will be in approximately the same environment macroscopic solvent effects on the chemical shifts should be minimized.

All of the fluorine atoms would be expected to exert a substantive electron acceptor polar effect (I), thus accounting for the observed decrease in basicity.[11] However, as indicated by eqn. (3), some of the fluorines could also exert an electron donor π delocalization effect (R) which would stabilize the positive charge on the conjugate acid and thus tend to increase the basicity of these compounds. Pushkina, Stepanov, Zhukov and Naumov [12] have developed correlation equations between fluorine shifts and the Taft [13] constants for *ortho*-, *para*- and *meta*-fluorines in pentafluorophenyl amines (eqns. 4–6). Similar correlations have also been observed by other workers.[14]



$$\delta(ortho) = 73.82\sigma_I - 24.35\sigma_R^\circ - 177.63 \quad (4)$$

$$\delta(meta) = 17.51\sigma_I - 4.73\sigma_R^\circ - 169.66 \quad (5)$$

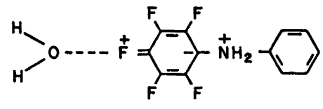
$$\delta(para) = 86.65\sigma_I - 22.30\sigma_R^\circ - 189.89 \quad (6)$$

The fact that the coefficient for σ_R° is much greater in eqn. (6) than in eqn. (5) indicates that the π delocalization effect is more pronounced for the *para*- than the *meta*-substituents. The correlation coefficient for eqn. (4) was unsatisfactory probably because the *ortho*-position is subject to large steric effects.[12] Consequently the differences between the chemical shifts of the *para*- and the *meta*-fluorines were used in this study.

Examination of the spectra indicated that the *para*-fluorines did indeed shift relative to the *meta*-fluorines as the compound underwent protonation (Table 2, Fig. 1). It was then possible to calculate values of log I as has been done with proton spectra [4] ($\log I = [BH^+]/[B] = (\Delta - \Delta_B)/(\Delta_{BH^+} - \Delta)$ where Δ_B is the chemical shift difference between the *meta*- and *para*-fluorines of the unprotonated amine, Δ_{BH^+} is the difference between the chemical shifts of the same fluorines in the completely protonated amine, and Δ the corresponding value when the amine is partially protonated.

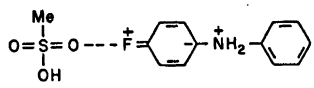
A plot of log I *vs.* $-H_0$ (Fig. 3) proved to be linear with an approximately unit slope up to about 85 % methanesulfonic acid, whereupon it began to exhibit a definite curvature. Nevertheless the intercept (which fell on the linear portion of the curve) gave an accurate estimate of the pK_{BH^+} value (-4.45 ± 0.11).

The curvature of the plot in Fig. 3 begins at a point where the mol ratio of methanesulfonic acid to water is approximately unity. This suggests that the change in slope at higher acidities may be connected with a decrease in the amount of water available for solvation (assuming that each molecule of acid is strongly solvated by one molecule of water as long as sufficient solvent is available). The change in slope of Fig. 3 may thus be associated with a loss of hydrogen bonding to the *meta*-fluorines as the amount of available water decreases. Alternatively it is possible that at lower acidities the *para*-fluorine is solvated extensively by water (as in *2*) which would not



2

be available at higher acidities. Solvation would then be absent or by methanesulfonic acid as in *3*. Such a change in solvation would likely



3

affect the ^{19}F chemical shifts and could be an explanation for the non-linearity of the plot in Fig. 3.

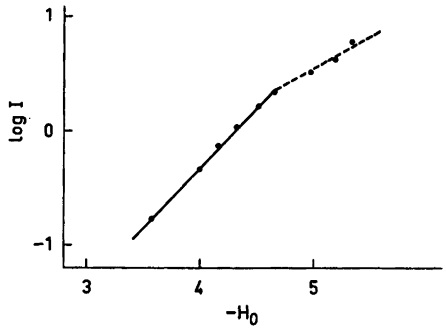


Fig. 3. Plot of log I *vs.* $-H_0$ based on ^{19}F NMR measurements.

In summary it has been found that (1) the pK_{BH^+} value for 2,3,4,5,6-pentafluorodiphenylamine is -4.35 ± 0.13, (2) the H_0 acidity function adequately describes the protonation behaviour of 2,3,4,5,6-pentafluorodiphenylamine and (3) ^{19}F NMR chemical shifts, while providing a good estimate of the pK_{BH^+} value, are apparently sensitive to solvation effects.

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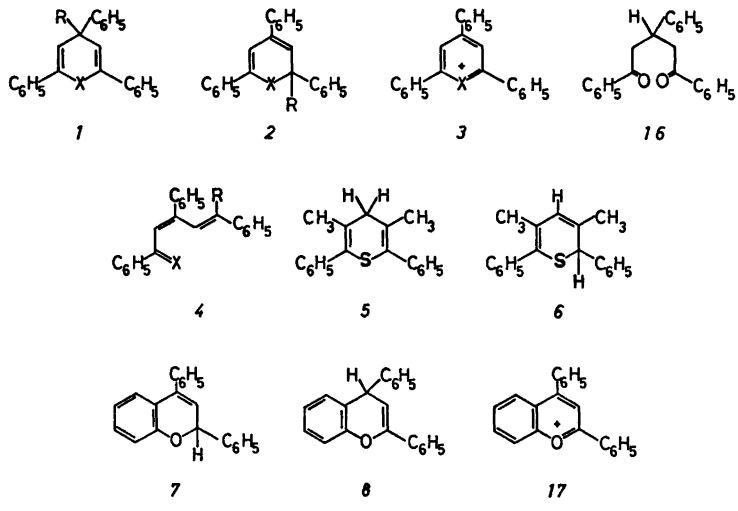
Intermolecular Hydride Transfer Reactions. VIII. Isomerisation of 2*H*- and 4*H*-Pyran Derivatives in Acetic Acid

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Isomerisation of 2*H*- and 4*H*-pyran derivatives in the presence of pyrylium cations has been studied. 2,4,6-Triphenyl-4*H*-thiopyran was quantitatively converted to the 2*H*-thiopyran in the presence of 2,4,6-triphenylthiopyrylium perchlorate in acetonitrile solution. The isomerisation was also obtained from treatment of the 4*H*-thiopyran with acetic acid. The 2,4,6-triphenylthiopyrylium cation was formed under the latter conditions. Isomerisation of 2,4,6-triphenyl-4*H*-pyran and 2,4-diphenylbenzo-2*H*-pyran to 1,3,5-triphenylpenta-2,4-dienone and 2,4-diphenylbenzo-4*H*-pyran, respectively, took place in boiling acetic acid. The mechanisms of these isomerisations are discussed.

Recently we reported [1] that interconversions of 2*H*- and 4*H*-pyran derivatives are catalysed by the corresponding pyrylium cation. Thus, 2,4,6-triphenyl-4*H*-pyran (*1a*, X = O) was isomerised to the 2*H*-pyran *2a* (X = O) in the presence of 2,4,6-triphenylpyrylium perchlorate (*3*, X = O) in acetonitrile solution. This isomerisation takes place through an intermolecular hydride transfer reaction (Scheme 1, and 2D). The 2*H*-pyran *2a* (X = O) is further con-



Scheme 1.

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verted to the open chain dienone structure *4* (X=O) through valence isomerisation. Earlier, the isomerisation of 3,5-dimethyl-2,6-diphenyl-4*H*-thiopyran (*5*) to the corresponding 2*H*-thiopyran *6* in acetic acid/HCl mixture was reported.[2] Intramolecular hydride transfer is suggested for this isomerisation (Scheme 1, and 2B). Oxidation of 2,4,6-triphenyl-4*H*-thiopyran (*1a*, X=S) to the pyrylium cation *3* (X=S) was observed in acetic acid/HCl solution.[2] However, formation of neither a pyrylium cation in addition to the isomerisation product nor an isomerisation product in addition to the oxidation product, was reported from reaction of *5* and *1a* (X=S), respectively, in acetic acid/HCl solution.

The purpose of the present work is to achieve isomerisation of 2*H*- and 4*H*-pyrans under the influence of acids with concurrent acid induced oxidation of the pyrans to pyrylium cations.

RESULTS

2,4,6-Triphenylthiopyrylium perchlorate (*3*, X=S) was quantitatively reduced with $NaBH_4$ to a 1:1 mixture of 2,4,6-triphenyl-4*H*-thiopyran (*1a*, X=S) and 2,4,6-triphenyl-2*H*-thiopyran (*2a*, X=S) in acetonitrile solution. Reduction with $NaBD_4$ afforded the γ-deuterated 4*H*-thiopyran *1b* (X=S) and the α-deuterated 2*H*-thiopyran *2b* (X=S).

The 4*H*-thiopyran *1a* (X=S) was isomerised to the 2*H*-thiopyran *2a* (X=S) in boiling acetic acid solution. Similar isomerisation of the γ-deuterated 4*H*-thiopyran *1b* (X=S) afforded exclusively the α-deuterated 2*H*-thiopyran *2b* (X=S).

A small amount of 2,4,6-triphenylthiopyrylium cation (*3*, X=S) was isolated as the perchlorate salt after prolonged boiling of *1a* (X=S) in acetic acid solution.

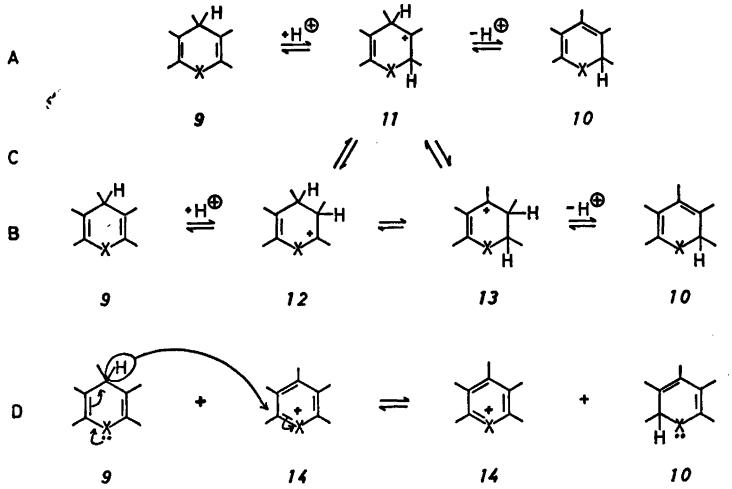
Isomerisation of the 4*H*-thiopyran *1a* (X=S) to the 2*H*-thiopyran *2a* (X=S) was not achieved in boiling acetonitrile solution. However, addition of the thiopyrylium perchlorate *3* (X=S) resulted in complete isomerisation. The thiopyrylium salt *3* (X=S) was recovered unchanged.

The rate of isomerisation of the 4*H*-thiopyran *1a* (X=S) in acetic acid solution was increased upon addition of the thiopyrylium salt *3* (X=S).

2,4,6-Triphenyl-4*H*-pyran *1a* (X=O) was isomerised to 1,3,5-triphenylpenta-2,4-dienone *4a* (X=O) in boiling acetic acid 1,3,5-Triphenylpenta-1,5-dione was observed as a by-product. 2,4-Diphenylbenzo-2*H*-pyran (*7*) was isomerised to 2,4-diphenylbenzo-4*H*-pyran (*8*) under similar conditions (Scheme 1).

DISCUSSION

Four mechanisms can be put forward to explain the isomerisation of 4*H*-pyrans *9* to 2*H*-pyrans *10* (and *vice versa*) (Scheme 2).



Scheme 2.

Acid catalysed isomerisation

A. Protonation at the α position of the 4*H*-pyran *9* with formation of the carbonium ion *11* which splits off the γ proton could give the 2*H*-pyran *10*.

B. Protonation at the β position of the 4*H*-pyran *9* with formation of the carbonium ion *12* and subsequent rearrangement (1,3 hydride shift) of *12* to the carbonium ion *13* which splits off the β proton could give *10*.

C. 1,2-Hydride shift in *11* to form *13* or two successive 1,2-hydride shifts in *12* to form *11* and *13* could give *10*.

Pyrylium cation catalysed isomerisation

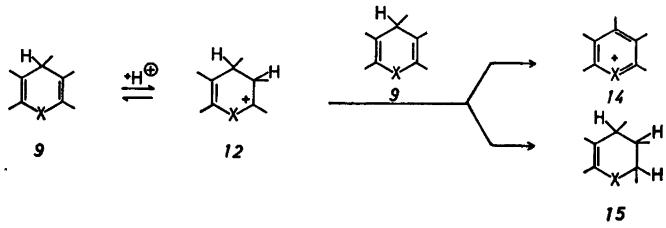
D. Intermolecular hydride transfer from the γ position of the 4*H*-pyran *9* to the α position of the pyrylium cation *14* could regenerate the pyrylium cation *14* and give the 2*H*-pyran 10.

Since the γ-deuterated 4*H*-thiopyran *1b* (X=S) afforded only the α-deuterated 2*H*-thiopyran *2b* (X=S) in boiling acetic acid, mechanisms A and C can be neglected. Deuterium/proton exchange with formation of the α-undeuterated 2*H*-pyran *2a* (X=S) in detectable amount (^{1}H NMR) would have been expected if these mechanisms were important. The isomerisation of the 4*H*-thiopyrans *1* (X=S) to the 2*H*-thiopyrans *2* (X=S) in acetonitrile solution in the presence of 2,4,6-triphenylthiopyrylium perchlorate (*3*, X=S) demonstrates that isomerisation is possible through mechanism D. Isolation of the thiopyrylium cation *3* (X=S) as the perchlorate salt (3 %) after prolonged boiling of the starting 4*H*-thiopyran *1a* (X=S) in acetic acid shows that the catalyst with respect to mechanism D, is formed under the condition of isomerisation.

Following the isomerisation of the 4*H*-thiopyran *1a* (X=S) in acetic acid (^{1}H NMR) it was observed that the rate was considerably increased after addition of a small quantity of the thiopyrylium perchlorate *3* (X=S).

A tentative explanation can be suggested to account for the formation of the pyrylium cation (*3*, X=S) from the 4*H*-thiopyran *1a* (X=S) in acetic acid solution (Scheme 3). Pyran derivatives *9* undergo acid induced disproportionation to give the corresponding pyrylium cations *14* and reduced pyran derivatives *15*.[3–5] The acids used for these purposes have been much stronger than acetic acid (HCl/CH_3COOH, $HClO_4/CH_3COOH$, CF_3COOH). Under such conditions the disproportionation is usually very fast and has resulted in isolation of pyrylium salts *14* in high yields. It seems likely that the rate of disproportionation is influenced by the equilibrium position of the protonation step (Scheme 3). The rate in weak acid may be slow due to a low concentration of the cation *12* which acts as a hydride acceptor in the redox step. The existence of an equilibrium between *9* and *12* was supported by ^{1}H NMR analysis of the products from treatment of the 4*H*-thiopyran *1a* (X=S) with deuterioacetic acid, which suggested a high degree of deuterium incorporation in the β positions. In acetic acid the disproportionation of *1a* (X=S) to *3* (X=S) seems to be slow, so slow that a concurrent isomerisation of *1a* (X=S) to *2a* (X=S) catalysed by *3* (X=S) can be observed.

Isomerisation of the 2,4,6-triphenyl-4*H*-pyran (*1a*, X=O) in boiling acetic acid solution resulted in isolation of the 1,3,5-triphenylpenta-2,4-dienone *4a* (X=O) (Scheme 1). In addition, the 1,3,5-triphenylpenta-1,5-dione (*16*) was observed as a byproduct. Formation of the latter compound can be explained from acid catalysed hydrolysis of the 4*H*-pyran *1a*



Scheme 3.

(X=O), due to the small concentration of water in the acetic acid.

Treatment of the 2,4-diphenylbenzo-2*H*-pyran (*7*) with acetic acid yielded the 2,4-diphenylbenzo-4*H*-pyran (*8*). The formation of *4a* (X=O) and *8* through isomerisation of *1a* (X=O) and *7*, respectively, is in agreement with the reported relative stabilities of the isomers.[1] It should be mentioned that the dienone *4a* (X=O) is supposed to be formed through valence isomerisation of the 2*H*-pyran *2a* (X=O). In this connection it is interesting to note that the dienone isomer *4a* is more stable than the 2*H*-pyran *2a* at 25 °C when X=O and that the relative stability seems to be reversed when X=S. Preliminary results (^{1}H NMR) from heating of the 2*H*-thiopyran *4a* (X=S) in neutral solution indicate ring cleavage above 90 °C.

EXPERIMENTAL

Reduction of 2,4,6-triphenylthiopyrylium perchlorate (*3*, X=S).

$NaBH_4$ (3.6 g, 0.096 mol) was gradually added (0.5 h) during stirring to a solution of 2,4,6-triphenylthiopyrylium perchlorate [6,7] (*3*, X=S) (10.0 g, 0.024 mol) in dry acetonitrile solution (300 ml). The reaction mixture was concentrated, added dry ether and filtered. The ethereal solution was extracted with water, dried and evaporated. The residual oil (7.5 g) was examined by ^{1}H NMR (see Table 1). Crystallisation from methanol gave pure 2,4,6-triphenyl-4*H*-thiopyran (*1a*, X=S). Yield 3.7 g (48 %): m.p. 110 °C:[8] ^{1}H NMR (60 MHz, $CDCl_3$):[9] δ 4.5 (1 H, *t*), 6.0 (2 H, *d*, *J* 4.0 Hz), 7.3 (15 H, *m*).

Similar reduction of *3* (X=S) with $NaBD_4$ gave 4-deuterio-2,4,6-triphenyl-4*H*-thiopyran (*1b*, X=S); m.p. 109 °C: ^{1}H NMR (60 MHz, $CDCl_3$): δ 6.0 (2 H, *s*), 7.3 (15 H, *m*).

Isomerisation of 2*H*- and 4*H*-pyrans in acetonitrile solution.

The reported method [1] for isomerisation of pyran derivatives catalysed by pyrylium salts was used.

2,4,6-Triphenyl-4H-thiopyran (*1a*, X=S) was quantitatively isomerised to 2,4,6-triphenyl-2*H*-thiopyran (*2a*, X=S). The product was obtained as an oil and was identified by ^{1}H NMR.[9] ^{1}H NMR (60 MHz, $CDCl_3$: δ 4.97 (1 H, *d*), 6.0 (1 H, *d*, *J* 6 Hz), 6.93 (1 H, *s*), 7.3 (15 H, *m*).

Table 1. Preparation and isomerisation of 2*H*- and 4*H*-pyrans. Reaction period 12 h, reflux.

Hetero atom	Reactants (1:1)	Products	
Solvent CH_3CN			
S	*3*, $NaBH_4$ [a,b]	*1a*, *2a*	(1:1)
S	*3*, $NaBD_4$ [b]	*1b*, *2b*	(1:1)
S	*3*, *1a*	*3*, *2a*	(1:1)
S	*3*, *1b*	*3*, *2b*	(1:1)
S	*1a*	*1a* [d]	
O	*3*, *1a*	*3* *4a*	(1:1)
O	*17*, *7*	*17*, *8*	(1:1)
Solvent CH_3CO_2H			
S	*1a*	*2a*	
S	*1b*	*2b*	
S	*1a* [c]	*3* [d], *2a*	
S	*1a* [e]	*1a*, *2a*	(1:2)
S	*3*, *1a* [e,f]	*1a*, *2a*	(1:9)
O	*1a*	*16*, *2a*	(1:3)
O	*7* [g]	*8*	

[a] Excess. [b] 20 °C, 1 h. [c] 150 h. [d] 3 %. [e] 30 min. [f] 1:25. [g] 75 h.

4-Deuterio-2,4,6-triphenyl-4H-thiopyran (*1b*, X=S) yielded 2-deuterio-2,4,6-triphenyl-2*H*-thiopyran (*2b*, X=S). ^{1}H NMR (60 MHz, $CDCl_3$): δ 6.0 (1 H, *s*), 6.93 (1 H, *s*), 7.3 (15 H, *m*).

Isomerisation of 2,4,6-triphenyl-4*H*-pyran (*1a*, X=O) and 2,4-diphenylbenzo-2*H*-pyran (*7*) by means of pyrylium catalysis has been reported earlier.[1] The results are included in Table 1.

Isomerisation of 2*H*- and 4*H*-pyrans in acetic acid solution.

2,4,6-Triphenyl-4*H*-thiopyran (*1a*, X=S) (0.96 g, 0.003 mol) in acetic acid solution (20 ml) was refluxed for 12 h. The solution was evaporated and the residue was examined by ^{1}H NMR. Only signals due to the 2*H*-thiopyran (*2b*, X=S) could be detected.

A similar solution of *1a* (X=S) was refluxed for 6 days. Addition of a hot saturated solution of $KClO_4$ in H_2O afforded a crystalline material after cooling. The precipitated material was collected, washed with hot water and recrystallised from acetic acid to yield 2,4,6-triphenylthiopyrylium perchlorate (*3*, X=S). Yield 3 %, m.p. 213 °C.

Two identical solutions of (*1a*, X=S) in acetic acid were prepared. To one of them was added the thiopyrylium perchlorate *3* (X=S) in molar ratio; *3*:*1a*=25:1. The solutions were refluxed for 0.5 h, evaporated and residues

were examined by ^{1}H NMR ($CDCl_3$). Results see Table 1.

*4-Deuterio-2,4,6-triphenyl-4*H*-thiopyran* (*1b*, X=S) (0.14 g) in [^{1}H]acetic acid solution (20 ml) was refluxed for 12 h. The solution was evaporated and the residue examined by ^{1}H NMR ($CDCl_3$) Only signals due to the 2-deuterio-2,4,6-triphenyl-2*H*-thiopyran (*2b*, X=S) were observed. No signals at δ 4.97 due to α protons could be detected.

*2,4,6-Triphenyl-4*H*-pyran* (*1a*, X=O)[1] (0.8 g, 0.0025 mol) in acetic acid solution (20 ml) was refluxed for 12 h. The solution was evaporated and the residue examined by ^{1}H NMR (CD_3COCD_3). Signals due to the 4*H*-pyran *1a* (X=O) could not be detected. All signals could be attributed to a mixture of 2,3,5-triphenylpenta-2,4-dienone *4a* (X=O)[1] and 1,3,5-triphenylpenta-1,5-dione (*16*). The former compound was isolated as a crystalline product on treatment of the residue obtained from the ^{1}H NMR sample with methanol. Yield 0.5 g, 62 %; m.p. 125 °C. Authentic 1,3,5-triphenylpenta-1,5-dione (*16*) (m.p. 85 °C)[10] was prepared for identification of this compound in the mixture mentioned above. ^{1}H NMR ($CDCl_3$): δ 3.31, 3.49, 4.07; AA'BB'X: J_{AB} −15 Hz, $J_{AX}=J_{BX}$ 7 Hz.

*2,4-Diphenylbenzo-2*H*-pyran* (*7*)[11] (0.9 g, 0.003 mol) in acetic acid solution was refluxed for 3 days. The solution was evaporated and the residue examined by ^{1}H NMR ($CDCl_3$). Only signals due to the 2,4-diphenylbenzo-4*H*-pyran (*8*)[12] could be detected. The 4*H*-pyran *8* was isolated as a crystalline product on treatment of the residue obtained from the ^{1}H NMR sample, with ethanol. Yield 0.72 g; 78 %; m.p. 109 °C.

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Lignin Model Compounds. I. The Synthesis of Glyceraldehyde 2-Aryl Ethers

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Two improved synthetic routes to the title compounds are described. The reaction of a substituted phenolate ion with 2-bromo-3-buten-1-ol (or its acetate, or the acetate quaternised with *N*,*N*-dimethylaniline) gives a mixture of S_N2 and S_N2' substitution products along with the 2-butene-1,4-bisaryl ether. Ozonation of 2-aryloxy-3-buten-1-ol produces the dimeric glyceraldehyde 2-aryl ether. More conveniently, the same product is obtained by addition of the phenolate ion to 4,4-dimethyl-3,5,7-trioxabicyclo[5.1.0]octane (*i.e.*, epoxidised *cis*-2-butene-1,4-diol acetonide), followed by acetonide cleavage and periodate oxidation.

Structural units in wood lignins seem to include some detached glyceraldehyde units. Based on what is known of the reactions in lignin biosynthesis, these units should mainly be linked to the rest of the lignin macromolecule through a 2-aryl ether bond (*1*).[1] Experimental evidence for the existence of such groupings in lignins has been provided by the formation of pyruvic aldehyde both from lignin and from an appropriate model compound (*2*) in mild acidolysis [2,3] and in an acid sulfite cook,[4] and by the formation of 2-(2-methoxy-4-propyl) phenoxy-1-propanol in wood hydrogenolysis.[5] Lundquist *et al.*[2,3] have synthetised the lignin model compound *2* in a low yield by condensing 2-aryloxy acetaldehyde with formaldehyde.

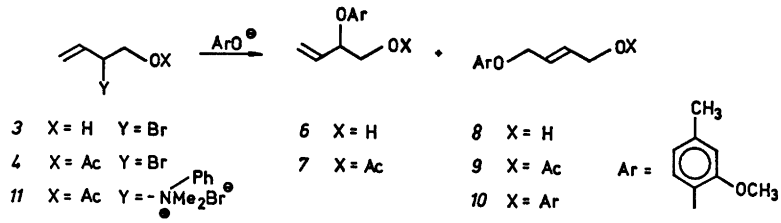
We have developed alternative synthetic routes to lignin model compounds of type *2*. In the first of these, the aryl alkyl ether linkage is formed in a substitution reaction of *3* or *4* with the phenolate ion ArO^- (*5*). Subsequent ozonation of the product gives the glyceraldehyde unit in good yield.

The substitution of a halide in 2-halo-3-buten-1-ol with 2-methylphenolate anion in 32 % yield has been described by Yale *et al.*[6] They do not, however, comment on the S_N2' substitution which we found to accompany the main reaction (see Scheme). Along with *6* and *8* the diether *10* was unexpectedly also isolated from the reaction of *3* with *5*.

The highest yield of the S_N2 substitution product *7* was obtained when the acetoxy bromide *4* was converted into a quaternary ammonium salt (*11*) prior to the reaction. Significantly more of the S_N2' substitution product (*8* or *9*, characterised by oxidation to the corresponding aldehyde) resulted from the direct use of *3* or *4*.

Ozonation of *6* (from hydrolysis of *7*) gave the aldol *2* as a viscous oil without C=O absorption bands in the IR spectrum as previously reported.[2,3] The aldol was reduced by sodium borohydride to the corresponding 2-aryl glycerol ether.[3]

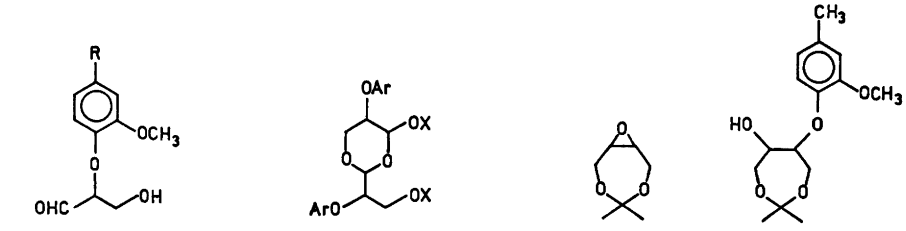
The aldol *2* actually has the dimeric 1,3-dioxane structure *12*; structurally simpler aldols are well known [7] to exist as 1,3-dioxan type cyclic dimers. This was shown by silylation of the aldol to give the protected derivative *13* having spectral data in accord with the structure indicated. In particular, the Eu-FOD resolved ^1H NMR spectrum showed that the sample had four different aromatic methyl groups in pairs of two (ratio *ca.* 1:2). As the silicon-bonded oxygens of *13* will not coordinate europium,[8] there remain six other potential coordinative oxygen atoms in the molecule. However, it is unnecessary to know where exactly the metal atom is located as it is known



3 X = H Y = Br
4 X = Ac Y = Br
11 X = Ac Y = -NMe2Br (Ph)

6 X = H
7 X = Ac

8 X = H
9 X = Ac
10 X = Ar



1 R = the rest of the lignin macromolecule
2 R = CH_3

12 X = H
13 X = $SiMe_2Bu$-t

14

15

that Eu(DPM)$_3$ induced shifts are simply additive with bioxygen functional molecules.[9] Thus the observed four aromatic methyl signals undoubtedly indicate that at least two stereoisomers of the substituted 1,3-dioxane ring are present. Although large shifts were observed for the dioxan ring protons (and incidentally, less so for the methoxyl protons, showing that these groups are not primarily involved in Eu coordination) the overlapping signals of the isomers remain sufficiently complex to obscure the stereochemistry of the dioxane substitution.

The above lengthy route to *2* requires a tedious separation of intermediates. Therefore, another route to *2* was examined. The oxido ketal *14*, readily available [10] from *cis*-2-butene-1,4-diol, undergoes attack by the phenolate ion *5* to give *15* in 60 % yield. Owing to the symmetric nature of *14*, no problems of regioselectivity of epoxide opening are encountered. The acetonide protective group is then removed and the *vic*-diol system cleaved by periodate oxidation in a one-pot reaction to give the aldol *2* (91 % yield from *15*).

EXPERIMENTAL

The following analytical instruments were used: Perkin Elmer 125 (IR), Varian A-60 or Jeol PMX 60 (^{1}H NMR, $CDCl_3$ solvent), Perkin-Elmer 270 B (MS) and F & M CHN Analyser 185.

2-Bromo-3-buten-1-ol (3).[11] NMR: δ 5.9 (1 H, m), 5.2 (2 H, m), 4.5 (1 H, dt, $J_1 = 8.5$ Hz, $J_2 = 6$ Hz), 4.7 (2 H, d, $J = 6$ Hz), 3.2 (1 H, s).

2-Bromo-3-buten-1-yl acetate (4).[11] NMR: δ 6.1 (1 H, m), 5.25 (2 H, m), 4.7 (1 H, dt, $J_1 = 8$ Hz, $J_2 = 5.5$ Hz), 4.35 (2 H, d, $J = 5.5$ Hz), 2.05 (3 H, s).

2-(2-Methoxy-4-methylphenoxy)-3-buten-1-ol (6). 2-Methoxy-4-methylphenol (9.1 g) was dissolved in 100 ml of dry dioxane (dried over CaH_2), 3.2 g of a 50 % Na-dispersion in paraffin oil were added, and the mixture was stirred for 2 h. After adding 9.1 g of *3* in 20 ml of dry dioxane, the stirring was continued overnight. The mixture was poured into water and extracted with ether. The ether solution was washed with 10 % KOH, and with brine, dried, and concentrated. Dry column chromatography on Silica gel Woelm with $CHCl_3$-light petroleum (1:1) gave three fractions:

(a) 1.19 g of *10*, m.p. 106 °C (EtOH), NMR: δ 2.27 (6 H, s), 3.78 (6 H, s), 4.47 (4 H, br s), 6.00 (2 H, m), 6.58 (6 H, br s) [reported [12] for 1,4-diphenoxy-2-butene: 4.5 (s), 6.0 (t), 6.7 – 7.4 (m)], MS (70 eV): *m/e* 328 (48.8 %, M^+), 191 (47.2, M – ArO), 190 (46.8, M – ArOH), 138 (61.4, ArOH), 137 (100, ArO), 109 (80, methyl-methoxy-cyclopentadienyl).

(b) 5.5 g (40 %) of *6* as an oil. Anal. $C_{12}H_{16}O_3$: C, H. IR (film): 3640, 1030, 990, and 925 cm^{-1}. NMR: δ 6.6 (3 H, m), 5.8 (1 H, m), 5.2 (2 H, m), 4.5 (1 H, dt, $J_1 = 5.5$ Hz, $J_2 = 5$ Hz), 3.65 (3 H, s), 3.7 (2 H, m), 2.2 (3 H, s).

(c) 3.45 (25 %) of *8* as an oil. Anal. $C_{12}H_{16}O_3$: C, H. IR (film): 3400, 1000, 965 (*trans*), and

750 (*cis*) cm^{-1}. NMR: δ 6.65 (3 H, m), 5.85 (2 H, m), 4.45 (2 H, m), 4.05 (2 H, m), 3.75 (3 H, s), 2.25 (3 H, s).

2-(2-Methoxy-4-methylphenoxy)-3-butenyl acetate (7). A. 2-Methoxy-4-methylphenol (7.75 g) was treated as above with 10.0 g of *4*. Dry column chromatography on Silica gel Woelm with $CHCl_3$-light petroleum (1:5) gave two fractions:

(a) 4.2 g (30 %) of *7* as an oil. Anal. $C_{14}H_{18}O_4$: C, H. IR (film): 1740, 990, and 930 cm^{-1}. NMR: δ 6.8 (3 H, m), 5.9 (1 H, m), 5.3 (2 H, m), 4.85 (1 H, q, $J=6$ Hz), 4.25 (2 H, d, $J=6$ Hz), 3.8 (3 H, s), 2.2 (3 H, s), 1.95 (3 H, s).

(b) 2.1 g (15 %) of *9* as an oil. Anal. $C_{14}H_{18}O_4$: C, H. IR (film): 1740, 970, and 715 cm^{-1}. NMR: δ 6.7 (3 H, m), 5.8 (2 H, m), 4.55 (2 H, m), 4.0 (2 H, m), 3.7 (3 H, s), 2.2 (3 H, s), 2.0 (3 H, s).

B. *4* (8.0 g) was dissolved in 50 ml of dried dioxane. 5.5 g of *N,N*-dimethylaniline were added, and the mixture was left to stand overnight. The mixture was added to the phenolate-solution *5*, made from 6.3 g of 2-methoxy-4-methylphenol as above. The mixture was stirred overnight and worked up as above. Dry column chromatography yielded 6.85 of *7* (60 %) and 2.6 g of *9* (23 %).

4-(2-Methoxy-4-methylphenoxy)-2-buten-1-al. 8 (2.0 g) was dissolved in acetone and treated with Jones' reagent. Normal work-up yielded 1.4 g (70 %) of 4-(2-methoxy-4-methylphenoxy)-2-buten-1-al as an oil. Anal. $C_{12}H_{14}O_3$: C, H. IR (film): 1690, 965 cm^{-1}. NMR; δ 9.4 (1 H, d, $J=7.5$ Hz), 6.6 (5 H, m), 4.55 (2 H, dd, $J_1=4$ Hz, $J_2=2.5$ Hz), 3.75 (3 H, s), 2.25 (3 H, s).

Hydrolysis of 2-(2-methoxy-4-methylphenoxy)-3-buten-1-yl acetate (7). 7 (5.75 g) was refluxed for 2 h in 50 ml of 80 % EtOH, which contained 0.5 g of KOH. Normal work-up gave 4.5 g (94 %) of *6*.

2-(2-Methoxy-4-methylphenoxy)-3-hydroxypropanal (2). 6 (4.9 g) was dissolved in 50 ml of $CH_3OH-CH_2Cl_2$ (1:1). An ozone-oxygen mixture was led through the solution at -78 °C until the starting material was consumed, as determined by TLC. Excess O_3 was removed by an N_2-stream, and 4.0 g of tris-dimethylaminophosphine were added to the solution. The mixture was allowed to stand for 2 h at room temperature, whereafter it was poured into water, the organic layer washed with dilute H_2SO_4 and water, dried, and evaporated. An oil (4.05 g, 82 %) was obtained after dry column chromatography on Silica gel Woelm with $CHCl_3$-EtOAc (2:1). Anal. $C_{11}H_{14}O_4$: C, H. IR (film): 3370 cm^{-1}. NMR: δ 6.6 (3 H, m), 5.4–3.6 (8 H, m), 2.2 (3 H, s).

Silylation of 2. *2* (0.42 g) was dissolved in 5 ml of dry DMF containing 0.64 g of imidazole. 1.34 g of *t*-butylchlorodimethylsilane was added and the mixture stirred for 14 h. It was then poured into water (0 °C), the solution extracted twice with light petroleum (b.p. 40–50 °C) and the organic phase dried and evaporated. TLC and NMR showed that the residue consisted of a 1:1 mixture of *13* and the dehydration product of *2*, *i.e.*, 2-aryloxyacrolein.[3] Preparative chromatography failing to separate these two compounds, pure *13* was isolated as follows. The mixture (0.69 g) in 10 ml of dry THF was stirred with 0.1 g of $NaBH_4$. After 4 h, 2-aryloxyacrolein was no longer detectable by TLC. Filtration through alumina provided pure *13* (0.19 g). NMR (CCl_4): δ 0.07 (12 H, s), 0.85 (9 H, s), 0.90 (9 H, s), 2.30 (6 H, br s), 3.82 (6 H, s), 3.5–4.5 (6 H, m) (a), 4.90 (2 H, m) (b), 6.5–7.15 (6 H, m). Successive additions of 0.1 equivalents of EuFOD to the solution produced large shifts with the signals (a) and (b) while all methyls and the aromatic multiplet remained fairly constant. In particular, at a 1:1 mol ratio of *13* and EuFOD, aromatic methyl singlets appeared as follows: 1.95 (c), 2.04 (d), 3.11 (e) and 3.29 (f) with intensities c:d:e:f = 2:1:2:1. MS (70 eV): 648 (15 %, M^+), 591 (2, M–*t*-Bu), 381 (9.9, M–(ArO + $BuMe_2SiOH$)), 353 (14.2, M–$ArOCHCH_2OSiMe_2Bu$), 307 (87.5), 190 (100), 175 (100). Anal. $C_{34}H_{56}O_8Si_2$: C, H.

2,2-Dimethyl-5-(2-methoxy-4-methylphenoxy)-6-hydroxy-1,3-dioxepane (15). The oxido ketal *14* [10] (5.0 g) was mixed with 6.0 g (1.25-fold excess) of 2-methoxy-4-methylphenol and 0.1 ml of 20 % ethanolic KOH. The solution was held at 110–120 °C for 10 h, cooled and taken up in ether. The ether solution was washed twice with 10 % aq. KOH, dried and concentrated. Crystallisation from ethanol gave 3.8 g of *15*, and the mother liquor furnished an additional 2.05 g of *15* on silica gel (Woelm) dry column chromatography with $CHCl_3$:EtOAc (9:1), the total yield thus being 60 %. 0.95 g of cresol was recovered from the alkaline washings. The analytical sample was recrystallised from ethanol, m.p. 91–92 °C, IR (KBr pellet): 3430 cm^{-1}. NMR: δ 1.33 (6 H, s), 2.25 (3 H, s), 3.1–4.1 (9 H, m), 6.7 (3 H, m). MS *m/e*: 282 (7.1 %, M^+), 267 (1.2, M–CH_3), 145 (3.5, M–ArO), 138 (38.1, ArOH). Anal. $C_{15}H_{22}O_5$: C, H.

2-(2-Methoxy-4-methylphenoxy)-3-hydroxypropanal (2) from 15. *15* (3.8 g) was dissolved in 150 ml of methanol, 0.1 g of TsOH added and the solution held for 20 min at 60 °C. TLC showed that no unreacted *15* remained at this stage. The solution was then cooled to 0 °C and 150 ml of water added followed by 2.9 g of $NaIO_4$. Stirring was continued for 2 h at 0 °C and 8 h at 20 °C. The bulk of methanol was removed (Rotavap), the residue poured on brine and extracted 4 times with ether. The combined extracts were dried and evaporated to give 2.57 g (91 %) of *2*, freed from minor impurities by silica gel (Woelm) dry column chromatography with $CHCl_3$–EtOAc 3:1. IR and NMR were identical with those of the material prepared as described above.

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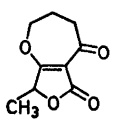
Use of Singly and Doubly Labelled [^{13}C]Acetate in the Elucidation of the Structure of Carolic Acid

JENS PETER JACOBSEN, TORSTEN REFFSTRUP and PER M. BOLL

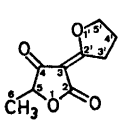
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^{13}C NMR spectra of unenriched and ^{13}C enriched carolic acid, isolated from *Penicillium charlesii* NRRL 1887, have been measured in deuteriochloroform and in alkaline solution. Carolic acid is shown to be a mixture of two isomers. The only acceptable assignments of the ^{13}C NMR signals is achieved by considering carolic acid as a mixture of (*E*)- and (*Z*)-5-methyl-3-(2′-tetrahydrofurylidene)tetrahydrofuran-2,4-dione (*2*). The structure proposal containing a seven-membered ring can, therefore, be rejected.

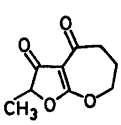
Carolic acid,[1] a major metabolite of *Penicillium charlesii* and *P. fellutanum*,[2] possesses the molecular formula $C_9H_{10}O_4$ and has been formulated as *1* by Clutterbuck *et al.*[3] The seven-membered ring is an unusual feature in itself. It has been argued that *1* most satisfactory explains the IR absorptions at 1760 cm^{-1} (γ-lactone) and 1710 cm^{-1} (ketonic carbonyl) [4] as well as the ^{1}H NMR spectrum.[5] Dean [6] has suggested that none of the published evidence suffices to eliminate structure *2* for carolic acid and states that if carolic acid is indeed an α,β-unsaturated ketone, then carbonyl absorption at 1710 cm^{-1} favours the idea that the carbonyl group is contained in a five-membered ring. Burrows and Turner [7] find that some of the signals in the ^{1}H NMR spectrum of carolic acid are doubled indicating the existence of at least two isomers. The absolute configuration of carolic acid has been established to be *R*.[8]



1



2



3

In order to clarify the problems related to the structure of carolic acid we have recorded the ^{13}C NMR spectra in deuteriochloroform of carolic acid as well as of carolic acid enriched with [1-^{13}C]-, [2-^{13}C]- and [1,2-^{13}C]acetate. In aqueous solution the non-lactonic ring in carolic acid is opened and an α-acyltetronic acid is formed. In non-aqueous media carolic acid appears as a compound having no active hydrogen. For the sake of completeness we also report the ^{13}C NMR spectra of the unenriched and the enriched samples in alkaline solutions.

RESULTS AND DISCUSSION

The ^{13}C NMR spectrum of carolic acid in $CDCl_3$ clearly demonstrates the existence of two isomers in the ratio of approximately 4:5. Each isomer shows only one signal downfield to 195 ppm. Since normal α,β-unsaturated ketonic carbon atoms always give values of chemical shift in this area,[9] it can be concluded that carolic acid is either a mixture of *1* and an isomer of *2* or a *cis/trans* mixture of *2*. The possibility of carolic acid existing as a mixture of either *1* or *2* together with a third structural possibility *3* can be excluded since such mixtures will give rise to the presence of three signals downfield to 195 ppm.

Feeding experiments with ^{14}C-labelled acetate added to *P. charlesii* [10,11] have demonstrated that C-2 and C-3 together with the seven-membered ring in *1* or *3*, or the tetrahydrofurylidene ring in structure *2*, is formed from a subunit bearing the normal polyketide labelling pattern. This work has been repeated using [1-^{13}C]-, [2-^{13}C]-, and [1,2-^{13}C]acetate as precursors.[12]

Table 1. ^{13}C NMR chemical shifts, δ_i(ppm), [a] and $^{13}C,^{13}C$ spin spin coupling constants $^1J_{ij}$ (Hz) [b] of carolic acid (*2*), enriched with [1,2-^{13}C]acetate, in $CDCl_3$.

	C-2	C-3	C-4	C-5	C-6	C-2′	C-3′	C-4′	C-5′
Z-form									
δ_i	167.24	95.64	195.70	79.27	17.02	187.31	33.46	21.70	77.51
$^1J_{ij}$	76.2					37.1		32.2	
E-form									
δ_i	170.29	95.05	199.01	79.66	17.02	186.61	33.27	21.70	78.03
$^1J_{ij}$	78.1					38.1		32.2	

[a] Uncertainty: ± 0.07 ppm. [b] Uncertainty: ± 0.5 Hz.

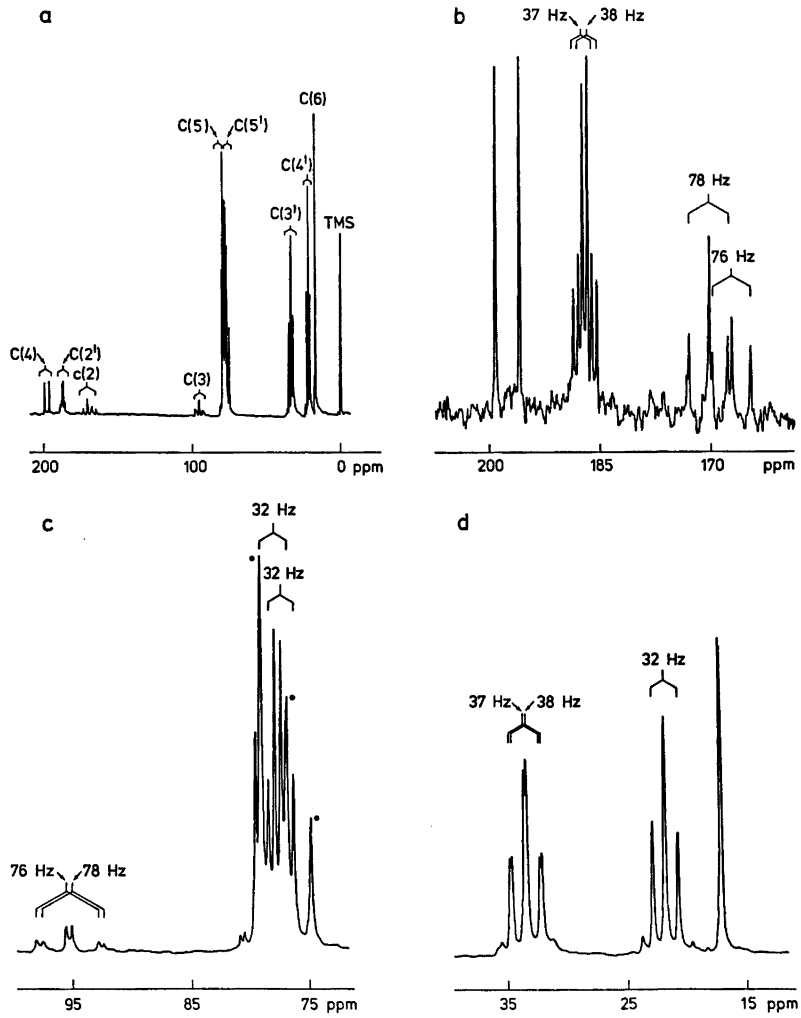
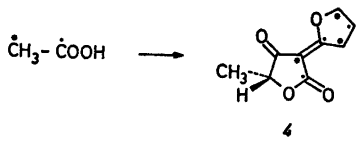


Fig. 1. ^{13}C NMR spectrum in $CDCl_3$ of carolic acid enriched with [1,2-^{13}C]acetate. Trace b–d are details of the spectrum shown in trace a. The lines marked with an asterisk contain signals from $CDCl_3$.

The ^{13}C spectrum of the enriched carolic acid shows that none of the carbon atoms responsible for the lines downfield to 195 ppm are enriched, giving rise to the conclusion that the two isomers of carolic acid both possess an α,β-unsaturated carbonyl group at C-4. The only possibility open is, therefore, to consider carolic acid as a *cis/trans* mixture of *2*. This structure proposal makes it possible to explain the various aspects of the ^{13}C NMR spectra and gives, as further evidence, the only reasonable assignments of the observed signals.

The ^{13}C chemical shifts obtained for carolic acid (*2*) are listed in Table 1. The assignments of the lines are based upon the gated decoupled spectrum and the spectra of the ^{13}C enriched molecules. The spectrum of *2* enriched with [1-^{13}C]acetate exhibits an increase of the intensity of the lines at 21.70, 167.24, 170.29, 186.61 and 187.31 ppm compared to the intensity of the lines in the spectrum of the unenriched compound. The spectrum of *2* enriched with [2-^{13}C]acetate shows increased intensity of the lines at 33.27, 33.46, 77.51, 78.03, 95.05 and 95.64 ppm. The degree of enrichment amounts in both cases to approximately 1.0 %.

The ^{13}C NMR spectrum of *2* enriched with [1,2-^{13}C]acetate is shown in Fig. 1. The signals from the enriched carbon atoms are composed of doublets arising from the ^{13}C,^{13}C spin spin coupling constants (given in Table 1). The two lines in the doublets are distributed symmetrically around the lines from the carbon in natural abundance carolic acid. The only carbon atoms, unaffected by introduction of ^{13}C-labeled acetate, are C-4, C-5, and C-6. In accordance with the proposed biosynthesis [12] the reaction pathway may, therefore, be rationalized as shown in Scheme 1.



4

Scheme 1.

Independently, Holker [13] has reached the same conclusion concerning the biosynthesis of a structurally related tetronic acid, terrestric acid, using the same technique.

The ^{13}C chemical shift of C-2 differs about 3 ppm in the two isomers. Brouwer and Stothers [14] have reported ^{13}C chemical shifts of the methyl esters of (*Z*)- and (*E*)-3-methoxyacrylic acid and concluded that the chemical shift of the ester carbon atom in the *E*-isomer is shifted 3 ppm downfield compared to the corresponding value in the *Z*-isomer. If this result is extended to *2*, the assignment of the lines to the two isomers are as given in Table 1. The differences of the ^{13}C,^{13}C spin spin coupling constants and the small differences in intensity of the lines given by the two isomers have been used for this assignment. The possibility of errors in this assignment caused by change of the nuclear Overhauser effect between the two isomers may therefore exist, except in case of C-2 and C-3.

The variation in ^{13}C chemical shift between the two isomers of *2* can be explained in terms of change in the electronic environment of the carbon atoms. The distances from C-2 and C-4 to O-1′ are distinctly different for the two isomers, thus giving rise to quite different contributions to the chemical shifts for the respective carbon atoms in the two isomers. The electronic environment around the other carbon atoms is expected to be only slightly different in the two isomers. It must, therefore, be possible to conclude that the difference in ^{13}C chemical shifts between the two isomers should be much larger for C-2 and C-4 than for the other carbon atoms. As can be seen from Table 1 this is in agreement with the experimental data.

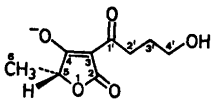
The chemical shifts observed for the carbon atoms in *2* are in agreement with results obtained from related compounds.[9,15] In case of C-2′ the shift value is exceptionally high for an ethylenic carbon atom, but reasonable when taking into consideration that the π-electrons are highly delocalized and that C-2′ is attached to the strongly electron-withdrawing alkoxy group.

The $^1J_{CC}$ coupling constants of *2* given in Table 1 show the usual values for sp^3 hybridized carbon atoms [15] in case of $^1J_{C-2',C-3'}$ and $^1J_{C-4',C-5'}$. The values found for $^1J_{C-2,C-3}$ are in the range normally found for coupling between sp^2 hybridized carbon atoms [15] supporting the idea of having a very high degree of delocalization of the π-electrons in *2*.

Table 2. ^{13}C NMR chemical shifts, δ_i(ppm), [a] and $^{13}C,^{13}C$ spin spin coupling constants $^1J_{ij}$ (Hz), [c] of the open form (*5*) of carolic acid in alkaline solution.

	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′
δ_i	177.46	96.09	200.00	77.64	16.70	199.35	33.35	27.55	61.53
$^1J_{ij}$	76.2					41.0		37.0	

[a] Uncertainty: ±0.07 ppm. [b] Uncertainty: ±0.5 Hz.



5

In alkaline solution carolic acid is hydrolyzed to give the open structure *5*. The chemical shifts of the carbon atoms have been determined for *5* as well as for the three ^{13}C enriched analogues. The results are given in Table 2. The assignment of the lines have been performed by use of the lines in the ^{13}C spectra of the ^{13}C enriched molecules and the gated decoupled spectrum. The lines due to C-2′ and C-5 show the existence of an exchange of the directly attached protons with deuterium. The rate of exchange differed in the two cases ranging from a few hours for H-2′ to a few days for H-5.

The chemical shifts of the carbon atoms in *5* are characterized by showing two signals due to α,β-unsaturated carbonylic carbon atoms and assigned to C-4 and C-1′. This implies that the excess charge is delocalized to a high degree in the bonds between C-1′, O-2, and C-4. From the $^1J_{CC}$ coupling constants in *5*, given in Table 2, it can be seen that the degree of delocalization is recognized in the value of $^1J_{C-2,C-3}$.

The ^{13}C NMR spectrum of the open structure of carolic acid in acid solution is complicated due to the existence of two forms, related to two different ways of forming hydrogen bonds in α-acyltetronic acids. This problem is currently under investigation.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded on a Jeol FX60 spectrometer. 8 K data points were used with a pulse length of 6 μs corresponding to a 60° flip angle. The spectral width was 4000 Hz. The magnetic field was stabilized by internal deuterium lock on the signal from the solvents. The probe temperature was 30 °C. All chemical shifts were measured as δ (ppm downfield to TMS). The sample concentrations used were 100 mg carolic acid in either 1.3 ml $CDCl_3$ or 1.3 D_2O + 0.05 ml 40 % NaOD.

Carolic acid was isolated in adequate amounts from single flask cultures of *Penicillium charlesii* NRRL 1887. Details concerning addition of ^{13}C-labeled acetate and isolation will be discussed in another paper.[13]

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The Orthoester Glycosylation Method. Part II.* Variation in the Anomeric Composition of the Product with Aglycone Basicity and Steric Accessibility

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The previously observed influence of aglycone basicity on the steric outcome of the two-step orthoester glycosylation procedure is further substantiated by the observation that 2-nitroethanol yields anomeric D-glucopyranosides in an $\alpha:\beta$ ratio of 44:56. Fluoroethanol yields the β-D-glucopyranoside only; this is ascribed to the *gauche* effect. A comparison of the products of glycosylation of cyclohexanol and 2,2,6,6-tetramethylcyclohexanol shows that steric hindrance may inhibit catalysis by electrophilic attack at the alkoxyl group, leading to an increase in the α-D-glucopyranoside produced.

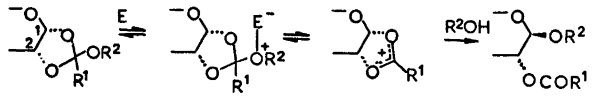
In the orthoester glycosylation method, developed by Kochetkov and co-workers for the synthesis of glycosides with 1,2-*trans*-configuration,[2–6] the usual procedure is to treat a 1,2-orthoester in which the alkoxyl residue is the desired aglycone, with a Lewis acid, in the presence of the alcohol. The 1,2-dioxolonium ions thus formed react with the alcohol producing a 1,2-*trans*-glycoside.

Other products may, however, be produced if the electrophile instead attacks O-1 or O-2. This possibility was studied in a previous investigation,[1] in which we demonstrated that the product composition varies with the basicity of the aglycone oxygen (R^2OH in Scheme 1). Low electron density rendering the oxygen less susceptible to electrophilic attack, gave rise to competing attack at O-1 and consequently other products, *e.g.* 1,2-*cis*-glycosides, were also formed. Competing attack at O-2 should lead to glycosides with an unsubstituted hydroxyl group in the 2-position. This, however, was not observed.

We now report some studies on the influence of steric factors in the choice of reaction paths. The stereospecific formation of 1,2-*trans*-glycosides depends on the electrophile attacking the alkoxyl oxygen only and the resulting formation of the required acyloxonium ion (Scheme 1). One might therefore anticipate that bulky substituents on the α-carbon atom(s) of the alkoxyl residue would direct the electrophile to O-1 (or, possibly O-2) with concomitant loss of stereospecificity.

The various orthoesters required for the study (*1*–*4*) were made as previously described. Only one stereoisomeric form, presumably the *exo* isomer was obtained for each orthoester. The glycosylations were carried out by allowing the various orthoesters to react with the appropriate alcohol, in nitromethane, in the presence of mercury(II) bromide.

* Part I, Ref. 1.



Scheme 1.

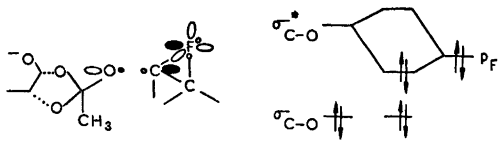


Fig. 1. Gauche effect in 2′-fluoroethyl orthoesters.

In order to investigate a possible steric effect on the reaction, the products of glycosylation of 2,2,6,6-tetramethylcyclohexanol were examined and compared to those obtained from cyclohexanol. Studies on the proton catalyzed glycosylation of the cyclohexyl orthoester *1* with cyclohexanol have shown that a mixture of anomeric glycopyranosides with extensive deacetylation in the 2-position are obtained.[7,8] Under the conditions used in the present study, the following products and yields were obtained: Cyclohexyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside (*5*, 62 %), cyclohexyl 3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (*6*, 16 %) and cyclohexyl 3,4,6-tri-*O*-acetyl-*β*-D-glucopyranoside (*7*, 15 %). The position of the free hydroxyl group in *6* and *7* was demonstrated by methylation analysis. Cyclohexyl acetate in an amount corresponding to *6* and *7* was found present in the original reaction mixture. Glycosylation of the 2′,2′,6′,6′-tetramethylcyclohexyl orthoester *2* afforded a 40 % yield of a mixture of the α-D-anomer *8* and the *β*-D-anomer *9* in a ratio of 26:74 by optical rotation of the mixture and 22:78 by weighing the separated components. Products with a free hydroxyl group in the 2-position were not observed. A comparison of the ratio of α-D-glucopyranoside to *β*-D-glucopyranoside obtained in this latter reaction to that obtained in the corresponding glycosylation of cyclohexanol (17:83) would implicate steric hindrance as a factor in these reactions. The effect, however, seems to be smaller than that obtained by varying the aglycone basicity.

Secondly, as a continuation of our previous investigation on the effect of polar substituent in the alcohol component (R^2OH, Scheme 1), we have examined the products obtained using 2-fluoroethanol and 2-nitroethanol. The former gave rise to an 85 % yield of the *β*-D-glucopyranoside *10*. The corresponding α-anomer was not detected in the reaction mixture. This result contrasts strikingly with those previously obtained with 2-chloroethanols. For these, the polar chlorine substituents gave rise to α-D- as well as to *β*-D-glucopyranosides.[1] A possible explanation for this apparent anomaly is provided by the more pronounced *gauche* effect expected for the fluoro substituent.[9–11] A *gauche* arrangement of the alkoxyl oxygen and the adjacent fluoro *p* orbital involves the mixing of an occupied fluorine *p* orbital and the carbon-oxygen antibonding orbital as depicted in Fig. 1 [11] (and additionally the corresponding mixing of an occupied *p* orbital on the alkoxyl oxygen and the antibonding carbon-fluorine orbital, not shown in Fig. 1). This orbital mixing results in a lower-lying highest occupied

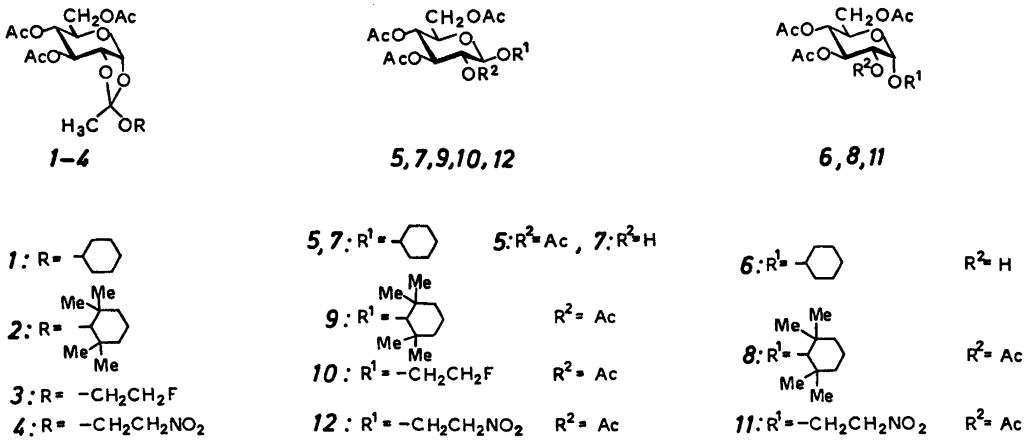


Fig. 2. Orthoesters and glycosides obtained.

molecular orbital for the electrophile to attack and thereby a lower-energy transition state.

For 2-nitroethanol this orbital effect is precluded. As anticipated, this alcohol gave rise to a mixture of anomeric 2′-nitroethyl D-glucopyranosides (*11* and *12*), in which the ratio of α- to β-D-pyranoside was 44:56. Deacetylation at the 2-position [7,8] was not observed in the above two examples.

EXPERIMENTAL

General methods were the same as those described before.[1]

Preparation of orthoesters. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (10 mmol) in dry nitromethane (10 ml) was treated with the appropriate alcohol (20 mmol) in the presence of 2,6-lutidine (20 mmol) at 37 °C for 20 h with respectively cyclohexanol, 2-fluoroethanol or 2-nitroethanol and for 96 h with 2,2,6,6-tetramethylcyclohexanol as the alcohol component. Aqueous silver nitrate (7.5 ml 2 M), water (12.5 ml) and acetone (25 ml) were added with stirring. The mixture was filtered and the filtrate extracted three times with chloroform (25 ml). The chloroform extract was shaken three times with water (25 ml), dried over magnesium sulfate, filtered and concentrated.

*3,4,6,-Tri-*O*-acetyl-1,2-*O*-(cyclohexyloxyethylidene)-α-D-glucopyranose* (*1*) was obtained in pure form by means of chromatography on a silica gel column (toluene–ethyl acetate 2:1) and then recrystallized from diethyl ether–light petroleum to yield 3.0 g (70 %), m.p. 83–84 °C, $[\alpha]_D$ +26° (*c*, 1.0, $CHCl_3$), (lit.[7] m.p. 83 °C, $[\alpha]_D$ +25° ($CHCl_3$).

*3,4,6-Tri-*O*-acetyl-1,2-*O*-(2′,2′,6′,6′-tetramethylcyclohexyloxyethylidene)-α-D-glucopyranose* (*2*) was obtained in pure form by means of chromatography on a silica gel column (toluene–ethyl acetate 4:1) in a yield of 2.2 g (45 %), $[\alpha]_D$ +24° (*c* 1.0, $CHCl_3$). Anal. $C_{24}H_{38}O_{10}$: C, H. NMR ($CDCl_3$): δ 0.88, 0.91 and 0.97 (12 H, s, cyclohexyl CH_3), 1.0–1.8 (6 H, cyclohexyl H), 1.76 (3 H, s, O–C(=O)–CH_3), 2.10 and 2.12 (9 H, s, OAc), 3.11 (1 H, s, cyclohexyl-O-C-H), 4.00 (1 H, m, H-5), 4.19 and 4.23 (1 H each, H-6, H-6′), 4.42 (1 H, dd, $J_{1,2}$ 5 Hz, $J_{2,3}$ 3 Hz, H-2), 4.92 (1 H, dd, $J_{3,4}$ 3 Hz, $J_{4,5}$ 9 Hz, H-4), 5.20 (1 H, t, *J* 3 Hz, H-3), 5.76 (1 H, d, *J* 5 Hz, H-1).

*3,4,6-Tri-*O*-acetyl-1,2-*O*-(2′-fluoroethoxyethylidene)-α-D-glucopyranose* (*3*) was recrystallized from ethanol to yield 2.8 g (71 %) m.p. 115–116 °C $[\alpha]_D$ +30° (*c* 1.0 $CHCl_3$). Anal. $C_{16}H_{23}O_{10}F$: C, H. NMR ($CDCl_3$): δ 1.76 (3 H, s, CCH_3), 2.12 and 2.14 (9 H, s, OAc), 3.64 and 3.94 (1 H each, OCH_2CH_2F), 4.00 (1 H, m, H-5), 4.2–4.3 (2 H, m, H-6, H-6′), 4.36 and 4.80 (1 H each, OCH_2CH_2F), 4.40 (1 H, dd, $J_{1,2}$ 5 Hz, $J_{2,3}$ 3 Hz, H-2), 4.94 (1 H, dd, $J_{3,4}$ 3 Hz, $J_{4,5}$ 9 Hz, H-4) 5.20 (1 H, t, *J* 3 Hz, H-3), 5.77 (d, *J* 5 Hz, H-1).

*3,4,6-Tri-*O*-acetyl-1,2-*O*-(2′-nitroethoxyethylidene)-α-D-glucopyranose* (*4*) was recrystallized from ethanol to yield 3.0 g (71 %), m.p. 119.5–120.5 °C, $[\alpha]_D$ +32° (*c* 1.1, $CHCl_3$). Anal. $C_{16}H_{23}NO_{12}$: C, H, NMR ($CDCl_3$): δ 1.76 (3 H, s, CCH_3), 2.12 and 2.14 (9 H, s, OAc), 4.00 (1 H, m, H-5), 4.10 (2 H, $OCH_2CH_2NO_2$). 4.24 and 4.28 (1 H each, H-6, H-6′), 4.34 (1 H, dd, $J_{1,2}$ 5 Hz, $J_{2,3}$ 3 Hz, H-2) 4.60 (2 H, $OCH_2CH_2NO_2$), 4.96 (1 H, dd, $J_{3,4}$ 3 Hz, $J_{4,5}$ 9 Hz, H-4), 5.24 (1 H, t, *J* 3 Hz, H-3), 5.78 (1 H, d, *J* 5 Hz H-1).

Preparation of glycosides. The appropriate orthoester (3 mmol), the corresponding alcohol (6 mmol) and mercury(II) bromide (1 mmol) in dry nitromethane (12 ml) was refluxed for 15 min. The various products were obtained as described below.

*Cyclohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside* (*5*). Chromatographic separation by means of silica gel column chromatography (toluene–ethyl acetate 1:1) of the product of glycosylation of 3,4,6-tri-*O*-acetyl-1,2-*O*-(cyclohexyloxyethylidene)-α-D-glucopyranose (*1*) and crystallisation from diethyl ether–light petroleum afforded 0.80 g of *5* (62 %), m.p. 119–120 °C $[\alpha]_D$ −24° (*c* 1.0, $CHCl_3$) in agreement with literature values.[13]

*Cyclohexyl 3,4,6-tri-*O*-acetyl-α- and β-D-glucopyranosides* (*6*, *7*). A slower-moving fraction, 0.37 g (32 %) containing the two anomers *6* and *7* was also obtained from the above separation. The optical rotation, $[\alpha]_D$ +66° (*c* 1.0, $CHCl_3$) indicated a ratio of 50:50 of *6* and *7* in the mixture. Further separation by means of silica gel column chromatography (toluene–ethyl acetate 4:1) afforded the pure anomers. Cyclohexyl 3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (*6*), 0.18 g (16 %) had $[\alpha]_D$ +133° (*c* 0.9, $CHCl_3$). Anal. $C_{18}H_{28}O_9$: C, H. NMR ($CDCl_3$): δ 1.1–2.0 (10 H, m, cyclohexyl H) 2.06 and 2.10 (9 H, s, OAc), 2.30 (1 H, OH), 3.5–3.9 (2 H, m, H-5 and cyclohexyl O-C-H), 4.0–4.4 (3 H, m, H-2, H-6 and H-6′), 5.02 (1 H, t, $J_{3,4}$ and $J_{4,5}$ 9 Hz, H-4), 5.10 (1 H, d, $J_{1,2}$ 4 Hz, H-1), 5.28 (1 H, t, $J_{2,3}$ 9 Hz, H-3). Cyclohexyl 3,4,6-tri-O-acetyl-β-D-glucopyranoside (*7*) was recrystallized from diethyl ether–light petroleum to yield 0.17 g (15 %), m.p. 108–109 °C $[\alpha]_D$ −2° (*c* 1.0, $CHCl_3$). Anal. $C_{18}H_{28}O_9$: C, H. NMR ($CDCl_3$): δ 1.1–2.0 (10 H, cyclohexyl H), 2.06 and 2.10 (9 H, s, OAc), 2.9 (1 H, OH), 3.5–3.9 (3 H, m, H-2, H-5 and cyclohexyl O-C-H), 4.14 (1 H, dd, $J_{5,6}$ 3 Hz, $J_{6,6'}$ 12 Hz, H-6), 4.35 (1 H, dd, $J_{5,6}$ 5 Hz, H-6′), 4.54 (1 H, d, $J_{1,2}$ 8 Hz, H-1), 4.9–5.3 (2 H, m, H-3, H-4).

The position of the free hydroxyl group in the above two anomers was demonstrated by methylation with methyl triflate [14,15] followed

by deacetylation (sodium methoxide in methanol), acidic hydrolysis (0.25 M H_2SO_4 at 100 °C overnight), reduction with sodium borohydride and acetylation.[16] The two products were examined by GLC-MS and were in both instances identical to 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-D-glucitol. Examination of the original reaction mixture by GLC and comparison to authentic material revealed the presence of cyclohexyl acetate. The amount was related to that of the cyclohexanol present. A known amount of authentic cyclohexyl acetate was added and the new ratio of cyclohexanol to cyclohexyl acetate was determined. From these results the amount of cyclohexyl acetate produced in the reaction was calculated and found to correspond stoichiometrically to *6* and *7*.

2',2',6',6'-Tetramethylcyclohexyl 2,3,4,6-tetra-O-acetyl-α-D- and -β-D-glucopyranosides (8, 9). Chromatographic separation by means of silica gel column chromatography (toluene–ethyl acetate 2:1) of the product of glycosylation of 3,4,6-tri-*O*-acetyl-1,2,*O*-(2',2',6',6'-tetramethylcyclohexyloxyethylidene)-α-D-glucopyranose (*2*) afforded a mixture of the anomeric glycosides *11* and *12*, 0.58 g (40 %). The optical rotation $[\alpha]_D$ +2° (*c* 1.2, $CHCl_3$), indicated a ratio of 26:74 of the α-D- and β-D-anomers, *11* and *12*, respectively. Further separations on a silica gel column (toluene–ethyl acetate 2:1) afforded each of the two anomers in pure form. 2',2',6',6'-Tetramethylcyclohexyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside, 0.12 g (8 %) was obtained, m.p. 113.5–114.5 °C, $[\alpha]_D$ +66° (*c* 0.9, $CHCl_3$). Anal. $C_{24}H_{38}O_{10}$: C, H. NMR ($CDCl_3$): δ 0.95, 0.98 and 1.02 (12 H, s, cyclohexyl CH_3), 1.0–1.6 (6 H, m, cyclohexyl H), 2.00, 2.04 and 2.06 (12 H, s, OAc), 3.00 (1 H, s, cyclohexyl O–Ċ–H), 4.0–4.4 (3 H, m, H-5, H-6, H-6'), 4.9–5.2 (3 H, m, H-1, H-2, H-4), 5.48 (1 H, t, *J* 9 Hz H-3). 2',2',6',6'-Tetramethylcyclohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, 0.43 g (30 %) was obtained, m.p. 141–142 °C, $[\alpha]_D$ −21° (*c* 1.0, $CHCl_3$). Anal. $C_{24}H_{38}O_{10}$: C, H. NMR ($CDCl_3$): δ 0.87, 0.94 and 1.00 (12 H, s, cyclohexyl CH_3), 1.0–1.6 (6 H, m, cyclohexyl H), 2.00, 2.02 and 2.06 (12 H, s, OAc), 2.98 (1 H, s, cyclohexyl O–Ċ–H), 3.66 (1 H, m, H-5), 4.16 (2 H, m, H-6 and H-6'), 4.62 (1 H, d, $J_{1,2}$ 8 Hz, H-1), 4.9–5.3 (3 H, m, H-2, H-3, H-4).

2'-Fluoroethyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (10). Chromatographic purification by means of silica gel column chromatography (toluene–ethyl acetate 1:1) of the product of glycosylation of 3,4,6-tri-*O*-acetyl-(2'-fluoroethoxyethylidene)-α-D-glucopyranose (*3*) and crystallization from diethyl ether afforded 1.0 g (85 %), m.p. 108.5–109.5 °C, $[\alpha]_D$ −16° (c 1.0, $CHCl_3$). Anal. $C_{16}H_{23}O_{10}F$: C, H. NMR ($CDCl_3$): δ 2.03, 2.05, 2.07 and 2.11 (12 H, s, OAc), 3.6–4.0 (2 H, m, H-5 and O*C*HCH_2F), 4.10 (1 H, m, −*C*HCH_2F), 4.2–4.4 (3 H, m, H-6, H-6' and OCH_2*C*HF), 4.66 (d, $J_{1,2}$ 8 Hz, H-1), 4.82 (1 H, m, OCH_2*C*HF), 4.9–5.2 (3 H, m, H-2, H-3 and H-4). The corresponding α-anomer was not detected in the reaction mixture.

2'-Nitroethyl 2,3,4,6-tetra-O-acetyl-α-D and β-D-glucopyranosides (11, 12). Chromatographic separation by means of silica gel column chromatography (toluene-ethyl acetate 1:1) of the product of glycosylation of 3,4,6-tri-*O*-acetyl-1,2-*O*-(2'-nitroethoxyethylidene)-α-D-glucopyranose (*4*) afforded 0.94 g (75 %) of an anomeric mixture which from the optical rotation, $[\alpha]_D$ +35° (*c* 1.0, $CHCl_3$), was calculated to contain the α-D- and the β-D-anomer in a ratio of 44:56. The anomers could be distinguished by TLC on silica gel plates impregnated with dimethyl sulfoxide using diethyl ether saturated with dimethyl sulfoxide as solvent. The chromatographically pure anomers were obtained by crystallization of the β-anomer from a diethyl ether–light petroleum solution. 2'-Nitroethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (*11*), 0.40 g (32 %) was obtained, $[\alpha]_D$ +100° (*c* 1.0, $CHCl_3$). Anal. $C_{16}H_{23}NO_{12}$: C, H. NMR ($CDCl_3$): δ 2.02, 2.04, 2.07 and 2.12 (12 H, s, OAc), 4.04 (1 H, m, H-5), 4.1–4.3 (4 H, H-6, H-6', $OCH_2CH_2NO_2$), 4.63 (2 H, m, $OCH_2CH_2NO_2$), 4.86 (1 H, dd, $J_{1,2}$ 4 Hz, $J_{2,3}$ 9.5 Hz, H-2), 5.06 (1 H, t, $J_{3,4}$ and $J_{4,5}$ 9.5 Hz, H-4), 5.16 (1 H, d, H-1), 5.41 (1 H, t, H-3). 2'-Nitroethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (*12*), 0.50 g (40 %) was obtained, m.p. 114–115 °C, $[\alpha]_D$ −16° (*c* 1.1, $CHCl_3$) (lit.[12] m.p. 119–120 °C $[\alpha]_D$ −16° $CHCl_3$). NMR ($CDCl_3$): δ 2.02, 2.05 and 2.11 (12 H, s, OAc), 3.76 (1 H, m, H-5), 4.2–4.3 (4 H, m, H-6, H-6' and $OCH_2CH_2NO_2$), 4.58 (2 H, $OCH_2CH_2NO_2$), 4.60 (1 H, d, $J_{1,2}$ 8 Hz), 4.9–5.4 (3 H, m, H-2, H-3 and H-4).

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Lithium Methyl-, Butyl- and Phenyl-[(±)-2-(1-dimethylaminoethyl)phenyl]cuprate Reagents and their Conjugate Addition to Methyl Cinnamate

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Lithium methyl-, butyl- and phenyl-[(±)-2-(1-dimethylaminoethyl)phenyl]cuprate reagents have been obtained from 2-(1-dimethylaminoethyl)phenyllithium and the appropriate organocopper compounds or from 2-(1-dimethylaminoethyl)-phenylcopper and the organolithium compounds. Methyl cinnamate on treatment with these reagents in ether gives methyl 3-phenylbutyrate, methyl 3-phenylheptanoate and methyl 3,3-diphenylpropionate, respectively, in 50 to 70 % yields.

Organocopper reagents are finding increasing use in organic synthesis since they are selective and react preferentially with "soft" groups in organic substrates.[1–4] Common examples are reactions with organic halides to form substitution products (eqn. 1) and 1,4-addition to α,β-unsaturated carbonyl compounds (eqn. 2).

$$R-Cu+X-R' \rightarrow R-R'+CuX \qquad (1)$$

The formation of Meisenheimer compounds from organocopper reagents and 1,3,5-trinitrobenzene [5,6] can also be looked upon as 1,4-addition reactions.

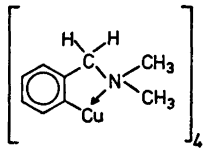
Special interest has been devoted to the lithium diorganocuprates (LiRR'Cu). The homocuprates have been widely used in conjugate addition (eqn. 2), and lithium dimethylcuprate is a standard reagent. With more precious groups an inexpensive or re-usable R' group can be used, *e.g.* acetylide.[4,7]

$$\begin{array}{l} Ph-CH=CH-COOCH_3+LiRR'Cu \rightarrow \\ \qquad\qquad\quad Li^+ \\ Ph-CH(R)^-CH-COOCH_3+R'Cu \\ \qquad\quad \downarrow H^+ \\ Ph-CH(R)-CH_2-COOCH_3 \end{array} \qquad (2)$$

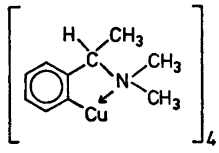
The reaction of lithium diorganocuprates may be facilitated by soft complexing agents.[7,8]

Thermally stable, chelated arylcopper compounds such as 2-(dimethylaminomethyl)phenylcopper (*1*) have been described by van Koten and Noltes.[9,10] Such compounds appear to be interesting components in mixed lithium diorganocuprates. The organocopper compound *1* could be expected to be regenerated or retained in a cuprate when a mixed lithium diorganocuprate is added to an α,β-unsaturated carbonyl compound. Also, simple modifications of compound *1* could give chiral organocopper compounds, such as 2-(1-dimethylaminoethyl)-phenylcopper (R*Cu, *2*) for preparation of chiral mixed organocuprates. We are therefore investigating lithium diorganocuprate reagents containing this component and their addition to α,β-unsaturated carbonyl compounds.

In the course of the work we learned about research on the optically active 2-(1-dimethylaminoethyl)phenylcopper.[11]



1



2

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RESULTS

Organocuprate reagents. Treatment of (±)-*N*,*N*-dimethyl-1-phenylethylamine with butyllithium gives 2-(1-dimethylaminoethyl)phenyllithium, characterized by deuteration and by trimethylsilylation, in at least 87 % yield. Further reaction of the lithium compound with copper(I) iodide in ether gave a 2-(1-dimethylaminoethyl)phenylcopper (*2*). Compound *2* was thermally stable and was only slowly hydrolyzed by water. It was more sensitive to water and oxygen in the presence of organic solvents. Compound *2* reacted with allyl bromide and with iodine to give the expected products.

Addition of slightly less than an equimolar amount of methyllithium to a preparation of *2* gave a black product which was not very soluble in ether. We used this reagent *in situ* assuming it to be a lithium methyl[2-(1-dimethylaminoethyl)phenyl]cuprate. The reagent was more conveniently obtained from 2-(1-dimethylaminoethyl)phenyllithium and methylcopper. In analogous ways we obtained the corresponding mixed butyl- and phenylcuprates. These mixed cuprate reagents seem to be thermally more stable than lithium dimethylcuprate and lithium dibutylcuprate, respectively.

Conjugate addition. Preliminary experiments confirmed [2] that an excess of the dimeric [12] lithium dimethylcuprate is necessary to obtain good yields in conjugate additions to α,β-unsaturated esters in ether, and accordingly 4 mol of cuprate (calc. as a monomer) was used per mol of cinnamate in the subsequent reactions.

Methyl cinnamate added to an excess of lithium (±)-methyl[2-(1-dimethylaminoethyl)phenyl]cuprate in ether at 0 to −5 °C, was rapidly consumed (GLC). A greenish-yellow precipitate, characteristic of compound *2*, appeared. Hydrolysis and separation gave the conjugate addition product, methyl 3-phenylbutanoate, in a fair yield. Pure *N*,*N*-dimethyl-1-phenylethylamine could be recovered in *ca.* 65 % yield.

The results for various cuprates prepared in different ways are summarized in Table 1. The yields of the 1,4-addition products obtained from the mixed cuprates are somewhat lower than those obtained from lithium dimethyl-, dibutyl- and diphenylcuprates. The table also shows that the yield is higher if the reagent is prepared from 2-(1-dimethylaminoethyl)phenyllithium and methylcopper. GLC measurements indicate that the actual yields of the conjugate addition products before distillation are in the order of 85 %. According to GLC, some high-boiling (nonbasic) material is also formed, which so far has not been further investigated. All reagents contain lithium bromide or iodide from the preparation of lithium compounds or from the reactions with copper(I) iodide. This may be important for the course and rate of the reactions (*cf.* Ref. 13).

Table 1. 1,4-Addition of racemic lithium diorganocuprates, LiRR'Cu, to methyl *trans*-cinnamate (molar ratio 4:1) in diethyl ether. Yields refer to distilled and isolated products. R* stands for the 2-(1-dimethylaminoethyl)phenyl group.

LiRR'Cu	LiRR'Cu prepared from	Reaction time/min	Reaction temp./°C	Main product	Isolated yield/%
$LiMe_2Cu$	MeLi + CuI	40	−5 to +5	$PhCH(CH_3)CH_2CO_2CH_3$	79 [a]
LiMeR*Cu	R*Cu + MeLi	45	−5 to 0	$PhCH(CH_3)CH_2CO_2CH_3$	55
LiMeR*Cu	R*Li + MeCu	50	−5 to 0	$PhCH(CH_3)CH_2CO_2CH_3$	68
$LiBu_2Cu$	BuLi + CuI	45	−20	$CH_3(CH_2)_3CH(Ph)CH_2CO_2CH_3$	77
LiBuR*Cu	R*Li + BuCu	312	−45		[b]
	and	60	−20	$CH_3(CH_2)_3CH(Ph)CH_2CO_2CH_3$	56
$LiPh_2Cu$	PhLi + CuI	30	−5 to 0	$Ph_2CHCH_2CO_2CH_3$	70
LiPhR*Li	R*Li + PhCu		−5 to 0	$Ph_2CHCH_2CO_2CH_3$	60

[a] Ref. 24. [b] GLC indicated *ca.* 45 % yield of methyl diphenylpropionate after 312 min at −45 °C. After additional stirring at −20 °C for 60 min the yield was >90 % according to GLC.

DISCUSSION

The main observation in the present work is that the methyl, butyl and phenyl groups are selectively transferred from the organocopper reagents to the α,β-unsaturated esters in fair yields. 2-(1-Dimethylaminoethyl)phenylcopper, R*Cu, is regenerated in the reaction mixture, and no product from the transfer of the 2-(1-dimethylaminoethyl)phenyl group has been observed.

Although we have assumed the presence of the dimeric lithium alkyl[2-(1-dimethylaminoethyl)phenyl]cuprate, $(LiRR^*Cu)_2$, this may not be the main organocopper cluster in the reaction mixture. Equilibria between several different organocopper clusters can be expected. Such equilibria are being studied by van Koten *et al.*, using NMR spectroscopy.[14]

In earlier work [2] it has been assumed that the mixed cuprate, LiRR'Cu, is the main organocopper cluster present after mixing RLi and R'Cu. Some experimental support for this assumption has been published by Posner *et al.*[15]

In the reactions of the mixed cuprates one group is transferred to the substrate regenerating the more stable or less reactive organocopper compound.[7] The thermal stability and low reactivity of the 2-(1-dimethylaminoethyl)-phenylcopper tetramer probably reflect its thermodynamic stability. The low reactivity of this copper compound, R*Cu, was well established during the attempts to prepare derivatives of R*Cu and R*Li. Relatively low yields of derivatives were obtained after long reaction periods. This may reflect a high thermodynamic stability but also a kinetic stability due to steric hindrance at the position next to the bulky 1-dimethylaminoethyl group.

EXPERIMENTAL

Organometallic reactions were carried out under dry, oxygen-free nitrogen. Dry diethyl ether and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl, and toluene from $LiAlH_4$. Commercial butyllithium in hexane and methyllithium in ether were used at the strength determined by the supplier. All organometallic reagents contained lithium halides from their preparation. Infrared spectra were recorded on a Beckman IR 9 spectrometer, NMR spectra on a Bruker WH 270 or a Varian A 60 spectrometer, mass spectra on an AEI MS 902 or a Varian 1200 GC-AEI MS 20 combination, and gas chromatograms on a Perkin Elmer 900 instrument using SE 30, OV 17 or OV 225 on Chromosorb W.

(±)-2-(Dimethylaminoethyl)phenyllithium. *N,N*-Dimethyl-1-phenylethylamine [16] (10 mmol) was lithiated [10,17] with butyllithium (10 mmol) in ether (50 ml) by stirring the components together for about 50 h at room temperature. The solution became pale yellow and gave a positive response to Gilman test I.[16] (Gilman test II remained positive for about 44 h.[19])

2-(1-Dimethylaminoethyl)phenyllithium and deuterium oxide. Deuterium oxide (2 ml) was added to the lithium reagent thus prepared. The yellow colour of the lithium reagent disappeared within 1 min. After stirring overnight at room temperature, the ether layer was separated and extracted with 1 M HCl. The acidic extract was made alkaline, the free amine collected in ether, and the ether layer dried over anhydrous sodium sulfate. After removal of the ether at reduced pressure 2-(1-dimethylaminoethyl)deuteriobenzene (1.3 g, 87 %) was isolated. NMR data: δ 7.15–7.35 (3.94 H, m), 3.20 (1 H, q), 2.15 (6 H, s) and 1.34 (3 H, d).

When methyllithium was used in place of butyllithium in the above lithiation reaction, the yield of the lithium reagent was about 5 % after 6 h as determined by derivatizing with chlorotrimethylsilane (see below).

2-(1-Dimethylaminoethyl)phenyllithium and chlorotrimethylsilane. An excess of trichlorotrimethylsilane (*ca.* 10 mmol) was added to 2-(1-dimethylaminoethyl)phenyllithium (prepared from 6.66 mmol of *N,N*-dimethyl-1-phenylethylamine in 50 ml of ether) and the mixture was stirred for 4 h at room temperature, followed by 1 h at 34 °C. The mixture was hydrolysed with aqueous ammonia/ammonium chloride solution and worked up in the usual way to give a yellowish oil. This oil, when purified by column chromatography and cup-distillation, gave 0.9 g (60 %) of 2-(1-dimethylaminoethyl)phenyltrimethylsilane. (Found: C 70.9, H 10.7. Calc. for $C_{13}H_{23}NSi$: C 70.5; H 10.5. The IR spectrum showed a strong absorption at 763 cm^{-1} indicating 4 adjacent hydrogens. NMR data: δ 7.59 (1 H, d), 7.46 (1 H, d), 7.35 (1 H, t), 7.19 (1 H, t), 3.39 (1 H, q), 2.22 (6 H, s), 1.30 (3 H, d), 0.35 (9 H, s). MS: *m/e* 221 (M^+, 4 %); 206 (87 %); 72 (100 %). The measured mass at *m/e* 221 was 221.160; calc. for $C_{13}H_{23}NSi$ 221.160.

General procedure for the preparation of 2-(1-dimethylaminoethyl)phenylcopper reagent. Copper(I) iodide (3.4 mmol) was added to a cold (−20 °C) ether solution of 2-(1-dimethylaminoethyl)phenyllithium reagent (prepared from 3.3 mmol of *N,N*-dimethyl-1-phenylethylamine in 50 ml of ether) over a period of 2 h (with the help of a solid addition tube). With the addition of the first portion of copper(I) iodide,

a black precipitate appeared which rapidly disappeared to give a white precipitate with subsequent additions of CuI. When approximately half of the CuI had been added, a green precipitate began to appear. The mixture was stirred at −20 °C for 1 h after the addition of CuI, and then at room temperature for 2 h during which period Gilman test I became negative. This copper reagent was used without isolation for subsequent reactions. In one instance it was isolated by filtration under nitrogen. The green precipitates were washed with 3 × 30 ml of dry ether and subsequently recrystallised from ether. This procedure gave lemon-green crystals which were stable in the presence of air or water. Several weeks of exposure to air at room temperature changed the colour to dark green. The copper compound, however, reacted much faster with both water and oxygen in the presence of an organic solvent. The solid copper compound started to decompose without melting at 228 °C and the decomposition was complete at 260 °C as determined visually.

2-(1-Dimethylaminoethyl)phenylcopper and deuterium oxide. Deuterium oxide (0.7 ml) was added to the green slurry of 2-(1-dimethylaminoethyl)phenylcopper (prepared from 3.33 mmol of *N,N*-dimethyl-1-phenylethylamine), and the mixture was stirred at room temperature. No immediate reaction took place. Some green precipitates of the copper compound could be seen even after 24 h. The mixture was worked up after 66 h of stirring to give 2-(1-dimethylaminoethyl)deuteriobenzene (0.45 g, 89 %). This deuterio compound was identical to that obtained on deuteriolysis of 2-(1-dimethylaminoethyl)phenyllithium.

2-(1-Dimethylaminoethyl)phenylcopper and iodine. Solid iodine (20 mmol) was added to a cold (−78 °C) slurry of 2-(1-dimethylaminoethyl)phenylcopper (prepared from 20 mmol of *N,N*-dimethyl-1-phenylethylamine in 50 ml of ether) and the mixture was stirred overnight at this temperature.[2] After warming to room temperature the mixture was worked up in the usual way to give a brown liquid (2.4 g). TLC showed three components. Separation by preparative TLC gave 2-(1-dimethylaminoethyl)iodobenzene (2.05 g, 37 %). The IR spectrum was as expected. NMR data: δ 7.8 (1 H, d), 7.5 (1 H, d), 7.3 (1 H, t), 6.9 (1 H, t), 3.55 (1 H, q), 2.25 (6 H, s), 1.27 (3 H, d). MS: $m/e = 275$, (M^+, 11 %); 260 (100 %); 132 (37 %); 104 (20 %); 72 (83 %).

2-(1-Dimethylaminoethyl)phenylcopper and allyl bromide. Allyl bromide (10 mmol) was added to 2-(1-dimethylaminoethyl)phenylcopper (prepared from 10 mmol of *N,N*-dimethyl-1-phenylethylamine in 50 ml of ether) at −20 °C and the mixture was stirred for 0.5 h. The mixture was then stirred overnight at room temperature. Usual work-up gave 0.95 g of a liquid which was a 1:3 mixture (GLC area ratio) of *N,N*-dimethyl-1-phenylethylamine and *N,N*-dimethyl-1-(2-allylphenyl)ethylamine. The mixture was separated on an SE 30 column and the MS was obtained for the latter component. MS: $m/e = 189$ (M^+).

Preparation of mixed cuprates

Lithium methyl[2-(1-dimethylaminoethyl)-phenyl]cuprate was prepared by gradually adding 2-(1-dimethylaminoethyl)phenyllithium (20 mmol) to methylcopper (prepared by adding 20 mmol of methyllithium to 21 mmol of copper(I) iodide in ether) at −5 °C and stirring the mixture until Gilman Test I was negative.

Lithium phenyl[2-(1-dimethylaminoethyl)-phenyl]cuprate was prepared by using phenylcopper in place of the methylcopper. Phenylcopper was prepared as described previously.[21]

Lithium butyl[2-(1-dimethylaminoethyl)phenyl]cuprate was prepared by using butylcopper and the temperature was kept at −40 °C. Butylcopper was prepared as described before.[22]

Lithium dibutylcuprate and lithium diphenylcuprate were prepared from copper(I) iodide and butyllithium and phenyllithium, respectively, as described before.[23]

1,4-Addition reactions to methyl cinnamate

Solid methyl cinnamate (5 mmol) was added all at once to the cuprate, LiRR'Cu, (20 mmol) in 50 ml of ether. For LiRR'Cu = lithium diphenylcuprate, lithium methyl[2-(1-dimethylaminoethyl)phenyl]cuprate and lithium phenyl-[2-(1-dimethylaminoethyl)phenyl]cuprate the reaction temperature was 0 to −5 °C. For LiRR'Cu = lithium butyl[2-(1-dimethylaminoethyl)phenyl]cuprate and lithium dibutylcuprate the reaction temperature was −20 °C. After stirring for 1 h the mixture was poured into a saturated solution of aqueous ammonia and ammonium chloride (pH 8), shaken well and the organic material extracted into ether. The ether extract was washed with 3 × 50 ml portions of NH_3/NH_4Cl solution or until all the copper(I) halide was removed, and then once with 50 ml of a dilute sodium thiosulfate solution. The basic compounds were removed from this ether solution by extracting with 2 M HCl (3 × 50 ml). From the acid extract the starting amine, *N,N*-dimethyl-1-phenylethylamine, was recovered in about 65 % yield. The ether extract was dried over sodium sulfate and the ether removed under reduced pressure. The 1,4-addition products were isolated after distillation of the crude oil. Small amounts of high-boiling by-products were observed.[24] Main products, yields, and reaction conditions are summarised in Table 1.

Products

Methyl 3-phenylbutyrate, b.p. 133–135 °C/22 mm, lit.[24] b.p. 74–78 °C/0.5 mm, identical with an authentic sample by comparison of NMR spectra.

Methyl 3-phenylheptanoate, b.p. 67–68 °C/0.06 mm. Mol. weight determined by exact mass measurement 220.1456, calc. for $C_{14}H_{20}O_2$ 220.1463. NMR data: δ 7.25 (2 H, m), 7.18 (3 H, m), 3.56 (3 H, s), 3.07 (1 H, quin.), 2.60 (2 H, dd), 1.56–1.69 (2 H, m), 1.04–1.35 (4 H, m) and 0.82 (3 H, t).

Methyl 3,3-diphenylpropionate, m.p. 42–45 °C, lit.[25] m.p. 48 °C, b.p. 144 °C/0.15 mm. Mol. weight determined by exact mass measurements was 240.1123, calc. for $C_{16}H_{16}O_2$ 240.1150. NMR data: δ 7.13–7.27 (10 H, m), 4.56 (1 H, t), 3.54 (3 H, s), 3.05 (2 H, d).

Acknowledgements. This work has been supported by a research fellowship to M.T.R. from the International Seminar of Chemistry and Physics at Uppsala University supported by the Swedish International Development Agency and by a grant from the Trygger foundation. We thank Professor Jan G. Noltes and Dr. Gerard van Koten, TNO, Utrecht, for interesting discussions and communications of unpublished material and Professor H. O. House for a sample.

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Short Communications

Derivatives and Reactions of Glutacondialdehyde. VIII. ^{13}C NMR Studies

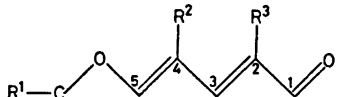
JENS PETER JACOBSEN and JAN BECHER

Department of Chemistry, University of Odense, DK-5000 Odense, Denmark

The glutacondialdehyde anion (*1*) first prepared by Baumgarten [1] has been assumed to have the all-*trans* configuration. This is mainly based



1



2-6

Scheme 1. *2*, $R^1=CH_3$, $R^2=R^3=H$; *3*, $R^1=C_6H_5$, $R^2=R^3=H$; *4*, $R^1=C_6H_5$, $R^2=CH_3$, $R^3=H$; *5*, $R^1=C_6H_5$, $R^2=CH_3O$, $R^3=H$; *6*, $R^1=C_2H_5O$, $R^2=R^3=H$.

on the X-ray structure of the derivative 5-hydroxy-2,4-pentadienal acetate (*2*) [2] and the existence of only three sets of lines in the ^1H NMR spectrum of *1*.[3,4] However, several other configurations are able to explain the equivalence in the proton spectrum of *1*. The H,H coupling constants [4] of *1* do not provide any conclusive evidence for the all-*trans* configuration, but do in fact indicate the possibility of having *cis* configuration of at least one of the double bonds. The number of known cyclization reactions of *1* may also be taken as an obstacle for the conception of *1* having the all-*trans* configuration. The assignment of *1* to a *cis* configuration or the depicting of *1* with an uncertain structure can be seen in numerous text-books and reviews.[5]

In order to clarify the problem related to the structure of *1* we have recorded the ^{13}C NMR spectra of this compound and of some simple derivatives. Furthermore, we have measured the ^{13}C spin lattice relaxation times, $T_1(^{13}C)$, of *1* and shown that these dynamical parameters in this case can be used in structure elucidation.

The ^{13}C chemical shifts of *1* and some simple derivatives in DMSO-d_6 are given in Table 1. The assignments are based upon gated decoupled spectra. In the case of *5* the pentadeuteriophenyl analogue has been used as an aid in the assignment. The spectrum of *1* consists in accordance with the proton spectrum of three lines only. The value of C-1 is shifted upfield to the area of normal α,β-unsaturated aldehydes [6] due to the delocalization of the excess charge. Wolkowski *et al.*[7] have established a correlation [$\delta_C=-220P(C)+1020$] between ^{13}C chemical shift, δ_C, and the charge density, $P(C)$, on the carbon atoms for α,β-unsaturated aldehydes. By use of this correlation the following charge densities in *1* can be evaluated from the ^{13}C chemical shifts: $P(C\text{-}1)=3.81$, $P(C\text{-}2)=4.16$ and $P(C\text{-}3)=3.92$. These values show the same trend as the ones calculated by Becher *et al.*[2] without known geometry of the molecule. A positive charge is associated with C-1 and C-3, a negative charge is associated with C-2. The excess charge in *1* does therefore not merely induce an increasing negative charge on all the carbon atoms, but do in fact reduce the electron density on C-3, thus, explaining the various reactions of *1*.[8]

The ^{13}C chemical shifts of *2* to *6* show the usual pattern of α,β-unsaturated aldehydes as well as the one of enol esters.[6] A downfield shift of 25 ppm of C-2 is observed when *1* is acylated. Under the assumption of unchanged value of the excitation energy [9] this shift indicates that the pronounced negative charge of C-2 in *1* is greatly diminished by acylation. The upfield shift of C-3 on acylation can likewise be taken as a reduction of the positive charge on this carbon atom.

The one bond C,H spin spin coupling constants in *1* are given by: $^1J_{C-1H-1}=155.3\pm0.5$ Hz, $^1J_{C-2H-2}=152.1\pm1.0$ Hz and $^1J_{C-3H-3}=155.8\pm1.0$ Hz. These values indicate an almost identical percentage *s*-character in all the C—H bonds.[10] The $^1J_{C-1H-1}$ coupling constant in *1* can be compared to the corresponding value in *2* of 171.5 ± 0.5 Hz. The increasing *p*-character in the C—H bonds between *2* and *1* is clearly demonstrated. Two long range C,H coupling constants have been found greater than 1.0 Hz in *1* and assigned to $^2J_{C-1H-3}=6.8\pm0.5$ Hz and

Table 1. ^{13}C Chemical shift, δ_i (ppm),[a] of glutacondialdehyde anion (*1*) and some simple derivatives (*2* – *6*).

Compound	C-1	C-2	C-3	C-4	C-5	$-C\langle^{O}_{O-}$	C(ω)[b]	C(*o*)	C(*m*)	C(*p*)
1	184.79	106.75	160.29	106.75	184.74					
2[c]	193.17	131.18	145.80	113.12	149.11	166.98				
3	193.29	131.70	146.06	114.29	148.92	163.37	127.67	129.88	129.04	134.50
4	193.36	128.32	142.49	120.20	152.82	162.11	127.80	129.82	129.04	134.43
5	192.71	129.94	127.22	141.19	146.77	161.59	–	129.55	128.97	134.30
6[d]	193.43	131.57	147.49	113.12	148.85	151.39				

[a] Uncertainty: ± 0.07 ppm. [b] Quaternary ring carbon. [c] δ 20.14 ppm (CH_3). [d] δ 13.84 ppm (CH_3), 65.30 ppm (CH_2).

$^2J_{C-^2H-1} = 21.3 \pm 0.5$ Hz. The corresponding values in *2* cannot be determined because of influence of second order effects in the proton spectrum. The value of $^2J_{C-^2H-1}$ in *1* is close to the values reported for various aldehydes.[11]

The spin lattice relaxation rate, $1/T_1$, due to intra-molecular dipole dipole mechanism is given by:[12]

$$1/T_1 = \hbar^2 \gamma_C^2 \gamma_H^2 (1/r_{CH}^6) \tau_c$$

in which γ_C and γ_H are the magnetogyric ratios of carbon and hydrogen, and r_{CH} the distance between the two nuclei. τ_c is the rotational correlation time for the C – H axis. If association tendency, where aggregation is long-lived compared to the rotational correlation time, is neglected, the relaxation behaviour of *1* can be discussed in terms of free molecules. Since *1* is a highly non-spherical molecule τ_c will differ amongst the various C – H axes, unless these axes are parallel.[13] This would give rise to different values of T_1, if the carbon atoms possess non-parallel C – H axes. Such a behaviour has been observed in several cases for instance in substituted benzenes.[6]

The $T_1(^{13}C)$ relaxation times have been measured in a saturated solution of *1* in DMSO-d_6 with the following results: T_1(C-1) = 2.7 ± 0.15 s T_1(C-2) = 2.5 ± 0.3 s and T_1(C-3) = 2.6 ± 0.3 s. Nuclear Overhauser effect measurements of the carbon atoms in *1* revealed that the relaxation is completely dominated by the intra-molecular dipole dipole mechanism. T_1 measurements in non-saturated solutions were less accurate but did confirm that the relaxation times for all the carbon atoms in *1* are identical. This implies that all C – H axes in *1* are parallel since only a small deviation caused by different C – H bonds and different C – C – H angles would be possible in this case. As it can be established that the C – H axes in *1* are parallel the all-*trans* configuration has been proved.

The possibility that *1* may exist as a rapid equilibrium between the all-*trans* and one or more of the other configurations may, *a priori*, not be neglected. However, it seems unlikely that such a situation should give rise to identical relaxation times for all the carbon atoms.

Experimental. The ^{13}C proton noise decoupled NMR spectra were recorded on a JEOL FX60 FT NMR spectrometer with internal deuterium lock. 8 K data points and a spectral width of 4000 Hz were used. The pulse length was 6 μs corresponding to a flip angle of 60°. The T_1 measurements were performed by the usual 180° – τ – 90° pulse sequence. The nuclear Overhauser effect was measured by comparing the gated decoupled spectrum with no spin spin splitting and the normal noise decoupled spectrum.[14] The compounds used have been prepared according to the methods described earlier.[15]

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Synthesis of [2$_4$](2,5)Thiopheneophanetetraene or [24]Annulene Tetrasulfide

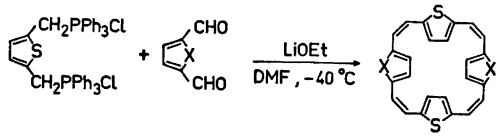
ANDERS STRAND, BENGT THULIN and OLOF WENNERSTRÖM

Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, Fack, S-402 20 Göteborg, Sweden

We have recently developed a convenient method for the preparation of various [2$_4$]-cyclophanes and related compounds from aromatic dialdehydes and bistriphenylphosphonium salts of bis(halomethyl)arenes by Wittig reactions at low temperatures.[1,2] Such cyclophanes are interesting starting materials for the synthesis of circulenes,[3] helicenes,[4] and annulenes. [2$_4$]Paracyclophanetetraene and the thiophene and furan derivatives contain a conjugated perimeter with 24 electrons and could formally be regarded as derivatives of [24]annulene. Although reasonably planar,[5] [2$_4$]paracyclophanetetraene does not show a paramagnetic ring current, *i.e.* it is not paratropic.[6] Among the bridged [18]annulenes, [18]annulene trioxide or [2$_3$]furanophanetriene has been characterized as aromatic.[8] In the corresponding sulfur compound, the three sulfur atoms are too large to be accomodated in a planar conformation. Thus the compound cannot sustain a diamagnetic ring current over the perimeter,[7] nor can [2$_3$]paracyclophanetriene.[9]

The heterobridged [24]annulenes have been studied less. Two isomers of [2$_4$]furanophanetetraene or [24]annulene tetroxide have been reported, both being similar to [24]annulene.[10] Some uncertainty in the structural assignment (*cis*/*trans* isomerism) has made conclusions less firm.

We now report the synthesis of [2$_4$](2,5)-thiophenophanetetraene, *1*, from 2,5-thiophenedicarbaldehyde and the bistriphenylphosphonium salt of 2,5-bis(chloromethyl)thiophene. An unstable [2](2,5)furano[2](2,5)thiopheno[2]-(2,5)furano[2](2,5)thiophenophanetetraene, *2*, was also prepared analogously from 2,5-furandicarbaldehyde (Scheme 1). The symmetrical structure of the compounds follows from their simple ^{1}H NMR spectra. Both cyclophanes were isolated as the all-*cis* isomers. [2$_4$](2,5)-Thiophenophanetetraene, *1*, shows a simple mass spectrum with the singly and doubly charged molecular ions as the major peaks. The absorption maximum (354 nm) is shifted 51 nm towards longer wavelengths as compared with [2$_4$]paracyclophanetetraene.[1] An attempted photocyclization of [2$_4$](2,5)thiophenophanetetraene in the presence of air and traces of iodine to give tetrathia[8]circulene was not successful, nor was the photocyclization of [2$_4$]paracyclophanetetraene,[3] [2](2,5)-thiopheno[2]paracyclo[2](2,5)thiopheno[2]paracyclophanetetraene or [2](2,5)furano[2]paracyclo[2](2,5)furano[2]paracyclophanetetraene.[2]



Scheme 1. 1 X=S, *2* X=O.

Molecular models show that [2$_4$](2,5)thiophenophanetetraene should be a rather flexible molecule. A conformation in which the sulfur atoms point outwards from the ring (A in Scheme 2) could be planar enough to allow for some overlap between the *p*-orbitals in the thiophene rings and the olefinic bridges. The thiophene protons are located inside the ring and the olefinic protons outside the ring in this conformation. A delocalized 24 π-electron system should result in a downfield shift of the inner protons and a smaller upfield shift of the outer protons, as is observed in the NMR spectrum of [2$_4$](2,5)thiophenophanetetraene. The effect, which is enhanced on cooling, is much smaller than in [24]annulene, however.

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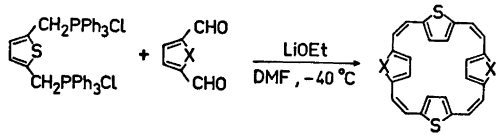
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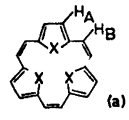
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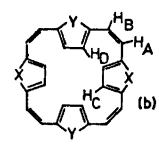


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Table 1. ^{1}H NMR data of some annulenes, [2_3]cyclophanetrienes (a), and [2_4]cyclophanetetraenes (b).

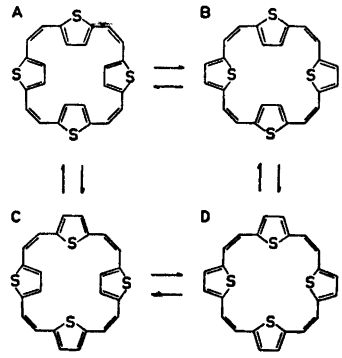




Compound	Temp. °C	Shift of protons outer			inner		Remark	Ref.
[18]Annulene	20	8.94			−2.0		Diatropic	11
	−60	9.28			−2.99			
[24]Annulene	40	7.25			7.25		Paratropic	12
	−80	4.73			12.9 − 11.2			
		H_A	H_B					
a, X = HC = CH	25	6.79	6.67				Nonplanar	7, 9
X = O	25	8.68,	8.66	not assigned			Diatropic	8
X = S	25	6.73,	6.67	not assigned			Nonplanar	9
b, X Y		H_A	H_B	J_{AB}	H_C	H_D		
HC = CH HC = CH	25	6.45	6.45		7.32	7.32		1
HC = CH S	25	6.55	6.38	11.8	7.24	6.80	No ring-	2
	−60	6.58	6.42		7.28	6.86	current	
HC = CH O	25	6.34	6.21	12.7	7.08	6.41		2
S O	25	6.17	5.94	12.3	7.23	6.73	Paratropic ?	
S S	25	6.19	6.19		7.33	7.33	Paratropic	
	−60	6.09	6.09		7.57	7.57		

A conformation in which two sulfur atoms point outwards from, and two towards the center of the cyclophane could be more planar (B and C in Scheme 2). Some deviations from the normal bond angles around the bridging double bonds, of the same magnitude as is observed in [2_4]paracyclophanetetraene,[5] are necessary, however. Four internal hydrogens and two sulfur atoms could be better accommodated in a planar conformation than eight internal hydrogens (compare A and B in Scheme 2). Such a favoured conformation should well agree with the observed UV spectrum. However, rapid rotation around the single bonds must occur even at −60 °C to rationalize the simple NMR spectrum (Scheme 2). The chemical shift for the thiophene protons is then an average of the shift for the internal and external protons. The observed temperature effect is explained by a large paramagnetic shielding of the inner protons and a smaller paramagnetic deshielding of the outer protons.

A conformation in which all four sulfur atoms point towards the center of the cyclophane (D in Scheme 2) could not be planar and show the observed chemical shifts and their temperature dependence.



Scheme 2.

Conclusions. All-*cis* [2_4](2,5)thiophenophanetetraene or [24]annulene tetrasulfide can readily be prepared in a one-step reaction from simple starting materials. Over the 24 π-electron periphery, the compound shows a small but significant paramagnetic ring current which is enhanced on cooling. Thus planar conformations have lower energy as is the case

for [24]annulene. Planarity is apparently the most important factor for the delocalization of the π-electrons. The presence of a local aromatic nucleus like thiophene does not interfere with the paratropic behaviour of the molecule.

Experimental. *[2$_4$](2,5)Thiophenophanetetraene*, 1. 2,5-Thiophenedicarbaldehyde (5 mmol) and the bistriphenylphosphonium salt of 2,5-bis(chloromethyl)thiophene (5 mmol) were mixed in dry dimethylformamide (250 ml) in a three-necked flask equipped with a mechanical stirrer and a dropping funnel. The temperature was kept at -40 °C with a thermostat-controlled cooling bath, and oxygen-free nitrogen was slowly flushed through the system. Lithium ethoxide in ethanol (*ca.* 0.3 M) was added dropwise at a rate allowing the coloured ylid to react between successive additions. The rate of reaction was fast in the beginning but slow after *ca.* half of the required base had been added. The addition took 24 h. When no colour change was observed on addition of base, the reddish solution was warmed to room temperature, diluted with water, and extracted with ether. The ether solution was washed with water, dried, and the solvent evaporated. The residue was chromatographed on silica gel with tetrachloromethane as eluent. The first reddish fractions yielded the desired product, [2$_4$](2,5)-thiophenophanetetraene, *1* (50 mg, 4.6 %, m.p. 210–215 °C). Later fractions, according to their NMR spectra, contained complex mixtures and were not further investigated. The product was assigned as the all-*cis* isomer. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 7.32$ and 6.19 (8 H, s, thiophene) (8 H, s, olefin), assigned by comparison with other cyclophanes [2]. On cooling the sample to -60 °C, the two singlets separated further to $\delta = 7.57$ and 6.09. UV (C_6H_{12}): 370 nm (sh), 354 (log $\varepsilon = 4.78$) and 298 (4.46). IR (KBr): 1595 cm^{-1} (m), 1445 (m), 1402 (m), 1235 (m), 1108 (m), 842 (m) and 816, 804, 800 (sh), 795 (s). MS (70 eV): *m/e* 432 (M^+, 100 %), 399 (4.0), 398 (4.2), 366 (4.4), 365 (5.8), 364 (4.2), 333 (5.1), 332 (3.6) and 216 (M^{2+}, 9.6). Abs. mass 432.0145 ± 0.002; calc for $C_{24}H_{16}S_4$ 432.0135.

[2$_4$]Thiophenophanetetraene, dissolved in cyclohexane or benzene with traces of iodine, was photolyzed in a Rayonet reactor. Light with maximum intensity at 254 or 300 nm was used. Only a slow decomposition of the cyclophane was observed.

[2](2,5)Furano[2](2,5)thiopheno[2](2,5)furano[2](2,5)thiophenophanetetraene, 2, prepared by the above method from 2,5-furandicarbaldehyde and the bistriphenylphosphonium salt of 2,5-bis(chloromethyl)thiophene, was found to be rather unstable and difficult to purify due to rapid decomposition. ^{1}H NMR (270 MHz, $CDCl_3$) of freshly chromatographed sample: $\delta =$ 7.23 (4 H, s, thiophene protons), 6.73 (4 H, s, furan protons), 6.19 (4 H, d) and 5.92 (4 H, d, $J = 12.3$ Hz, olefinic protons).

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Preferred Conformational Angles in Peptides Unperturbed by Hydrogen Bonding and α-Substituents

JOHANNES DALE, PER GROTH and KIRSTEN TITLESTAD

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With the recent X-ray structure determinations of cyclohexasarcosyl [1] and cyclodecasarcosyl,[2] which turned out to have the configuration sequences, *cis,cis,trans,cis,cis,trans* and *cis,cis,cis,trans,trans,cis,cis,cis,trans,trans*, respectively, some striking common features and

for [24]annulene. Planarity is apparently the most important factor for the delocalization of the π-electrons. The presence of a local aromatic nucleus like thiophene does not interfere with the paratropic behaviour of the molecule.

Experimental. *[2$_4$](2,5)Thiophenophanetetraene*, 1. 2,5-Thiophenedicarbaldehyde (5 mmol) and the bistriphenylphosphonium salt of 2,5-bis(chloromethyl)thiophene (5 mmol) were mixed in dry dimethylformamide (250 ml) in a three-necked flask equipped with a mechanical stirrer and a dropping funnel. The temperature was kept at -40 °C with a thermostat-controlled cooling bath, and oxygen-free nitrogen was slowly flushed through the system. Lithium ethoxide in ethanol (*ca.* 0.3 M) was added dropwise at a rate allowing the coloured ylid to react between successive additions. The rate of reaction was fast in the beginning but slow after *ca.* half of the required base had been added. The addition took 24 h. When no colour change was observed on addition of base, the reddish solution was warmed to room temperature, diluted with water, and extracted with ether. The ether solution was washed with water, dried, and the solvent evaporated. The residue was chromatographed on silica gel with tetrachloromethane as eluent. The first reddish fractions yielded the desired product, [2$_4$](2,5)-thiophenophanetetraene, *1* (50 mg, 4.6 %, m.p. 210–215 °C). Later fractions, according to their NMR spectra, contained complex mixtures and were not further investigated. The product was assigned as the all-*cis* isomer. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 7.32$ and 6.19 (8 H, s, thiophene) (8 H, s, olefin), assigned by comparison with other cyclophanes [2]. On cooling the sample to -60 °C, the two singlets separated further to $\delta = 7.57$ and 6.09. UV (C_6H_{12}): 370 nm (sh), 354 (log $\varepsilon = 4.78$) and 298 (4.46). IR (KBr): 1595 cm^{-1} (m), 1445 (m), 1402 (m), 1235 (m), 1108 (m), 842 (m) and 816, 804, 800 (sh), 795 (s). MS (70 eV): *m/e* 432 (M^+, 100 %), 399 (4.0), 398 (4.2), 366 (4.4), 365 (5.8), 364 (4.2), 333 (5.1), 332 (3.6) and 216 (M^{2+}, 9.6). Abs. mass 432.0145 ± 0.002; calc for $C_{24}H_{16}S_4$ 432.0135.

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With the recent X-ray structure determinations of cyclohexasarcosyl [1] and cyclodecasarcosyl,[2] which turned out to have the configuration sequences, *cis,cis,trans,cis,cis,trans* and *cis,cis,cis,trans,trans,cis,cis,cis,trans,trans*, respectively, some striking common features and

Table 1. Sets of torsion angles for cyclic sarcosine peptides.

	Number of possible isomers	In crystal	In $CDCl_3$ soln. at room temp.	*cis* N ϕ	*cis* CO ψ	*cis* N ϕ	*trans* CO ψ	*trans* N ϕ	*cis* CO ψ	*trans* N ϕ	*trans* CO ψ	Ref.
Sar_4	6	ctct	100 % ctct			−94	170	(−121	66)			14
Sar_5	8	cctt	>90 % cctt	−103	173	−69	172	(−123	70)	−64	148	15
				−89	176							
Sar_6	14	cctct	mixture	−75	170	−74	167	−75	138			1
Sar_7	20	ccctttt	mixture	−87	180	−72	177	(−134	69)	−87	−176	16
				−92	−164					−64	−169	
				−82	180							
Sar_8	38	cctcctt	100 % cctcctt	−107	180	−77	−167	−93	179	−71	167	17
				−92	−176	−72	−167	−83	173	−77	173	
Sar_{10}	100	ccctcccctt	–	−74	−165	−72	−174	−84	−173	−82	−178	2
				−101	−176							

regularities have become apparent for a whole series of cyclic peptides of sarcosine, excluding the lowest members (cyclodisarcosyl [3] and cyclotrisarcosyl [4]) which are constrained to an all-*cis* amide configuration and have torsion angles largely imposed by the ring structure.

These simple model substances were synthesized [5,6] in the hope of revealing the intrinsically preferred conformational angles unperturbed by the skeletal adjustments required to obtain good internal or external hydrogen bonding and by the trivial steric effects of large α-substituents. Because of the similar energy of *cis*- and *trans*-amide configurations and the observed absence of transannular interactions, the particular *cis,trans* sequence chosen by each ring among the great number of possible ones (Table 1) can be expected to be optimal. This is supported by the fact that the crystal conformation, at least as defined by the *cis,trans* sequence, is in several cases found to be alone, or dominant, also in solution.[7,8]

The table shows all thirty independently observed pairs of torsion angles, ϕ and ψ, for adjacent NC_α and $C_\alpha C$ bonds with the relative signs indicated,[9] choosing for each pair a negative sign of ϕ so as to allow easy comparison with proteins and poly-α-amino acids where the L-configuration generally favours this sign. It is seen that the values are largely concentrated in the region $\phi = 70 - 95°$ and ψ around 180°, and it makes no significant difference whether the adjoining amide groups are in *cis*- or *trans*-configuration, except possibly that the highest ϕ values are obtained when the amide on the carbonyl side is *cis*. The three exceptions form a second group with ϕ in the region $120 - 135°$ and ψ close to 70° with opposite sign, and this occurs exclusively when the amide on the carbonyl side is *cis* and on the nitrogen side *trans*.

The data of the table have been plotted in a conformational map (Fig. 1) displaced along both coordinates by 180° from the usual peptide conformational map.[9] This has the advantage of showing conformational minima grouped on both sides of ϕ or $\psi = 180°$ in a single area, torsion angles grouped on both sides of 0° being more rare. It is also more generally suitable for other compounds, for example in showing all easy interconversion paths for pentane unbroken within the map area.

The major group of torsion angle pairs for cyclosarcosyl peptides falls in the general area broadly defined [10] for protein structures as "extended chain", but is distinctly separated from the main region for hydrogen-bonded β-structures of α-substituted L-amino-acids in proteins [10] and homopolymers [9] and above all for β-structures involving glycine.[10] It is closer to the region for the non-hydrogen-bonded L-proline in proteins [10] and in helical homopolymers, both all-*cis* [11] and all-*trans*,[12] and to the metastable polyglycine helix [13] having no internal hydrogen bonds. The positions of

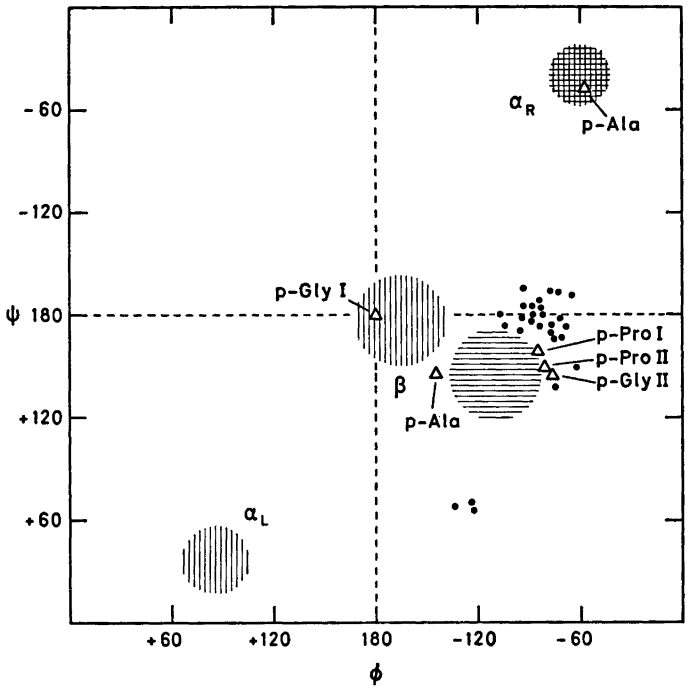


Fig. 1. Conformational map showing sets of torsion angles (sign of ϕ chosen negative) for cyclic sarcosine peptides (black dots), compared with those for poly-α-L-aminoacids (triangles), and the most common regions for glycine (vertical hatching) and α-alkyl-L-aminoacids (horizontal hatching) in proteins. Extended chain = β, righthanded α-helix = α_R, lefthanded α-helix = α_L.

various extended chains seen on this map suggest that the effect of the L-α-substituent, including the proline ring structure, is to decrease the ψ-angle from $\sim 180°$ to $+150°$; the effect of replacing *N*-methyl with NH and establishing hydrogen bonds is to increase the ϕ angle from about $-80°$ to near 180°; and the combined effect of both is to give the same decrease of ψ but intermediate increase of ϕ.

The minor group of torsion angle pairs for cyclosarcosyl peptides is completely isolated in the map. Since this combination occurs only in the smaller of the rings, it must be of lower stability and used only when the ring structure requires the sharp bend possible by the sequence $\omega = 0$ (*cis*), $\psi \sim 70°$. However, if the ϕ value were then to remain near $-80°$ the bend would be too sharp, and the transannular repulsion widens the ϕ angle to $\sim -130°$. It is also easy to see that for simple steric reasons the sequence $\omega = 0$, $\psi = 60°$, $\phi = 120°$, $\omega' = 0$ is impossible (except in a cyclic tripeptide) and thus explains why the amide configuration on the nitrogen side must be *trans* ($\omega' = 180°$).

It thus looks as if there is a substantial ethane-like intrinsic barrier in the $C_\alpha - C$ bond and that the ψ-angle is preferentially *anti*, the alternative *gauche* being found only in the smaller rings. The total spread of ϕ angles over a single wider range ($64 - 134°$) suggests the absence of an intrinsic barrier in the $N - C_\alpha$ bond, and a simple steric repulsive balance between the carbonyl oxygen and the two substituents on the nitrogen of the same residue (methyl and the main chain).

The general occurrence of sequences of only *cis*- or only *trans*-amide configuration in the larger rings (Table 1) is difficult to understand. It is striking, however that in these crystal conformations the carbonyl oxygen of all *cis*-amide groups tend to point out of the ring whereas oxygen of *trans*-amide groups point into the ring, orienting themselves against the positive end of *cis*-amide groups across the ring. Similar and well-known examples of induction of identical configuration are shown by poly-L-proline, which crystallizes as all-*trans* from polar solvents and as all-*cis* from less polar solvents. It may be significant that in the all-*trans* helix [12] the carbonyl oxygens point outwards (to the polar solvent) whereas in the all-*cis* helix [11] each oxygen is oriented towards the positive end of other amide groups further along the axis.

The synthesis of cyclodecasarcosyl has not been reported before. The *N*-protected decapeptide was obtained by coupling two pentapeptides, using the same general methods as already described,[6] and the trichlorophenyl ester cyclized in pyridine, yield 50 %, m.p. 280 – 282 °C.

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On the Fluorescence of Propellicene

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Certain crowded polyaromatic molecules, most notably 9,9′-bianthracene,[1,2] exhibit a surprising charge-transfer emission in many solvents. Although the theory of the emission has not been worked out in detail, the observation of a charge-transfer emission may be ascribed to (a) fluctuations in the solvent arrangements within a sufficiently polar solvent environment which permit the evolution of a cybotactic region [3] that stabilizes a charge-transfer state and (b) lack of rapid quenching pathways for nonradiative decay of the charge-transfer state. In 9,9′-bianthracene, a perpendicular relationship of the two rings leads to decay rates of the charge-transfer state which are lower than the radiative rate.[4] It was thus of interest to find whether the unusual cyclic helicene, propellicene (see Fig. 1; the formula),[5] could give rise to charge-transfer emissions.

Fluorescence spectra were measured in dioxane and dioxane-water mixtures, and in glycerol. The insolubility of propellicene in glycerol forced us to examine a dispersion of a dioxane solution of the compound in glycerol. The fluorescence lifetimes and quantum yields of fluorescence were also measured in dioxane and 33 % dioxane-water solutions.

In no case was a charge-transfer emission observed. The fluorescence quantum yield is not particularly high, but the quenching mechanisms were not further investigated. A structured fluorescence spectrum with peaks at 413 and 433 nm (shoulders at 463 and 495 nm) can be observed in dioxane. Addition of water does not affect the emission spectrum until a composition of 33 % dioxane-water is reached, at which point, the spectrum shifts to 10 nm longer wavelengths without change in excitation spectrum. The excitation spectrum in all cases was identical with the absorption spectrum.

Lifetime data and derived rate constants are shown in Table 1. The fluorescence lifetimes of helicenes are extremely long [6] and propellicene is no exception. The absorption and fluorescence spectra of propellicene resemble those of hexahelicene.[6]

The change in position of the fluorescence maxima in 33 % dioxane-water from those found for pure dioxane is probably due to an aggregate (dimer?) of propellicene. The spec-

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Table 1. Fluorescence lifetimes, quantum yields and derived rate constants for propellicene.

	Dioxane-water mixtures Vol. % dioxane	
	100	33.3
$E_T(30)$ [a]	36.0	56.5
Φ_F [b]	0.034	0.012
τ_{obs}/ns	12.2	3.58
$k_r/10^7$ s^{-1} [c]	0.279	0.235
τ_r/ns	358	293
$k_{nr}/10^7$ s^{-1} [d]	7.93	27.6

[a] Solvent polarity parameter, see Ref. 8. [b] Quantum yield of fluorescence. [c] Radiative rate constant, $k_r=\Phi_F/\tau_{obs}$. [d] Nonradiative rate constant, $k_{nr}=(1-\Phi_F)/\tau_{obs}$.

trum in dioxane dispersed in glycerol resembled that in dioxane.

The lack of a charge-transfer emission probably implies that the nonradiative rate for a charge-transfer state is too high. Overlap between the positive and negative ends of a charge-transfer state are apparently too great to allow the separated charges to be stabilized by solvent traps. Appropriately substituted propellicenes (with donor and acceptor groups at opposite ends of the molecule; appropriately substituted helicenes might also serve the purpose) might give rise to charge-transfer emissions.

Experimental. Propellicene was available from a previous synthesis.[5] UV spectra were measured with a Cary model 17 recording spectrometer. Fluorescence spectra (corrected) (temperature controlled at 25.0 °C) were recorded with a Hitachi-Perkin-Elmer MPF-4 with a corrected spectra attachment. Fluorescence lifetimes were measured by the single photon counting technique with a setup similar to that described by Berson and Horowitz.[7] Dioxane was Spectrograde and glycerol was for fluorescence microscopy (E. Merck and Co., Darmstadt).

Acknowledgements. Support from the United States-Israel Binational Science Foundation (BSF) (EMK, HD) and the Swedish Natural Science Research Council (BT, OW) is appreciated.

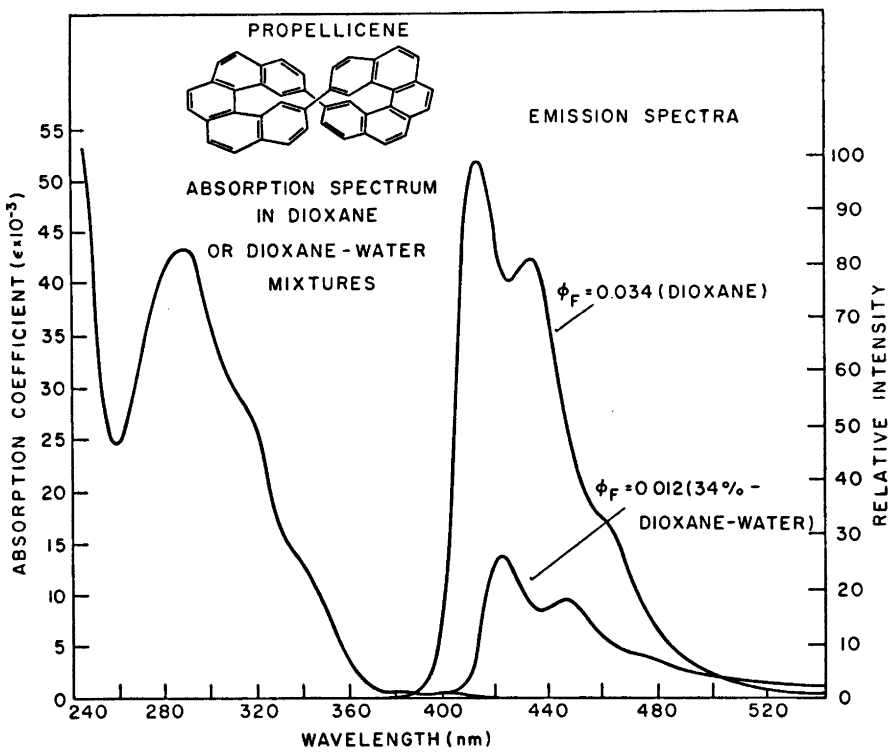


Fig 1. Absorption spectrum and emission spectra for propellicene.

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Mass Spectral Differentiation of Some Unsymmetrically Substituted Isomeric Dihydrobarbiturates

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Recently it has been shown that the sodium borohydride reduction of some unsymmetrically substituted barbituric acid derivatives such as 1-methyl-5-ethyl-5-phenylbarbituric acid (MEPBA, *1*) leads to the formation of two different dihydrobarbiturates.[1] From a mass spectral investigation of the products formed it was found that mass spectrometry provides a method by which they can be easily distinguished. In the present communication we describe the results mainly obtained using the dihydrobarbiturates *2* and *3*, derived from *1*.

The mass spectral fragmentation of unreduced barbituric acid derivatives is well-known and it has been shown that the preferential fragmentation is strongly influenced by the nature of the C-5 substituents.[2-4] See also Refs. 5–11.

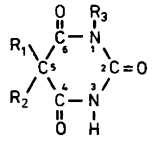
Fig. 1 shows the mass spectrum of 1-methyl-5-ethyl-5-phenyl-6-dihydrobarbiturate *2*. The fragmentation is strongly dominated by the formation of an abundant ion corresponding to *m/e* 146 (base peak). Its formation and further fragmentation are depicted in Scheme 1. The loss of water from the molecular ion has also taken place to a certain extent.

Fig. 2 shows the mass spectrum of 1-methyl-5-ethyl-5-phenyl-4-dihydrobarbiturate *3*. The formation of the base peak at *m/e* 146 can be depicted in a manner analogous to the previous case (Scheme 2, Route A). The elimination of water leads to the ion of *m/e* 230* which may fragment to the ion of *m/e* 146. However, its main fragmentation path is of *retro*-Diels-Alder type, leading to an abundant ion of *m/e* 173. Its formation and further fragmentation are depicted in Scheme 2 (Route B).

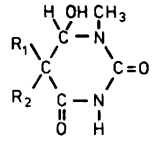
It is worthy of note that the McLafferty rearrangement of the C-5 ethyl substituent, dominating the mass spectral fragmentation of similar unreduced barbituric acid derivatives (*e.g.* 5-ethyl-5-phenylbarbituric acid, Luminal® *4*),[3] is not present in any appreciable amount in either case.

The importance of the *retro*-Diels-Alder process in the mass spectral behaviour of *3*, in all probability due to the vicinity of the OH- and NH-groups permitting the thermal 1,2-elimination of water (*vide supra*), can be used successfully to differentiate between *2* and *3*. In an analogous manner, the peaks at *m/e* 159 and *m/e* 149 in the mass spectra of *6* and *8*, respectively, permit the differentiation of *6* and *8*

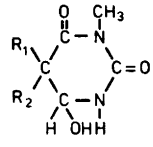
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2. $R_1 = C_6H_5$; $R_2 = C_2H_5$
5. $R_1 = C_6H_5$; $R_2 = CH_3$
7. $R_1 = R_2 = CH_2{=}CH{-}CH_2$



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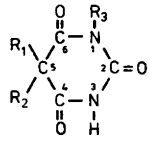
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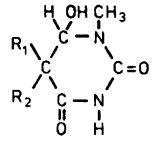
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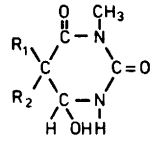
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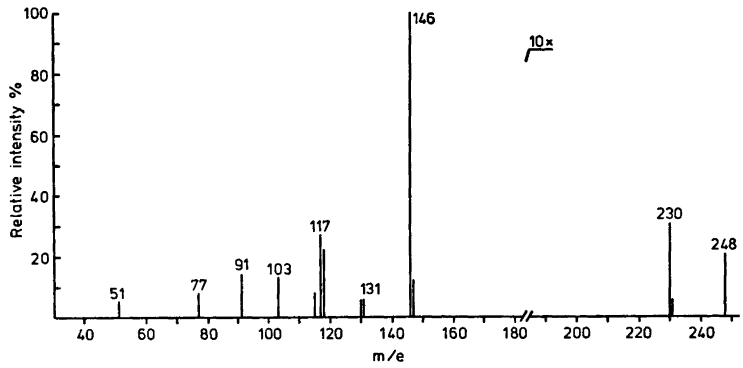


Fig. 1. Mass spectrum (70 eV) of 1-methyl-5-ethyl-5-phenyl-6-dihydrobarbiturate *2*.

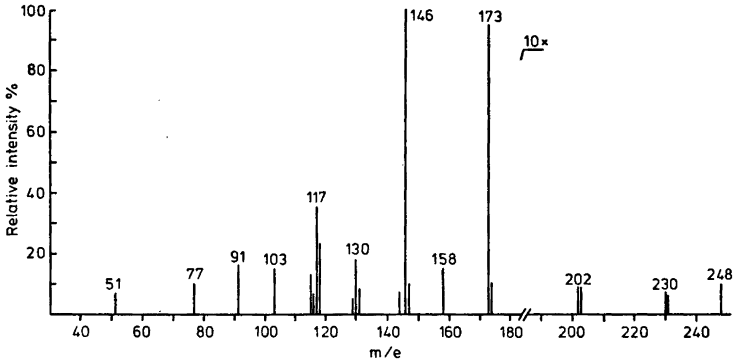
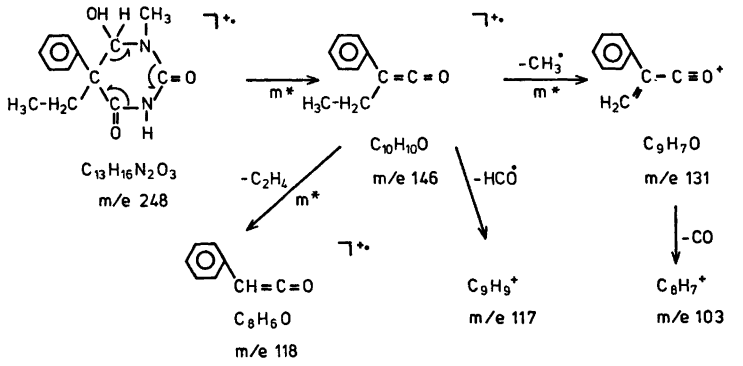
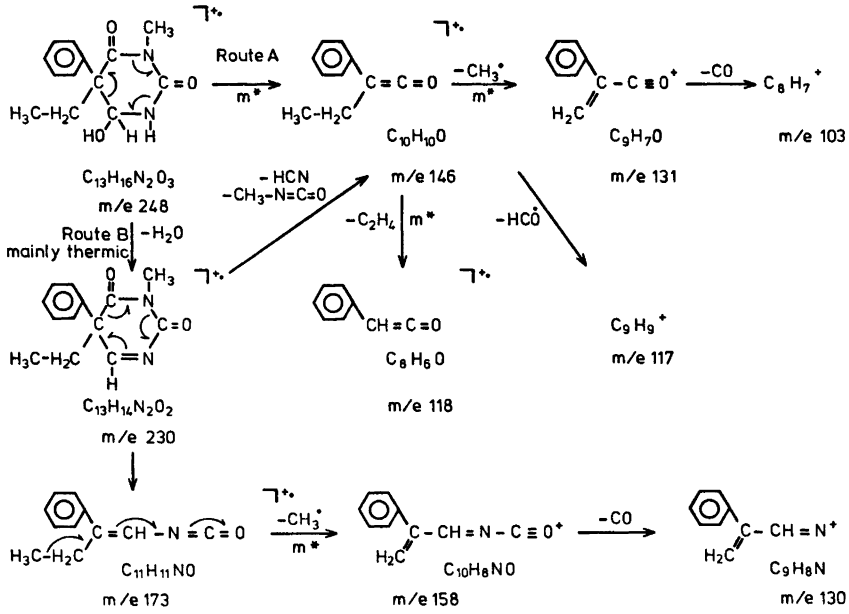


Fig. 2. Mass spectrum (70 eV) of 1-methyl-5-ethyl-5-phenyl-4-dihydrobarbiturate *3*.



Scheme 1.



Scheme 2.

from their corresponding C-6-OH isomers *5* and *7*.

Experimental. The preparation of dihydrobarbiturates *2*, *3*, *5*, *6*, *7* and *8* has been described earlier.[1] The mass spectra were recorded on a Jeol JMS-D-100 Mass Spectrometer at 70 eV using direct sample insertion into the ion source, whose temperature was 110–120 °C. The presence of metastable ions, when indicated, was confirmed by measurements performed using a Hitachi Perkin-Elmer RMU 6E instrument.

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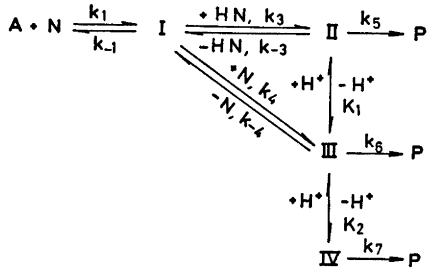
Acta Chemica Scandinavica B 31 (1977) 531—536

Hydroxylaminolysis of Anilides. VI. Kinetic Investigation of the Reaction with Monofluoroacetanilide. Evidence for the Existence of a Reaction Path Involving Three Hydroxylamine Molecules

BARBRO ARIANDER OHLSON and ULF JONSSON

Department of Inorganic and Physical Pharmaceutical Chemistry, Biomedical Centre, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden

The reaction between hydroxylamine and monofluoroacetanilide has been studied by means of kinetic methods in the pH interval $3.62 \leqslant -\log [H^+] \leqslant 9.79$. The concentration of the hydroxylamine system (C) varied between 0.2 M and 3.0 M ($\mu = 3.0$ with KCl). In the pH interval studied, the kinetics are mainly in accord with the mechanism previously postulated for formanilides with and without electron withdrawing substituents, from which was derived a mathematical expression for the observed reaction rate which is first order in C at low pH values (pH ~ 4) and second order in C at high pH values (pH > 7). In addition to this expression the kinetics of the monofluoroacetanilide reaction include a term first order in C at $-\log [H^+] = 9.79$, and a term third order in C in the interval $6.50 \leqslant -\log [H^+] \leqslant 7.27$. This is to our knowledge the first reported evidence of an aminolysis reaction which is third order in the amine. Some of the rate constants for the separate steps in the overall mechanism have been evaluated and the influence of the fluoro substitution on these steps is discussed.



Scheme 1. A, anilide; P, products.

In two previous papers [1,2] from this Laboratory, the kinetics of the hydroxylaminolysis of acetanilide and of trifluoroacetanilide were studied. The mechanism depicted in Scheme 1 was postulated for the reaction with acetanilide, and eqn. (1) was derived for the relationship between the observed pseudo-first order rate constant, k_{obs}, and the concentration of the hydroxylamine system. ($N = H_2NOH$, $HN = H_3N^+OH$, $C = [N] + [HN]$).

$$k_{obs} = k_1[N]pC/(1 + pC) \quad (1)$$

In eqn. (1), k_1 is the rate constant for the nucleophilic attack by N on the carbonyl carbon, and p is a pH dependent parameter. At high pH values, the product $pC \ll 1$ and $C \sim [N]$. Thus eqn. (1) is reduced to $k_{obs} = k_1 p[N]^2 = k[N]^2$. In this pH range, the breakdown of the anilide is thought to proceed entirely *via* the steps k_1, k_4, (K_2) and k_7 in Scheme 1.

The kinetics of the trifluoroacetanilide reaction,[2] on the other hand, could be described simply by eqn. (2).

$$k_{obs} = k_I[N] + k_{II}[N]^2 \quad (2)$$

In the pH range studied, the term first order in $[N]$ was of little importance. According to the second term in the equation, the hydroxylaminolysis reaction is catalyzed by a second hydroxylamine molecule, but the kinetics could not be used as a basis for determination of where and when the catalysis takes place. However, there is nothing in the kinetics to contradict the assumption that the k_{II} path

in the trifluoroacetanilide equation represents the $k_1-k_4-K_2-k_7$ path in the mechanism in Scheme 1.

In an attempt to find a connection between the kinetics of acetanilide and trifluoroacetanilide, we have now investigated the hydroxylaminolysis of monofluoroacetanilide, which may be expected to combine the properties of the other two anilides.

MATERIALS AND METHODS

Monofluoroacetanilide (MFA) was synthesized as described in Ref. 3. All other chemicals used were of analytical grade.

The hydroxylaminolysis reaction was studied in the range $3.62 \leqslant -\log [H^+] \leqslant 9.79$. The relation between measured pH values and molar concentration of hydrogen ion, $[H^+]$, in 3 M KCl at 25 °C ($-\log [H^+] = pH + 0.20$), has been determined previously.[4,5] Because of the different medium effects in different concentrations of the hydroxylamine system,[1] $-\log [H^+]$ is used instead of pH when a precise denotation is desired.

The kinetic experiments and the assay were performed as in our previous work.[7] The initial concentration of MFA was $(1-7.5) \times 10^{-3}$ M. The reaction was followed until maximally 40 % of the anilide had reacted.

The k_{obs} values were determined from plots of log[remaining anilide] against time, except in a few cases where less than 4 % of the anilide

Table 1. Observed and theoretical pseudo-first order rate constants (k_{obs} and $k_{calc}/min^{-1} \times 10^{-5}$) for the formation of aniline from monofluoroacetanilide with different fractions of the hydroxylamine system present as base. The values of k_{calc} have been obtained by means of eqn. (3) using $k_1 = 3 \times 10^{-3}$ min^{-1} M^{-1} and the values of p' and q listed in Table 2.

C/M	0.2 %		1.0 %		3.0 %		5.0 %		10.0 %	
	k_{obs}	k_{calc}	k_{obs}	k_{calc}	k_{obs}	k_{calc}	k_{obs}	k_{calc}	k_{obs}	k_{calc}
0.2	0.087	0.108	0.10	0.10	0.15	0.16	0.17	0.17	0.49	0.25
0.6	0.35	0.33	0.41	0.37	0.62	0.60	0.66	0.70	1.12	1.15
1.0	0.57	0.55	0.69	0.67	1.20	1.19	1.48	1.48	2.49	2.55
1.4	0.82	0.77	1.01	1.02	1.96	1.91	2.40	2.50	4.64	4.42
1.8	0.98	1.00	1.43	1.42	2.81	2.82	3.68	3.73	6.87	6.73
2.2	1.24	1.23	1.89	1.85	3.64	3.71	5.16	5.16	9.62	9.45
2.6	1.47	1.46	2.30	2.32	4.82	4.78	6.87	6.78	12.5	12.6
3.0	1.70	1.69	2.74	2.83	5.96	5.95	8.75	8.58	16.1	16.0

C/M	20.0 %		40.0 %		60.0 %		80.0 %	
	k_{obs}	k_{calc}	k_{obs}	k_{calc}	k_{obs}	k_{calc}	k_{obs}	k_{calc}
0.2	0.37	0.20	0.44	0.49	1.10	0.74	0.93	0.94
0.6	1.72	1.75	3.56	4.27	5.48	6.40	6.50	8.15
1.0	4.56	4.70	11.0	11.4	16.4	17.1	20.0	21.8
1.4	8.80	8.93	21.5	21.5	32.0	32.3	41.4	41.3
1.8	14.3	14.3	36.0	34.3	52.4	51.5	69.8	65.9
2.2	20.3	20.8	52.0	49.5	87.8	74.3	106	95.2
2.6	28.0	28.2	70.2	66.9	122	100	160	129
3.0	37.4	36.6	94.0	86.2	175	129	200	166

C/M	90.0 %		C/M	99.0 %		99.9 %		99.97 %	
	k_{obs}	k_{calc}		k_{obs}	k_{calc}	k_{obs}	k_{calc}	k_{obs}	k_{calc}
0.2	1.46	0.88	0.994	–	–	20.7	21.1	23.9	22.7
0.58	7.20	7.17	0.997	20.7	20.8	–	–	–	–
0.99	19.3	20.3	1.78	60.8	63.0	65.7	64.3	–	–
1.38	39.0	38.3	1.79	–	–	–	–	68.6	69.4
1.78	65.6	61.8	2.54	–	–	125	125	–	–
2.17	102	89.4	2.58	–	–	–	–	139	137
2.55	147	120	2.59	129	126	–	–	–	–
2.95	192	157							

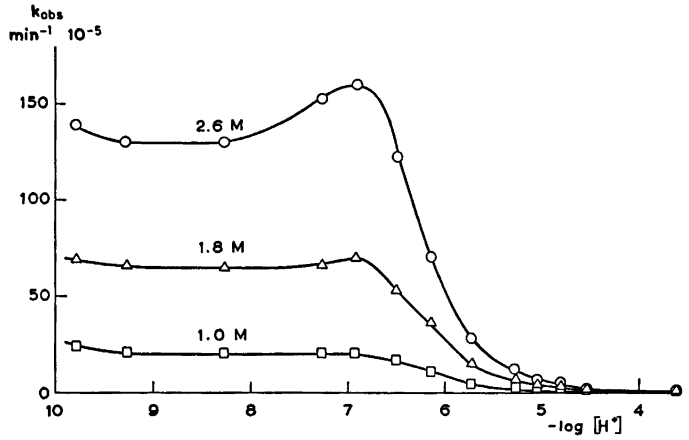


Fig. 1. Plots of k_{obs} *vs.* $-\log[H^+]$ at three different C values. The points are experimental. The curves are not calculated.

had reacted. In these cases the k_{obs} values were evaluated from plots of concentration of product against time.

RESULTS

Table 1 lists the pseudo-first order rate constants, k_{obs}, for the formation of anilide from MFA at different concentrations of the hydrox-

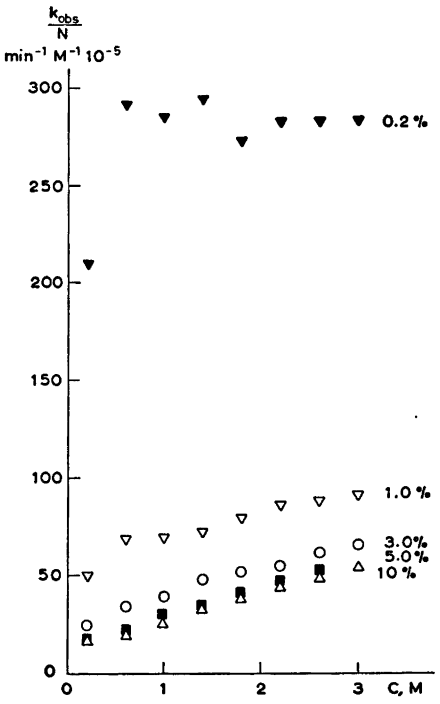


Fig. 2. Plots of $k_{obs}/[N]$ *vs.* C at 0.2, 1.0, 3.0, 5.0 and 10.0 % base.

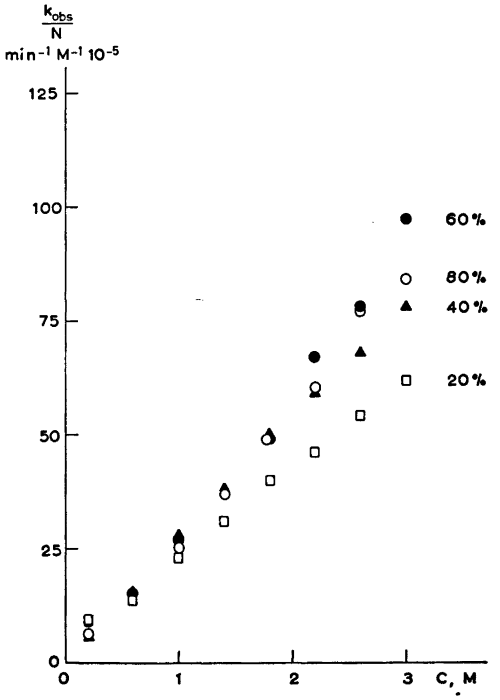


Fig. 3. Plots of $k_{obs}/[N]$ *vs.* C at 20.0, 40.0, 60.0 and 80.0 % base.

ylamine system (C) and with different fractions of the system present as base. Fig. 1 shows the variation of k_{obs} with pH at 3 different concentrations of the hydroxylamine system. This kind of pH profile is typical of most anilides, except for the rise at the highest pH value which has not been encountered earlier, and will be commented upon below.

There is no significant contribution to the reaction rate from uncatalyzed or HO^- catalyzed alkaline hydrolysis.[3] Acid hydrolysis is noticeable only at $C = 0.2$ M at 0.2 % base where it has been calculated to amount to about 4 % of k_{obs}.[6] The value given in Table 1 has been corrected for the acid hydrolysis. In all other cases, the contribution was 1 % or less, and the corresponding k_{obs} values have not been corrected.

Figs. 2 – 4 show plots of $k_{obs}/[N]$ *vs.* C. These plots will be commented upon in the Discussion.

DISCUSSION

The rate data for MFA show the characteristics that we have earlier found[1,7–9] to be typical of the hydroxylaminolysis of anilides. Also, the pH-dependent intercepts of the plots

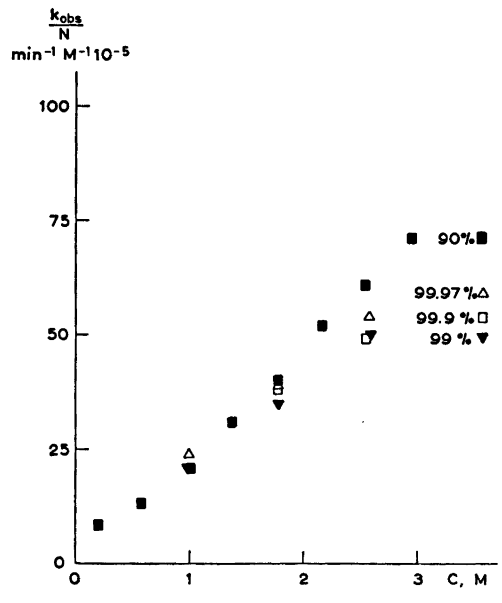
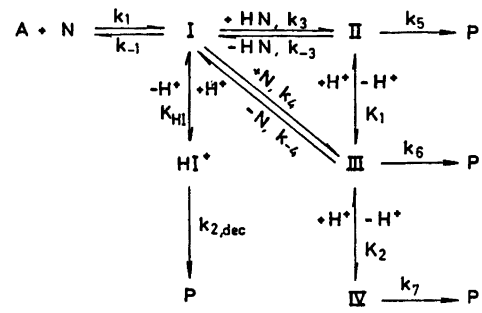


Fig. 4. Plots of $k_{obs}/[N]$ *vs.* C at 90.0, 99.0, 99.9 and 99.97 % base.



Scheme 2. A, anilide; P, products.

of $k_{obs}/[N]$ *vs.* C indicate that the hydroxylaminolysis of MFA involves H+ catalysis. In that respect MFA differs from acetanilide [1] and trifluoroacetanilide [2] but is similar to formanilides.[9] The mechanism proposed for formanilides is shown in Scheme 2. Eqn. (3), which is derived from this mechanism, generally gives a good fit to the experimental data of MFA (Table 1).

$$k_{obs} = k_1[N](p'C + q)/(1 + p'C) \quad (3)$$

The parameter p' in eqn. (3) is a function of [H+] and the microconstants in Scheme 2 [9] and is closely related to p in eqn. (1). The parameter q is also pH-dependent and is defined by eqn. (4).

$$q = \frac{k_{2,dec}[H^+]/K_{HI}k_{-1}}{1 + k_{2,dec}[H^+]/K_{HI}k_{-1}} \quad (4)$$

Eqn. (3) has been fitted to the experimental data by the method described in Ref. 9. The value of k_1 has been estimated from the plot of $k_{obs}/[N]$ *vs.* C at 0.2 % base. From the intercepts of the plots of $k_{obs}/[N]$ *vs.* C (Fig. 2) approximate values of q have been obtained. Different values of p' and q have then been entered into eqn. (3) and the calculated and experimental k_{obs} values plotted together, until the best fit was obtained. Since the plots of k_{obs} *vs.* C at 60, 80 and 90 % base are slightly more than second order in C, the uppermost part of these plots ($C > 2$ M) have been disregarded in the search for the optimal p' and q values. A variation of p' by 3 – 5 % and of q by 10 % gives a less good fit to the experimental k_{obs} values. The optimal p' and q values are listed in Table 2.

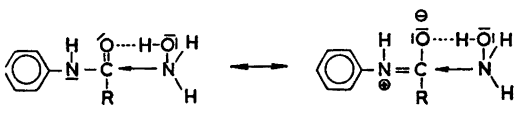
Table 2. Values of p' and q in eqn. (3) which give the best fit to the experimental k_{obs} values.

% Base	− log [H+]	p'/M^{-1}	q
0.2	3.62	0.20	0.90
1.0	4.52	0.07	0.17
3.0	4.80	0.06	0.08
5.0	5.04	0.06	0.45
10.0	5.27	0.06	0.03
20.0	5.72	0.085	–
40.0	6.14	0.105	–
60.0	6.50	0.105	–
80.0	6.92	0.100	–
90.0	7.27	0.083	–
99.0	8.27	0.076	–
99.9	9.27	0.077	–
99.97	9.79	0.083	–

Formation of Intermediate I

The rate constant k_1 for the formation of the intermediate I may be evaluated directly from a plot of $k_{obs}/[N]$ *vs.* C at low pH if the plot is parallel to the abscissa.[9] The value of k_1 for monofluoroacetanilide, evaluated by this method from the plot at 0.2 % base, is 3×10^{-3} min^{-1} M^{-1}. (For a more detailed comment on the method of evaluation, see Ref. 9). In the hydroxylaminolysis of acetanilide, $k_1 = 7 \times 10^{-4}$ min^{-1} M^{-1}.[1] Thus k_1 is increased fourfold when the fluorine atom is introduced into the acyl group. The effect is small compared to the effect in the corresponding k_1 values in alkaline hydrolysis, where the fluorosubstitution results in a 200-fold increase.[3,10]

In the nucleophilic attack on the amide group the hydroxylamine molecule is suggested [7,11] to be hydrogen bonded to the carbonyl oxygen, thus forming a cyclic structure which has two mesomeric forms that help to stabilize the system long enough for the C−N bond to be formed. See Scheme 3. This may explain why hydroxylamine is a far more active nucleophile than is warranted by its basicity.[12] Consequently, if the hydrogen bond is weakened, the rate of formation of the hydroxylamine-



Scheme 3.

anilide addition intermediate would be lowered. It is known [13,14] that the hydrogen bond to the carbonyl oxygen is weaker in fluoroacetic acid than in acetic acid. Thus the small increase in k_1 following upon fluoro substitution is the result of two opposed substituent effects: an increase due to lower electron density at the carbonyl carbon and a decrease due to weaker hydrogen bonding to the oxygen.

H+ Catalyzed breakdown of I

The value of the ratio $k_{2,dec}/K_{HI}k_{-1}$ estimated from the q values in Table 2 is about 6×10^4 min^{-1} M^{-1}, which is the same as for *p*-chloro- and *p*-nitroformanilide.[9]

The H+ catalyzed path has earlier been found only with formanilides, either unsubstituted or with electron-withdrawing substituents in the *para* position, but not with acetanilides.[9] The fact that an electron-withdrawing substituent in the acyl part of an acetanilide makes this path kinetically detectable supports the conclusion that the ratio reflects the influence of the substituent on the breaking of the amide bond rather than the influence on the protonation of intermediate I.

Additional catalysis by the hydroxylamine system

At 60 − 90 % base, the experimental plots of k_{obs} *vs.* C (not shown here) are slightly more than second order in C. Thus a third N or HN must be involved in the reaction in this pH range. Since a more than second order dependence on C has not been found with any of the anilides studied earlier in the same C and pH ranges the phenomenon cannot be ascribed to general medium effects but must be due to some specific properties of the MFA structure.

Assuming that Scheme 2 is valid for the hydroxylaminolysis of MFA, the third N or HN must act as a catalyst in the k_1 step or in one or more of the k_5, k_6 and k_7 steps, in order to result in a term third order in C, unless it is to be ascribed to a totally different mechanism. However, the relatively small influence of the third order term suggests that a large part of the reaction takes place *via* a path where the additional catalysis is not operating. Thus the possible ways for the additional catalysis are

an *N* or *HN* catalyzed nucleophilic attack parallel to the uncatalyzed k_1 path, and an *N* or *HN* catalyzed breakdown of II, III and/or IV parallel to the k_6, k_8 and k_7 paths. At this stage we cannot distinguish between the two possibilities.

HO^- Catalyzed hydroxylaminolysis

At high pH (99 – 99.9 % base) the overall reaction rate reaches a constant value (Fig. 1), which rises as the pH is further increased to give 99.97 % base. According to Ref. 3 this rise cannot be due to alkaline hydrolysis.

The plot of $k_{obs}/[N]$ *vs.* *C* at 99.97 % base is parallel to the plots at 99 and 99.9 % base (see Fig. 4), but it has an intercept which makes it lie above the other two. The intercept indicates that at this pH there is a path in the reaction mechanism which involves one *N* and one HO^-. This path may consist of HO^- catalyzed breakdown [15] of I or of hydroxylamine catalyzed breakdown of an anilide-hydroxyl ion addition intermediate.

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Studies on the Autoxidation of *t*-Butyl-substituted Phenols in Alkaline Media. 1. Reactions of 4-*t*-Butylguaiacol

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The course of the alkaline autoxidation of 4-*t*-butylguaiacol is described. Initial oxygenations in the 4- and 6-positions are followed by alkaline-oxidative conversions of the resulting intermediates, *viz.* an epoxidated hydroxycyclohexadienone and an *o*-quinone, into carboxylic acids and lactones. Oxidative coupling to give a dimer competes with the degradation routes.

Numerous studies on the autoxidation of organic compounds [1] in alkaline media have revealed that the process proceeds *via* peroxy anions (R—O—O⁻). The formation of these important intermediates may be formulated as a chain reaction involving free radicals [2–4] or as a direct reaction between a carbanion and oxygen involving electron spin inversion in a radical pair intermediate.[5–8] Experimental support for both formulations has been supplied and in many instances it seems difficult to prove or disprove either of the two alternatives.

Phenols constitute substrates which are easily autoxidized in alkaline media due to the facile electron transfer from phenolate anions to oxygen. There exists a well-established correlation between the rate of autoxidation and the standard [9] or critical [10] redox potential in a series of mono-, di- and trivalent phenols. When dimeric products can be isolated, the autoxidation is usually interpreted as proceeding *via* phenoxy radicals.[10]

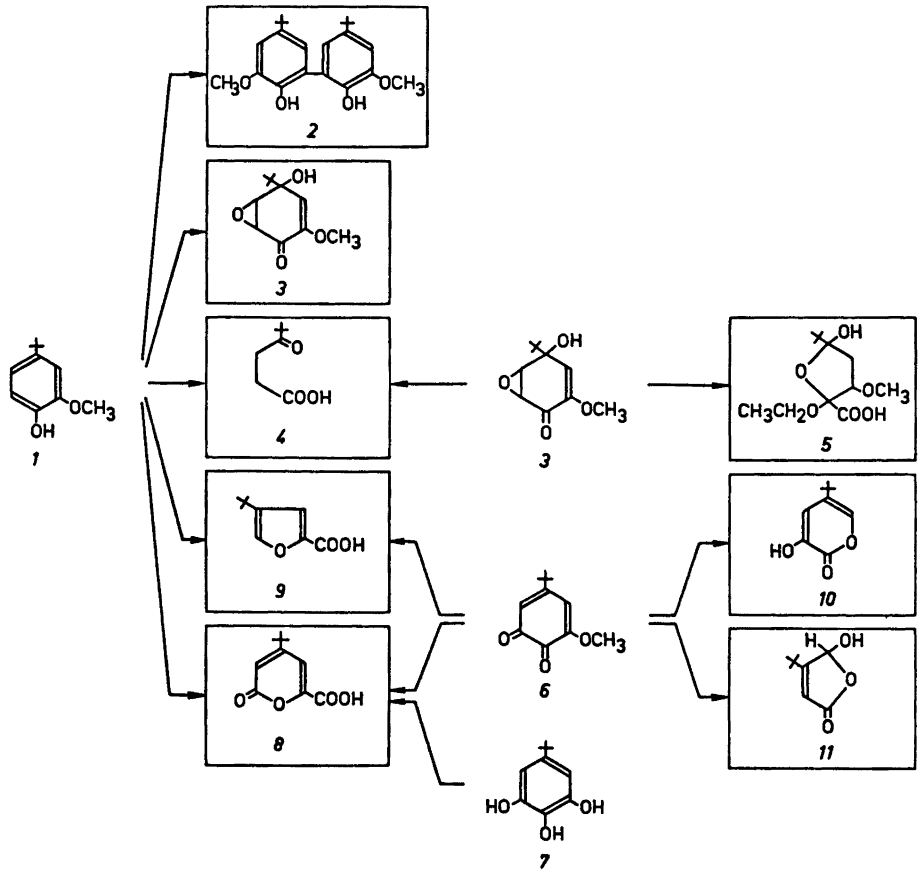
The autoxidation of phenols has been extensively studied using various types of substituted compounds and mild conditions.[10–14] The results of these studies have been explained in terms of reaction mechanisms involving the formation of cyclohexadienone hydroperoxides.[10–14] In a few favourable cases these intermediates have been isolated and their alkaline and thermal reactions have been investigated.[6,15,16] In general, the primary reactions of cyclohexadienone hydroperoxides can be classified as follows: (a) conversion to quinols,[17] (b) dehydration to quinones,[18] (c) rearrangement to epoxidated quinols [12,13,16] and (d) rearrangement with cleavage of the ring to muconic acid derivatives.[12,13]

The present work deals with the autoxidation of 4-*t*-butylguaiacol (*1*) in alkaline media. The reactions involved in the autoxidation of this compound should illustrate the behaviour of certain types of "uncondensed" phenolic units in lignins [19] and residual lignins under the conditions existing in oxidative-alkaline delignification processes.

RESULTS AND DISCUSSION

The autoxidation of 4-*t*-butylguaiacol (*1*) in alkaline (0.2 M NaOH) aqueous ethanol (EtOH:H_2O = 1:3) at 40 °C yielded a dimer *2*, an epoxide *3* and the acids *4*, *8* and *9* as main products (Scheme 1). The acid *4* was also obtained by autoxidation of epoxide *3* and the acids *8* and *9* by autoxidation of the *o*-quinone *6*. In addition the epoxide *3* yielded *5*, and the *o*-quinone *6* gave *10* and *11*. The pyrogallol derivative *7* gave only compound *8*. The experimental conditions used and the results

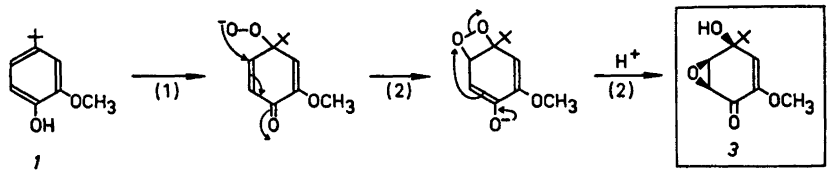
* Present address: Research Laboratory, Aktieselskapet Borregaard, N-1701 Sarpsborg, Norway.



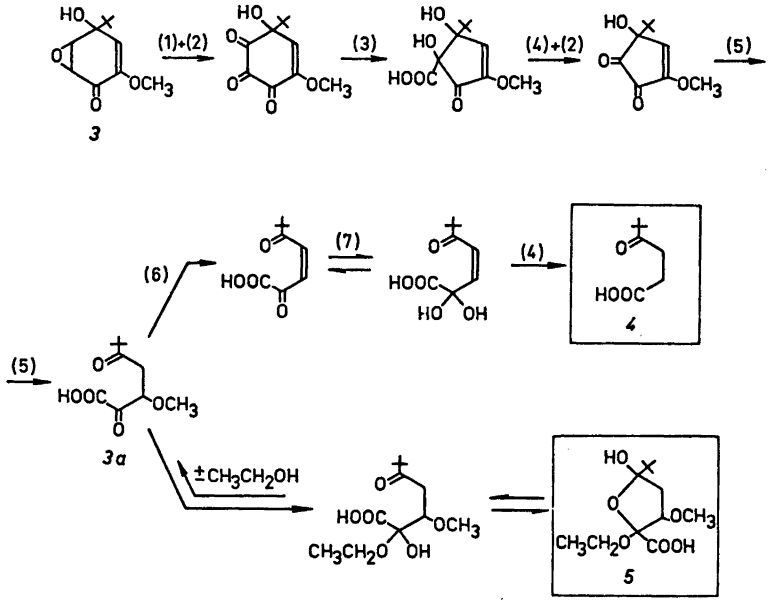
Scheme 1. Autoxidation of *1* and intermediates. (In this and the following Schemes, the products isolated are framed).

obtained are summarized in Table 1 (see EXPERIMENTAL).

Formation of 2. Compound *2*, a product of oxidative coupling, is also formed when *1* is treated in weakly alkaline solution with one-electron oxidants, such as potassium hexacyanoferrate(III).[20] It has moreover been shown [21] that other 4-alkyl substituted guaiacols react in the same way when exposed to similar conditions. The biphenyl structure of *2* exhibits remarkable stability toward oxygen in alkaline media. A possible explanation is that *2*, under the conditions used, exists as the monoanion in which the phenolic oxygens are protected against electron abstraction by intramolecular hydrogen bonding.[22,23] The monomethyl ether of *2* was oxidized at a rate comparable to that of compound *1*. This finding is in accordance



Scheme 2. Formation of *3* from *1*: (1) oxygenation,[6,13,15,16], (2) rearrangement.[13,16]



Scheme 3. Formation of *4* and *5* from *3*: (1) opening of epoxide ring, (2) enediol oxidation,[25,26] (3) benzilic acid rearrangement,[18,25–28] (4) decarboxylation of (vinylogous) β-keto acid,[25] (5) alkaline rearrangement,[15,26–28] (6) β-elimination, (7) hydration.

with the view that the degree of hydrogen bonding of the phenolate anion determines the oxidizability of the parent phenol.[22,23]

Formation of 3, 4 *and* 5. The reaction sequence leading to the formation of epoxide *3* is outlined in Scheme 2, in analogy to the mechanism proposed for the base-catalyzed autoxidation of other alkyl-substituted phenols.[13,16,24]

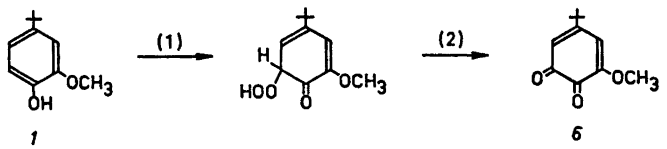
Compound *1* gave only a low yield of *3* under the reaction conditions used. In a separate experiment, using ethanol-water (1:1) as solvent, it was shown that this epoxide suffered alkaline-oxidative degradation. The main products were the γ-keto acid *4* and the α,δ-diketo acid *3a* isolated as its cyclic ethyl acetal *5* (Scheme 3). The formation of *4* from *3* indicates that the latter may be an intermediate in the alkaline-oxidative degradation of *1* (Scheme 1).

A plausible route for the formation of *4* and *5* from *3* is presented in Scheme 3. Analogous reactions found in the literature are referred to in the legend of the scheme.

The intermediacy of compound *3a* in the formation of *4* was supported by the isolation of the cyclic ethyl acetal *5*. The validity of Scheme 3 is further supported by the behaviour of 4,6-di-*t*-butylguaiacol during a similar oxidative-alkaline treatment.[24]

Formation of 6. The formation of the *o*-quinone *6* is outlined in Scheme 4. The route is analogous to that suggested for the formation of *p*-quinones upon autoxidation of phenols [16] and resorcinols [18] which are unsubstituted in the *para* position(s).

Formation of 8 – 11. When *6* was oxidized under the conditions given in Table 1, a rapid

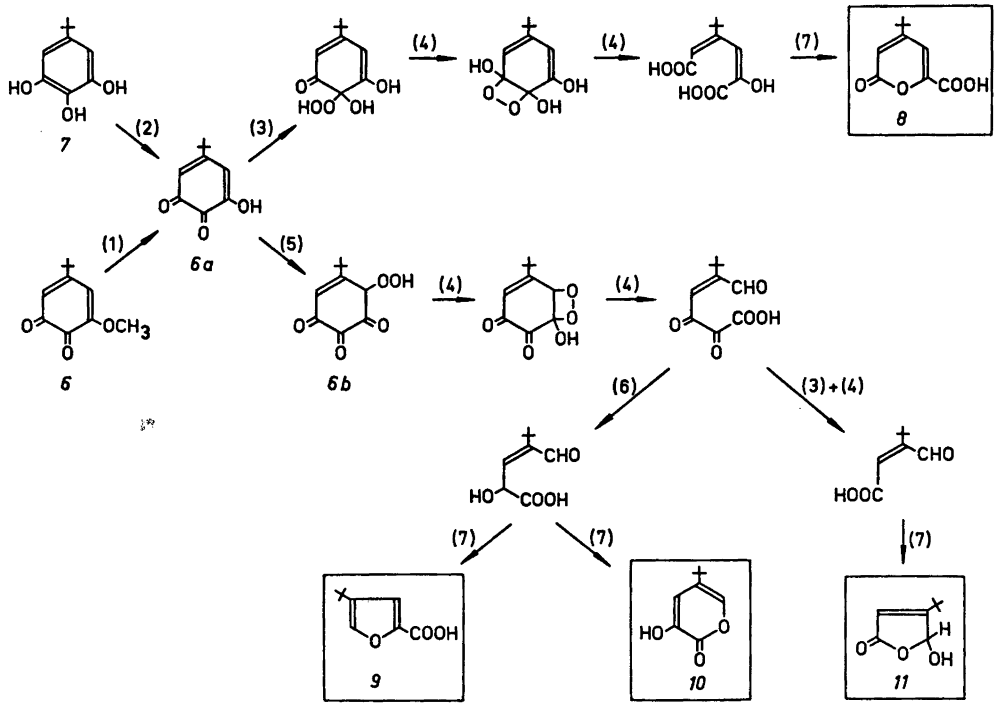


Scheme 4. Formation of *6* from *1*: (1) oxygenation,[29] (2) dehydration.[29]

Table 1. Alkaline autoxidation of model compounds at 40 °C in 0.2 – 0.3 M NaOH and with an oxygen consumption of 1 – 1.5 mol O_2/(mol model compound).

Compound (mol/l)	Solvent EtOH:H_2O [a]	Reaction time/h	Products (yielded % of theoretical)	Starting material recovered %
1 (0.1)	1:3	17	*2* (7), *3* (1.5), *4* (6), *8* (1), *9* (5), *12* [e] *13* [e]	35
2 (0.05)	6:1	48		90
2-Me ether (0.05)	6:1	20		60
3 (0.05)	1:1	11	*4* (12), *5* (15)	–
6 (0.05)	3:1	0.10	*8* (4), *9* (31), *10* (18), *11* (15)	–
7 (0.04)	1:1	0.15	*8* (85)	–
14 [b] (0.04)	1:3	20		*90* [c]
15 (0.05)	3:1	15		50 [d]

[a] The different ratios of EtOH:H_2O had to be used in order to ensure complete solubility of the compounds during the treatments. [b] Disodium salt of the *cis-trans*-forms. [c] Isolated as γ-lactone. [d] Determined by GC-analysis of the acidic fraction after methylation with diazomethane (reference: authentic compound). [e] Yield not determined.



Scheme 5. Formation of *8* – *11* from *6*: (1) alkaline demethoxylation, (2) enediol oxidation,[25,26] (3) hydrogen peroxide addition,[24] (4) rearrangements *via* dioxetanes,[31,32] (5) oxygenation, (6) decarboxylation of β-keto acid [25] after hydration, (7) cyclizations.

reaction took place and compounds *8*–*11* were formed. Compounds *8* and *9* were also isolated after autoxidation of *1* and it is therefore suggested that the *o*-quinone *6*, like the epoxide *3*, constitutes an intermediate in the alkaline-oxidative degradation of *1*. The remaining compounds (*10* and *11*) were not detected in the reaction mixture from *1*, probably due to further alkaline-oxidative conversions of these hydroxy lactones during the long period of treatment of *1* (Table 1). Thus, it has been shown[30] that 3-hydroxypyran-2-ones similar to compound *10* are converted into 2-furoic acids similar to *9* upon treatment with alkali.

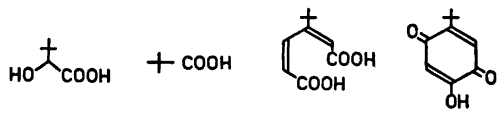
The proposed pathways of the oxidative-alkaline degradation of *6* are presented in Scheme 5.

The initial step of the reaction sequence (1) involves alkaline demethoxylation resulting in the formation of the hydroxy-*o*-quinone *6*a. Oxidation of *6*a by the action of hydrogen peroxide (3) gives ultimately *8* whereas oxygenation of *6*a (5) leads to *9*–*11*. The hydrogen peroxide required for step (3) may be generated by alkaline hydrolysis of *6*b to give the corresponding hydroxy triketone (*cf.* Refs. 9 and 17) and by subsequent autoxidation of the resulting dihydroxyquinone tautomers. A similar source of hydrogen peroxide should be the alkaline conversion of *6*a into the *vic*-tetrahydroxybenzene derivative followed by autoxidation of the latter. However, products expected to arise from *6*a and *6*b *via* such reactions have not been found so far in the autoxidation mixtures from *1* and *6*. During the autoxidation of 4-*t*-butylpyrogallol (*7*), hydrogen peroxide is produced in the first step (2) of the sequence and competes successfully with oxygen for the intermediate *6*a (3). Thus, a high yield of *8* is obtained (Table 1).

The pathways proposed for the formation of 8–11 (Scheme 5) also explain the formation of analogous products after similar oxidative treatments of 4,6-di-*t*-butylpyrogallol[33,34] and 4,6-di-*t*-butyl-3-ethoxy-*o*-quinone.[24]

An alternative to the route of carbon-carbon bond cleavage in Scheme 5 [step (4)] would involve addition of a hydroxide or alkoxide ion to the carbonyl adjacent to the hydroperoxy group, followed by rearrangement with heterolytic fission of the O–O bond without formation of dioxetanes (*cf.* Refs. 24 and 32).

The total yield of identified acidic and neutral products from *1* is low (Table 1). This may be partly due to further oxidation to simple aliphatic acids and carbon dioxide. Autoxidation of *1* and GC-MS analysis of the acidic fraction after esterification showed the presence of a great number of acidic degradation products. Compounds *12* and *13* were two of the main components in this fraction. Formic and acetic acids have not been taken into account in this study.



12 *13* *14* *15*

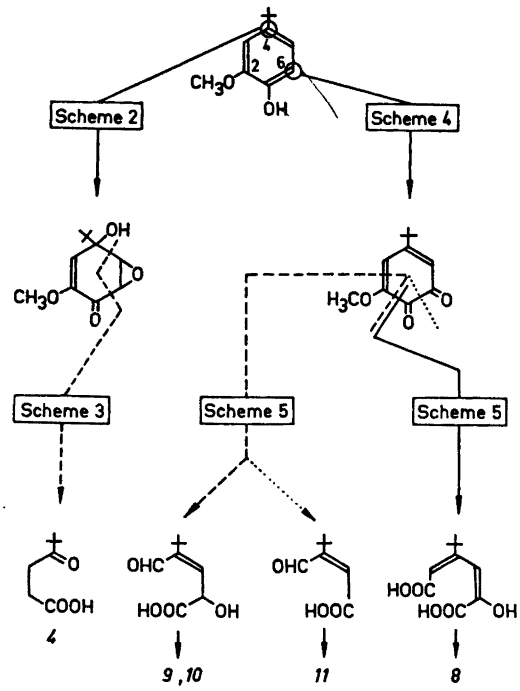
An alternative pathway starting with oxygenation of *1* in the 2-position (*cf.* behaviour of 4,6-di-*t*-butylguaiacol[24]) would lead to the muconic acid derivative *14* and/or to the hydroxyquinone *15*.[35] These oxidation products were, however, not found in the reaction mixture obtained from *1* although their stability under similar oxidative conditions was demonstrated in separate treatments of authentic compounds.

CONCLUSIONS

The *fragmentation* reactions of compound *1* are tentatively summarized in Scheme 6, in which the following steps are suggested.

The initial oxidative attack involves oxygenation preferentially in the 4- and 6-positions. Oxygenation in the *4-position* gives an epoxidated cyclohexadienone derivative (*3*, Scheme 2). This derivative undergoes alkaline and oxidative degradation (Scheme 3) resulting in the loss of the carbon atoms 5 and 6 by decarboxylations and of the methoxy substituent by β-elimination. The γ-keto acid *4* is the predominant product of this degradation route.

Oxygenation in the *6-position* affords an *o*-quinone (*6*, Scheme 4), which after alkaline demethoxylation undergoes two different oxidative conversions (Scheme 5). The first involves cleavage of the C^1-C^2 (or C^1-C^6) bond by hydrogen peroxide and leads to compound *8*. The second entails the cleavage of the orig-



Scheme 6. Fragmentations of *1*.

inal C^2-C^3 (or C^5-C^6) bond *via* oxygenation, followed by decarboxylation (loss of C^3 or C^6, formation of *9* and *10*) or by oxidative fragmentation (loss of C^1 and C^2 or C^1 and C^6, formation of *11*).

The oxidative degradation reactions of *1* compete with each other and with the oxidative coupling yielding the dimeric compound *2*. This product is more stable than the parent compound toward the standard treatment with oxygen-alkali. Thus, the oxidative coupling of *1* limits the extent of oxidative degradation.

EXPERIMENTAL

Materials

Synthesis of model compounds. The following compounds were prepared as previously described: *1*,[36] *2*,[37] *2*-monomethyl ether [20] and *7*.[38]

5,6-Epoxy-4-hydroxy-2-methoxy-4-t-butylcyclohex-2-enone (3). 4-*t*-Butylguaiacol (20 mmol) was treated with molecular oxygen in dimethylsulfoxide (200 ml) for 2 h using potassium *t*-butoxide (21 mmol) as base. After consumption of 1.2 equiv. of oxygen the mixture was neutralized with CO_2, diluted with water (500 ml) and extracted with chloroform (2 × 200 ml). The chloroform extract was washed with water, dried with sodium sulfate and evaporated. The residue (3.0 g) was purified by recrystallization from light petroleum (40 – 60 °C)-benzene (5:1) to give *3* as colourless crystals, yield 1.5 g (35 %). The compound was identical with the product isolated after alkaline autoxidation of *1* in ethanol-water (^{1}H NMR, MS, m.p.).

5-t-Butyl-3-methoxy-o-quinone (6). Sodium metaperiodate (0.05 mol) in water (250 ml) was added to a cooled (+ 5 °C) solution of 4-*t*-butyl-2,6-dimethoxyphenol [39] (0.05 mol) in 85 % acetic acid (600 ml). After 15 min, the reaction mixture was diluted with water and extracted with methylene chloride. The extract was washed with water, dried with sodium sulfate and evaporated to give 10 g of crude product. Crystallization from light petroleum gave *6* as dark-red crystals. Yield 3 g (30 %), m.p. 78 – 80 °C; Lit. 79 – 80 °C [40] and 86 – 88 °C.[20]

5-t-Butyl-2-hydroxy-p-quinone (15). 4-*t*-Butylcatechol (20 mmol) was treated with molecular oxygen [35] in an alkaline solution (0.3 M NaOH) containing ethanol-water (3:1) (200 ml) at 0 °C. After consumption of approximately 1.2 equiv. of oxygen (15 min), the reaction mixture was acidified (pH 1 – 2), diluted with water (300 ml) and extracted with chloroform. The chloroform extract was dried with sodium sulfate, filtered and evaporated to give 3.6 g of crude product. Recrystallization from toluene gave *15* as yellow crystals. Yield 1.7 g (51 %); m.p. 100 – 102 °C; Lit.[35] 101 – 103 °C. Methylation (CH_2N_2) of *15* gave the methyl ether. Yield 80 %; recrystallization from light petroleum, m.p. 162 – 164 °C; Lit.[20] 162 – 163 °C.

β-t-Butylmuconic acid (14). The method described for the preparation of β-methylmuconic acid [41] was adopted. Thus, 2-nitro-4-*t*-butylphenol [42] was treated with concentrated sulfuric acid at 110 °C yielding a γ-lactone (2,5-dihydro-5-oxo-3-*t*-butylfuran-2-acetic acid). Yield 22.5 %, m.p. 143 – 146 °C (benzene-light petroleum 2:1). Anal. $C_{10}H_{14}O_4$: C, H, O. ^{1}H NMR (60 MHz $CDCl_3$): δ 1.30 (s, 9 H, *t*-Bu), 3.07 (A), 2.57 (B), 5.42 (X), [CH_2 and CH; ABX; J_{AB} 16, J_{AX} 3 and J_{BX} 9 Hz], 5.90 (d, 1 H, ol, J 1.3 Hz, coupled to X). This lactone was converted to *14* (*cis-trans* form) as described for the corresponding methyl derivative.[41] *14* was characterized as the disodium salt. ^{1}H NMR (60 MHz, D_2O): δ 1.10 (s, 9 H, *t*-Bu), 5.90 (s, 1 H, ol), 6.08 (d, 1 H, ol, J 16 Hz), 7.28 (d, 1 H, ol, J 16 Hz).

Methods

Autoxidation of model compounds. The alkaline autoxidation of the model compounds was carried out in a three-necked 500 ml flask con-

nected to a gas burette. The quantity of oxygen consumed was measured. The reaction conditions and results are given in Table 1.

Work-up procedure. The reaction mixture was diluted with water, neutralized with carbon dioxide and extracted with ether to give the *neutral products.* The aqueous solution was then concentrated under reduced pressure (40 °C) to remove ethanol and acidified to pH 1 – 2 with hydrochloric acid or sulfuric acid. Subsequent extraction with ether afforded the *acidic products.*

The ether extracts were dried with sodium sulfate and evaporated under reduced pressure to give the crude reaction mixtures. The acidic products were methylated (in one instance ethylated) with diazomethane (diazoethane) prior to separation. Acetylation of several neutral compounds was performed with pyridine-acetic anhydride (1:1) (25 °C, 24 h).

Spectroscopy. The ^{1}H NMR spectra were obtained on a Perkin Elmer R-12 Spectrometer using deuteriochloroform ($CDCl_3$) as solvent.

The mass spectra were recorded on a Perkin Elmer 270 instrument at 25 eV using the direct inlet system or in combination with a gas chromatograph (Perkin Elmer 900).

The IR spectra were recorded on a Perkin Elmer 221 instrument and the UV and visible spectra on a Cary-118 C instrument.

Chromatography. Preparative separations were carried out by column chromatography using silica gel 60 (120 – 230 mesh ASTM, Merck) as adsorbant and mixtures of chloroform-ethyl acetate (19:1) or light petroleum (40 – 60 °C)-ethyl acetate in proportions from 9:1 to 7:3 as solvent systems. If necessary, the products were further purified by preparative thin-layer chromatography on silica gel H_{254} (Merck) using the same solvent systems as in column chromatography.

Gas chromatography. Analytical separations were carried out with a Perkin Elmer (F-30) gas chromatograph. The column material was OV-1 (10 %) on Chromosorb Q. In general, the temperature program, 100 – 200 °C, 4 °C/min, was used during a gas chromatographic run.

Product identification. MS, IR and UV data as well as m.p.'s and elemental analyses of the isolated products are given below. The analyses agree within ±0.3 % units with the calculated values unless otherwise stated. All mass fragments (max. 8) having $m/e > 100$ and intensities > 10 % of the base peak are listed.

3,3'-Dimethoxy-5,5'-di-t-butylbiphenyl-2,2'-diol (2), m.p. 166 – 168 °C. MS *m/e* (rel. int.): 358 (M, 80), 343 (M – 15, 95), 302 (M – 56, 20), 287 (M – 71, 100), 154 (25). IR (KBr): 3535 (m, OH), 1590 (m, aromatic). UV [MeOH (ε)]: 284 (7050).

5,6-Epoxy-4-hydroxy-2-methoxy-4-t-butylcyclohex-2-enone (3), m.p. 100 – 102 °C. Anal. $C_{11}H_{16}O_4$: C, H, O. MS *m/e* (rel. int.): 156 (M – 56, 75), 155 (M – 57, 70), 141 (M – 71, 65), 138 (M – 74, 30), 137 (70), 113 (100), IR (KBr): 3480 (s, OH), 1690 (s, C=O), 1625 (m, C=C–C=O). UV [MeOH (ε)]: 273 (4540).

Methyl 5,5-dimethyl-4-oxo-hexanoate (methyl ester of 4), amorphous. Anal. $C_9H_{16}O_3$: C, H, O. MS *m/e* (rel. int.): 141 (M – 31, 10), 115 (M – 57, 100), 113 (M – 59, 10), 87 (M – 85, 10). IR ($CHCl_3$): 1730 (s, C=O), 1700 (s, C=O).

Methyl tetrahydro-5-t-butyl-2-ethoxy-5-hydroxy-3-methoxy-2-furoate (methyl ester of 5), amorphous. (Found: C 58.16; H 8.44; O 33.33. Calc. for $C_{13}H_{24}O_6$: C 57.39; H 7.82; O 34.78). MS *m/e* (rel. int.): 230 (M – 46, 16), 212 (M – 64, 14), 169 (M – 107, 30), 167 (M – 109, 20), 157 (15), 129 (25), 127 (85), 115 (100). IR ($CHCl_3$): 3420 (m, OH), 1740 (s, C=O).

4-t-Butyl-6-methoxycarbonyl-(2H)-pyran-2-one (methyl ester of 8), m.p. 84 – 86°C. Anal. $C_{11}H_{14}O_4$: C, H, O. MS *m/e* (rel. int.): 210 (M, 25), 182 (M – 28, 10), 167 (M – 43, 15), 151 (M – 57, 100). IR (KBr): 1730 (s, C=O), 1715 (s, C=O), 1640 (w, C=C–C=O) 1545 (s, C=C–C=O). UV [MeOH (ε)]: 298 (7000).

Methyl 4-t-butyl-2-furoate (methyl ester of 9), amorphous. Anal. $C_{10}H_{14}O_3$: C, H, O. MS *m/e* (rel. int.): 182 (M, 15), 167 (M – 15, 100), 151 (M – 31, 10), 139 (M – 43, 12). IR ($CHCl_3$): 1710 (s, C=O), 1590 (s, C=C), 1505 (m, C=C). UV [MeOH (ε)]: 263 (–).

3-Acetoxy-5-t-butyl-(2H)-pyran-2-one (acetate of 10), m.p. 97 – 99 °C. Anal. $C_{11}H_{14}O_4$: C, H, O. MS *m/e* (rel. int.): 168 (M – 42, 70), 153 (M – 57, 80), 140 (M – 70, 15), 125 (M – 85, 100). IR (KBr): 1760 (m, C=O). 1710 (s, C=O), 1635 (m, C=C–C=O), 1545 (m, C=C–C=O). UV [MeOH (ε)]: 295 (6520).

γ-Acetoxy-β-t-butyl-$\Delta^{\alpha,\beta}$-butenolide (acetate of 11), amorphous. (Found: C 61.33; H 7.07; O 31.68. Calc. for $C_{10}H_{14}O_4$: C 60.60; H 7.07; O 32.32). MS *m/e* (rel. int.): 156 (M – 42, 12), 141 (M – 57, 17), 139 (M – 59, 30), 138 (M – 60, 38), 111 (50), 110 (90), 95 (100). IR ($CHCl_3$): 1765 (s, C=O), 1630 (m, C=C–C=O).

^{1}H NMR data are presented in Table 2.

The presence of the low molecular weight acids *12* (3,3-dimethyl-2-hydroxybutyric acid) and *13* (2,2-dimethylpropionic acid) in the reaction mixture obtained on autoxidation of *1* was shown by GC-MS analyses of the ethyl esters (ethylation with diazoethane) and in case of *12* also as the acetate of the methyl ester.

Ethyl ester of 12. MS *m/e* (rel. int.): 104 (M – 56, 55), 87 (M – 73, 40), 75 (56), 69 (70), 57 (100).

Methyl ester of 12 *(acetate).* MS *m/e* (rel. int.): 157 (M – 31, 5), 132 (M – 56, 40), 129 (M – 59, 25), 90 (100), 87 (55), 69 (20), 67 (30), 57 (60).

Ethyl ester of 13. MS *m/e* (rel. int.): 130 (M, 5), 115 (M – 15, 3), 87 (M – 43, 10), 57 (100).

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Table 2. ^{1}H NMR of products isolated from alkaline autoxidation of *1*, *3*, *6*, and *7*. Types of protons: ac, acetyl; ar, aromatic; es, ester; et, ether; ol, olefinic.

Compound	δ (*t*-Bu) (s, 9 H)	δ (Me) (s, 3 H)	δ other protons
2	1.31 [a]	3.90 [b] (et)	6.91 (s, 4 H, ar), 5.98 (s, 2 H, OH)
3	1.03	3.59 (et)	2.42 (broad s, 1 H, OH), 3.75 (A), 3.51 (B), 5.29 (X) [ol, CH, CH; ABX; J_{AB} 4.7, J_{AX} 2.7, $J_{BX}\sim 0$ Hz]
4 [c]	1.16	3.63 (es)	2.59 (A) and 2.79 (B) [CH_2 and CH_2; A_2B_2; J_{AB} 6.4 Hz]
5 [c]	0.94	3.73 (es)	2.36 (broad s, 1 H, OH); 2.26 (A), 2.02 (B) and 3.96 (X) [CH_2 and CH; ABX; J_{AB} 14.3, $J_{AX}\sim 6$, $J_{BX}\sim 9$ Hz], 3.61 (q, 2 H, CH_2), 1.03 (t, 3 H, CH_3), J_{CH_2,CH_3} 7.3 Hz
8 [c]	1.25	3.90 (es)	6.36 (d, 1 H, ol), 7.13 (d, 1 H, ol), $J_{ol,ol}$ 1.7 Hz
9 [c]	1.23	3.85 (es)	7.10 (d, 1 H, ol), 7.28 (d, 1 H, ol), $J_{ol,ol}$ 0.6 Hz
10 [d]	1.21	2.29 (ac)	7.21 (s, 2 H, ol)
11 [d]	1.21	2.13 (ac)	5.90 (d, 1 H, CH), 7.0 (d, 1 H, ol), $J_{ol,CH}$ 0.8 Hz

[a] 18 H. [b] 6 H. [c] Methyl ester. [d] Acetate.

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Studies on the Autoxidation of *t*-Butyl-substituted Phenols in Alkaline Media. 2.* Reactions of 4,6-Di-*t*-butylguaiacol and Related Compounds

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The courses of the main reactions of 4,6-di-*t*-butylguaiacol (*1*) during alkaline autoxidation are described. Initial oxygenations in the 2- or 4-positions are followed by alkali-promoted rearrangements and conversions of the resulting cyclohexadienone hydroperoxide intermediates. The 3,5-di-*t*-butyl-*o*-quinone formed in one of these conversions undergoes oxidative-alkaline degradation yielding the same fragmentation products as are obtained from *1*.

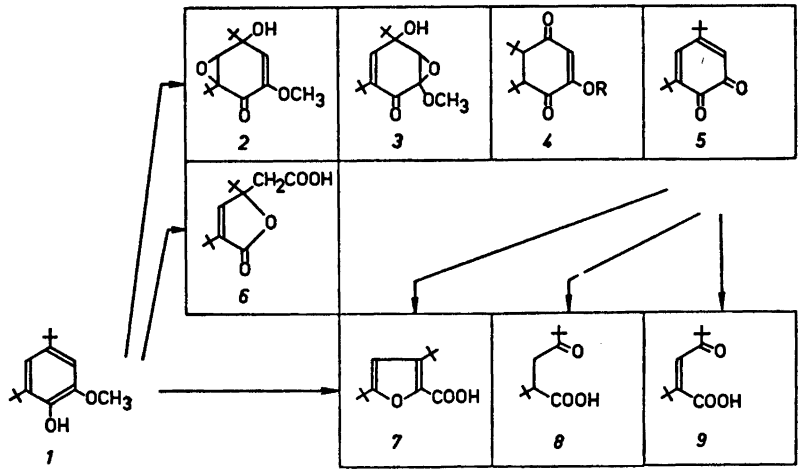
In the first part of this series the alkaline autoxidation of 4-*t*-butylguaiacol, which serves as a model for "uncondensed" phenolic units in lignins, was studied. The present work is concerned with the autoxidation of 4,6-di-*t*-butylguaiacol and 4-*t*-butyl-2,6-dimethoxyphenol which constitute models for the "condensed" guaiacol and the syringyl-type units, respectively.

* Part 1, see Ref. 1.

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RESULTS AND DISCUSSION

The conditions of the treatments and the results are summarized in Table 1 (see EXPERIMENTAL). The reaction products obtained by autoxidation of 4,6-di-*t*-butylguaiacol (*1*) are depicted in Scheme 1.



Scheme 1. Autoxidation of *1* and *5*. (In this and Schemes 2 – 12, the products isolated are framed).

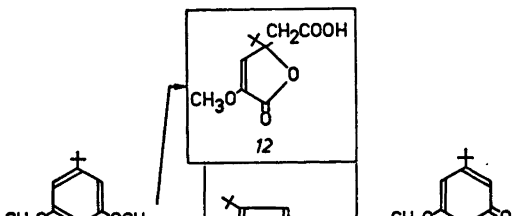
Table 1. Alkaline autoxidation of model compounds. Temperature 40 °C. Alkalinity 0.2 – 0.3 M NaOH. Oxygen consumption 1 – 1.5 mol O_2/mol model compound.

Compound (mol/l)	Solvent EtOH:H_2O	Reaction time, min	Products (Yields, % of theoretical)	Starting material recovered, %
1 (0.1)	3:1	15 × 60	2 (60), *3* (2), *4* (7), *5* (2), *6* (7), *7* (1), *8* (2), *9* (8)	–
2 (0.08)	3:1	14 × 60		90
5 (0.1)	1:3	10	*7* (3), *8* (2), *9* (14), *15* (1), *20* (17), *23*+*24* (2), *25* (2), *26* (8), *27* (3), *28* (2)	–
10 (0.01)	3:1	24 × 60	*12* (8), *13* (20)	50
14 (0.1)	1:3	10	*5* (1), *7* (3), *8* (1), 9 (16), *15* (2), *20* (7), *23*+*24* (2), *25* (8), *26* (14), *26* – ethyl ester (traces), *27* (1), 28 (2)	–
15 (0.08)	1:3	12	*7* (traces), *8* (15), *20* (30), *21*A+*21*B (~10, GC), *25* (10), *26* (traces), *27* (3)	–
16 (0.05)	1:3	10	*7* (10), *9* (15), *25* (20), *28* (20). Yields determined from NMR-spectra.	–
17 (0.03)	1:2	15	*8* (10), *20* (60).	
18 (0.1)	1:3	10	*19*,[a] *25* (60) and other products.	
19 (0.06)	1:3	15		95
24 (0.1)	1:3	30		90

[a] Yield not determined.

Compounds *2*, *3* and *6* have previously been isolated [2,3] after a similar treatment of *1*. The acids *7*, *8* and *9* were also obtained by a similar treatment of 3,5-di-*t*-butyl-*o*-quinone (*5*) which gave six additional compounds as shown in Scheme 6.

Autoxidation of 4-*t*-butyl-2,6-dimethoxyphenol (*10*) (Scheme 2) yielded *12* (*cf.* also Ref. 3) and *13* as main products. The acid *13*, together with three other major products, was also obtained by a similar treatment of 5-*t*-butyl-3-methoxy-*o*-quinone (*11*).[1]

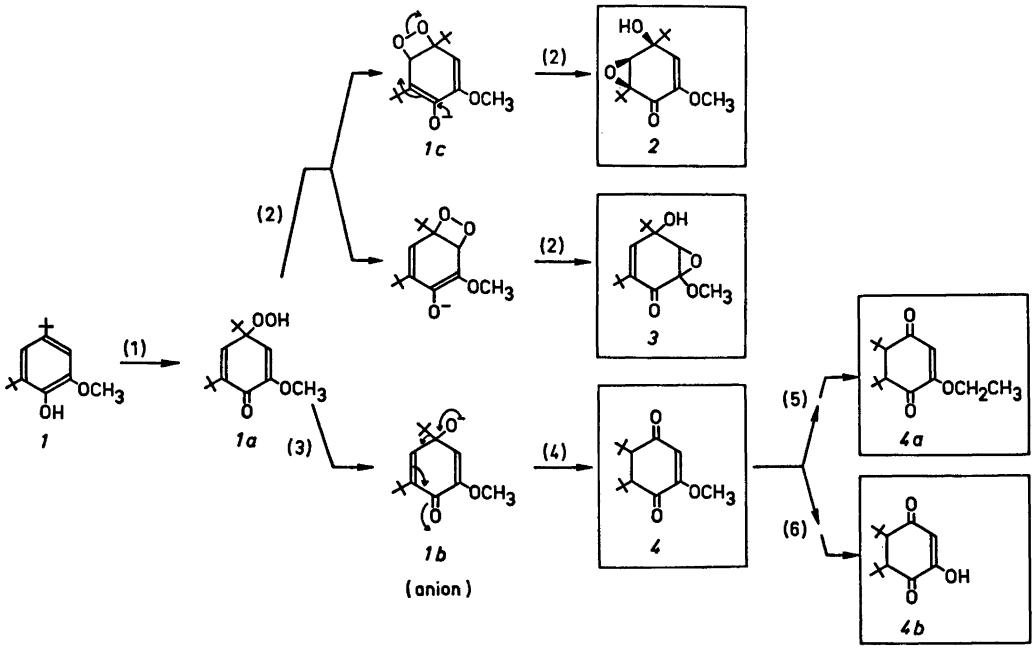


Scheme 2. Autoxidation of *10* and *11*.

Formation of 2, 3 *and* 4. The mechanism of formation of the predominating product *2*, isolated in 60 % yield, and of compounds *3* and *4* is outlined in Scheme 3.

Steps (1) and (2) have been previously suggested.[3,4] Experimental support for these steps has now been provided by the following findings. When the alkaline autoxidation of *1* was carried out in an $^{18}O_2$ atmosphere, the resulting epoxidated quinol *2* contained two atoms of ^{18}O. Furthermore, a structure determination by X-ray crystallography [6] showed that the hydroxyl group and the epoxide ring in *2* bear a *cis* relationship to each other.

These results exclude an alternative mechanism of formation of *2* [2,3] involving alkaline conversion of *1*a into the corresponding hydroxy cyclohexadienone, *1*b, followed by hydrogen



Scheme 3. Formation of *2*, *3* and *4* from *1*: (1) oxygenation,[3,4] (2) intramolecular epoxidations by rearrangement *via* dioxetanes,[3,4] (3) conversion into hydroxy cyclohexadienone,[5] (4) dienone-phenol rearrangement with migration of the 4-*t*-butyl substituent,[5] (5) transetherification by the solvent (ethanol), (6) demethoxylation by alkali.

peroxide oxidation (epoxidation). Formation of *2* through epoxidation of *1*b by *1*a (*cf.* Ref. 7) appears unlikely due to the steric hindrance exerted by the *t*-butyl group.

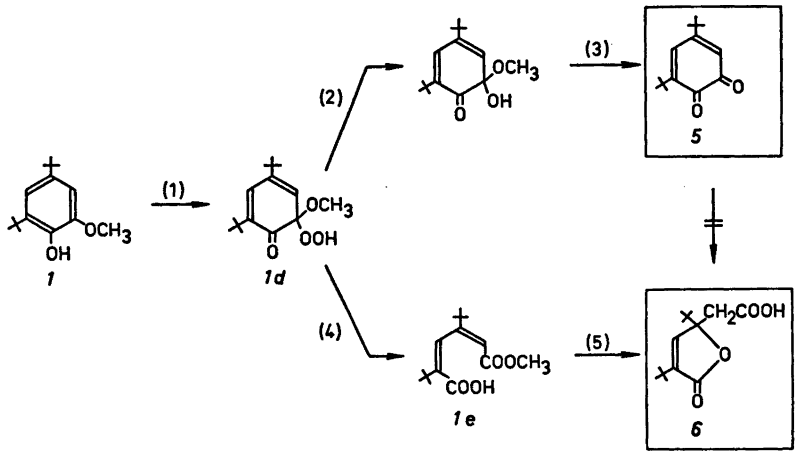
The lower yield of *3* compared to that of *2* indicates that the anion from *1*a preferentially attacks the *t*-butyl-substituted enone moiety (formation of *1*c). However, the lower yield of *3* may also be due to a partial alkaline-oxidative degradation of this compound.

The epoxidated *p*-quinol *2* essentially resists the oxidative-alkaline treatment. This behaviour is in contrast to the extensive degradation of the corresponding product from 4-*t*-butylguaiacol taking place under similar oxidative-alkaline conditions.[1] Obviously, steric factors cause this difference.

The formation of *4* through steps (3) and (4) is formulated in analogy to a previously described [5] anionic dienone-phenol rearrangement involving a 1,2-migration of a *t*-butyl group. The expected final step, enolization of the cyclohexenedione *4* to give the corresponding hydroquinone, is prevented by steric factors (*cf.* Ref. 5).

Transetherification and demethoxylation of *4* [steps (5) and (6)] proceed *via* conjugate 1,4-additions of ethoxide and hydroxide ions, respectively (Michael additions, *cf.* also the alkaline demethoxylation of methoxyquinones [1]). The acidic product *4*b has been previously isolated after alkaline autoxidation of 2,6-di-*t*-butyl-4-methylphenol,[8] 2,6-di-*t*-butyl-*p*-quinone [8] and 4,6-di-*t*-butyl resorcinol,[9] as well as after alkaline treatment of 3,5-di-*t*-butyl-*o*-quinone (*5*) in the absence of oxygen.[5] These reactions involve also alkali-promoted migrations of a *t*-butyl group, similar to step (4) in Scheme 3.

Formation of 5 and 6. The formation of *5* from *1* involves oxidative demethoxylation (Scheme 4). An analogous mechanism *via* a cyclohexadienone hydroperoxide has been previously suggested [10] for the demethoxylation of the isomeric 2,6-di-*t*-butyl-4-methoxyphenol during base-catalyzed autoxidation which gives

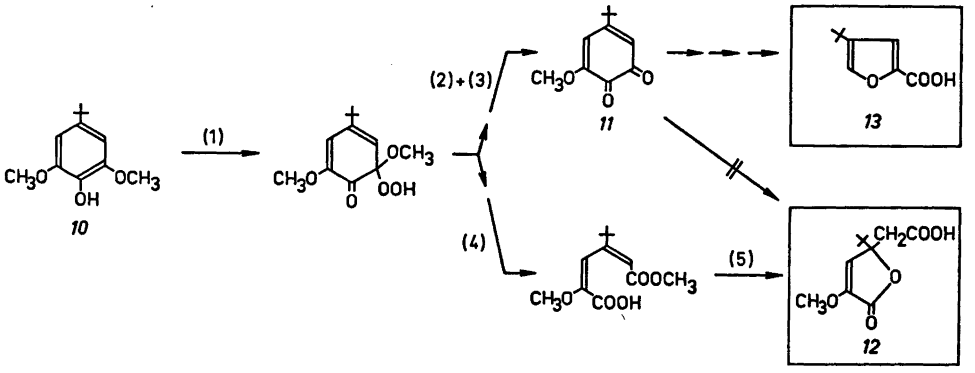


Scheme 4. Formation of *5* and *6* from *1*: (1) oxygenation, (2) alkaline conversion, (3) elimination of methanol (cleavage of hemiketal), (4) ring cleavage by rearrangement *via* dioxetane,[1,3,11] (5) hydrolysis and lactonization.

the corresponding *p*-quinone (*cf.* also Ref. 2). In this instance, the hydroperoxide intermediate could be isolated using mild conditions.[10]

The lactone acid *6* was the main component of the acidic fraction obtained by autoxidation of *1*. Obviously, this acid is formed by oxidative cleavage of the linkage between the carbon atoms carrying the hydroxyl and methoxyl groups of *1*. Two possible routes may be envisaged.[2,3] The initially formed cyclohexadienone hydroperoxide *1*d may undergo rearrangement resulting in ring opening and formation of the monomethyl ester *1*e [step (4)[3] *cf.* also Ref. 11]. The latter is then hydrolyzed before or after lactone formation (5). Alternatively, *6* could arise from the *o*-quinone *5* by oxidative cleavage of the carbon-carbon linkage between the carbonyl groups and subsequent ring closure of the resulting muconic acid.[2,3] However, the latter pathway was excluded by treatment of *5* with oxygen under similar conditions. Only traces of *6* were detected. In contrast to this result, compound *5* has been reported[12] to give *6* in high yield upon treatment with oxygen in a Bu^tOH-Bu^tOK system. This discrepancy is obviously due to the different solvent systems used.

Formation of 12 *and* 13. The reaction routes presented in Scheme 4 also explain the formation of *12* and *13* upon autoxidation of 4-*t*-butyl-2,6-dimethoxyphenol (*10*, Scheme 5).



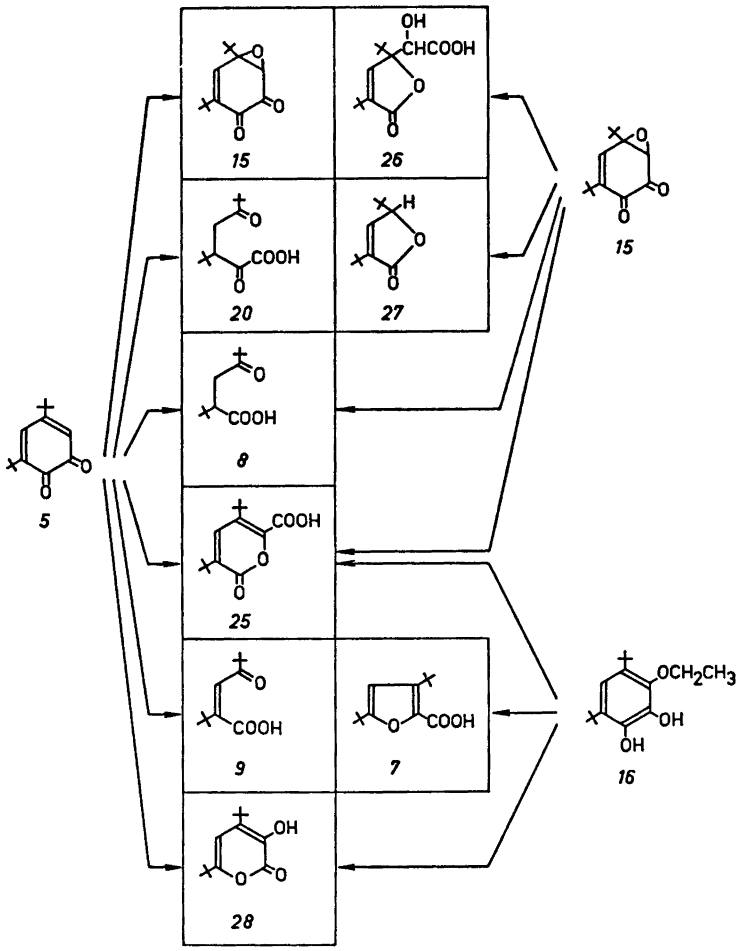
Scheme 5. Formation of *12* and *13* from *10*. (For numbering of reaction steps, see Scheme 4).

Compound *12* corresponds to compound *6* obtained from *1* (Scheme 4), and compound *13* formed *via* *11*[1] corresponds to compound *7* formed *via* *5* (Schemes 7 and 12). Again, *o*-quinone *11* was not converted into the lactone acid *12* during autoxidation. Apparently, the alkaline autoxidation of guaiacol and syringol derivatives to give muconic acid derivatives proceeds *via* internal rearrangement of cyclohexadienone hydroperoxide intermediates rather than *via* oxidative cleavage of *o*-quinoid intermediates.

Formation of 7, 8 *and* 9. The fact that the *o*-quinone *5* was isolated in a small amount (2 %) after autoxidation of *1*, together with the facile oxidation of 5, to 7, 8 and 9 (Scheme 1), indicates that compound *5* is an intermediate in the formation of *7*–*9* from *1*.

In view of the general importance of *o*-quinoid intermediates in the autoxidation of compounds of the guaiacyl type (*cf.* also Ref. 1), all the reaction products isolated after alkaline autoxidation of *5* were investigated. In addition to *7*–*9*, compounds *15*, *20* and *25*–*28* were identified (Scheme 6). Plausible routes for the formation of all identified products are presented in Schemes 7–12. These routes imply that hydrogen peroxide, in addition to oxygen, functions as oxidant (*cf.* also Ref. 1) whereas the formation of the products *2*–*6* from *1* only requires oxygen (Schemes 3 and 4).

The description of the alkaline-oxidative



Scheme 6. Autoxidation of *5*, *15* and *16*.

conversions of *5* is based on the finding that the epoxidated *o*-quinone *15* and the catechol derivative *16*, upon appropriate oxidative treatments, give together all the products obtained from *5* (Scheme 6). *15* and *16* may therefore be considered as possible intermediates in the reactions of *5* (see below).

Formation of the intermediates 15 *and* 16. Compound *15* was isolated in excellent yields after treatment of *5* with hydrogen peroxide or of *14* with oxygen in a 0.2 M solution of sodium hydroxide in aqueous ethanol at 0 °C (Scheme 7). When the oxidative alkaline treatments of *5* and *14* were carried out at 40 °C, the yields of *15* were considerably lower. The oxidations of *5* with hydrogen peroxide and of *14* with oxygen are rapid processes whereas the oxidation of *5* with oxygen under similar conditions proceeds slowly. When *5* was treated with a 0.25 M solution of sodium hydroxide in aqueous ethanol with exclusion of oxygen, a 60 % yield of catechol *14* was obtained. No oxidation products from ethanol were found in the reaction mixture.

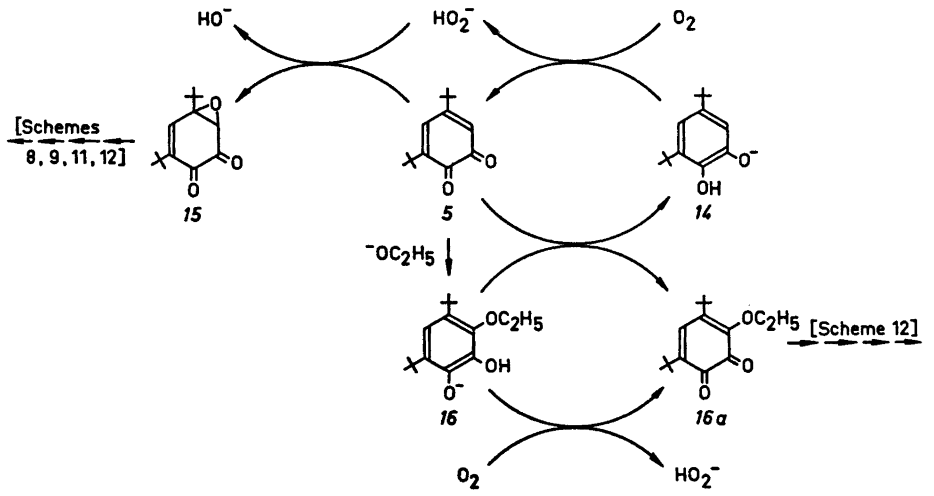
These experimental findings are explained in terms of the redox and addition reactions described in Scheme 7.

The rapid oxidation of catechol *14* with molecular oxygen in alkaline media, yielding *o*-quinone *5*, generates hydrogen peroxide.[13,14] This oxidant reacts rapidly with *5* to give epoxide *15* [15] (*cf.* also Ref. 16).

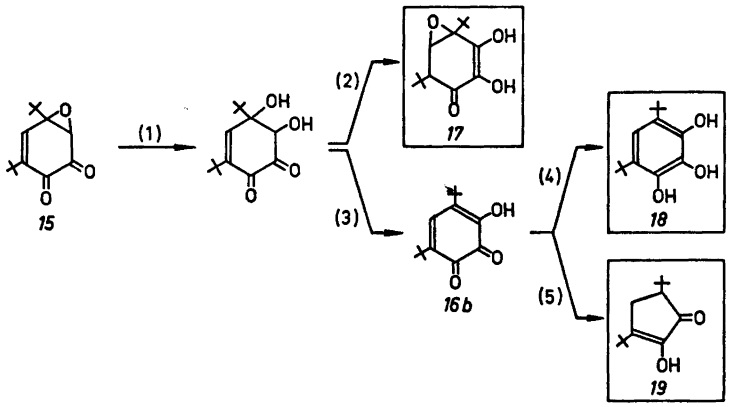
The slow autoxidation of *o*-quinone *5* in alkaline aqueous ethanol is suggested to proceed as follows: Nucleophilic 1,6-addition of ethoxide anions (*cf.* Ref. 17) gives catechol *16* which, however, was not isolated from the reaction mixture. The suggested role of *16* as an intermediate in the autoxidation of *5* is solely indicated by the behaviour towards oxygen in alkali. Autoxidation of *16* gives *16*a and hydrogen peroxide which is available for the conversion of *5* to *15*. Deethoxylation of *16*a gives *16*b which may undergo alkaline-oxidative conversions as outlined in Scheme 12. When the alkaline treatment of *5* is carried out in the absence of oxygen, *16* is obviously oxidized by *5* [18], thereby producing *16*a and *14*. The high yield of the latter compound (60 %) indicates that compound *16*a or the deethoxylation product *16*b is further oxidized by *5*. This reaction has however not been studied so far. The formation of *15* and *16* as intermediates in the oxidative-alkaline degradation of *5* can thus be explained rationally by the competition of hydroperoxide and ethoxide anions for *5*.

In the following sections, the oxidative-alkaline degradations of the intermediates *15* and *16* are briefly described.

Treatment of *15* with alkali in aqueous ethanol in the absence of oxygen afforded



Scheme 7. Redox and addition reactions of *5*.



Scheme 8. Formation of *17*–*19* from *15*: (1) opening of the epoxide ring, (2) rearrangement and tautomerization, (3) elimination of water, (4) reduction by enediols, (5) benzylic acid rearrangement, followed by decarboxylation and tautomerization.[19]

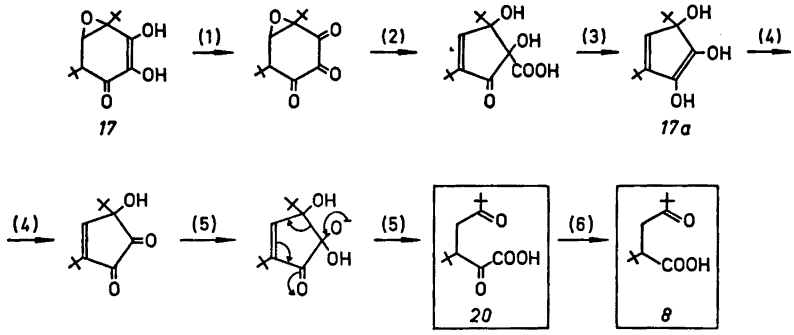
compounds *17*–*19*. The autoxidation of *15* in alkaline media may therefore be preceded by the alkaline (non-oxidative) transformations shown in Scheme 8.

In the presence of oxygen, compounds *17* and *18* undergo further conversions whereas *19* is essentially stable under the conditions used (Table 1).

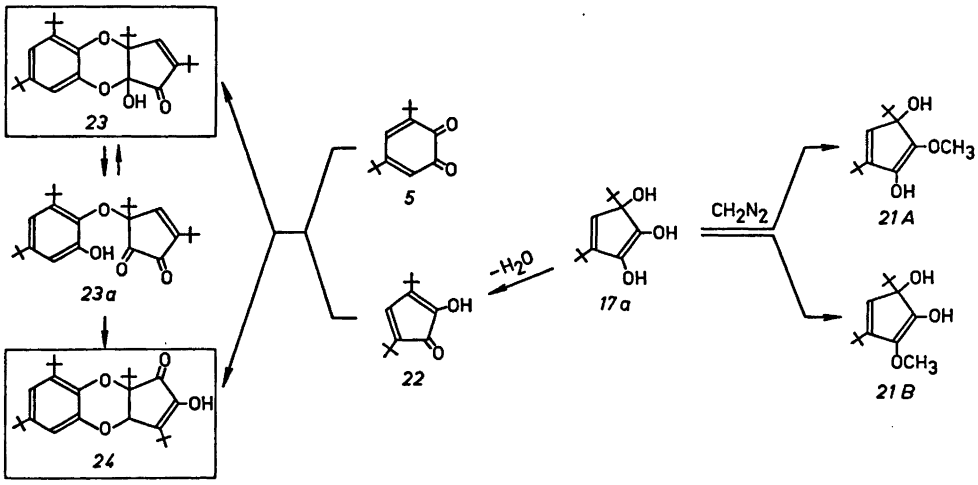
Formation of 20 *and* 8. When *17*, or each of the compounds *5*, *14* and *15*, were treated with alkali in the presence of oxygen, *20* and *8* were formed in varying amounts (Table 1). Compound *17* may therefore be regarded as a possible intermediate in the formation of *20* and *8* from *5*, *14* and *15*. A plausible route for the conversion of *17* into *20* and *8* is outlined in Scheme 9. A similar pathway has been proposed for the formation of the 4-keto acid corresponding to *8* which is obtained on autoxidation of 4-*t*-butylguaiacol.[1]

The validity of the reaction sequence presented in Scheme 9 was supported by demonstrating the intermediary formation of *17*a during the autoxidation of *5*, *14* or *15*. This was achieved in two different ways (Scheme 10).

1. Column chromatography of the reaction mixtures obtained after autoxidation of *5* or *14* gave, among other products, compounds *23* and *24*. The formation of these products is interpreted as a Diels-Alder reaction between *5* acting as "diene" and cyclopentadienone *22* acting as dienophile.[23] *22* is formed from *17*a by elimination of water. The 1,8-addition of



Scheme 9. Formation of *20* and *8* from *17*. (1)=(4) enediol oxidations,[20] (2) benzilic acid rearrangement, (3) decarboxylation, (5) alkaline rearrangement,[21] (6) oxidation by hydrogen peroxide.[21,22]



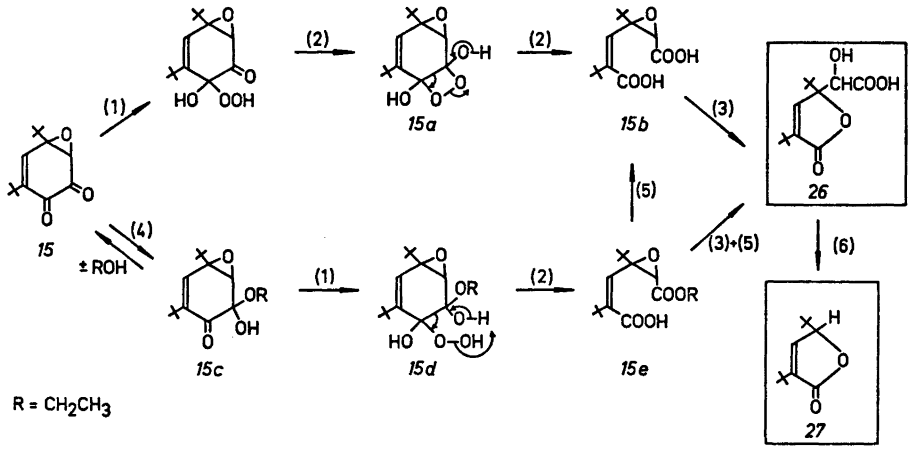
Scheme 10. Trapping of intermediate *17*a (Scheme 9) and its dehydration product *22*.

22 to *5* was demonstrated by reacting authentic *5* and *22* under similar alkaline conditions. A quantitative yield of the dimers *23* and *24* in a ratio of 1:1 was obtained.

Acetylation of the unseparated reaction mixture of *23* and *24* afforded the acetate of *24* only. This shows that *23* easily rearranges probably *via* ring opening by tautomerization (formation of *23*a), followed by intramolecular 1,4-addition of the phenol moiety to the cyclopentenedione part of the molecule. Compound *24* proved stable towards further alkaline-oxidative treatment under the conditions used.

2. GC-MS analysis of the diazomethane-methylated acidic fraction after autoxidation of *15* revealed the presence of two isomeric mono-methyl ethers *21*A and *21*B. Methylation of *17*a is expected to give two isomeric monomethyl ethers due to the enediol nature of this compound.

The oxidative decarboxylation of *20* by the action of hydrogen peroxide [21,22] (Scheme 9) was shown in a separate experiment.



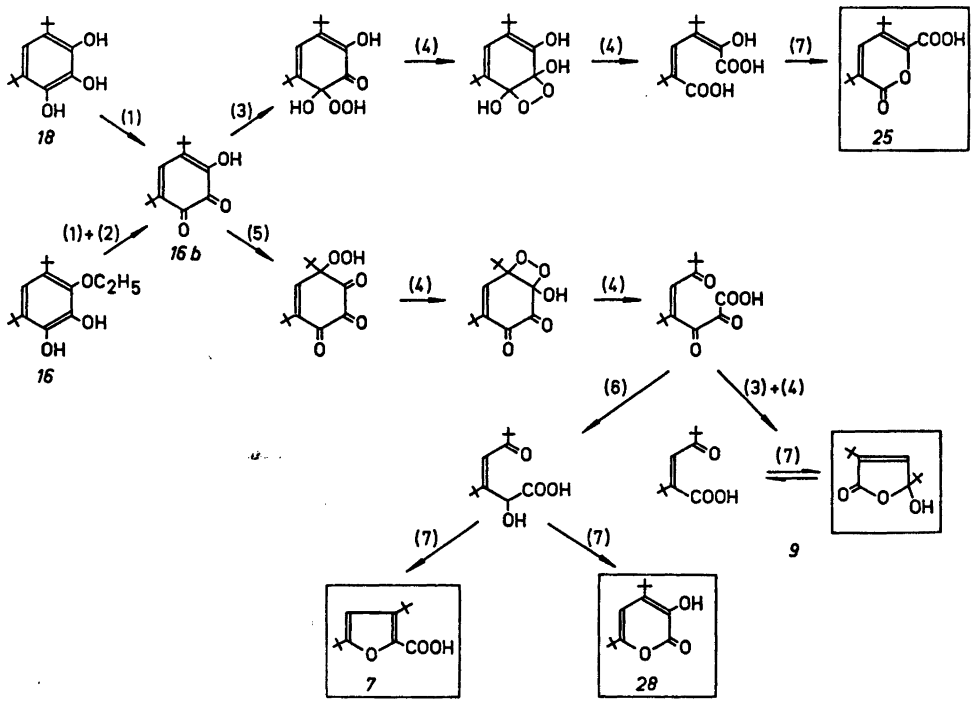
Scheme 11. Formation of *26* and *27* from *15*: (1) addition of hydrogen peroxide, (2) rearrangement, (3) lactone formation with opening of the epoxide ring, (4) addition of ethanol, (5) alkaline hydrolysis of the ester group, (6) alkali-promoted elimination of glyoxylic acid.

Formation of 26 *and* 27. Oxidation of *15* with alkaline hydrogen peroxide in aqueous ethanol at 0 °C gave a 85 % yield of *26*. Treatment of *26* with alkali at 40 °C afforded *27*. These results suggest that *15* is an intermediate, not only in the formation of *8* and *20* (see above) but also in the formation of *26* and *27* from compound *5* (Scheme 6). Two possible pathways for the conversion of *15* into *26* and *27* are outlined in Scheme 11.

Both routes involve addition of hydrogen peroxide to one of the carbonyl groups. The first route then proceeds *via* a dihydroxy dioxetane intermediate (*15*a) which rearranges to give the epoxidated muconic acid *15*b. The second route includes formation of a hemiacetal (*15*c) prior to hydrogen peroxide addition. The hydroperoxy hemiacetal *15*d undergoes a rearrangement with elimination of water and formation of the corresponding epoxidated muconic acid half ester (*15*e). Compound *15*e is hydrolyzed before or after cyclization. The latter reaction entails the opening of the epoxide ring.

Some support has been provided indicating that the conversion of *15* into *26* may, to a certain extent, follow the second route: The facile acetalization of *15* was shown by dissolving this compound in chloroform-ethanol (20:1) and isolating *15*c in a quantitative yield (^{1}H NMR). Furthermore, autoxidation of *14* in an alkaline solution containing ethanol and water (1:3) gave traces of the ethyl ester of *26* together with *26*, *27* and many other compounds (*cf.* also Ref. 24 and 25). Apparently, the ester group, to a certain extent, resists alkaline hydrolysis. The methyl ester of *26* has previously been isolated [25] in 20 % yield after treatment of compound *5* with alkaline (0.1 M Na_2CO_3) hydrogen peroxide in aqueous methanol.

Formation of 7, 9, 25 *and* 28. Autoxidation of *16* gave the lactones *9*, *25* and *28*, and the furoic acid *7* (Scheme 12). These compounds were



Scheme 12. Formation of *7*, *9*, *25* and *28* from *16*: (1) enediol oxidations, (2) alkaline deethoxylation, (3) addition of hydrogen peroxide, (4) hydroperoxide rearrangements *via* dioxetanes, (5) oxygenation, (6) decarboxylation, (7) cyclizations.

also present in the reaction mixtures obtained on autoxidation of *5* (Scheme 6). Compound *25* was also formed upon autoxidation of *14*, *15* or *18*. When treated under suitable conditions, compound *18* afforded *25* in high yield together with varying amounts of *19* (*cf.* also Ref. 19).

Plausible routes leading from compounds *16* and *18* to *7*, *9*, *25* and *28* are presented in Scheme 12.

The oxidative ring cleavages described in this Scheme are suggested to proceed by rearrangement *via* dioxetane intermediates. However, as in the oxidation of *15* (Scheme 11), an alternative mechanism of ring cleavage, involving rearrangement of a hydroperoxy hemiacetal intermediate, may also be operative to a certain extent (*cf.* also Ref. 1).

Scheme 12 can also be used to explain the following findings:

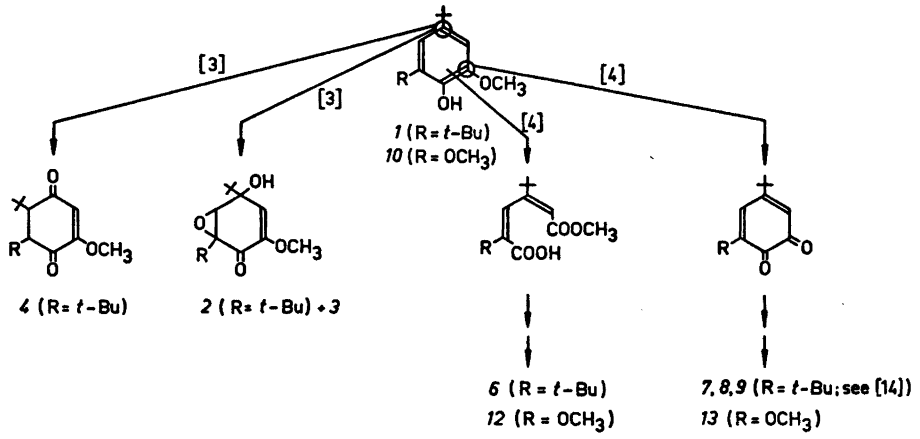
1. Products corresponding to *7*, *9*, *25* and *28* are obtained upon a similar autoxidation of 3-methoxy-5-*t*-butyl-*o*-benzoquinone (*11*) arising *via* oxygenation of 4-*t*-butylguaiacol[1] or by oxidative demethoxylation of 4-*t*-butyl-2,6-dimethoxyphenol (*10*, Scheme 5). The formation of these products has been formulated analogously,[1] 3-hydroxy-5-*t*-butyl-*o*-benzoquinone being the quinoid intermediate instead of *16*b.

2. The hydroxylactone *9* has previously been obtained by autoxidation of *5* in *t*-butanol[12] and of 2,6-dimethoxy-3,5-di-*t*-butylphenol in aqueous ethanol.[3] The formation of *9* from the latter substrate should also proceed *via* *16*b arising by oxidative demethoxylation (*cf.* also behaviour of *10*, Scheme 5), followed by alkaline demethoxylation.[1]

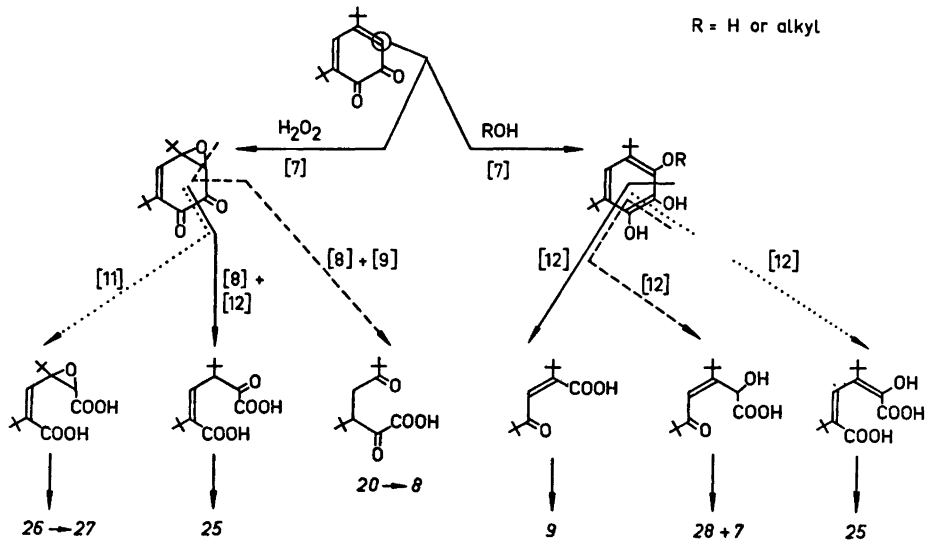
CONCLUSIONS

The reaction taking place during the autoxidation of 4,6-di-*t*-butylguaiacol (*1*) can be divided into two categories: The first includes oxygenations in the 2 and 4-positions followed by alkali-promoted rearrangements and conversions of cyclohexadienone hydroperoxide intermediates (Schemes 3 and 4). The second comprises nucleophilic additions of hydroperoxy, hydroxy and alkoxy anions in the 6- (originally 3-) position of the *ortho*-quinone intermediate (Scheme 7), followed by alkaline and oxidative conversions and degradations of the addition products (Schemes 8, 9, 11 and 12). The courses of the alkaline-oxidative transformations of *1* and of intermediate *5* are summarized in Schemes 13 and 14, respectively. The figures within brackets in these schemes refer to previous schemes in the text. The arrows indicate the sites of alkline-oxidative attack, ring cleavage and also, in the case of Scheme 14, fragmentation.

The other compound investigated, 4-*t*-butyl-2,6-dimethoxyphenol (*10*), reacts in a similar fashion, the 2-position constituting the main site of primary oxygenation (Schemes 5 and 13).



Scheme 13. Course of degradation of *1* (summary).



Scheme 14. Course of degradation of *5* (summary).

EXPERIMENTAL

Materials

Syntheses of model compounds. The following compounds were prepared as previously described: *1*,[26] *10*,[27] *11*,[1] *18*,[28] *19* [21] and *22*.[29]

3,5-Di-t-butyl-o-quinone (5). A method described for the synthesis of 4-methyl-*o*-quinone [30] from 4-methylcatechol was adopted. Sodium metaperiodate (50 mmol) in water (200 ml) was added to a cooled solution (+5 °C) of 3,5-di-*t*-butylcatechol (50 mmol) in 60 % acetic acid (300 ml). The reaction mixture was extracted with methylene chloride (2 × 400 ml) after 1 min. The coloured extract was washed free of acid, dried with sodium sulfate and evaporated to yield 11 g of crude material. Crystallization from light petroleum (40–60 °C) yielded dark red needles (9 g, 82 %), m.p. 116–117 °C (lit.[24] 113–114 °C).

4,5-Epoxy-6-oxo-2,4-di-t-butylcyclohex-2-enone (15). 3,5-Di-*t*-butyl-catechol (*14*) (20 mmol), dissolved in a 0.2 N solution of sodium hydroxide containing ethanol-water (3:1) (200 ml), was autoxidized at 0 °C. The reaction mixture was neutralized with carbon dioxide after 20 min (oxygen consumption: 1.2 equiv.) diluted with water and extracted with methylene chloride. The yellow extract was dried with sodium sulfate and evaporated to yield crude material (4.7 g) which was dissolved in benzene (20 ml). After concentration under reduced pressure, light petroleum (20 ml) was added precipitating pale yellow needles (3.1 g, 65 %). For spectroscopic data and elemental analyses, see Table 3 and data below.

4,6-Di-t-butyl-3-ethoxycatechol (16). 3-Ethoxycatechol [31] (1.0 mmol) in acetic acid (2 ml) and *t*-butanol (3 ml) were treated with sulfuric acid (0.85 ml) at 20 °C. The reaction mixture was kept at room temperature for 24 h. After dilution with water, the solution was extracted with methylene chloride. Evaporation of the dried (Na_2SO_4) extract yielded 0.27 g of crude material. Recrystallization from light petroleum (40–60 °C) gave white crystals (0.18 g, m.p. 151–153 °C). Anal. $C_{16}H_{26}O_3$: C, H, O. ^1H NMR (60 MHz, $CDCl_3$): δ 1.34 (s, 9 H, *t*-Bu), 1.37 (s, 9 H, *t*-Bu), 1.42 (t, 3 H, CH_3, *J* 7.5), 3.93 (q, 2 H, CH_2, *J* 7.5), ~5.15 (broad s, 1 H, OH), ~5.50 (broad s, 1 H, OH), 6.73 (s, 1 H, ar).

Methods

Autoxidation of model compounds. The treatment of the model compounds with oxygen and the work-up procedure were carried out as previously [1] described. The conditions and results are given in Table 1.

Autoxidation of 4,6-di-t-butylguaiacol (1) with $^{18}O_2$. The reaction was performed under the conditions given in Table 1 except that $^{18}O_2$ mixed with N_2 (~80 vol %) was used as oxidant. The resulting epoxide (*2*) was identified by GC-MS analysis (see p. 000).

Autoxidation of 3,5-di-t-butyl-o-quinone (5) at 0 °C. Compound *5* (10 mmol) was autoxidized in a 0.2 M solution of sodium hydroxide in ethanol-water (4:1) (100 ml). 1.2 equivalents of oxygen were consumed within 8 h. The reaction mixture was not investigated in detail. The

presence of *15* in the neutral fraction was shown by TLC.

Autoxidation of 3,5-di-t-butylcatechol (14) at 0 °C, see preparation of compound *15* above.

Oxidation of model compounds with alkaline hydrogen peroxide. The oxidation of model compounds with alkaline hydrogen peroxide was carried out in an atmosphere of nitrogen. The work-up procedure was essentially the same as that described for the autoxidation experiments.[1] The extractions were carried out with methylene chloride instead of ether. The conditions and results are given in Table 2.

Alkaline treatment of model compounds. All treatments were carried out in an atmosphere of nitrogen.

Table 2. Oxidation of model compounds with alkaline hydrogen peroxide. Alkalinity 0.2–0.3 M NaOH. Temperature 40 °C.

Compound (mol/l)	Solvent EtOH:H_2O	Equivalents of H_2O_2	Reaction time, min	*t*, °C	Products (Yields % of theoretical)
5 (0.1)	4:1	1.2	10	0	*15* (55), *26* (20)
15 (0.1)	4:1	1.5	10	0	*26* (85)
17 (0.1)	1:1	2	30	40	*8* (80), *20*[a]
20 (0.1)	1:1	2	120	40	*8* (60)

[a] Yield not determined.

Table 3. ^{1}H NMR of products isolated from alkaline autoxidation of *1*, *5*, *10*, *14*, *15* and *16*. Types of protons: ac, acetyl; ar, aromatic; es, ester; et, ether; ol, olefinic.

Compound	δ (*t*-Bu) (s, 9 H)	δ (Me) (s, 3 H)	δ other protons
2	1.02, 1.11	3.58 (et)	3.74 (d, 1 H, CH), 5.18 (d, 1 H, ol), $J_{CH,ol}$ 2.7 Hz, 2.44 (s, 1 H, OH)
3	0.99, 1.20	3.55 (et)	3.85 (d, 1 H, CH), 6.10 (d, 1 H, ol), $J_{CH,ol}$ 2.7 Hz, 2.27 (s, 1 H, OH)
4 R = CH_3	0.95[a]	3.75 (et)	3.68 (s, 1 H, CH), 3.76 (s, 1 H, CH), 5.93 (s, 1 H, ol)
4 R = C_2H_5	0.95[a]		1.43 (t, 3 H, CH_3), 3.96 (q, 2 H, CH_2), J_{CH_3,CH_2} 7.3 Hz, 2.67 (s, 1 H, CH), 2.75 (s, 1 H, CH), 5.93 (s, 1 H, ol)
6[b]	0.97, 1.22	3.57 (es)	3.01 (A) and 2.73 (B) [CH_2; AB; J_{AB} 14.0 Hz]
7[b]	1.19, 1.26	3.82 (es)	6.03 (s, 1 H, ol)
8[b]	1.00, 1.15	3.63 (es)	2.30–3.30 (m, 3 H, CH+CH_2)
9[b]	1.16, 1.20	3.80 (es)	6.43 (s, 1 H, ol)
9 (lactone)	1.02, 1.23		6.76 (s, 1 H, ol), 3.32 (broad s, 1 H, OH)
12	1.00	3.75 (et)	2.89 (s, 2 H, CH_2), 6.07 (s, 1 H, ol), 9.37 (broad s, 1 H, OH)
15	1.10, 1.20		3.80 (d, 1 H, CH), 7.07 (d, 1 H, ol), $J_{CH,ol}$ 0.9 Hz
20[b]	0.99, 1.12	3.90 (es)	2.60–3.40 (m, 2 H, CH_2), 3.50–3.80 (q, 1 H, CH)
23	1.08, 1.18 1.27[a]		6.83 (s, 2 H, ar), 7.08 (s, 1 H, ol), 4.09 (s, 1 H, OH)
24	0.97, 1.25 1.38, 1.44		5.00 (s, 1 H, CH), 6.87 (broad s, 2 H, ar), 5.65 (broad s, 1 H, OH)
25	1.35[a]		7.40 (s, 1 H, ol), 9.70 (broad s, 1 H, OH)
26	1.07, 1.20		4.68 (s, 1 H, CH), 7.06 (s, 1 H, ol), 5.15 (s, 2 H, OH)
27	0.95, 1.25		4.48 (d, 1 H, CH), 6.92 (d, 1 H, ol) $J_{CH,ol}$ 1.5 Hz
28	1.25, 1.33		6.00 (s, 1 H, ol), 6.45 (s, 1 H, OH)

[a] 18 H. [b] Methyl ester.

Alkaline treatment of 5. Compound *5* (5 mmol) was treated with a 0.25 M solution of sodium hydroxide in ethanol-water (3:1) (100 ml) at 40 °C for 30 min. 3,5-Di-*t*-butylcatechol (*14*) was the main product (~60 %, determined from the ^{1}H NMR spectrum of the neutral fraction).

Alkaline treatment of 15. Compound *15* (6 mmol) was treated with a 0.3 M solution of sodium hydroxide in ethanol-water (4:1) (100 ml) at 40 °C for 25 min. The reaction mixture consisted of the acidic products *17* (30 %) and *20* (10 %) and the neutral products *18* (35 %) and *19* (10 %). The yields were determined from ^{1}H NMR spectra of the crude acidic and neutral fractions. The yield of *17* was increased to 70 % by treating *15* with a stronger alkaline solution (3 M sodium hydroxide) containing ethanol-water (3:1) under otherwise identical conditions.

Alkaline treatment of a mixture of 5 *and* 22. Compound *5* (0.5 mmol) and compound *22* (0.5 mmol) were dissolved in a 0.3 M solution of sodium hydroxide containing ethanol-water (4:1) (10 ml) and kept at 40 °C. The solution became colourless within 15 min. After neutralization (CO_2) and dilution with water (50 ml), a white precipitate (0.17 g, 80 %) was formed. The ^{1}H NMR spectrum of this product revealed the presence of *23* and *24* in an approximate ratio of 1:1. Acetylation of this mixture gave *24*-acetate (yield 91 %).

Chromatography. The preparative separations by column or thin-layer chromatography and the gas chromatography separations combined with mass spectral analyses were carried out as previously [1] described.

Product identification

The reaction products were identified by usual spectrometric methods (types of instruments used are given in Ref. 1). The ^{1}H NMR data are presented in Table 3. IR, UV and MS data are given in the following. Only mass fragments (max. 8) with $m/e > 100$ and relative intensities > 10 % of the base peak are included.

5,6-Epoxy-4-hydroxy-2-methoxy-4,6-di-t-butylcyclohex-2-enone (*2*). m.p. 102–103 °C. Anal. $C_{15}H_{24}O_4$: C, H, O, MS *m/e* (rel. int.): 212, (M–56, 45), 211 (M–57, 100), 194 (M–74, 10), 179 (M–89, 10), 156 (95), 151 (30), 143 (30), 141 (30). IR (KBr): 3505 (s, OH), 3490 (m, OH), 1695 (s, C=O), 1635 (m, C=C–C=O). UV [MeOH (ε)]: 272 (3400).

2,3-Epoxy-4-hydroxy-2-methoxy-4,6-di-t-butylcyclohex-5-enone (*3*), m.p. 100–100.5 °C. Anal. $C_{15}H_{24}O_4$: C, H, O. MS *m/e* (rel. int.): 240 (M–28, 15), 239 (M–29, 90), 212 (M–56, 90), 211 (M–57, 65), 209 (85), 183 (25), 179 (75), 153 (100). IR (KBr): 3470 (m, OH), 1685 (s, C=O). UV [EtOH (ε)]: 228 (16.000).

1-Methoxy-4,5-di-t-butyl-cyclohex-1-enedi-3,6-one (4, $R = CH_3$), m.p. 150 °C. MS *m/e* (rel. int.): 171 (M–81, 10), 140 (M–112, 100), 125 (15). IR (KBr): 1685 (s, C=O), 1655 (s, C=C–C=O), 1610 (C=C–C=O). UV [MeOH (ε)]: 271 (–).

1-Ethoxy-4,5-di-t-butyl-cyclohex-1-enedi-3,6-one (4, $R = C_2H_5$), m.p. 95–97 °C. Anal. $C_{16}H_{26}O_3$: C, H. MS *m/e* (rel. int.): 154 (M–112, 100), 126 (35). IR (KBr): 1680 (s. C=O), 1650 (s, C=C–C=O), 1600 (s, C=C–C=O). UV [EtOH (ε)]: 274 (10 750).

Methyl 2,5-dihydro-5-oxo-2,4-di-t-butylfuran-2-acetate (methyl ester of 6), m.p. 67–69 °C. Anal. $C_{15}H_{24}O_4$: C, H, O. MS *m/e* (rel. int.):* 212 (M–56, 100), 197 (M–71, 75), 153 (M–115, 38), 137 (10), 109 (30), IR (KBr): 1745 (s, C=O), 1735 (s, C=O), 1640 (w, C=C–C=O). UV [MeOH (ε)]: 215 (sh, –).

Methyl 3,5-di-t-butyl-2-furoate(methyl ester of 7), amorphous: Anal. $C_{14}H_{22}O_3$: C, H, O. MS *m/e* (rel. int.): 238 (10), 223 (100), 207 (10), 191 (20), 165 (20). IR ($CHCl_3$): 1710 (s, C=O), 1585 (m, C=C), 1520 (m, C=C).

Methyl 5,5-dimethyl-2-t-butyl-4-oxohexanoate (methyl ester of 8), amorphous. MS *m/e* (rel. int.): 197 (M–31, 10), 171 (M–57, 100), 143 (M–85, 40), 125 (15), 111 (80). IR ($CHCl_3$): 1730 (s, C=O), 1710 (s, C=O).

Methyl 5,5-dimethyl-4-oxo-2-t-butyl-hex-2-enoate (methyl ester of 9), amorphous. Anal. $C_{13}H_{22}O_3$: C, H, O. MS *m/e* (rel. int.): 195 (M–31, 10), 170 (M–56, 100), 169 (M–57, 65), 155 (85), 141 (20), 109 (20). IR ($CHCl_3$): 1725 (s, C=O), 1685 (m, C=O), 1610 (m, C=C–C=O).

α,γ-Di-t-butyl-γ-hydroxy-Δ-α,β-butenolide (lactone of 9), m.p. 98–100 °C. Anal. $C_{12}H_{20}O_3$: C, H, O. MS *m/e* (rel. int.): 156 (M–56, 100), 155 (M–57, 20), 141 (M–71, 40), 138 (10), 125 (10), 109 (15). IR (KBr): 3460 (s, OH), 1730 (s, C=O), 1635 (w, C=C–C=O). UV [EtOH (ε)]: 212 (9700).

2,5-Dihydro-5-oxo-4-methoxy-2-t-butylfuran-2-acetic acid (*12*), m.p. 144–145 °C. Anal. $C_{11}H_{16}O_5$: C, H, O. MS *m/e* (rel. int.): 172 (M–56, 100), 127 (M–101, 50). IR (KBr): 1765 (s, C=O), 1690 (s, C=O), 1655 (s, C=C–C=O). UV [EtOH (ε)]: 227 (12 300).

3,4-Epoxy-3,4-dihydro-4,6-di-t-butyl-1,2-benzoquinone (*15*), m.p. 100–101 °C. Anal. $C_{14}H_{20}O_3$: C, H, O. MS *m/e* (rel. int.): 208 (M–28, 20), 207 (M–29, 25), 193 (M–43, 95), 180 (M–56, 100), 165 (75), 151 (25), 150 (55), 149 (17). IR (KBr): 1730 (s, C=O), 1680 (s, C=O), 1610 (w, C=C–C=O). UV [EtOH (ε)]: 247 (4600).

Methyl 6,6-dimethyl-3-t-butyl-2,5-dioxoheptanoate (methyl ester of 20), m.p. 89–90 °C. Anal. $C_{14}H_{24}O_4$: C, H, O. MS *m/e* (rel. int.): 238 (M–18, 10), 223 (M–33, 35), 197 (M–59, 100), 169 (20), 151 (25), 143 (20), 113 (15). IR (KBr): 1735 (s, C=O), 1715 (s, C=O), 1685 (s, C=O).

9a-Hydroxy-3a,9a-dihydro-2,3a,5,7-tetra-t-butyl-1H-cyclopenta-[b] [*1,4*]*benzodioxin-1-one*

* Yield not determined.

(23), m.p. 196–197 °C. MS *m/e* (rel. int.): 428 (M, 55), 221 (M–207, 50), 207 (M–221, 45), 165 (100), 152 (60), 151 (35), 137 (45). IR (KBr): 3410 (s, OH), 1725 (s, C=O), 1605 (w, C=C–C=O), 1585 (m, aromatic C=C).

2-Hydroxy-3a,9a-dihydro-3,6,8,9a-tetra-t-*butyl-1H-cyclopenta*-[b] *[1,4]benzodioxin-1-one (24)*, m.p. 192–194 °C. Anal. $C_{27}H_{40}O_4$: C, H, O. IR (KBr): 3360 (s, OH), 1705 (s, C=O), 1645 (m, C=C–C=O), 1580 (m, aromatic C=C). UV [Ethyl ether (ε)]: 262 (10 000).

3,5-Di-t-*butyl-6-carboxy-2H-pyran-2-one (25)*, m.p. 188–190 °C. MS *m/e* (rel. int.): 252 (M, 35), 237 (M–15, 20), 209 (M–43, 95), 195 (M–57, 40), 193 (35), 192 (35), 178 (35), 167 (100). IR (KBr): 2800–2500 (m, OH), 1754 (s, C=O), 1660 (s, C=O), 1630 (m, C=C–C=O), 1565 (s, C=C–C=O).

2,5-Dihydro-5-oxo-2,4-di-t-butylfuran-2-glycolic acid (26), m.p. 137–139 °C. Anal. $C_{14}H_{22}O_5$: C, H, O. MS *m/e* (rel. int.): 209 (M–61, 15), 197 (M–73, 100), 169 (M–104, 30), 165 (M–105, 40), 151 (35), 129 (15), 125 (25), 113 (65). IR (KBr): 3500–3200 (m, OH), 1740–1700 (s, C=O), 1630 (w, C=C–C=O). UV [MeOH (ε)]: 215 (9200).

α,γ-Di-t-*butyl-Δ^{α,β}-butenolide (27)*, m.p. 96–97 °C. Anal. $C_{12}H_{20}O_2$: C, H, O. MS *m/e* (rel. int.): 181 (M–15, 10), 140 (M–56, 80), 125 (M–71, 100). UV [MeOH (ε)]: 211 (12 300).

3-Hydroxy-4,6-di-t-*butyl-2H-pyran-2-one (28)*, m.p. 121–122 °C. Anal. $C_{13}H_{20}O_3$: C, H, O. MS *m/e* (rel. int.): 224 (M, 30), 209 (M–15, 100), 181 (M–43, 20). IR (KBr): 3335 (s, OH), 1680 (s, C=O), 1640 (s, C=C–C=O), 1555 (m, C=C–C=O). UV [EtOH (ε)]: 305 (9300).

Further identification of products and intermediates

Mass spectrum of *2* obtained in $^{18}O_2$ atmosphere: Mass fragments having *m/e* > base peak and rel. int. > 5 % are listed. *m/e* (% rel.int.): 221 (14), 216 (5), 215 (10), 213 (5), 212 (7), 211 (6), 210 (10), 208 (5), 196 (8), 195 (10), 193 (7), 185 (12), 184 (100). Mass spectrum of *2* obtained in $^{16}O_2$ atmosphere: *m/e* (% rel. int.): 221 (14), 212 (10), 211 (22), 209 (8), 208 (22), 207 (7), 194 (9), 193 (22), 182 (10), 181 (40), 180 (100).

High resolution mass spectrometry of *2* ($^{16}O_2$) showed that the fragment with mass number 212 is $C_{11}H_{16}O_4$ (M–C_4H_8). (Calc.: *m/e* = 212.1048. Found: *m/e* = 212.1035). The corresponding fragment from *2* ($^{18}O_2$), *m/e* = 216, was found and it is therefore concluded that two atoms of ^{18}O were incorporated during autoxidation (Scheme 3).

Treatment of 15 *with diazomethane. 15* was treated with diazomethane in ether to give a quantitative yield of *2,4-di-t-butyl-4,5-epoxy-6-spiroepoxy-cyclohex-2-enone*, m.p. 83–85 °C. Anal. $C_{15}H_{22}O_3$: C, H, O. IR (KBr): 1675 (s, C=O), 1620 (s, C=C–C=O). UV [MeOH, (ε)]: 252 (4850). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.05 (s, 9 H, *t*-Bu), 1.18 (s, 9 H, *t*-Bu), 3.18 and 3.44 (dd, 2 H, CH_2, J_{gem} 6.6 Hz), 3.38 (d, 1 H, CH, *J* ~ 1 Hz), 7.12 (d, 1 H, ol, *J* ~ 1 Hz). The same compound was isolated after autoxidation of *5*, methylation and separation of the reaction mixture.

2,3-Dihydroxy-4,5-epoxy-4,6-di-t-*butylcyclohex-2-enone (17)*, UV [MeOH, (ε)]: 271 (9000). ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.97 (s, 9 H, *t*-Bu), 1.14 (s, 9 H, *t*-Bu), 2.70 (d, 1 H, CH, *J* 1.5 Hz), 4.02 (d, 1 H, CH, *J* 1.5 Hz), 7.10 (broad s, 2 H, OH, exchangeable). Acetylation of *17* followed by methylation (diazomethane) gave two isomeric acetylated monomethyl ethers.

Separation by preparative TLC afforded: *2-Acetoxy-4,5-epoxy-3-methoxy-4,6-di*-t-*butylcyclohex-2-enone*, colourless crystals, m.p. 82–85 °C (light petroleum). Anal. $C_{17}H_{26}O_5$: C, H, O. IR (KBr): 1765 (s, C=O), 1710 (s, C=O), 1690 (s, C=C–C=O), 1625 (m, C=C–C=O). UV [EtOH (ε)]: 253 (10 700). ^{1}H NMR (60 MHz, $CDCl_3$): 0.97 (s, 9 H *t*-Bu), 1.13 (s, 9 H, *t*-Bu), 2.19 (s, 3 H, ac), 4.05 (s, 3 H, OMe), 2.87 (d, 1 H, CH, *J* 3 Hz), 4.05 (d, 1 H, CH, *J* 3 Hz).

2-Acetoxy-5,6-epoxy-3-methoxy-4,6-di-t-*butylcyclohex-2-enone*, colourless crystals, m.p. 100 °C (light petroleum). Anal. $C_{17}H_{26}O_5$: C, H, O. IR (KBr): 1770 (s, C=O), 1705 (s, C=O), 1695 (s, C=C–C=O), 1635 (m, C=C–C=C). UV [EtOH (ε)]: 248 (12200). ^{1}H NMR (60 MHz, $CDCl_3$): 1.00 (s, 9 H, *t*-Bu), 1.20 (s, 9 H, *t*-Bu), 2.20 (s, 3 H, ac), 3.96 (s, 3 H, OMe), 2.28 (d, 1 H, CH, *J* 3 Hz), 4.06 (d, 1 H, CH *J* 3 Hz).

1,3-Dihydroxy-2-methoxy-1,4-di-t-*butylcyclopentadiene (21A) and 1,2-dihydroxy-3-methoxy-1,4-di*-t-*butylcyclopentadiene (21B)*. GC-MS analysis (molecular ion and mass fragments having *m/e* > 100 and rel. int. > 10 % of the base peak are given):

*17*a-monomethyl ether (form 1): 240 (M, 5), 238 (M–2, 10), 223 (M–17, 55), 191 (M–49, 10), 183 (M–57, 100), 170 (20), 169 (15), 165 (17), 155 (20), 141 (35), 127 (27), 109 (55).

*17*a-monomethyl ether (form 2): 240 (M, 7) 223 (M–17, 15), 183 (M–57, 100), 165 (20), 141 (30), 127 (25), 109 (40). On the basis of the mass spectra, however, it was not possible to attribute structures *21*A and *21*B to forms 1 and 2.

2-Acetoxy-3a,9a-dihydro-3,6,8,9a-tetra-t-*butyl-1H-cyclopenta*[b] *[1.4]-benzodioxin-1-one (acetate of 24)*. In addition to the ^{1}H NMR data given in Table 3, the structure of *24* was confirmed by the ^{13}C NMR spectrum (20 MHz, $CDCl_3$) of *24*-acetate: δ 195.49 (C 1), 166.73 (C=O in acetate), 161.79 (C 3), 148.97, 145.27, 144.96, 139.75, 137.98 (C 2, C 5, C 6, C 7, C 8), 116.01, 112.59 (C 4 a, C 8 a), 84.22 (C 3 a), 74.80 (C 9 a), 38.26, 34.55, 34.36, 34.05 (–C–)

in *t*-Bu), 31.41, 30.15, 28.74 and 25.87 (CH_3 in *t*-Bu), 20.26 (CH_3 in acetate). The location of *t*-butyl groups at the carbon atoms 6 and 8 in the benzodioxin ring is uncertain. They may possibly be linked to carbon atoms 5 and 7.

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Studies on the Degradation of Phenolic Lignin Units of the β-Aryl Ether Type with Oxygen in Alkaline Media*

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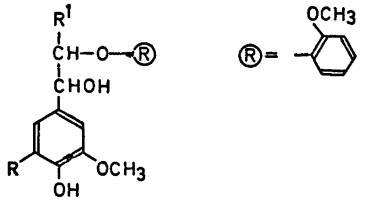
The degradation of *p*-hydroxy-arylglycerol-β-aryl ether structures in lignins by oxygen in alkaline media has been studied using appropriate model compounds. Three pathways (A, B, C, Schemes 1–3), explaining the formation of the various degradation products under different conditions, have been elucidated. Pathways A and B involve oxidative elimination of the side chain by oxygen and hydrogen peroxide, respectively. Hydrogen peroxide may be generated by autoxidation of certain lignin structures and intermediates. Pathway C entails the oxidative cleavage of the $C_\alpha - C_\beta$ bond by oxygen.

The primary oxidation products (aliphatic and aromatic aldehydes and *p*-quinones) undergo further oxidative-alkaline degradation to give ultimately aliphatic acids.

It is also shown that the oxidative-alkaline degradation of certain enolizable phenacyl aryl ether structures follows similar routes.

In recent years, the treatment of wood and pulps with oxygen in alkaline media has become an attractive alternative to conventional delignification processes.[1,2] Numerous studies have therefore been carried out to elucidate the breakdown of lignins by oxygen in alkali and plausible mechanisms have been proposed.[3–8] These studies deal primarily with the oxidative attack on phenolic lignin nuclei and do not account for the reactions involving also lignin side chains.

The main subject of the present work is the degradation of structural elements of the *p*-hydroxy-arylglycerol-β-aryl ether and related types with oxygen in alkali. The reactions involved were studied using model compounds *1*–*6* representing "uncondensed" [9] (*1* and *2*) and "condensed" [9] (*3* and *4*) guaiacyl type units, as well as syringyl (*5* and *6*) type units.



	R	R¹
1	H	H
2	H	CH_2OH
3	*t*-butyl	H
4	*t*-butyl	CH_2OH
5	OCH_3	H
6	OCH_3	CH_2OH

Unless otherwise stated, the oxidative treatments were carried out under standard conditions. The term "standard conditions" refers here and in the following to an approximately 0.05 M concentration of the compound in 0.2–0.3 M sodium hydroxide, a reaction temperature of 40 °C and an oxygen pressure of 1 bar. The standard treatment was stopped when

* Preliminary reports on the results of this work have been given (by J. G.) at the Annual ACS-Meeting, Chicago, Ill., August 26–31, 1973, Abstracts of papers, Cell 4, and at the International Symposium on Delignification with Oxygen, Ozone and Peroxides, Raleigh, N.C., May 27–29, 1975, Abstracts of papers, p. 13.

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1.0 – 1.5 mol of oxygen per mol of compound had been consumed. The mild conditions and the length of the treatment were chosen to facilitate the isolation of intermediates.

RESULTS AND DISCUSSION

The results from the treatments of the model compounds with oxygen in alkali are summarized in Table 1 (see EXPERIMENTAL).

Treatment of *p*-hydroxy-aryl-glycerol- and *p*-hydroxy-aryl-ethyleneglycol-β-aryl ethers with oxygen in alkaline media

Compounds *1* and *2*, when autoxidized under standard conditions for 48 h, were essentially stable and gave only small amounts of guaiacol. No vanillin was detected in the reaction mixtures. However, when the oxidative treatment of *2* was carried out at 100 °C using an oxygen

Table 1. Autoxidation of model compounds. Temperature: 40 °C. Alkalinity: 0.2 – 0.3 M NaOH. Oxygen consumption: 1 – 1.5 mol O_2/mol compound.

Compound	Reaction time, h	Reaction products isolated and/or identified (Yield %)	Starting material %
1	48	G(8)	80
1-arom. methyl ether	48		97
2	48	G(8)	81
2 [a]	1	G(3), V(5), M[b] acids: FA(4), AA(29.0), GA(15.0), OA(31.0), MA(4.2), SA (3.6), FuA(1)	9
2-triacetate	48	G(27), V(14), *21*(16), acids: VA(1), FA(64), other acids (5)	25
3	2	G(22), *11*(28)	38
4	1	G(5), *11*(15), *15*(3)	80
5-α-methyl ether	48		93
6	1	G(14), *15*(15) acids: FA(21), AA(2.7), GA(5)	35
6-triacetate	3	G(52), syringaldehyde(4), *15*(3) acids: FA(40), GA(15)	8
7	0.5	M(41) acids: FA(17), AA(2.8), GA < (0.1), OA(6.6), MA(1.6), SA(0.3), FuA(0.1)	0
8	2	*11* (96)	0
9	1.5	acids: FA(3.8), AA(3.5), OA(7.9), MA(1.7)	0
Vanillin	40	acids: FA(103), AA(12), OA(5), MA(2), FuA(0.5), SA(3)	44
14 (R^1 = H)	2	G(66) acidic products (20); not identified	0
Guaiacoxy-AA	18.5	G(0)	100
20 (R = H, *trans* + *cis*)	16	G(55), V(21)	6
22	28	G(55) acid: Ver A(53)	45
23	28	G(trace)	89
24	28	dimerization products [15]	0
25	28	*23*(100)	0
26	48	G(7) acids: VA(2), guaiacoxy-AA(2)	90
27	28	*28* (90)	0

[a] Reaction conditions: 100 °C, 7 bar. O_2. [b] Yield not determined.

AA = acetic acid, FA = formic acid, FuA = fumaric acid, G = guaiacol, GA = glycolic acid, M = methanol, MA = malonic acid, OA = oxalic acid, SA = succinic acid, SM = starting material, V = vanillin, VA = vanillic acid, VerA = veratric acid.

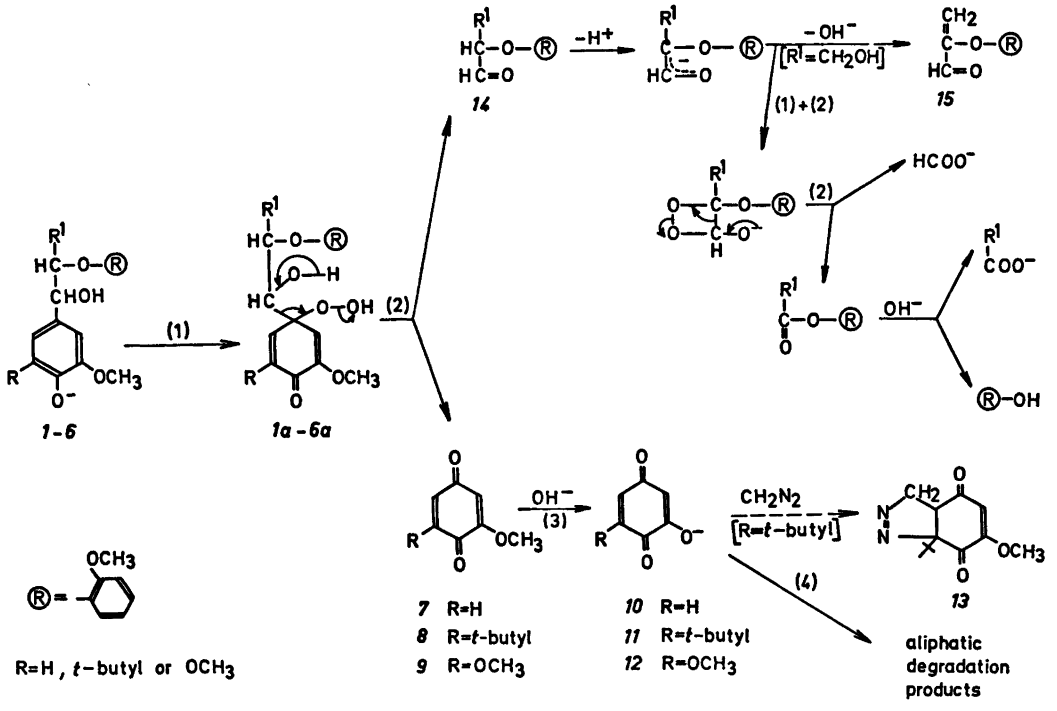
pressure of 7 bar, an extensive degradation took place within one hour. The reaction mixture contained small amounts of guaiacol and vanillin and a great number of aliphatic acids (GLC).

The oxidations of the *t*-butyl-substituted analogues *3* and *4* under standard conditions proceeded considerably faster than those of *1* and *2*. Compound *3* yielded considerable amounts of guaiacol and of the *p*-benzoquinone derivative *11* after treatment for only 2 h. Compound *4* gave, in addition to guaiacol and *11*, α-guaiacoxyacrolein (*15*) after autoxidation for 1 h. No 5-*t*-butylvanillin was detected in the reaction mixtures from *3* and *4* which contained large amounts of the starting compounds.

A still more rapid autoxidation under standard conditions was observed with the 2,6-dimethoxy-substituted phenols *5* and *6*. After a reaction period of 1 h, the reaction mixture from compound *6* contained appreciable amounts of guaiacol and α-guaiacoxyacrolein (*15*). No syringaldehyde was detected.

The different behaviour of the model compounds on treatment with oxygen in alkali and the formation, in varying yields, of the reaction products listed in Table 1 can be explained by suggesting three possible pathways of oxidative-alkaline degradation:

Pathway A involves elimination of the side chain by an alkali-promoted rearrangement of a cyclohexadienone hydroperoxide intermediate (*1a–6a*) and a further oxidative degradation of the quinoid and aldehydic fragments (Scheme 1). The first two steps in Scheme 1 have previously been proposed [4] on the basis of results from the autoxidation of simple *p*-hydroxybenzyl alcohols. These steps have now been confirmed by the isolation of the appropriate fragmentation products from *4*, *viz.* *11* and *14* ($R^1 = CH_2OH$). The first product was characterized as the pyrazoline derivative *13* formed by reaction with diazomethane and the second as



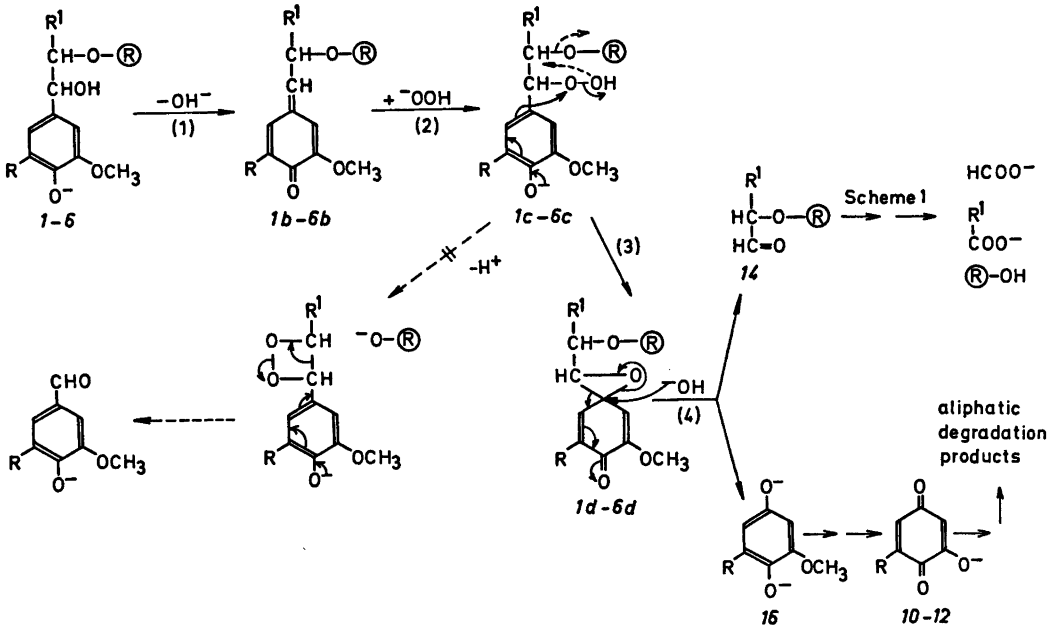
Scheme 1. Pathway A. (1) oxygenations, (2) alkaline rearrangements with fragmentation, (3) alkaline demethoxylation, (4) further oxidative degradation. Compounds *1–6* and *10–12* are depicted as anions.

the dehydration product *15* which arises in a reaction competing with the oxidative degradation of *14*. Further support for the validity of oxidative side chain elimination according to Scheme 1 was supplied by the behaviour of methylated model compounds. Thus, the aromatic methyl ether of *1* and the α-methyl ether of *5* were found to be essentially stable towards treatment under standard conditions. Methylation of the phenolic hydroxyl group in *1* may be expected to block step (1) and methylation of the α-hydroxyl group in *5* should inhibit step (2). If it is assumed that the α-methyl ether of *5*, like the parent compound, is oxygenated, its recovery after the oxidative treatment would indicate that the oxygenation step (1) is reversible.[10]

Step (3) was shown by treating *8* under standard conditions and isolating *11* in high yield. The remarkable stability of *11* is obviously due to the *t*-butyl substituent and to hybridization between the *para*- and *ortho*-quinoid resonance structures of the anion. On prolonged treatment with alkali, the *ortho*-quinoid tautomer of *11* undergoes benzilic acid rearrangement followed by decarboxylation and various oxidation and addition reactions.[11] When R = H or R = OCH_3, the quinoid intermediates (*10* and *12*) could not be isolated. It was shown in a separate experiment that the authentic compound *7*, when exposed to standard conditions, gave among other products a mixture of aliphatic acids having the same qualitative composition as that of the mixture obtained from *2* after treatment under more drastic conditions (Table 1).

These results are in agreement with the assumption that *para*-quinones are intermediates in the oxidative degradation of phenolic nuclei in compounds of the β-aryl ether type (*e.g.* *2*) according to Scheme 1. The reaction mixture from *7* also contained hydrophilic products not extractable with organic solvents. This fraction has not been investigated.

The further oxidative-alkaline degradation of the aldehydic fragments (*14*, R^1 = H or CH_2OH) also includes oxygenation (1) and alkali-induced rearrangements (2) proceeding *via* hydroxy dioxetanes [8,12] (*cf.* also Scheme 4)



Scheme 2. Pathway B. (1) quinone methide formation, (2) addition of hydrogen peroxide, (3) alkaline rearrangement with formation of a spiro epoxide, (4) alkali-promoted fragmentation. Compounds *1* – *6*, *1c* – *6c*, *10* – *12* and *16* are depicted as anions.

or *via* keto hydroperoxides in their hydrated or hemiacetal forms.[8,12] The formation of the appropriate fragmentation products, guaiacol, formic acid and glycolic acid, was demonstrated by autoxidation of *2* and *6*. The facile oxidative-alkaline degradation of α-aroxy aldehydes was confirmed by reacting guaiacoxy acetaldehyde (*14*, $R^1=H$) under standard conditions for 2 h and isolating guaiacol in 66 % yield. Alkaline treatment of *14* ($R^1=H$) in a nitrogen atmosphere under otherwise identical conditions did not afford any detectable amount of guaiacol which excludes purely alkaline degradation. As expected, guaiacoxyacetic acid, which does not enolize, proved stable when exposed to standard autoxidation conditions.

Pathway B. Although pathway A (Scheme 1) satisfactorily explains the formation of the quinoid and aldehydic intermediates, a further route of formation of these fragments, involving hydrogen peroxide as oxidant, must be considered (Scheme 2).

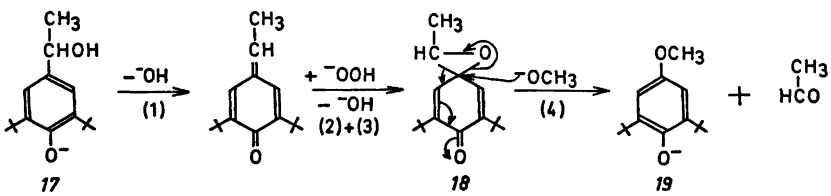
The possibility of the oxidative and alkali-promoted elimination of side chains following pathway B was indicated by the behaviour of *1*-diacetate towards alkaline hydrogen peroxide (Table 2). The sequence was illustrated by treating the *p*-hydroxybenzyl alcohol *17* with the same oxidant at room temperature and isolating the corresponding cyclohexadienone spiro epoxide *18* [steps (1)–(3), Schemes 2 and 3]. The elimination of the side chain from this intermediate by the action of alkali [step (4)] was demonstrated by treating compound *18* with a solution of sodium hydroxide in aqueous methanol at 60 °C. The hydroquinone monomethyl ether *19* thus expected was isolated in high yield. Compound *19* was also obtained when *17* was oxidized with hydrogen peroxide in the same solvent (Table 2).

Instead of rearranging to give spiro epoxides (*1*d–*6*d), the intermediates of the *p*-hydroxybenzyl hydroperoxide type in β-aryl ether structures (*1*c–*6*c, Scheme 2) could also be thought to form dioxetanes by neighbouring group participation with concomitant elimination of the β-aroxy substituent. This reaction would be reminiscent of the cleavage of β-aryl ether bonds involving the formation of oxirane[13] or thiirane [14] intermediates. However, treatment of the diacetate of compound *1* with alkaline hydrogen peroxide in a nitrogen atmosphere yielded a large amount of guaiacol (Table 2) but no vanillin (TLC). In a separate experiment it was shown that vanillin survives a similar oxidative treatment. The absence of vanillin in the reaction mixture from *1*-diacetate is in accordance with steps (3) and (4) and excludes the other fragmentation alternative in Scheme 2.

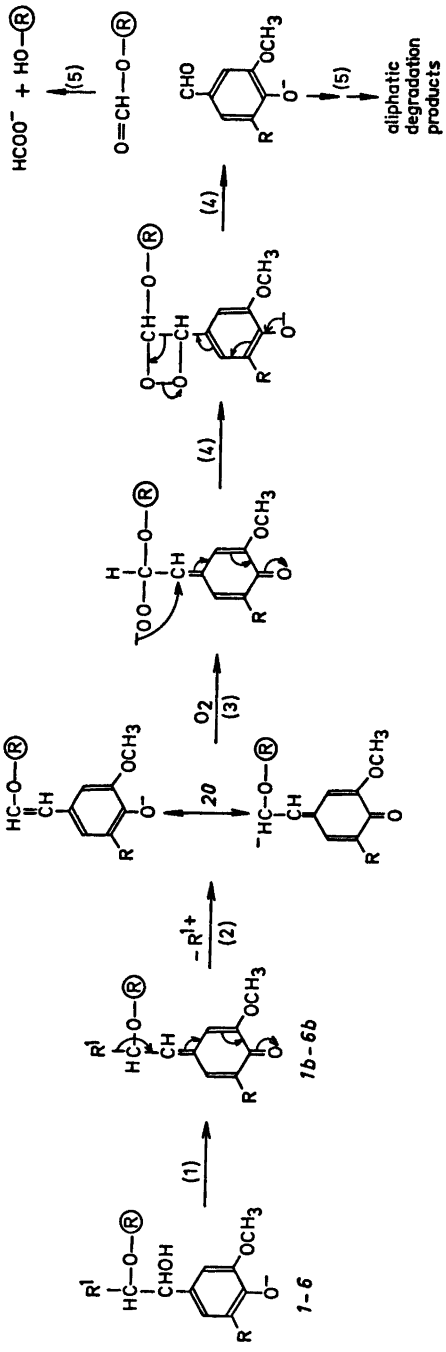
The initial oxidative steps of the degradation following routes A and B are due to oxygen and hydrogen peroxide, respectively. Both routes involve cleavage of the C_α-aryl bond and lead to the same aldehydic and quinoid intermediates. The further degradation of these intermediates may be common for both pathways.

Pathway C, presented in Scheme 4, accounts for the formation of aromatic aldehydes by cleavage of the $C_\alpha-C_\beta$ bond. Like pathway B, this route is initiated by alkaline conversion of the starting compound into the corresponding quinone methide which then gives the corresponding β-aroxystyrene intermediate.[13] In contrast to pathway B, however, the subsequent oxidative degradation is brought about by oxygen rather than by hydrogen peroxide.

The proposed oxidative-alkaline degradation of β-guaiacoxystyrene intermediates (*e.g.* *20*, R=H), formed from quinone methide intermediates (*1*b–*6*b), follows a route analogous to that for the degradation of the aldehydic fragments *14* (Scheme 1). This analogy in the



Scheme 3. (1) quinone methide formation, (2) addition of hydrogen peroxide, (3) formation of a spiro epoxide, (4) opening of the spiro epoxide ring with rearomatization and elimination of the side chain. Compounds *17* and *19* are depicted as anions.



Scheme 4. Pathway C. (1) quinone methide formation, (2) β-aroxystyrene formation, (3) oxygenation, (4) alkaline rearrangement with fragmentation, (5) further alkaline and/or oxidative degradations. Compounds *1 – 6* and *20* are depicted as anions.

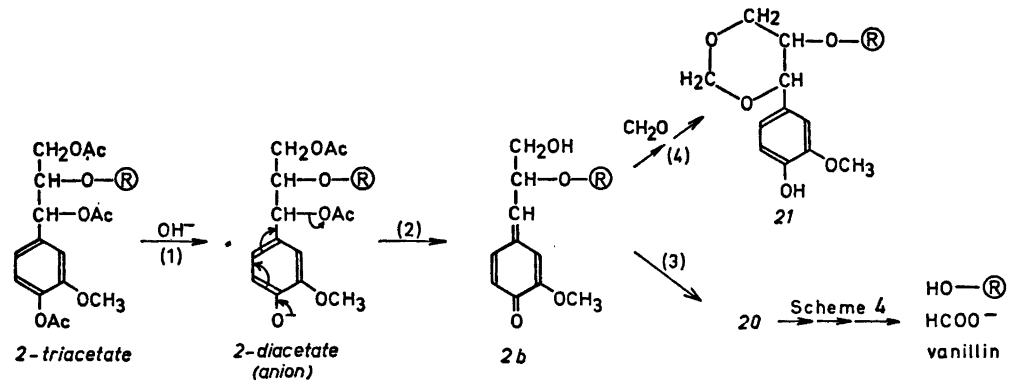
mechanism of degradation may be ascribed to the fact that styrene derivatives *20* in the carbanion form constitute conjugated analogues to the carbanions derived from the aldehydic fragments *14*. As in the case of the aldehydic intermediates (*14*), the fragmentation of β-guaiacoxystyrenes could also proceed *via* addition of hydroxide ions, followed by rearrangement of the resulting α-hydroxy hydroperoxides.

The suggested formation of β-aroxystyrene derivatives prior to the oxidative degradation was supported by treating a mixture of the *trans*- and *cis*-forms of *20* (R = H) (approximate ratio 2:1) under standard autoxidation conditions for 16 h. Guaiacol and vanillin were isolated as main reaction products. The starting material recovered (6 %) was a mixture of the *trans*- and *cis*-forms in an approximate ratio of 1:3.

The more facile degradation of the β-guaiacoxystyrene derivative *20* (R = H) compared to that of the parent β-guaiacyl ethers *1* or *2* shows that the formation of either the quinone methide intermediates [step (1)] or the styrene intermediates [step (2)] is the rate-determining step in the over-all reaction. When *2*-triacetate was treated under standard conditions (Scheme 5), considerable amounts of guaiacol, vanillin and the 1,3-dioxane derivative *21* were formed (Table 1). The extensive reaction may be attributed to the acetoxy group in the α-position which constitutes a better leaving group than the α-hydroxyl group in *2*. In this way, the conversion into *2*b, *i.e.* quinone methide formation [step (2)] is facilitated. Compound *21* arises from *2*b by an alkali-promoted condensation with liberated formaldehyde (4).[15] Reaction (4) competes with reaction (3) (formation of the β-aroxystyrene intermediate *20*, R = H).

Further oxidation of the aromatic aldehydes (*e.g.* vanillin) gives, in addition to formic acid, a mixture of other aliphatic acids which were also obtained after a similar treatment of the corresponding methoxy-*p*-quinones (*e.g.* *7*). This result is consistent with the view that the oxidative-alkaline degradation of aromatic aldehydes proceeds *via* a Dakin type of reaction to give the appropriate *p*-quinones.[4]

Some of the products listed in Table 1 can be considered to indicate the actual degradation route for the side chain of a phenolic aryl-glycerol-β-guaiacyl ether model compound. Thus, glycolic acid and α-guaiacoxyacrolein (*15*) should be generated by pathway A or B



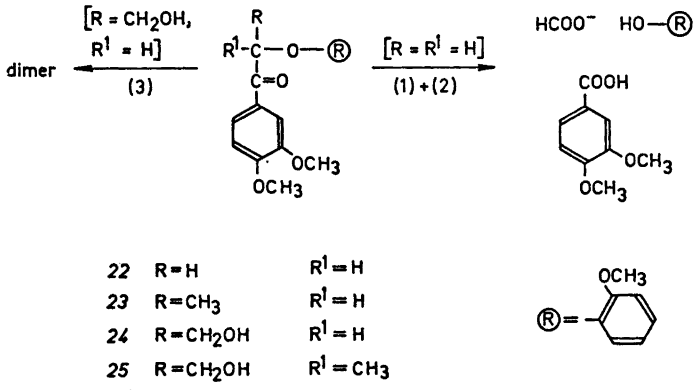
Scheme 5. (1) Alkaline hydrolysis, (2) quinone methide formation, (3) guaiacoxystyrene formation, (4) condensation with formaldehyde liberated in (3).

(Schemes 1 or 2) whereas aromatic aldehydes (*e.g.* vanillin) should only arise through pathway C. *p*-Quinones (*e.g.* 7—9), on the other hand, could be produced both directly (pathways A and B) and *via* aromatic aldehydes (pathway C). However, quantitative estimates of the significance of the different pathways based on the amounts of products isolated cannot be made because of the instability of some of these products under the conditions used. Furthermore, alkali-promoted non-oxidative reactions may contribute to the over-all degradation of phenolic β-aryl ether structures, provided that the formation of quinone methide intermediates is possible.[13,16]

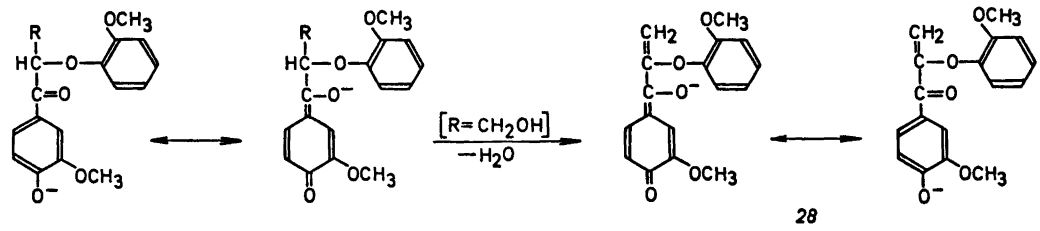
Treatment of phenacylaryl ethers with oxygen in alkaline media

When compound *22* was treated under standard conditions, extensive oxidation took place. Guaiacol and veratric acid were isolated from the reaction mixture in high yields. The methyl derivative of *22* (*23*) was not oxidatively degraded to any appreciable extent and was recovered in high yield.

The hydroxy derivative of *23* (*24*) also resisted oxygenation during the standard treatment but underwent alkali-promoted dimerization.[15] Compound *25* lost one molecule of formaldehyde upon treatment under standard



Scheme 6. Reactions of phenacyl aryl ethers: (1) oxygenation, (2) rearrangement with fragmentation, (3) dimerization.



26 R=H

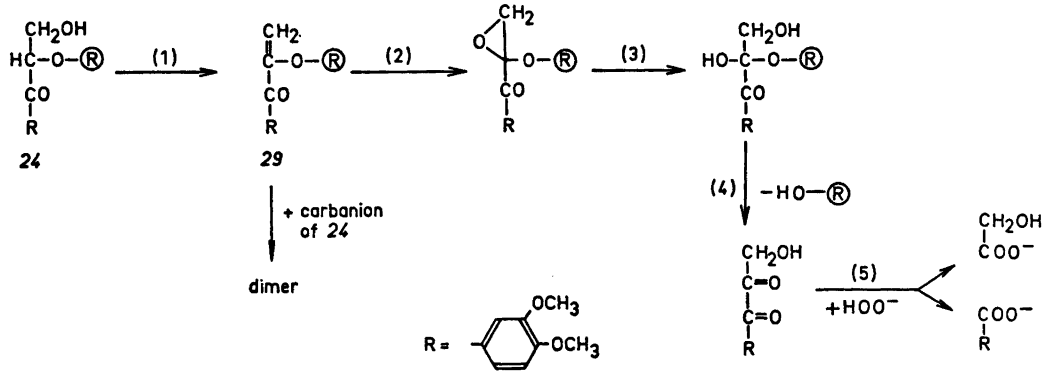
27 R=CH_2OH

Scheme 7. Compounds *26* – *28* are depicted as anions.

conditions and was converted quantitatively into *23* (*cf.* also Ref. 15).

The phenolic counterparts of *22* and *24* (*26* and *27*, respectively) exhibited considerable stability towards oxygen under standard conditions. After a 48 h treatment of *26*, the starting material was thus recovered in high yield. Only small amounts of guaiacol, guaiacoxyacetic acid and vanillic acid were formed. Similar treatment of compound *27* afforded the unsaturated carbonyl compound *28* (*cf.* also formation of *15*, Scheme 1) in a high yield. No detectable amounts of dimerization or oxidation products were found.

The behaviour of compounds *22* – *28* towards oxygen-alkali can be interpreted as follows: *22* is degraded following a route [steps (1) and (2), Scheme 6] analogous to the degradations of the aldehydic fragmentation products (Schemes 1 and 2) and of the aroxystyrene intermediates (Scheme 4). The slight oxygenation of *23* is due to a low degree of enolization while the stability of *25* towards oxygen is a result of its inability to enolize. Compound *24* does enolize but the carbanion formed is consumed by a competitive addition to an enone system [step (3)] arising from *24* by dehydration.[15] The phenolic phenacyl aryl ethers *26* and *27* have a greatly impaired ability to enolize due to the efficient stabilization of their phenolate anions by resonance. The small amounts of guaiacoxyacetic acid and vanillic acid obtained from *26* are indicative of pathway A (or B) and pathway C, respectively. Subsequent to enolization, *27* undergoes dehydration to give *28* rather than oxygenation



Scheme 8. Oxidation of phenacyl aryl ethers by hydrogen peroxide: (1) dehydration, (2) epoxidation by hydrogen peroxide, (3) alkaline opening of the epoxide ring, (4) cleavage of the hemiketal, (5) oxidative cleavage of the 1,2-diketone (cleavage of the original $C_\alpha - C_\beta$ bond).

or dimerization.[15] *28* remains unchanged when exposed to the standard autoxidation conditions.

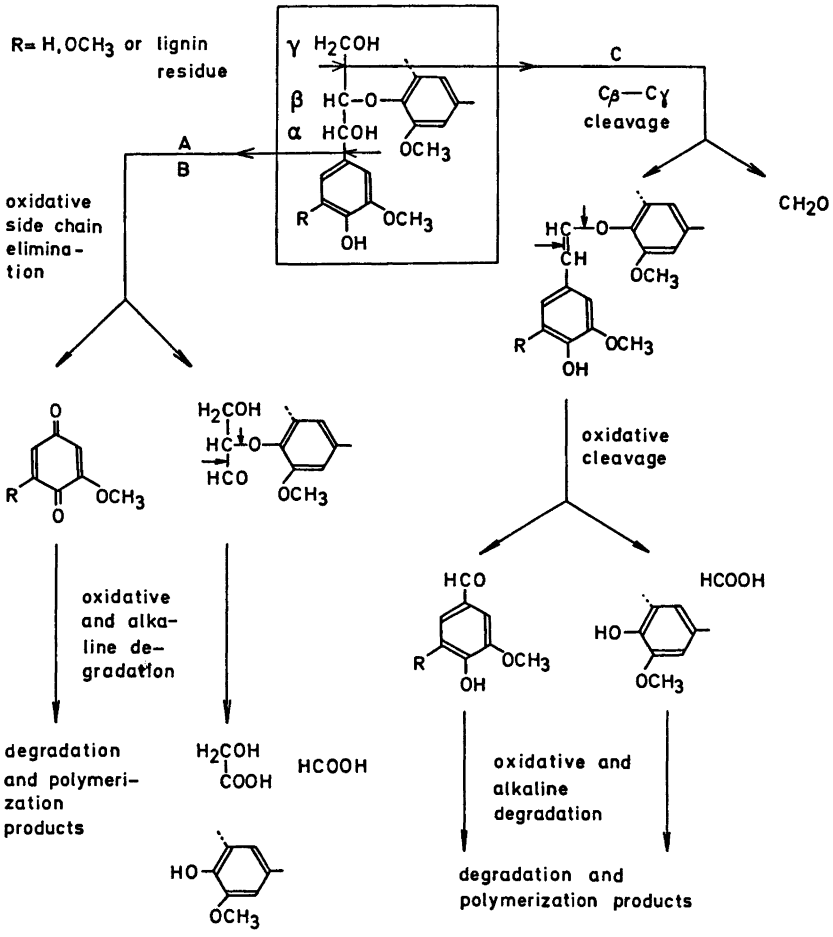
In alkaline solution the unsaturated phenolic keto compound *28* exhibits strong absorption at 370 nm ($\varepsilon = 18.440$ l mol^{-1} cm^{-1}) due to the extended conjugation. This chromophoric system is reminiscent of the chromophore formed from coniferaldehyde in alkaline solutions.[17]

Phenacylaryl ethers, which give enones by an alkali-promoted reaction,[15] are extensively degraded when alkaline hydrogen peroxide is used as oxidant instead of oxygen (Scheme 8). Compound *24* yielded guaiacol and veratric acid in varying yields depending on the amount of oxidant employed. Treatment with an equimolar amount afforded, in addition to guaiacol and veratric acid, the enone *29* and a dimerization product, both of which are formed merely by the action of alkali.[15]

The oxidative fragmentation of *24* by alkaline hydrogen peroxide may be explained by the reaction route outlined in Scheme 8.

CONCLUSIONS

The results listed in Table 1 suggest that *pathway A* is the dominant process under standard conditions. Phenols carrying sub-



Scheme 9. Pathways of oxidative-alkaline degradation of *p*-hydroxy-arylglycerol *β*-aryl ether structures (Summary).

stituents in both the 2 and 6-positions (*e.g.* 3 – 6) react with particular ease probably due to their relatively low acidity. The inverse correlation of oxidizability with acidity seems reasonable since weak acids give relatively unstable carbanions which are more sensitive to the electrophilic attack by oxygen.[18]

Extensive oxidative degradation of mono-*o*-substituted phenols (*e.g.* *1* and *2*) requires more drastic conditions. Under such conditions, quinone methide intermediates may be formed which can initiate also *pathways B and C*. The competition between B and C will be primarly determined by the alkalinity of the solution and the availability of hydrogen peroxide during the oxidative treatment.

All three pathways, outlined in Schemes 1, 2 and 4 and summarized in Scheme 9, lead ultimately to the formation of aliphatic degradation products arising from eliminated side chains and to aliphatic acids and hydrophilic polymerization products arising from intermediates of the *para*-quinone and aromatic aldehyde types. The alkaline-oxidative degradations of β-aryl ether structures also result in the liberation of the β-aroxy substituent as a phenolate ion. The stability of the latter (*e.g.* the guaiacolate ion liberated from *1* – *6*) towards oxygen in alkali under the conditions used shows that the oxidative degradation of phenolic units containing side chain structures of the benzyl alcohol type (*e.g.* *1* – *6*) starts with the oxidative elimination (pathways A and B) or fragmentation (pathway C) of the side chain rather than with the oxidative cleavage of the phenolic nucleus.

Once the aroxy substituent is liberated by one of the routes A – C, the degradation process may be repeated, provided the liberated phenolic unit has the same or a similar type of β-aryl ether structure. The oxidative lignin fragmentation ("oxidative lignin peeling") may thus continue until it reaches a unit containing a side chain which does not meet the structural requirements for at least one of the three pathways.

The degradation of phenacyl-aryl ethers by oxygen-alkali (Scheme 6) exhibits many formal analogies to that of the aldehydic fragments (Scheme 1) and the β-quaiacoxystyrene intermediates (Scheme 4). The degradation is restricted to structures having an easily enolizable keto group and lacking the ability to form an enone system for conjugate addition.[15]

The oxidative-alkaline degradation processes are accompanied and, in part, counteracted by oxidative coupling reactions[3–8] and alkali-promoted conjugated additions (condensations and polymerizations).[15]

EXPERIMENTAL

Materials

Syntheses of model compounds. The following compounds were prepared as previously described: *1*,[13] *2*,[19] *5* (analogous to *1*), *7*,[20] *8*,[21] *9*,[22] *14* (R^1 = H),[23] *17*,[24] *20* (R = H, *cis* and *trans*),[15] *22*,[25] *23*,[26] *24*,[25] *25*,[27] *26* (analogous to *27*), *27*.[15]

The β-aryl ethers *3* – *6* were prepared from the appropriate acetophenones as described for *1* and *2*.

All analyses agree within ±0.3 % units with the calculated values.

4-Acetoxy-5-t-butyl-3-methoxyacetophenone. 6-*t*-Butylguaiacol[28] (50 g) dissolved in acetyl chloride (100 ml) was added to a cooled solution (10 °C) of aluminium chloride (50 g) in acetyl chloride (200 ml). The temperature of the reaction mixture was kept below 15 °C. After the addition had been completed, the reaction mixture was stirred for 30 min, treated with cooled 0.5 M hydrochloric acid and extracted with chloroform. The excess of 6-*t*-butylguaiacol acetate was distilled off (142 – 146 °C/19 mmHg). Crystallization of the distillation residue from light petroleum yielded the pure compound (17 %), m.p. 83 – 85 °C. (Found: C 68.47; H 7.32; O 24.19. $C_{15}H_{20}O_4$ requires: C 68.18; H 7.58; O 24.24).

3,5-Dimethoxy-4-hydroxyacetophenone. This compound was prepared using a modification of a method previously[29] described. The acetate of 1,3-dimethoxyphenol (39 g) was treated with aluminium chloride (32 g) in nitrobenzene (200 ml) at 50 °C for 1 h. After addition of cooled 0.5 M hydrochloric acid, the reaction mixture was extracted with 2 M sodium hydroxide. The aqueous layer was acidified and extracted with methylene chloride. Evaporation of this extract and crystallization of the residue from ethanol gave the pure compound (20 %), m.p. 123 – 126 °C (lit. 110 – 120 °C).

Analytical data of β-aryl ethers.

1-(4-Acetoxy-5-t-butyl-3-methoxyphenyl)-2-O-(2-methoxyphenyl)ethyleneglycol (3-acetate), m.p. 97 – 99 °C (ligroin). (Found: C 68.03; H 6.91; O 24.99. $C_{22}H_{28}O_6$ requires: C 68.02; H 7.26; O 24.71).

1-(4-Acetoxy-5-t-butyl-3-methoxyphenyl)-2-O-(2-methoxyphenyl)-1,3-O-diacetylglycerol (4-triacetate), amorphous. The ^1H NMR spectrum

Table 2. Oxidation of model compounds with alkaline hydrogen peroxide. Alkalinity: 0.2 – 0.3 M NaOH, nitrogen atmosphere. Temperature: 40 °C.

Compound	Solvent	Reaction time, h	Equiv. of H_2O_2	Reaction products isolated and/or identified (yield % of theoretical)	Starting material, %
1-Diacetate	d-w (2:3)	1	1	G (60)	0
Vanillin	d-w (2:1)	1	1.5		100
17	d-w (2:1)	0.5	1.5	*18* (25)	
17	m-w (3:1)	2.5	1.2	*19* (26)	
24	e-w (3:1)	2.5	1	*29* (19) G (35), Ver A (31), Dimeric product (12)[15]	0
24	e-w (3:1)	24	3	G (88), Ver A (80)	0
27	e-w (3:1)	1	1		90

d-w dioxane-water, m-w methanol-water, e-w ethanol-water, G = guaiacol, VerA = veratric acid.

indicated a 1:1 mixture of the *erythro* and *threo*-forms. (Found: 64.60; H 7.00; O 28.40. $C_{27}H_{34}O_9$ requires: C 64.52; H 6.81; O 28.66).

1-(3,5-Dimethoxy-4-hydroxyphenyl)-2-O-(2-methoxyphenyl)-glycerol (6), m.p. 162 – 164 °C (chloroform). The ^{1}H NMR spectrum of the synthetic compound indicated the *threo*-configuration. The coupling constant of the α and β-protons in the side chain was $J_{\alpha\beta} = 7.0$ cps.[30] Anal. $C_{18}H_{22}O_7$: C, H, O.

Methods

Autoxidation of model compounds. The reaction conditions and results are given in Table 1. Autoxidation with oxygen under pressure was carried out in a 1 l stainless steel autoclave. The yields of the low molecular weight acids were determined by an ion-pair benzylation or methylation[31] technique in combination with GC analysis. The work-up procedure and preparative separation of the reaction mixtures were carried out as previously described.[8]

Oxidation of model compounds with alkaline hydrogen peroxide. The reaction conditions and results are given in Table 2.

Treatment of guaiacoxyacetaldehyde with alkali. Guaiacoxyacetaldehyde (*14*, $R^1 = H$) (2 mmol) was treated with a 0.3 M sodium hydroxide solution in ethanol-water (4:1) at 40 °C for 2 h (nitrogen atmosphere). No guaiacol could be detected in the reaction mixture (TLC).

Treatment of spiro epoxide 18 *with alkali.* Compound *18* (0.18 mmol) was treated with a 0.2 M sodium hydroxide solution in methanol-water (4:1) (10 ml) at 60 °C for 6 h (nitrogen atmosphere). 2,6-Di-*t*-butyl-4-methoxyphenol (*19*) was isolated in 91 % yield.

Product identification

6-t-Butyl-2-hydroxy-p-quinone (11), yellow crystals from ethanol, m.p. 135 – 138 °C (Lit.[11] 138 – 139.5 °C).

^{1}H NMR (60 MHz, $(CD_3)_2SO$): 1.24 (s, 9 H, *t*-Bu), 5.84 (d, 1 H, ol, *J* 2.7 Hz), 6.37 (d, 1 H, ol, *J* 2.7 Hz).

7a-t-Butyl-6-methoxy-3a,7a-dihydro-3H-indazolquinone (4,7) (13), pale yellow crystals from ether. Decomposes between 155 and 162 °C. Anal. $C_{12}H_{16}O_3N_2$: C, H, O, N. IR (KBr): 1690 (s, C=O), 1650 (s, C=O), 1605 (s, C=C–C=C). UV [MeOH (ε)]: 268 (9150). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.10 (s, 9 H, *t*-Bu), 3.80 (s, 3 H, OMe), 5.03 (A) and 4.39 (B) [CH_2], 3.06 (X) [CH]; ABX; J_{AB} 18.3; J_{AX} 11.1; J_{BX} 7.5; 5.88 (s,1 H, ol).

α-(2-Methoxyphenoxy)-acrolein (15). GC-MS analysis (fragments having $m/e > 100$ and intensities > 10 % of base peak are given: 178 (M, 100), 149 (M – 29, 14), 135 (M – 43, 13), 134 (M – 44, 12), 123 (M – 55, 30), 121 (M – 57, 25), 109 (35), 108 (50). ^{1}H NMR (60 MHz, $CDCl_3$) of the crude fraction (90 % *15*) obtained by autoxidation of *6* and ether extraction of the alkaline reaction mixture: δ 3.79 (s, 3 H, OMe), 5.02 (d, 1 H, ol, J_{gem} 2.7 Hz), 5.22 (s, 3 H, ol, J_{gem} 2.7 Hz), 7.00 (m, 3 H, ar), 9.43 (s, 1 H, CHO).

5,7-Di-t-butyl-2-methyl-1-oxaspiro[2,5]octa-4,7-diene-6-one (18), white crystals, m.p. 95 – 97 °C (ether). Anal. $C_{16}H_{24}O_2$: C, H, O. UV [MeOH (ε)]: 258 (15 200). ^{1}H NMR (60 MHz, $CDCl_3$): 1.23 (s, 18 H, 2 × *t*-Bu), 1.47 (d, 3 H, CH_3, J_{CH,CH_3} 5.3 Hz), 3.44 (*q*, 1 H, CH, J_{CH,CH_3} 5.3 Hz), 6.08 (d, 1 H, ol, $J_{ol,ol}$ 2.7 Hz), 6.33 (d, 1 H, ol, $J_{ol,ol}$ 2.7 Hz).

2,6-Di-t-butyl-4-methoxyphenol (19), white crystals, m.p. 102 – 104 °C (light petroleum) (lit.[32] 105 – 106 °C). GC-MS analysis (fragments having $m/e > 100$ and relative intensities > 10 % of base peak are given): 236 (M, 32), 222 (M – 14, 15), 221 (M – 15, 100), 220 (M – 16, 10), 205 (M – 31, 10), 193 (M – 43, 10), 179 (M – 57, 17), 177 (20), 165 (10), 163 (10), 161 (11), 151 (10), 149 (13), 135 (10). ^{1}H NMR (60 MHz, $CDCl_3$): 1.40 (s, 18 H, 2 × *t*-Bu), 3.73 (s, 3 H, OMe), 4.70 (s, 1 H, OH), 6.74 (s, 2 H, ar).

Compounds *21* and *28* were identified as previously [15] described.

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Tobacco Chemistry. 34. (3*E*,6*E*)-2,6-Dimethyl-10-oxo-3,6-undecadien-2-ol and (2*E*)-3-Methyl-4-oxo-2-nonen-8-ol. Two New Constituents of Greek *Nicotiana tabacum* L.

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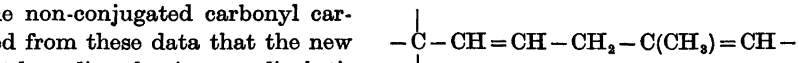
Two new compounds were isolated from Greek *Nicotiana tabacum* L. and shown to be (3*E*,6*E*)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol and (2*E*)-3-methyl-4-oxo-2-nonen-8-ol by spectroscopic methods.

Previous studies have disclosed that tobacco contains numerous volatile, and frequently odoriferous constituents, which in all probability arise by oxidative degradation of carotenoids, diterpenoids and other isoprenoids.[1] The present communication describes the isolation and structure elucidation of two new volatile compounds from sun-cured Greek tobacco. One of these (*1*) clearly belongs to the group of nor-compounds derived from acyclic isoprenoids, while the biogenetic origin of the other compound (*2*) is uncertain.

RESULTS

Accurate mass measurements of the $[M-18]^+$ ion established that the first compound (*1*) had the composition $C_{13}H_{22}O_2$. Its ^{13}C NMR spectrum confirmed the presence of thirteen carbon atoms and revealed that these comprise four methyl, three sp^3 methylene, one non-protonated, oxygen-carrying sp^3 carbon, three sp^2 methine, one non-protonated sp^2 carbon and one non-conjugated carbonyl carbon. It followed from these data that the new compound must be a diene having one disubstituted and one trisubstituted double bond, a conclusion supported by the fact that the ^1H NMR spectrum exhibited signals due to three olefinic protons. Addition of $Eu(dpm)_3$ made these amenable to decoupling experiments. Thus, irradiation at the frequency of a broadened triplet, which had been shifted from δ 5.11 to 5.24, sharpened a broadened methyl singlet at δ 1.69 and a broadened two-proton doublet at δ 2.82. This indicated that the olefinic proton is allylically coupled to a methyl and a methylene group, which are attached to the same olefinic carbon. The olefinic proton, giving rise to the broadened triplet ($J = 6.5$ Hz), must be vicinal to one of the sp^3 methylene groups.

The protons of the disubstituted double bond gave rise to overlapping signals at δ 5.6, which were resolved into the AB part of an ABX_2 system, δ 6.10 (A) and 6.26 (B), on addition of $Eu(dpm)_3$. Irradiation at δ 6.10 converted the broadened two-proton doublet at δ 2.82 into a broadened singlet and, conversely, the ABX_2 system changed into an AB system when the frequency of the two-proton doublet was irradiated. This demonstrated that the carbon atoms of the disubstituted double bond are adjacent to the non-protonated carbon atom and a methylene group, respectively. Since the latter is also allylic to the trisubstituted double bond it can be concluded that the diene (*1*) incorporates the partial structure



Since the IR spectrum exhibited absorption at 3600 and 3550 – 3200 cm^{-1}, it was evident that the non-protonated carbon carries a hy-

droxyl group (70.66 ppm). It also followed that the two methyl groups, giving rise to a six-proton singlet at δ 1.32 in the ^{1}H NMR spectrum, are attached to this carbon. Lanthanide induced shift (LIS) experiments, which demonstrated that these two methyl singlets and the signal due to one of the olefinic protons of the disubstituted double bond exhibited the largest relative shifts, 100 and 97, respectively, are in agreement with this formulation. Moreover, the largest LIS in the ^{13}C NMR spectrum of the diene *1* was observed for the signal due to the hydroxylated carbon (100) and the relative shifts of the two methyl carbon resonances were of the same magnitude as that of the sp^2 carbon signal at 139.54 ppm, 20 and 19, respectively.

The methyl ketone group, IR band at 1710 cm^{-1}, methyl singlet at δ 2.14, must be attached to the methylene group, which is adjacent to the tri-substituted double bond, *via* the remaining sp^3 methylene group, a conclusion supported by the fact that 4-oxopentanal was obtained on ozonolysis of the diene (*1*). The new tobacco constituent can therefore be formulated as 2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (*1*).

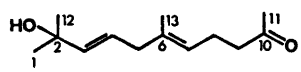
The configurations of the two double bonds in (*1*) were established as *E* on NMR evidence, *i.e.* the coupling constant between the olefinic protons of the disubstituted double bond was 16 Hz and the ^{13}C chemical shift value of the methyl group attached to the tri-substituted double bond was 16.02 ppm, which is diagnostic of *E*-configuration.[2,3]

According to the ^{13}C NMR data the second compound (*2*), $C_{10}H_{18}O_2$, incorporated three methyl, three sp^3 methylene, one oxygenated sp^3 methine, one sp^2 methine, one non-protonated sp^2 carbon and one carbonyl carbon. Since the IR spectrum showed absorption at 1660 cm^{-1} and the UV spectrum had maximum at 225 nm (ε = 9600) it was evident that the tri-substituted double bond in *2* is conjugated with the carbonyl group. As judged from the ^{1}H NMR spectrum, which contained a broadened three-proton singlet at δ 1.77 and a broadened three-proton doublet at δ 1.85, the two remaining substituents are methyl groups, aligned as shown in the partial structure

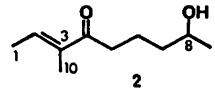
$$CH_3-CH=C(CH_3)-\overset{\overset{O}{\|}}{C}-$$

This was corroborated by decoupling experiments. Thus, the doublet at δ 1.85 collapsed into a singlet on irradiation at the frequency of an olefinic quartet. Conversely, irradiation at the frequency of the methyl doublet converted the quartet to a singlet. The presence of the proposed partial structure in compound *2* was further supported by the ^{13}C NMR spectrum. The signals due to the sp^2 methine and the sp^2 non-protonated carbon atoms appeared at 137.3 and 138.3 ppm, respectively, in the spectrum of the enone (*2*), which is close to the chemical shift values reported for the corresponding signals for 3-methyl-3-penten-2-one, 138.5 and 138.0 ppm.[4] Furthermore, the ^{13}C shieldings of the methyl groups attached to the double bond (11.0 and 14.8 ppm) are close to those published for the corresponding groups in the tigloyl residue *3*, (11.9 and 14.3 ppm),[5] but differ from those of the methyl groups in the angeloyl residue *4* (15.9 and 20.5 ppm),[6] which indicated that enone *2* has 2*E* configuration. This view was supported by the fact that the olefinic proton signal was present at δ 6.76 and 6.85 in the ^{1}H NMR spectra of enone *2* and the tigloyl residue *3*,[5] respectively, but at δ 6.20 for the angeloyl residue *4*.[6]

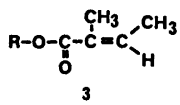
The remaining oxygen atom in the enone *2* was accommodated by a hydroxyl substituent



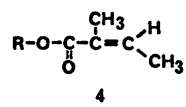
1



2



3



4

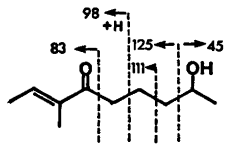


Fig. 1. MS cleavage of *2*.

attached to the sp^3 methine carbon atom as shown by IR absorption at 3600 – 3100 cm^{-1} and the presence of a one-proton sextet at δ 3.77 in the ^1H NMR spectrum. Irradiation at this frequency converted a methyl doublet at δ 1.18 to a singlet. Conversely, the sextet collapsed to a triplet on irradiation at the frequency of the methyl doublet, demonstrating that the hydroxyl-bearing carbon is linked to a methyl and a methylene group. The latter must be attached to the enone moiety *via* the remaining two sp^3 methylene groups, which established that the new tobacco constituent (*2*) is 3-methyl-4-oxo-2-nonen-8-ol. Additional evidence for this formulation was obtained by the examination of the mass spectrum of *2*, which contained diagnostically important peaks at *m/e* 170 (M), 125, 111, 98, 83 and 45 corresponding to species arising from the cleavage reactions outlined in Fig. 1.

The absolute configuration of the new tobacco isolate (*2*) could not be settled due to its high instability.

EXPERIMENTAL

Optical rotations were measured on a Perkin-Elmer 141 polarimeter, IR spectra on Digilab FTS-14 and Perkin-Elmer 257 instruments and UV spectra on a Varian Techtron 635 UV-visible spectrophotometer. Mass spectra were recorded on an LKB 2091 instrument and accurate mass measurements were carried out on a Varian MAT 311 instrument at the Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm.

Fourier transform ^1H NMR (100 Mz) and ^{13}C NMR (25.16 MHz) spectra were obtained in $CDCl_3$ solutions using TMS as internal standard and on a Varian XL-100-12 spectrometer, which was equipped with an S-124 FT and disk accessories and controlled by a Varian 620/L computer. Gas chromatography was performed on a Varian 1700 instrument using glass capillary columns (50 m × 0.37 mm) coated with HB 5100. High performance liquid chromatography was carried out using a Waters 6000 A solvent delivery system, a U6K injector and an R-401 differential refractometer.

Isolation of (3E,6E)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (1) and (2E)-3-methyl-4-oxo-2-nonen-8-ol (2). A volatile neutral fraction (B8) [7] of an extract obtained from 295 kg of sun-cured Greek *Nicotiana tabacum* L. was chromatographed over silica gel using a light petroleum/diethyl ether gradient. Two of the subfractions obtained were separated further by liquid chromatography using columns packed with Bondapak C18/Porasil (Waters) and μ-Porasil (Waters) to afford 32 mg of *1* and 16 mg of *2* as colourless oils.

(3E,6E)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (1) (Found: $[M-H_2O]^+$ 192.1500. Calculated for $C_{13}H_{20}O$: 192.1514) had IR bands at 3600 (m), 3550 – 3200 (m), and 1710 (s) cm^{-1}; ^1H NMR peaks at δ 1.32 (6 H, s), 1.60 (3 H, broad s), 2.14 (3 H, s), 2.18 – 2.58 (4 H, overlapping signals), 2.66 (2 H, broad d, J 6 Hz), 5.11 (1 H, broad t, J 6.5 Hz) and 5.53 – 5.64 (2 H, overlapping signals). On addition of $Eu(dpm)_3$ ^1H NMR peaks at δ 1.69 (3 H, broad s), 1.99 (6 H, s), 2.22 (3 H, s), 2.25 – 2.70 (4 H), 2.82 (2 H, broad d, J 6 Hz), 5.24 (1 H, broad t, J 6.5 Hz), 6.10 (1 H, dt, J_1 6 Hz, J_2 16 Hz), and 6.26 (1 H, d, J 16 Hz) (AB part of an ABX_2 system). ^{13}C NMR peaks and assignments: C-1 29.85; C-2 70.66; C-3 139.54; C-4 125.01; C-5 42.31; C-6 135.17; C-7 123.49; C-8 22.52; C-9 43.65; C-10 208.80; C-11 29.92; C-12 29.85; C-13 16.02 ppm. The relative shifts of the ^{13}C NMR signals on addition of $Yb(dpm)_3$ were: C-1 and C-12 20; C-2 100; C-3 19; C-4 11; C-5 5; C-6 3; C-7 3; C-8 3; C-9 3; C-10 6; C-11 3; C-13 2. The measurements were made within the linear LIS range (shift reagent/substrate ratio 0 – 0.5 and were normalized by arbitrarily assigning the value 100 to the carbon signal exhibiting the largest shift). MS peaks at *m/e* (composition, %): 210 (M, 1), 192 ($C_{13}H_{20}O$, 6), 177 ($C_{12}H_{17}O$, 3), 159 ($C_{12}H_{15}$, 4), 149 (6), 134 ($C_{10}H_{14}$, 44), 119 (C_9H_{11}, 28), 109 (C_7H_9O and C_8H_{13}, 18), 107 (C_8H_{11}, 20), 95 (C_7H_{11} and C_6H_7O, 22), 93 (C_7H_9, 39), 85 (18), 81 (16), 79 (18), 69 (C_5H_9, 19), 59 (18) and 43 (100).

Ozonolysis of (3E,6E)-2,6-dimethyl-10-oxo-3,6-undecadien-2-ol (1). The diene (*1*, 5 mg), dissolved in CH_2Cl_2 (15 ml) and pyridine (0.1 ml) was treated with excess ozone at – 60 °C for 15 min. Zinc powder (0.1 g) and acetic acid (0.5 ml) were added and the mixture was stirred for 1 h during which the temperature was slowly raised to 20 °C. The reaction mixture was filtered and the solution washed with aqueous $NaHCO_3$ and water. Evaporation of the solvent afforded 4-oxopentanal, which was identical to an authentic sample (GC_{RT} and GC-MS).

(2E)-3-Methyl-4-oxo-2-nonen-8-ol (2) (Found: M^+ 170.1278. Calc. for $C_{10}H_{18}O_2$: 170.1306) had IR bands at 3600 – 3100 (m) and 1660 (s) cm^{-1}; UV maximum at 225 nm

($\varepsilon = 9600$); ^{1}H NMR peaks at δ 1.18 (3 H, d, J 6 Hz), 1.77 (3 H, broad s), 1.85 (3 H, dd, J_1 7, J_2 1 Hz), 2.68 (3 H, t, J 7 Hz), 3.77 (1 H, sextet, J 6 Hz) and 6.76 (1 H, broad q, J 7 Hz); ^{13}C NMR peaks and assignments: C-1 11.03; C-2 137.29; C-3 138.26; C-4 202.03; C-5 36.89; C-6 20.87; C-7 38.87; C-8 67.48; C-9 23.41; C-10 14.76 ppm. MS peaks at *m/e* (composition, %): 170 (M, $C_{10}H_{18}O_2$, 2), 152 ($C_{10}H_{16}O$, 13), 137 ($C_9H_{13}O$, 12), 125 (2), 111 ($C_7H_{11}O$, 9), 98 ($C_6H_{10}O$, 7), 83 (C_5H_7O, 100), 69 (5), 55 (60) and 45 (8).

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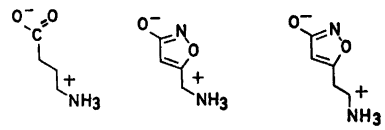
Acta Chemica Scandinavica B 31 (1977) 577—583

Muscimol Analogues. I. Syntheses of 4- and 8-Aminocyclohepteno[1,2-*d*]isoxazol-3-ols and 4-(3-Aminopropyl)-5-methyl-3-isoxazolol

POVL KROGSGAARD-LARSEN, LILIANA NATOVA * and SØREN BRØGGER CHRISTENSEN

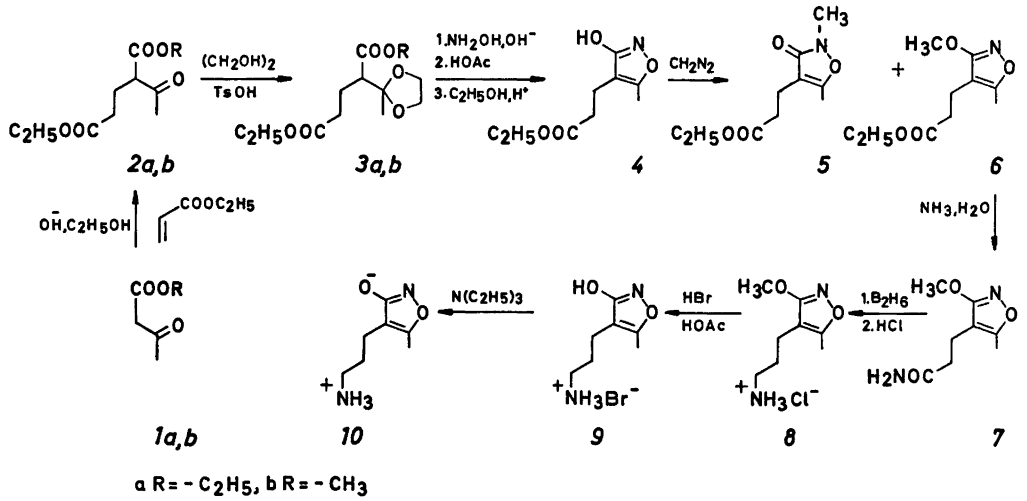
Royal Danish School of Pharmacy, Department of Chemistry BC, DK-2100 Copenhagen Ø, Denmark

The syntheses of the zwitterions 4-(3-aminopropyl)-5-methyl-3-isoxazolol (*10*) and 4- and 8-aminocyclohepteno[1,2-*d*]isoxazol-3-ols (*23*) and (*19*), which are structural analogues of muscimol (5-aminomethyl-3-isoxazolol) and γ-aminobutyric acid (GABA), are described. The 3-isoxazolol zwitterion *10* was synthesized *via* ethyl 3-(3-hydroxy-5-methylisoxazol-4-yl)-propionate (*4*) by using 1-methyl 5-ethyl 2-acetylglutarate (*2b*) as a starting compound. The zwitterions *19* and *23* were prepared *via* 3-methoxycyclohepteno[1,2-*d*]isoxazol-8-one (*15*) and 3-methoxycyclohepteno[1,2-*d*]isoxazol-4-one (*16*), respectively. *15* and *16* were prepared by chromic acid oxidation of 3-methoxycyclohepteno[1,2-*d*]isoxazole (*13*). The pK_A values of *10*, *19*, and *23* have been determined.



Scheme 1.

Muscimol [1,2] and related 5-aminoalkyl-3-isoxazolol zwitterions, including homomuscimol (Scheme 1), are potent γ-aminobutyric acid (GABA) agonists.[3] The development of conformationally restrained GABA analogues related to muscimol is part of an investigation of the relationship between structure, molecular



a R = - C_2H_5, b R = - CH_3

Scheme 2.

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flexibility, and biological activity of GABA agonists. This paper presents the synthesis of *10*, *19*, and *23*.

The key step in the reaction sequence for the preparation of *10* is formation of the 3-isoxazolol derivative *4* from *3a,b* (Scheme 2). A crude product formed by reaction of *3a* [4] with hydroxylamine, was treated with an ethanolic solution of hydrogen chloride to give *4* in a low yield. Moderate yields of *4* were obtained by using *3b* instead of *3a* as starting material. Treatment of *4* with diazomethane gave almost equal amounts of the *N*- and *O*-methylated products *5* and *6* in agreement with general findings.[5] The primary amine obtained by diborane reduction of *7*, prepared from *6*, was isolated as the hydrochloride *8* and deprotected by hydrogen bromide in glacial acetic acid.

The structure determinations of the new compounds *2b*, *3b*, and *4*–*10* are based mainly on ^{1}H NMR and IR spectroscopic methods and elemental analyses. For the compounds *4*–*10* this was supported by UV spectroscopy. The ^{1}H NMR and IR data obtained from the 4,5-disubstituted 3-oxygenated isoxazole moieties of *4* and *6*–*9* and from the 3-isoxazolone moiety of *5* are in accordance with general findings.[5] The spectroscopic data and protolytic properties of *10* are in agreement with those published for other 3-isoxazolol zwitterions.[6–10]

The reaction sequences for the preparations of *19* and *23* are outlined in Scheme 3. Cyclohepteno[1,2-*d*]isoxazol-3-ol (*11*) was prepared according to a described method,[11] but a considerably higher yield was obtained by using a different isolation procedure.

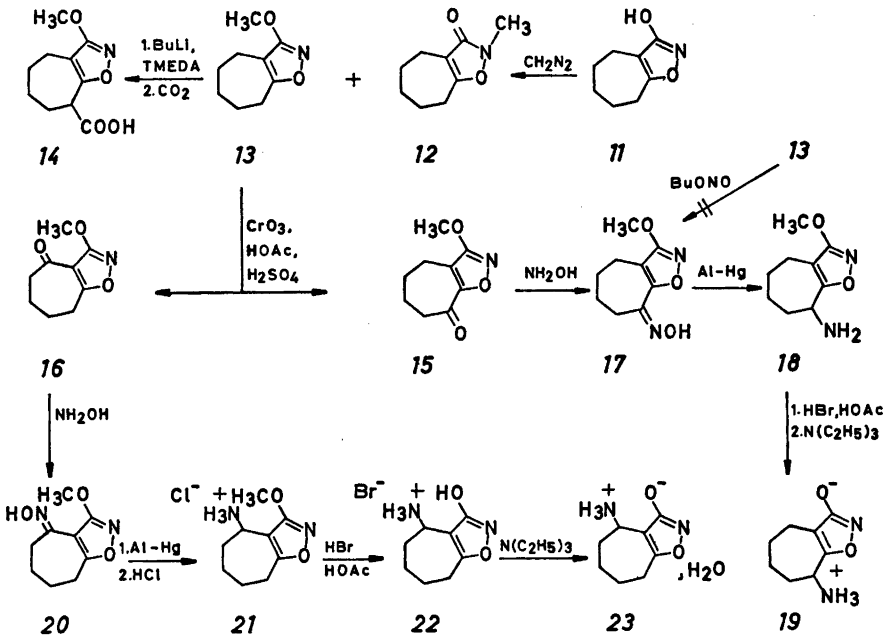
Attempts were made to synthesize *19* *via* the carboxylic acid *14*. Compound *13* was treated with butyllithium and carbon dioxide in analogy with the preparation of 3-methoxy-4-methylisoxazol-5-ylacetic acid from 3-methoxy-4,5-dimethylisoxazole.[12] Only the starting material (*13*) could be isolated from the reaction mixture. Lithiation of *13* in the presence of 1,2-bis(dimethylamino)ethane (TMEDA) and subsequent carboxylation gave *14* in a very low yield, and the planned reaction sequence from *14* to *19* involving a Curtius rearrangement was considered inapplicable.

Chromic acid oxidation of *13* gave the ketones *15* and *16* in yields of 15 and 44 %, respectively. Despite the consumption of excess of chromic acid during the oxidation process, starting material was always isolated from the reaction mixtures indicating that further oxidation of one or both of the ketones *15* and *16* must take place.

An attempt to synthesize *17* directly from *13* by oximation with butyl nitrite using a method analogous to that earlier described [13,14] was unsuccessful. The primary amine *18*, prepared by aluminium amalgam reduction of *17*, was demethylated, and the crude reaction product was treated with triethylamine to give the zwitterion *19*. A similar reaction sequence was utilized for the synthesis of *23* from the oxime *20*.

The structures of the new compounds *12*–*23* were established spectroscopically and by elemental analyses. The identities of the 3-isoxazolone moiety of *12* and the 3-oxygenated isoxazole moieties of *13*–*18* and *20*–*22* were established as mentioned above for *4*–*9*. By comparing the ^{1}H NMR spectra of *11*, which has not been described in connection with the previous preparations of this compound,[11,15] and *13* with the published ^{1}H NMR data of 4,5-dimethyl-3-isoxazolol [5] the signals at δ *ca.* 2.3 in the spectra of *11* and *13* are assigned to the protons in the positions 4 and the signals at δ *ca.* 2.7 to the protons attached to C-8. Based on these assignments the ^{1}H NMR data of *14* confirm the position of the carboxyl group as depicted in Scheme 3.

The structure determinations of the ketones *15* and *16* and the oximes *17* and *20* are based mainly on ^{1}H NMR and UV spectroscopy. In the ^{1}H NMR spectrum of *16* the absence of a signal at δ 2.3 indicates that no protons are attached to C-4. Correspondingly the ^{1}H NMR spectrum of *15* indicates that no protons are attached to C-8 in this compound. The relative positions of the UV absorption maxima of *15* and *16* are in agreement with those of the related monocyclic compounds 3-methoxy-5-acetylisoxazole (at 238 nm) [9] and 3-methoxy-4-acetyl-5-methylisoxazole [12] (at 233 nm). The ^{1}H NMR data of the oximes *17* and *20* are similar to those of the parent ketones *15* and *16*, respectively. The considerable difference between the wavelengths of the UV absorption maxima of *17* and *20* is similar to the difference between those of the related mono-



Scheme 3.

cyclic oximes 3-methoxy-5-(1-hydroxyiminoethyl)isoxazole (248 and 250 nm for the *Z*- and *E*-forms)[16] and 3-methoxy-4-(1-hydroxyiminoethyl)-5-methylisoxazole[12] (214 nm). The structures of the zwitterions *19* and *23* were established by spectroscopic methods as mentioned above for *10*.

The pK_A values of *10* (5.32 ± 0.01 and 10.6 ± 0.1) are significantly different from those of its structural analogue homomuscimol (5.12 ± 0.03 and 9.46 ± 0.02).[7] The pK_A values of *19* (4.51 ± 0.06 and 8.30 ± 0.05) are comparable with those of the structurally related compound muscimol (4.78 and 8.43).[17]

EXPERIMENTAL

Unless otherwise stated the determination of melting points, the recording of IR, UV, and ^1H NMR spectra, and the performance of microanalyses were accomplished as described in a previous paper.[18] Unless otherwise stated thin layer chromatography (TLC) and CC was accomplished by using silica gel F_{254} plates (Merck) and silica gel, 0.05 – 0.200 mm (Merck), respectively, and columns were developed by stepwise gradient elution. Compounds containing a 3-isoxazolol nucleus were visualized on TLC plates by using an iron(III) chloride spraying reagent (yellow colour) and ammonium derivatives and the amine *18* by using a ninhydrin spraying reagent. The pK_A values were determined as described in a previous paper.[7]

1-Methyl 5-ethyl 2-acetylglutarate (2b). *2b* was synthesized from 50.0 g (0.43 mol) of *1b* and 43.0 g (0.43 mol) of ethyl acrylate by using a method analogous to that described[19] for the preparation of *2a*. *2b* (60.2 g; 65 %) was obtained as a colourless oil, b.p. 127.0 – 127.5 °C/0.50 kPa. Anal. $C_{10}H_{16}O_5$: C, H. IR (film): 2980 (m), 2955 (m), 1730 (s), 1645 (w) cm^{-1}. ^1H NMR (CCl_4): δ 4.03 (2 H, q, J 7 Hz), 3.7 – 3.3 (m) and 3.68 (s) (a total of 4 H), 2.5 – 1.7 (m) and 2.18 (s) (a total of 7 H), 1.23 (3 H, t, J 7 Hz).

1-Methyl 5-ethyl 2-acetylglutarate ethylene acetal (3b). A mixture of 130 g (0.6 mol) of *2b*, 75 g (1.2 mol) of ethylene glycol, 1 g of 4-toluenesulfonic acid, and 1000 ml of benzene was refluxed for 6 h by using a Dean-Stark water separator. The solution was washed with two 200 ml portions of aqueous sodium carbonate (1 M) and two 200 ml portions of water, dried (K_2CO_3), and distilled to give *3b* (120 g; 77 %) as a colourless oil, b.p. 126 – 132 °C/53 Pa. Anal. $C_{12}H_{20}O_6$: C, H. IR (film): 2990 (m), 2955 (m), 2895 (m), 1735 (s) cm^{-1}. ^1H NMR (CCl_4): δ 4.02 (q, J 7 Hz) and 3.87 (s) (a total of 6 H), 3.63 (3 H, s), 2.7 – 2.3 (m) and 2.3 – 1.5 (m) (a total of 5 H), 1.33 (s) and 1.23 (t, J 7 Hz) (a total of 6 H).

Ethyl 3-(3-hydroxy-5-methylisoxazol-4-yl)propionate (4). To a stirred solution of 33.7 g (*ca.* 0.60 mol) of potassium hydroxide in methanol (80 ml) was added a solution of 31.3 g (0.45 mol) of hydroxylamine hydrochloride in methanol (300 ml). After stirring for further 90 min and subsequent cooling of the mixture to 0 °C a solution of 78.0 g (0.30 mol) of *3b* in methanol (50 ml) was added, and the mixture was allowed to stand at 8 °C for 5 d. Upon addition of glacial acetic acid (70 ml) the mixture was filtered. The filtrate was evaporated *in vacuo* and the residue submitted to CC [silica gel: 900 g; eluents: ethyl acetate containing methanol (8–22 %) and formic acid (1 %)]. 35.4 g of an oily product was obtained, which was almost homogenous on TLC plates [R_F: 0.40; eluent: ethyl acetate-methanol-formic acid (90:9:1); visualization with an iron(III) chloride spraying reagent (purple colour)]. The intermediate product (35.4 g) was dissolved in an ethanolic solution of hydrogen chloride (300 ml; 5 %), and the solution was refluxed for 7 h. Evaporation *in vacuo* gave 25.9 g (43 %, calculated on the basis of *3b*) of crude *4*. An analytical sample was purified by sublimation *in vacuo* (60 Pa) at 80 °C to give *4* as colourless crystals, m.p. 65.5–66.5 °C. Anal. $C_6H_{13}NO_4$: C, H, N. IR (KBr): 3600–2300 (m), 2990 (m), 1730 (s), 1665 (m), 1545 (m), 1530 (m) cm^{-1}. UV (methanol (log ε)]: 214 (3.71) nm. ^1H NMR ($CDCl_3$): δ 11.4 (1 H, s), 4.07 (2 H, q, J 7 Hz), 2.58 (4 H, s), 2.27 (3 H, s), 1.23 (3 H, t, J 7 Hz).

Ethyl 3-(3-methoxy-5-methylisoxazol-4-yl)propionate (6) and ethyl 3-(2-methyl-3-oxo-5-methylisoxazolin-4-yl)propionate (5). To a solution of 5.0 g (*ca.* 25 mmol) of crude *4* in ether (150 ml) was added with stirring a solution of *ca.* 1.4 g (32 mmol) of diazomethane [prepared from 9.8 g (45 mmol) of *N*-methyl-*N*-nitroso-4-toluenesulfonamide] in ether (100 ml). Stirring was continued for 4 h and unreacted diazomethane was destroyed by addition of formic acid (0.5 ml). The ether solution was evaporated *in vacuo* and the oily residue was submitted to CC [silica gel (Woelm 0.063–0.1 mm): 150 g; eluents: methylene chloride containing ethyl acetate (10–60 %)]. *6* (1.9 g; 36 %) was obtained as a colourless oil after ball-tube distillation at 35 Pa (oven temperature 130 °C). Anal. $C_{10}H_{15}NO_4$: C, H, N. IR (film): 3000–2800 (several bands, m), 1730 (s), 1650 (m), 1525 (s) cm^{-1}. UV [methanol (log ε)]: 214 (3.79) nm. ^1H NMR (CCl_4): δ 3.97 (q, J 7 Hz) and 3.83 (s) (a total of 5 H), 2.40 (4 H, s), 2.22 (3 H, s), 1.18 (3 H, t, J 7 Hz). *5* (1.4 g; 26 %) was obtained as a colourles oil after ball-tube distillation at 65 Pa (oven temperature 160 °C). Anal. $C_{10}H_{15}NO_4$: C, H, N. IR (film): 3000–2850 (several bands, w), 1730 (s), 1660 (s), 1420 (m) cm^{-1}. UV [methanol (log ε)]: 233 (3.97) nm. ^1H NMR ($CDCl_3$): δ 4.07 (3 H, q, J 7 Hz), 3.43 (3 H, s), 2.57 (4 H, s), 2.20 (3 H, s), 1.23 (3 H, t, J 7 Hz).

3-(3-Methoxy-5-methylisoxazol-4-yl)propionamide (7). A mixture of 1.78 g (8.4 mmol) of *6* and aqueous ammonia (100 ml; ϱ 0.87) was stirred at 25 °C for 19 h. The clear solution was evaporated *in vacuo* to give 1.67 g of crystalline product. Recrystallization (benzene) gave *7* (1.27 g; 83 %) as colourless crystals, m.p. 141.5–142.5 °C. Anal. $C_8H_{12}N_2O_3$: C, H, N. IR (KBr): 3385 (s), 3185 (s), 3010–2860 (several bands, w), 1655 (s), 1645 (s), 1520 (s), 1475 (s) cm^{-1}. UV [methanol (log ε)]: 213 (3.87) nm. ^1H NMR [$CDCl_3$–DMSO-d_6 (5:1)]: δ 7.0–6.5 and 6.3–5.8 (two broad signals, a total of 2 H), 3.87 (3 H, s), 2.7–2.3 (4 H, m), 2.23 (3 H, s).

3-(3-Methoxy-5-methylisoxazol-4-yl)propylammonium chloride (8). A solution of 1.11 g (6 mmol) of *7* in tetrahydrofuran (100 ml) containing diborane, externally generated[20] from 0.82 g (22 mmol) of sodium borohydride in diglyme (25 ml) and 4.8 g (34 mmol) of boron trifluoride etherate in diglyme (10 ml) was refluxed for 16 h. After cooling to 25 °C followed by careful addition of hydrochloric acid (10 ml; 5 M) the solution was evaporated to dryness *in vacuo*. Upon addition of water (1 ml) and aqueous potassium hydroxide (10 ml; 50 %) the mixture was extracted with two 10 ml portions of ether. The combined ether phases were dried (K_2CO_3) and evaporated *in vacuo* to give 0.95 g of an oil. The oily product was dissolved in an ethanolic solution of hydrogen chloride (5 ml; 5 %) and upon addition of ether (2 ml) *8* (0.88 g; 71 %) was obtained as colourless crystals, m.p. 153.0–154.0 °C. Anal. $C_8H_{15}ClN_2O_3$: C, H, Cl, N. IR (KBr): 3700–2500 (several bands, s), 2050 (w), 1655 (m), 1630 (w), 1525 (s), 1475 (s) cm^{-1}. UV [methanol (log ε)]: 213 (3.89) nm. ^1H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 4.77 (*ca.* 3.5 H, s), 3.93 (3 H, s), 3.2–2.8 (2 H, t), 2.6–2.2 (m) and 2.28 (s) (a total of 5 H), 2.2–1.6 (2 H, m).

3-(3-Hydroxy-5-methylisoxazol-4-yl)propylammonium bromide (9). To a solution of hydrogen bromide in glacial acetic acid (3 ml; 43 %) was added *8* (362 mg; 1.8 mmol), and the solution was refluxed for 15 min. Upon evaporation to dryness *in vacuo* the residue was treated as described above for further 15 min. Evaporation of the reaction mixture to dryness *in vacuo* followed by recrystallization (methanol-ether) of the residue gave *9* (315 mg; 76 %) as colourless crystals, m.p. 218–219 °C (decomp.). Anal. $C_7H_{13}BrN_2O_3$: C, H, Br, N. IR (KBr): 3700–2300 (several bands, s), 1660 (m), 1625 (w), 1605 (w), 1525 (s), 1455 (m) cm^{-1}. UV (methanol): < 210 nm. ^1H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 4.73 (*ca.* 5 H, s), 3.2–2.8 (2 H, t), 2.6–2.2 (m) and 2.27 (s) (a total of 5 H), 2.2–1.6 (2 H, m).

4-(3-Aminopropyl)-5-methyl-3-isoxazolol zwitterion (10). To a solution of 82 mg (0.35 mmol) of *9* in water (0.2 ml) was added a solution

of 36 mg (0.36 mmol) of triethylamine in ethanol (0.8 ml). Upon standing at 5 °C for several days *10* (30 mg; 56 %) was obtained as colourless crystals, m.p. 202 – 203 °C (decomp.). Anal. $C_7H_{12}N_2O_2$: C, H, N. IR (KBr): 3700 – 2350 (several bands, s), 2255 (m), 1665 – 1645 (several bands, m), 1505 (s), 1495 (s) cm^{-1}. UV [methanol (log ε)]: 213 (3.71) nm. pK_A values (H_2O, 26 °C): 5.32 ± 0.01, 10.6 ± 0.1.

*Cyclohepteno[1,2-*d*]isoxazol-3-ol (11).* 32.0 g (0.17 mol) of ethyl 2-oxocycloheptane-1-carboxylate [21] was treated with hydroxylamine under basic conditions as earlier described.[11] After standing at 5 °C for 18 h the reaction mixture was acidified with concentrated hydrochloric acid (22 ml) and extracted with three 200 ml portions of ether. The combined ether phases were dried (Na_2SO_4) and evaporated *in vacuo*. The oily residue was submitted to CC [silica gel: 300 g; eluents: benzene containing ethyl acetate (30 – 50 %) and formic acid (1 %)]. Appropriate fractions were mixed and evaporated *in vacuo* to give *11* (9.6 g; 36 %). Sublimation of an analytical sample *in vacuo*, (20 Pa) at 90 °C gave *11* as colourless crystals, m.p. 118.0 – 119.0 °C (Ref. 11, 120 °C; Ref. 15, 119 – 120 °C). ^{1}H NMR [CCl_4 – $CDCl_3$ (4:1)]: δ 12.5 (1 H, s), 2.8 – 2.6 (2 H, m), 2.4 – 2.2 (2 H, m), 1.9 – 1.5 (6 H, m). IR and UV data were in agreement with those published.[15]

*3-Methoxycyclohepteno[1,2-*d*]isoxazole (13) and 2-methylcyclohepteno[1,2-*d*]isoxazolin-3-one (12).* To a solution of 9.2 g (60 mmol) of *11* in ether (120 ml) was added with stirring a solution of *ca.* 3 g (75 mmol) of diazomethane [prepared from 21.5 g (100 mmol) of *N*-methyl-*N*-nitroso-4-toluenesulfonamide] in ether (200 ml). Stirring was continued for 18 h and the remaining diazomethane was destroyed by addition of glacial acetic acid (1 ml). The ether solution was evaporated *in vacuo* and the oily residue (11 g) submitted to CC [silica gel: 250 g; eluents: benzene containing ethyl acetate (40 – 60 %) and formic acid (1 %)]. *13* (4.7 g; 47 %) was obtained as a colourless oil, b.p. 123 – 125 °C/1.6 kPa. Anal. $C_9H_{13}O_2N$: C, H, N. IR (film): 2930 (s), 2855 (m), 1655 (m), 1520 (s), 1480 (s) cm^{-1}. UV [cyclohexane (log ε)]: 217 (3.73) nm. ^{1}H NMR (CCl_4): δ 3.88 (3 H, s), 2.8 – 2.6 (2 H, m), 2.4 – 2.2 (2 H, m), 1.9 – 1.5 (6 H, m). *12* (5.3 g; 53 %) was obtained as a colourless oil, b.p. 150 – 153 °C/1.7 kPa. Anal. $C_9H_{13}O_2N$: C, H, N. IR (film): 2935 (s) 2850 (m), 1675 (s), 1655 (s), 1440 (m) cm^{-1}. UV [cyclohexane (log ε)]: 244 (3.76) nm. ^{1}H NMR (CCl_4): δ 3.39 (3 H, s), 2.7 – 2.4 (2 H, m), 2.4 – 2.1(2 H, m), 1.9 – 1.5 (6 H, m).

*3-Methoxycyclohepteno[1,2-*d*]isoxazole-8-carboxylic acid (14).* To a solution of 334 mg (2 mmol) of *13* in tetrahydrofuran (7 ml) kept under nitrogen at – 70 °C was added 229 mg (2.5 mmol) of TMEDA and a solution of butyllithium in hexane (1.25 ml; 2 M). Upon standing at – 70 °C for 20 min the reaction mixture was added to a suspension of 1 g of freshly prepared carbon dioxide in ether (5 ml). The mixture was left for 30 min and after extraction with aqueous sodium hydroxide (5 ml; 2 M) the ether phase was dried (K_2CO_3) and evaporated *in vacuo* to give unreacted *13* (311 mg; 93 %), identified by IR spectroscopy. The aqueous phase was acidified with hydrochloric acid (5 ml; 4 M) and extracted with ether (10 ml). The ether phase was dried (Na_2SO_4) and evaporated *in vacuo* to give *14* (25 mg; 6 %), m.p. 164.0 – 165.5 °C (2-propanol – benzene – light petroleum). Anal. $C_{10}H_{13}O_4N$: C, H, N. IR (KBr): 3700 – 2400 (several bands, m), 1730 (s), 1660 (m), 1530 (s), 1480 (m) cm^{-1}. UV [methanol (log ε)]: 214 (3.80) nm. ^{1}H NMR [$CDCl_3$ – DMSO-d_6 (2:1)]: δ 9.72 (1 H, s), 4.0 – 3.8 (m) and 3.92 (s) (a total of 4 H), 2.4 – 2.2 (2 H, m), 2.2 – 1.3 (6 H, m).

*3-Methoxycyclohepteno[1,2-*d*]isoxazol-8-one (15) and 3-methoxycyclohepteno[1,2-*d*]isoxazol-4-one (16).* To a solution of 751 mg (4.5 mmol) of *13* in glacial acetic acid (20 ml) containing concentrated sulfuric acid (0.5 ml) and kept at 25 – 30 °C was added dropwise and with stirring a solution of 940 mg (3.2 mmol) of sodium dichromate dihydrate in glacial acetic acid (10 ml). To the green reaction mixture was added a suspension of sodium hydrogen carbonate (42 g) in water (100 ml). Extraction of the mixture with four 50 ml portions of ether, and evaporation *in vacuo* of the combined and dried (K_2CO_3) ether phases gave 720 mg of an oily product. CC [silica gel (Woelm 0.063 – 0.1 mm): 50 g; eluents: benzene containing ethyl acetate (15 – 31 %)] gave unreacted *13* (200 mg; 27 %) identified by IR spectroscopy. The yield of *15* was 120 mg (15 %), m.p. 88.0 – 89.0 °C (cyclohexane). Anal. $C_9H_{11}O_3N$: C, H, N. IR (KBr): 2945 (m), 2880 (w), 1670 (s), 1615 (w), 1530 (s) cm^{-1}. UV [methanol (log ε)]: 249 (3.94) nm. ^{1}H NMR (CCl_4): δ 3.80 (3 H, s), 2.7 – 2.3 (4 H, m), 2.1 – 1.7 (4 H, m). The yield of *16* was 360 mg (44 %), m.p. 76.0 – 77.0 °C (cyclohexane). Anal. $C_9H_{11}O_3N$: C, H, N. IR (KBr): 2950 (m), 2880 (w), 1670 (s), 1605 (s), 1525 (s), 1465 (s) cm^{-1}. UV [methanol (log ε)]: 232 (3.97) nm. ^{1}H NMR (CCl_4): δ 3.85 (3 H, s), 3.0 – 2.8 (2 H, m), 2.6 – 2.4 (2 H, m), 2.1 – 1.7 (4 H, m).

*3-Methoxycyclohepteno[1,2-*d*]isoxazol-8-one oxime (17).* A solution of 372 mg (2.1 mmol) of *15*, 174 mg (2.5 mmol) of hydroxylammonium chloride, and 340 mg (2.5 mmol) of sodium acetate trihydrate in ethanol-water (2:1) (5 ml) was refluxed for 45 min. Evaporation of the solution to *ca.* 1 ml followed by filtration and washing of the crystals with water (5 ml) afforded 282 mg (70 %) of crude *17* as colourless and TLC-pure crystals [R_F: 0.41; eluent: benzene-ethyl acetate (2:1)]. An analytical sample was recrystallized (ethanol-water) to give pure *17*, m.p. 177.0 – 178.0 °C. Anal. $C_9H_{12}O_3N_2$: C, H, N. IR (KBr): 3600 – 3000 (m), 2940 (m), 2860 (w), 1650 (m), 1615 (w), 1535 (s), 1475 (s) cm^{-1}. UV [methanol (log ε)]: 252

(4.04) nm. ^{1}H NMR ($CDCl_3$): δ 10.1 (1 H, s), 3.97 (3 H, s), 3.0–2.7 (2 H, m), 2.6–2.3 (2 H, m), 2.0–1.6 (4 H, m).

(±)-3-Methoxy-8-aminocyclohepteno[1,2-d]-isoxazole (18). To a solution of 343 mg (1.8 mmol) of *17* in methanol–water (6:1) (15 ml) was added aluminium amalgam. [The latter was prepared by treatment of 567 mg (21 mmol) of aluminium strips with an aqueous mercury-(II) chloride solution (18 ml; 5 %) for 30 s followed by washing with ethanol]. After stirring for 4 h at 25 °C the mixture was filtered and concentrated *in vacuo* to give an oil. Ball-tube distillation at 120 Pa (oven temperature 150 °C) gave *18* (245 mg; 77 %) as a colourless oil. Found: C 58.90; H 7.61; N 14.97. Calc. for $C_9H_{14}O_2N_2$: C 59.32; H 7.74; N 15.37. IR (film): 3380 (m), 3300 (m), 2935 (s), 2850 (m), 1655–1640 (three bands, m), 1520 (s) cm^{-1}. UV [methanol (log ε)]: 216 (3.84) nm. ^{1}H NMR (CCl_4): δ 4.0–3.7 (m) and 3.83 (s) (a total of 4 H), 2.3–2.1 (2 H, m), 2.0–1.4 (m) and 1.50 (s) (a total of 8 H).

(±)-8-Aminocyclohepteno[1,2-d]isoxazol-3-ol zwitterion (19). *18* (245 mg; 1.35 mmol) was demethylated as described above for the preparation of *9* by treatment with two 2 ml portions of the reagent. The evaporated reaction mixture was kept at 1.8 kPa over potassium hydroxide for 2 d. To a solution of the glassy residue in water (6 ml) was added a solution of 157 mg (1.55 mmol) of triethylamine in ethanol (1.5 ml). Upon standing at 5 °C for 10 d *19* (143 mg; 63 %) was isolated as colourless crystals, m.p. 206–207 °C (decomp.). Anal. $C_8H_{12}O_2N_2$: C, H, N. IR (KBr): 3700–3200 (m), 3200–2000 (several bands, s), 1665 (m), 1630 (m), 1565 (m), 1495 (s), 1485 (s) cm^{-1}. UV [methanol-water (99:1)]: < 210 nm. pK_A values (H_2O, 25 °C): 4.51 ± 0.06, 8.30 ± 0.05.

3-Methoxycyclohepteno[1,2-d]isoxazol-4-one oxime (20). *20* was synthesized as described for *17* by using 1.08 g (6.0 mmol) of *16*, 0.45 g (6.5 mmol) of hydroxylammonium chloride, and 0.89 g (6.5 mmol) of sodium acetate trihydrate as starting materials and 15 ml of solvent. Crude *20* (0.99 g; 85 %) was obtained as colourless and TLC-pure crystals [R_F: 0.32; eluent: benzene–ethyl acetate (2:1)]. An analytical sample was recrystallized (ethanol) to give pure *17*, m.p. 175.0–175.5 °C. Anal. $C_9H_{12}N_2O_3$: C, H, N. IR (KBr): 3600–3100 (s), 2940 (m), 2865(w), 1630 (s), 1525 (s) cm^{-1}. UV [methanol (log ε)]: 226 (4.02) nm. ^{1}H NMR ($CDCl_3$): δ 10.6–9.6 (*ca.* 1 H, broad s), 3.97 (3 H, s), 3.0–2.7 (4 H, m), 2.0–1.6 (4 H, m).

(±)-3-Methoxy-4-aminocyclohepteno[1,2-d] isoxazole hydrochloride (21). A solution of 0.98 g (5.0 mmol) of *20* in methanol–water (7:1) (80 ml) was treated with aluminium amalgam, prepared from 2.0 g (74 mmol) of aluminium, as described above for *18*. After stirring for 60 h at 25 °C the mixture was filtered and evaporated *in vacuo*. The oily residue was mixed with hydrochloric acid (30 ml; 1 M). Upon extraction with four 10 ml portions of ether the aqueous phase was evaporated to dryness *in vacuo*. Recrystallization of the residue (ethanol–ether) gave *21* (0.88 g; 81 %) of *21* as colourless crystals, m.p. 217–218 °C (decomp.). Anal. $C_9H_{15}ClN_2O_2$: C, H, Cl, N. IR (KBr): 3700–3200 (m), 3200–2700 (s), 2615 (w), 1640 (m), 1590 (m), 1530 (s) cm^{-1}. UV (methanol): < 210 nm. ^{1}H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 4.75 (*ca.* 4 H, s), 4.5–4.2 (1 H, m), 3.97 (3 H, s), 3.0–2.7 (2 H, m), 2.2–1.7 (6 H, m).

(±)-4-Aminocyclohepteno[1,2-d]isoxazol-3-ol hydrobromide (22). *22* was synthesized as described for *9* by using 730 mg (3.3 mmol) of *21* as a starting material and two 6 ml portions of the reagent. *22* (675 mg; 82 %) was obtained as colourless crystals, m.p. 195.0–197.0 °C (methanol–ether). Anal. $C_8H_{13}BrN_2O_2$: C, H, Br, N. IR (KBr): 3700–3300 (m), 3200–2200 (several bands, s), 1650 (s), 1595 (m), 1545 (s), 1525 (m), 1505 (s) cm^{-1}. UV (methanol): < 210 nm. ^{1}H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 4.77 (*ca.* 5 H, s), 4.5–4.2 (1 H, m), 3.0–2.7 (2 H, m), 2.2–1.6 (6 H, m).

(±)-4-Aminocyclohepteno[1,2-d]isoxazol-3-ol zwitterion hydrate (23). To a solution of 142 mg (0.57 mmol) of *22* in water (0.5 ml) was added a solution of 65 mg (0.64 mmol) of triethylamine in ethanol (0.5 ml). Upon standing at 25 °C for 5 min *23* (90 mg; 85 %) was obtained as colourless crystals, m.p. 191–194 °C (decomp.). Anal. $C_8H_{12}N_2O_2.H_2O$: C, H, N. IR (KBr): 3700–2300 (several bands, s), 2100 (w), 1650 (s), 1535 (s), 1505 (s) cm^{-1}. UV (methanol): < 210 nm. pK_A values (H_2O, 26 °C): 4.63 ± 0.01, 9.89 ± 0.02.

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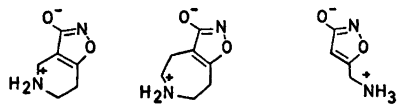
Muscimol Analogues. II. Synthesis of Some Bicyclic 3-Isoxazolol Zwitterions

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The syntheses of the 3-isoxazolol zwitterions 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (*7a*), 5,6,7,8-tetrahydro-4*H*-isoxazolo[5,4-*c*]-azepin-3-ol (*7b*), and 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol (*7c*) are described. The starting materials were the cyclic β-oxo-esters ethyl 1-methoxycarbonyl-3-oxopiperidine-4-carboxylate (*2a*), ethyl 1-methoxycarbonyl-3-oxoperhydroazepine-4-carboxylate (*2b*), and ethyl 1-methoxycarbonyl-4-oxoperhydroazepine-3-carboxylate (*2c*). The ethylene acetals of *2a*—*c* were treated with hydroxylamine, and deacetalization and cyclization of the intermediate β-oxohydroxamic acid ethylene acetals gave the respective 3-isoxazolol derivatives methyl 3-hydroxy-4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]pyridine-6-carboxylate (*5a*), methyl 3-hydroxy-4,5,6,8-tetrahydro-7*H*-isoxazolo[5,4-*c*]-azepine-7-carboxylate (*5b*), and methyl 3-hydroxy-4,6,7,8-tetrahydro-5*H*-isoxazolo[4,5-*c*]-azepine-5-carboxylate (*5c*), which were transformed into the zwitterions *7a*—*c*. The pK_A values of *7a*—*c* have been determined.

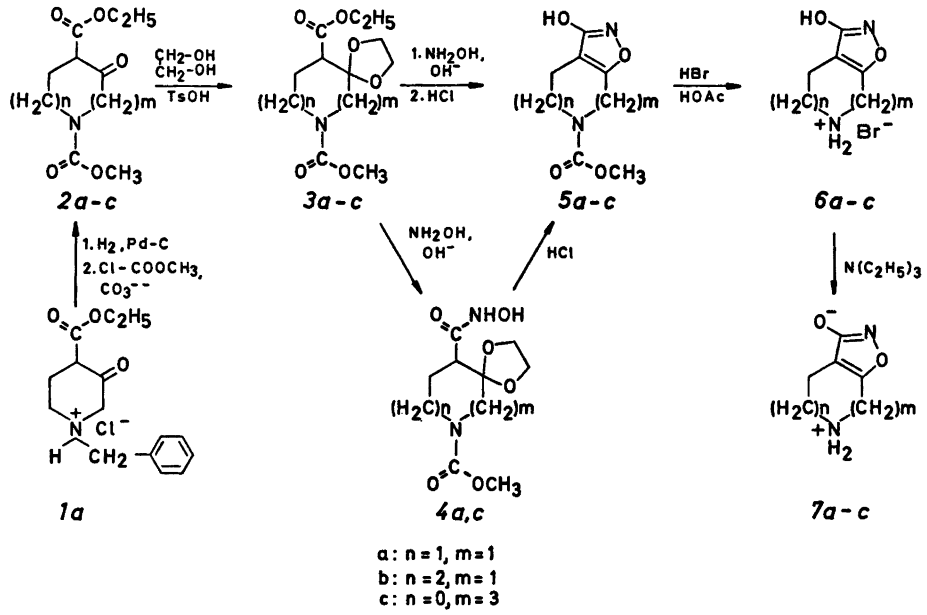
While muscimol (5-aminomethyl-3-isoxazolol) is a weak inhibitor of γ-aminobutyric acid (GABA) uptake,[1] the bicyclic muscimol analogue, 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (*A*) (Scheme 1), is a relatively potent inhibitor of GABA uptake.[2] The related compound 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*d*]-azepin-3-ol (*B*) is inactive.[2] In order to study in further detail the relationship between structure and biological activity of this type of muscimol analogues the related compounds *7a*—*c* have now been synthesized.



Scheme 1.

The reaction sequences for the preparation of *7a*—*c* are outlined in Scheme 2. The key steps in the reaction sequences are transformation of the β-oxoester ethylene acetals *3a*—*c* into the 3-isoxazolol derivatives *5a*—*c* *via* the corresponding hydroxamic acids. Only moderate yields of *5a*—*c* were obtained. The synthesis of *5a* required isolation of the hydroxamic acid *4a* in a pure state and subsequent heating of *4a* with concentrated hydrochloric acid, the latter reaction being accompanied by extensive degradation processes.

The depicted structures of the new compounds *2a* and *3*—*7* are based on the unequivocal structure determinations of the respective starting materials *1a* [3] and *2b,c* [4] and confirmed spectroscopically and by elemental analyses. The spectroscopic data of the 4,5-disubstituted 3-oxygenated isoxazole moieties of *5a*—*c* and *6a*—*c* are in accordance with general findings.[5] The spectroscopic and protolytic properties of *7a*—*c* are in agreement with those of other 3-isoxazolol zwitterions.[6—11] With the exceptions mentioned below the spectroscopic data of analogous compounds in Scheme 2 are very similar. The cyclic β-oxoester *2a* was shown by ^{1}H NMR and IR spectroscopy to be almost exclusively in the enol form, whereas this form of the related compound *2b* is hardly detectable by the same methods.[4] The 3-isoxazolol derivative *5c* was obtained in two crystal modifications with different IR spectra. In analogy with previous ^{1}H NMR spectroscopic findings for a series of cyclohepteno[1,2-*d*]isoxazole



Scheme 2.

derivatives[11] the resonance signals of the protons attached to C-4 in *5b*, *6b*, *5c*, and *6c* are observed at higher fields than those of the corresponding protons at C-8 in *5c*, *6c*, *5b*, and *6b*, respectively.

EXPERIMENTAL

Unless otherwise stated the determination 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.[12] Unless otherwise stated thin layer chromatography (TLC) and column chromatography (CC) was accomplished by using silica gel F_{254} plates (Merck) and silica gel 0.05–0.200 mm (Merck), respectively. Columns were developed by stepwise gradient elution. An iron(III) chloride spraying reagent was used to visualize on TLC plates hydroxamic acids (purple colour) and 3-isoxazolol derivatives (yellow colour). Ammonium groups were visualized by spraying with a ninhydrin reagent (yellow colour). The pK_A values were determined as described in a previous paper.[7]

*Ethyl 1-methoxycarbonyl-3-oxopiperidine-4-carboxylate (*2a*).* A solution of *1a*[3] (14.0 g; 47 mmol) in aqueous ethanol (300 ml; 50 %) was hydrogenated (*ca.* 300 kPa) in a PARR hydrogenation apparatus by using a 10 % Pd-C catalyst (1.4 g). The reaction mixture was filtered and evaporated to dryness *in vacuo*. To an ice cooled solution of the residue in water (50 ml) was added with stirring an iced solution of potassium carbonate (19.4 g; 140 mmol) in water (20 ml) followed by addition of methyl chloroformate (11.3 g; 120 mmol). Stirring was continued at 0 °C for 30 min and at 25 °C for 30 min. The mixture was extracted with three 100 ml portions of ether. The combined and dried (Na_2SO_4) ether phases were evaporated *in vacuo* to give 10.0 g of crude product. Ball-tube distillation at 40–130 Pa (oven temperature 170 °C) gave *2a* (9.0 g; 84 %) as a colourless oil, which slowly crystallized, m.p. 36–38 °C. Anal. $C_{10}H_{15}NO_5$: C, H, N. IR (film): 2980–2850 (several bands, m-s), 1700 (s), 1655 (s), 1620 (m) cm^{-1}. ^{1}H NMR (CCl_4): δ 12.3 (1 H, s), 4.13 (q, J 7 Hz) and 4.0–3.9 (m) (a total of 4 H), 3.62 (3 H, s), 3.43 (2 H, t, J 6 Hz), 2.4–2.1 (2 H, m), 1.30 (3 H, t, J 7 Hz).

*Ethyl 1-methoxycarbonyl-3-oxopiperidine-4-carboxylate ethylene acetal (*3a*).* A mixture of *2a* (9.0 g; 39 mmol), ethylene glycol (100 ml), 4-toluenesulfonic acid (0.7 g), and benzene (500 ml) was refluxed for 6 d using a Dean-Stark water separator. The mixture was washed with aqueous sodium carbonate (300 ml; 1 M), water (300 ml), and saturated aqueous sodium chloride (300 ml). The organic phase was dried (K_2CO_3) and evaporated *in vacuo* to give 8.6 g of an oil. CC [silica gel (Woelm 0.063–0.1 mm): 350 g; eluents: methylene chloride to which ethyl acetate (20–35 %) was added] followed by ball-tube distillation at 40 Pa (oven temperature 170 °C) gave *3a* (7.0 g; 65 %) as a colourless oil. Anal. $C_{12}H_{19}NO_6$: C, H, N. IR (film): 2970 (s), 2900 (s), 1730 (s)

cm^{-1}. ^{1}H NMR (CCl_4): δ 4.05 (q, J 7 Hz) and 3.92 (s) (a total of 6 H), 3.60 (s) and 3.7 – 3.0 (m) (a total of 7 H), 2.8 – 2.5 (1 H, t), 2.2 – 1.6 (2 H, m), 1.23 (3 H, t, J 7 Hz).

1-Methoxycarbonyl-3-oxopiperidine-4-carbohydroxamic acid ethylene acetal (4a). To a stirred and iced solution of potassium hydroxide (7.3 g; 130 mmol) in methanol (30 ml) was added hydroxylammonium chloride (6.9 g; 100 mmol). After stirring at 0 °C for a further 30 min a solution of *3a* (6.8 g; 25 mmol) in methanol (20 ml) was added, and the mixture was left at 8 °C for 8 d. Upon addition of glacial acetic acid (15 ml) and filtration the filtrate was evaporated *in vacuo* to give a treacly mass. CC [silica gel (Woelm 0.063 – 0.1 mm): 250 g; eluents: ethyl acetate to which methanol (15 – 26 %) and formic acid (1 %) was added] afforded *4a* (1.9 g; 29 %) as a crystalline and TLC-pure substance [R_F: 0.23; eluent: ethyl acetate – methanol – formic acid (90:9:1)]. An analytical sample was recrystallized (ethanol – benzene) to give *4a* as colourless crystals, m.p. 150.0 – 152.0 °C. Anal. $C_{10}H_{16}N_2O_6$: C, H, N. IR (KBr): 3700 – 3350 (m), 3280 (m), 3210 (s), 3055 (w), 3000 – 2870 (several bands, w-m), 1690 (s), 1640 (s), 1550 (w) cm^{-1}. ^{1}H NMR [$CDCl_3$ – DMSO-d_6 (1:1)]: δ 10.5 – 10.1 (1 H, m), 4.9 – 4.3 (1 H, m), 3.93 (s), 3.60 (s), and 4.1 – 3.1 (m) (a total of 11 H), 2.8 – 2.6 (1 H, m), 2.2 – 1.8 (2 H, m).

Methyl 3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-6-carboxylate (5a). A solution of *4a* (750 mg; 2.9 mmol) in concentrated hydrochloric acid (13 ml) was heated to 70 °C for 10 min. The mixture was evaporated *in vacuo* to give a black oil. CC [silica gel (Woelm 0.063 – 0.1 mm): 60 g; eluents: benzene to which ethyl acetate (40 – 70 %) and formic acid (1 %) was added] gave crystalline and TLC-pure *5a* (244 mg; 43 %) [R_F: 0.27; eluent: benzene – ethyl acetate – formic acid (50:50:1)]. An analytical sample was recrystallized (benzene – cyclohexane) to give pure *5a* as colourless crystals, m.p. 136.0 – 138.0 °C. Anal. $C_8H_{10}N_2O_4$: C, H, N. IR (KBr): 3700 – 3300 (m), 3300 – 2500 (several bands, w-m), 1655 (s), 1525 (m), 1490 (s) cm^{-1}. UV [methanol (log ε)]: 212 (3.64) nm. ^{1}H NMR ($CDCl_3$): δ 10.6 (1 H, s), 4.43 (2 H, s), 3.70 (s) and 3.8 – 3.5 (t) (a total of 5 H), 2.6 – 2.3 (2 H, t).

3-Hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridinium bromide (6a). A solution of *5a* (309 mg; 1.6 mmol) in a solution of hydrogen bromide in glacial acetic acid (3 ml; 43 %) was refluxed for 15 min. Upon evaporation to dryness *in vacuo* the residue was treated with the same reagent (3 ml) for further 15 min. Evaporation of the reaction mixture to dryness *in vacuo* and recrystallization (methanol – ether) of the residue gave *6a* (193 mg; 56 %) as faintly reddish crystals, m.p. 162 – 163 °C (decomp.). Anal. $C_6H_9BrN_2O_2$: C, H, Br, N. IR (KBr): 3700 – 3300 (m), 3070 (s), 3000 – 2300 (several bands, m-s), 1670 (m), 1580 (m), 1525 (s), 1505 (w) cm^{-1}. UV (methanol): < 210 nm. ^{1}H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 4.77 (*ca.* 5 H, s), 4.43 (2 H, t, J 1 Hz), 3.7 – 3.4 (2 H, q, J 6 and 7 Hz), 3.0 – 2.7 (2 H, t).

4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridin-3-ol zwitterion (7a). To a solution of *6a* (77 mg; 0.35 mmol) in water (0.6 ml) was added a solution of triethylamine (39 mg; 0.39 mmol) in ethanol (0.6 ml). The mixture was left at 25 °C for 2 h. *7a* (42 mg; 86 %) was isolated as colourless crystals, m.p. 242 – 244 °C (decomp.). Anal. $C_6H_8N_2O_2$: C, H, N. IR (KBr): 3700 – 2900 (s), 2900 – 1900 (several bands, m-s), 1670 (s), 1625 (m) cm^{-1}. UV [methanol (log ε)]: 212 (3.64) nm. pK_A values (H_2O, 25 °C): 4.44 ± 0.03, 8.48 ± 0.04.

Ethyl 1-methoxycarbonyl-3-oxoperhydroazepine-4-carboxylate ethylene acetal (3b). *3b* was synthesized as described above for *3a* by using *2b* [4] (4.1 g; 17 mmol) and ethylene glycol (20 ml). After reaction for 6 d the reaction mixture was worked up to give 6.0 g of crude product. CC [silica gel: 300 g; eluents: benzene to which ether (30 – 50 %) was added] followed by ball-tube distillation at 40 Pa (oven temperature 180 °C) gave *3b* (3.2 g; 66 %) as a colourless oil. Anal. $C_{13}H_{21}NO_6$: C, H, N. IR (film): 2980 – 2890 (several bands, m), 1730 (s), 1705 (s) cm^{-1}. ^{1}H NMR (CCl_4): δ 4.02 (q, J 7 Hz) and 3.9 – 3.8 (m) (a total of 6 H), 3.60 (s) and 3.6 – 3.2 (m) (a total of 7 H), 2.7 – 2.5 (1 H, m), 2.0 – 1.6 (4 H, m), 1.23 (3 H, t, J 7 Hz).

Methyl 3-hydroxy-4,5,6,8-tetrahydro-7H-isoxazolo[5,4-c]azepine-7-carboxylate (5b). *3b* (1.29 g; 4.5 mmol) was treated with hydroxylamine for 9 d as described above for *4a* by using potassium hydroxide (1.29 g; *ca.* 23 mmol), hydroxylammonium chloride (1.25 g; 18 mmol), and methanol (8 ml). After addition of methanol (10 ml) and glacial acetic acid (2 ml) the mixture was filtered and the filtrate evaporated *in vacuo*. A solution of the residue in methanol – concentrated hydrochloric acid (2:1) (12 ml) was heated to 70 °C for 5 min and evaporated *in vacuo* to give an oil. CC [silica gel: 60 g; eluents: benzene to which ethyl acetate (30 – 45 %) and formic acid (1 %) was added] gave *5b* (227 mg; 24 %) as TLC-pure crystals [R_F: 0.35; eluent: benzene – ethyl acetate – formic acid (50:50:1)]. An analytical sample was recrystallized (benzene) to give pure *5b* as colourless crystals, m.p. 162.0 – 162.5 °C. Anal. $C_9H_{12}N_2O_4$: C, H, N. IR (KBr): 3700 – 3300 (m), 3200 – 2500 (several bands, w-m), 1695 (s), 1655 (m), 1540 (s), 1475 (s) cm^{-1}. UV [methanol (log ε)]: 214 (3.79) nm. ^{1}H NMR ($CDCl_3$): δ 11.0 (1 H, s), 4.50 (2 H, s), 3.65 (s), and 3.7 – 3.4 (m) (a total of 5 H), 2.6 – 2.2 (2 H, t), 2.0 – 1.6 (2 H, m).

3-Hydroxy-5,6,7,8-tetrahydro-4H-isoxazolo[5,4-c]azepinium bromide (6b). *6b* was synthesized as described above for *6a* by using *5b* (400 mg; 1.9 mmol) and two 6 ml portions of

reagent. Recrystallization of crude *6b* (methanol – ether) gave pure *6b* (366 mg; 83 %) as faintly yellowish crystals, m.p. 222 °C (decomp.). Anal. $C_7H_{11}BrN_2O_2$: C, H, Br, N. IR (KBr): 3700 – 3300 (w), 3200 – 2200 (several bands, m-s), 1670 (m), 1535 (s) cm^{-1}. UV [methanol (log ε)]: 212 (3.81) nm. ^1H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 4.70 (*ca.* 4 H, s), 4.40 (2 H, s), 3.7 – 3.5 (2 H, t), 2.7 – 2.3 (2 H, m), 2.3 – 1.8 (2 H, m).

5,6,7,8-Tetrahydro-4H-isoxazolo[5,4-c]azepin-3-ol zwitterion monohydrate (7b). 7b was prepared as described above for *7a* by using *6b* (83 mg; 0.35 mmol). The mixture was left at 5 °C for 3 d. *7b* (41 mg; 68 %) was isolated as colourless crystals, m.p. 200 – 201 °C (decomp.). Anal. $C_7H_{10}N_2O_2 \cdot H_2O$: C, H, N. Anal. $C_7H_{10}N_2O_2$ (after heating of *7b* to 120 °C for 20 h): C, H, N. IR (KBr): 3700 – 3200 (s), 3100 – 1900 (several bands, m-s), 1660 (m), 1620 (s) cm^{-1}. UV [methanol (log ε)]: 215 (3.74) nm. pK_A values (H_2O, 26 °C): 4.56 ± 0.03, 8.56 ± 0.06.

Ethyl 1-methoxycarbonyl-4-oxoperhydroazepine-3-carboxylate ethylene acetal (3c). 3c was synthesized as described above for *3a* by using *2c*[4] (4.7 g; 19 mmol) and ethylene glycol (7 ml). After reaction for 2 d the reaction mixture was worked up to give 6.2 g of crude product. Ball-tube distillation at 70 Pa (oven temperature 170 °C) gave *3c* (4.6 g; 83 %) as a colourless oil. Anal. $C_{13}H_{21}NO_6$: C, H, N. IR (film): 2980 – 2890 (several bands, m), 1730 (s), 1700 (s) cm^{-1}. ^1H NMR (CCl_4): δ 3.98 (q, J 7 Hz) and 3.82 (s) (a total of 6 H), 3.55 (s) and 3.6 – 3.2 (m) (a total of 7 H), 2.9 – 2.6 (1 H, m), 1.9 – 1.4 (4 H, m), 1.23 (3 H, t, J 7 Hz).

1-Methoxycarbonyl-4-oxoperhydroazepine-3-carbohydroxamic acid ethylene acetal (4c). 4c was synthesized as described above for *4a* by using *3c* (2.5 g; 9 mmol), potassium hydroxide (2.5 g; *ca.* 45 mmol), hydroxylammonium chloride (2.5 g; 36 mmol), and methanol (16 ml). After reaction for 10 d glacial acetic acid (6 ml) was added. The mixture was filtered, and evaporation *in vacuo* of the filtrate gave a treacly mass. CC [silica gel (Woelm 0.063 – 0.1 mm): 320 g; eluents: ethyl acetate to which methanol (10 – 21 %) and formic acid (1 %) was added] gave crude crystalline *4c*. After recrystallization (ethanol – benzene) *4c* (510 mg; 21 %) was obtained as colourless crystals, m.p. 178.5 – 180.0 °C. Anal. $C_{11}H_{18}N_2O_6$: C, H, N. IR (KBr): 3700 – 3450 (m), 3290 (m), 3160 (m), 3000 – 2880 (several bands, m), 1705 (s), 1635 (s), 1530 (m) cm^{-1}. ^1H NMR [$CDCl_3$ – DMSO-d_6 (2:1)]: δ 10.2 (1 H, s), 8.9 – 8.5 (1 H, m), 3.85 (s), 3.60 (s), and 4.0 – 3.1 (m) (a total of 11 H), 2.6 – 2.4 (1 H, m), 2.0 – 1.5 (4 H, m).

Methyl 3-hydroxy-4,6,7,8-tetrahydro-5H-isoxazolo[4,5-c]azepine-5-carboxylate (5c). Method a. 5c was synthesized as described above for *5b* by using *3c* (2.5 g; 9 mmol), potassium hydroxide (2.5 g; *ca.* 45 mmol), hydroxylammonium chloride (2.5 g; 36 mmol), glacial acetic acid (4 ml), and methanol – concentrated hydrochloric acid (2:1) (20 ml). Purification of a crude reaction product by CC [silica gel: 60 g; eluents: benzene to which ethyl acetate (30 – 40 %) and formic acid (1 %) was added] followed by recrystallization (benzene) of slightly impure *5c* gave *5c* (442 mg; 24 %) as colourless crystals, m.p. 141.5 – 142.5 °C. Anal. $C_9H_{12}N_2O_4$: C, H, N. IR (KBr): 3600 – 3300 (w), 3200 – 2400 (several bands, w-m), 1710 (s), 1650 (m), 1550 (s), 1525 (m), 1480 (s), 1305 (s), 1250 (s), 1115 (s), 965 (s) cm^{-1}. UV [methanol (log ε)]: 213 (3.80) nm. ^1H NMR ($CDCl_3$): δ 11.2 (1 H, s), 4.23 (2 H, s), 3.63 (s) and 3.7 – 3.4 (m) (a total of 5 H), 2.9 – 2.6 (2 H, t), 2.1 – 1.6 (2 H, m). In another experiment a modification of *5c* with m.p. 144.5 – 146.0 °C was obtained. IR (KBr): 3700 – 3300 (w), 3300 – 3000 (m), 2980 – 2400 (several bands, w-m), 1665 (s), 1650 (s), 1520 (s), 1485 (s), 1440 (s), 1410 (s), 1270 (s), 1255 (s), 955 (s) cm^{-1}.

Method b. A solution of *4c* (436 mg; 1.6 mmol) in methanol – concentrated hydrochloric acid (2:1) (10 ml) was heated to 70 °C for 5 min. The solution was evaporated *in vacuo* to give an oil. Upon addition of a saturated aqueous solution of sodium chloride (5 ml) the mixture was extracted with three 20 ml portions of chloroform. The combined and dried (Na_2SO_4) organic phases were evaporated *in vacuo* to give TLC-pure *5c* (331 mg) [R_F: 0.32; eluent: benzene – ethyl acetate – formic acid (50:50:1)]. Recrystallization (benzene) gave *5c* (280 mg; 83 %) as colourless crystals, m.p. 144.5 – 145.5 °C. The IR spectrum was identical with that of the modification of *5c* with m.p. 144.5 – 146.0 °C prepared according to method a.

3-Hydroxy-5,6,7,8-tetrahydro-4H-isoxazolo-[4,5-c]azepinium bromide (6c). 6c was synthesized as described above for *6a* by using *5c* (381 mg; 1.8 mmol) and two 5 ml portions of reagent. Recrystallization of crude *6c* (methanol – ether) gave *6c* (320 mg; 76 %) as colourless crystals, m.p. 213 °C (decomp.). Anal. $C_7H_{11}BrN_2O_2$: C, H, Br, N. IR (KBr): 3700 – 3300 (w), 3200 – 2100 (several bands, w-s), 1665 (s), 1600 (s), 1540 (s), 1515 (w) cm^{-1}. UV [methanol (log ε)]: 211 (3.82) nm. ^1H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 4.70 (*ca.* 4 H, s), 4.04 (2 H, s), 3.7 – 3.5 (2 H, t), 3.1 – 2.8 (2 H, t), 2.3 – 1.9 (2 H, m).

5,6,7,8-Tetrahydro-4H-isoxazolo[4,5-c]azepin-3-ol zwitterion (7c). 7c was prepared as described above for *7a* by using *6c* (83 mg; 0.35 mmol). Upon addition of ether (0.1 ml) the mixture was left at 25 °C for 4 d. *7c* (45 mg; 83 %) was isolated as colourless crystals, m.p. 257 – 258 °C (decomp.). Anal. $C_7H_{10}N_2O_2$: C, H, N. IR (KBr): 3700 – 3300 (m), 3050 (m), 2940 – 2000 (several bands, m-s), 1665 (s), 1600 (m), 1550 (s), 1510 (m) cm^{-1}. UV [methanol (log ε)]: 212 (3.88) nm. pK_A values (H_2O, 26 °C): 4.50 ± 0.05, 9.76 ± 0.05.

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A Comparison of the Electron Density and Chemical Shift of Some 2,4-Dinitrophenylhydrazones

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The NMR chemical shift of the NH hydrogen is correlated with its electron density, as calculated by CNDO/2 method, for ten 2,4-dinitrophenylhydrazones. This gives a nearly linear plot, thus indicating that the chemical shift is largely decided by the electron density about the nucleus.

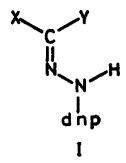
In recent years there has been found a linear relationship between the NMR chemical shift of the nucleus and the electron density as calculated by semi-empirical molecular orbital methods. Good correlations are found for the case of ^{13}C nuclei.[1,2] The ^{1}H shift has been correlated, on the basis of a formula developed by Fraenkel *et al.*,[3] with the π electron density on the carbon atom to which the hydrogen atom is bound.[4–10]

In some cases the ^{1}H NMR shift has been correlated with the electron density of the hydrogen atom. Hamer *et al.*[11,12] found a good correlation in their investigation of substituted styrenes. On the other hand, Roth [13] found no correlation between the corrected chemical shift and the electron density about the hydrogen atom in the investigation of vinyl monomers. In other investigations reasonable to good results were obtained.[14–17]

RESULTS AND DISCUSSION

This investigation deals with ten 2,4-dinitrophenylhydrazones (DNPH's) whereof two make up a *syn/anti* isomer pair. The general form of the compounds is given as I, where either X or Y is a nitro group.

The configuration of the compounds (see below), the NH shift (δ), the electron density (q_H) as calculated by the CNDO/2 method and NH stretch frequency (ν_{NH}) are given in Table 1.



I

Scheme 1. (dnp = 2,4-dinitrophenyl).

Assignments of configuration. Compound *1* showed two NH peaks in the IR and two absorptions in the NH area in the NMR spectrum, thus indicating a mixture of both isomers. The band at lowest frequency [18] and the absorption at lowest field [19] may be ascribed to the *syn*-nitro isomer.

By comparing the NH position in the IR and NMR spectra of *2* and *3* with the positions found for compound *1*, it was assumed that the *anti*-nitro isomer was present in each case.

The compound *4* showed exceptionally low NH frequency in the IR and showed a NH signal at low field in the NMR spectrum. The amide group is known [20] to form very strong intermolecular hydrogen bonds, and it was therefore assumed that the low NH frequency in the IR and the low field signal in the NMR spectrum were due to intra molecular hydrogen bonding from the amide group.

Compounds *5* and *6* existed in the form of both isomers in the ratio 9:1 as determined by the methyl signals in the NMR spectrum. Using Karabatsos' methods [21,22] the isomer making the greatest contribution in each case

Table 1. Spectral data and electron density of 2,4-dinitrophenylhydrazones.

No.	Tentative assignment X [a]	Y [a]	δ_{NH} [b]	q_H	ν_{NH}/cm^{-1}
1a	H	NO_2	14.333	0.7530	3241
1b	NO_2	H	11.666	0.8173	3283
2	NO_2	CH_3	11.000	0.8219	3302
3	NO_2	CH_3CH_2	11.166	0.8215	3304
4	NO_2	$CONH_2$	14.466 [c]	0.7543	[d]
5	NO_2	$COCH_3$	14.000	0.7706	3160
6	NO_2	CO_2CH_3	13.499	0.7675	3204
7	NO_2	NO_2	13.466	0.7436	3222
8	NO_2	F	11.350	0.8100	3261
9	NO_2	Cl	11.283	0.8095	3267

[a] X and Y refers to I. [b] ppm from internal TMS. [c] Uncertain by ±0.2 ppm due to a very weak and broad signal. [d] Seems to come as an asymmetry of CH(arom) stretch frequency.

was determined to be the *anti*-nitro compound. No NH signal of the *syn*-nitro isomers was detected due to the low concentration.

Compounds *8* and *9* gave NH absorptions at high frequencies in the IR and at high field in the NMR spectrum and, compared with compound *1*, it was assumed that these compounds existed as *syn*-halo isomers. This conclusion is supported by the fact that the *syn*-iodo isomer of 2-iodo-2-phenylhydrazonoacetonitrile could not be rearranged [23] into the *anti*-iodo isomer, which was expected to be the more stable form for steric reasons.

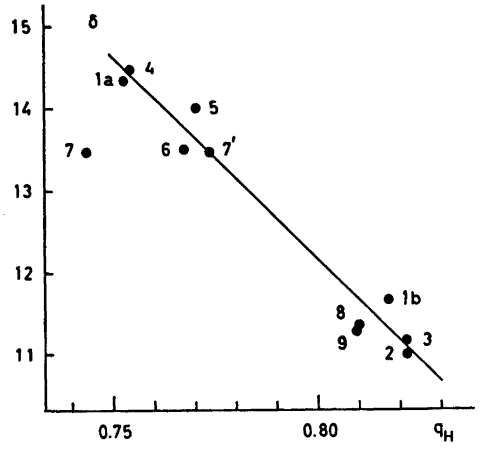


Fig. 1. Correlation of the NH proton shift (δ *vs.* the electron density (q_H) as calculated by the CNDO/2 method. The equation of the line is: $\delta = 51.86 - 49.7q_H$ where S.D. in the constant is 0.95, in the slope 3.6 and in δ 0.266.

When the configurations were assigned, the chemical shift was plotted against the electron density as shown in Fig. 1.

In spite of a number of uncertain factors (see below) the points lie close to a straight line. The relatively large discrepancy, in the case of compound *7*, may be the result of steric interaction between the nitro groups in the carbonyl moiety. As a result of this, one or both of the groups will be twisted somewhat out of the plane of conjugation. Calculations show that a twisting of the *syn*-nitro group of 30° out of the mesomeric plane leads to an increase in electron density such that the point for this conformer lies on the line (*7'*, Fig. 1).

The scatter of the points about the line can be the result of a series of factors: failure in the description of the real geometry, non-linear electric field effects,[24,25] bond anisotropic effects from groups in the neighbourhood of the NH hydrogen,[26] overestimation of charge dipole sigma polarization [27] and solvation effects as the shifts are not corrected to the gas phase due to the low solubility of the compounds. Such correction to the gas phase does not, however, necessarily lead to a better result.[8]

Nelson *et al.*[2] suggest that the geometry is a critical factor, since small differences in geometry (*ca.* 0.02 Å) can lead to "discrepancies comparable to the calculated charge densities". In the present work a different set of coordinates was used in addition to the coordinates determined by the COORD program (see experimental). These were found by geometrical constructions from standard bond lengths and

angles. Differences between the two sets were at most 0.07 Å, and this led to differences in electron density about the NH hydrogen of up to 0.0362. The points were still grouped around a straight line and had a correlation coefficient of 0.97. This suggests that the difference in geometry is not critical with respect to the linearity of the correlation, even if the individual points are somewhat changed in position.

Bond anisotropic effects are difficult to judge quantitatively as a result of the uncertain data available for bond susceptibilities.[28]

Linear regression (compound *7* excluded) gave $r = 0.98$. The slope of the line is 3060 Hz/electron or 50 ppm/electron. This is higher than the previously calculated scale-factors of 26.6 ppm/electron for the shielding of a proton in the hydrogen molecule,[29] and 17.8 ppm/electron for diamagnetic shielding in a hydrogen atom.[30] Sebastian and Grunwell [15] found a slope of 23.4 ppm/electron, while Hamer *et al.*[11,12] found values from 17.0 ppm/electron to 23.2 ppm/electron.

However, the slope of the line is sensitive to changes in geometry. Thus a value of 30.6 ppm/electron was found when using the second coordinate set (see above).

Lazzaretti and Taddei [14] give no slope values in their work, but by using their data it is possible to calculate a slope of about 130 ppm/electron in the series where the substituents belong to the second row of the periodic system. This large difference from the previously stated values can indicate that the semi-empirical method of del Re which Lazzaretti and Taddei used, underestimates charge differences.

The investigation of Sterk and Holzer [16] shows considerable differences in slope values as various semi-empirical methods are employed. In their correlation A1 they found a slope of 12.0 ppm/electron as calculated by MINDO/2, 13.9 ppm/electron by INDO, 1.9 ppm/electron by EHT and 21.4 ppm/electron as calculated by CNDO. The CNDO value is in excellent agreement with the previously stated values.[11,12,15,29,30]

In the work of Ohkubo *et al.*[17] no slope values are given, but one could calculate values ranging from ~12 ppm/electron to ~200 ppm/electron for the OH proton. Ohkubo *et al.* give no explanation of this great variation.

On the basis of the good correlation and the reasonable value of the slope, the conclusion is drawn that the chemical shift of the NH proton is mainly determined by its electron density.

An interesting aspect of the correlation is that it may be used in *syn/anti* assignment of hydrazones where a relatively large electron density variation about the NH hydrogen atom takes place, *cf.* compounds *1a* and *1b*. This will be further investigated in a following paper.

EXPERIMENTAL

Synthesis of the compounds NO_2-CH_2-X ($X = H$, CH_3, CH_3-CH_2, $CONH_2$, $COCH_3$, CO_2CH_3, NO_2). Nitromethane, nitroethane and 1-nitropropane were obtained from commercial sources (Fluka A.G.). The various steps in the synthesis of nitroacetamide,[31–33] nitroacetone,[34,35] methyl nitroacetate [31,32] and dinitromethane [36,37] are described in the literature.

Diazo couplings. For $X = H$, CH_3 and CH_3CH_2 a procedure given in Organic Reactions [38] was followed. In all other cases the method described by Juvvik and Solheim [39] was used. The hydrazones so formed were purified by recrystallization from a water/acetic acid mixture, or by preparative TLC.[40]

Compounds *8* and *9* were synthesized by allowing *7* to react with excess of the respective halogen acid in a dimethyl sulfoxide:water (2:1, v:v) solution. At the completion of the reaction the mixture was diluted with water and the product extracted with ethyl acetate and purified by preparative TLC.

Infrared spectra of the compounds dissolved in tetrachloroethylene (E. Merck, Uvasol) in 4.00 cm cells were obtained on a Unicam SP 200G instrument.

NMR spectra of the compounds dissolved in trichloroacetonitrile (EGA, 99 % purity) were recorded on a JEOL Minimar 60 or a JEOL HNM-C-60H high resolution instrument, both operating at 60 MHz. The position of the various signals was measured by a Hewlett-Packard 5326 A frequency counter in the frequency sweep mode and internal lock to the TMS signal.

The quantum mechanical calculations were performed using a modified CNDO/2 program.[41] The program uses *s* and *p* Slater orbitals for all atoms except hydrogen where only the *s* orbital is used. All parameters were taken from the original papers.[42,43]

Molecular geometry. On the basis of X-ray crystallographic data,[44–46] the bond length N–N was set at 1.40 Å. The element –NH–N=C was considered to be co-planar with the 2,4-dinitrophenyl ring.[44,45] The bond length

C–Cl was set at 1.73 Å.[47] In all other cases standard bond lengths and angles were used.[48] The actual transformations to coordinates were performed by the program COORD.[49]

The CNDO/2 and the COORD calculations were undertaken on a Univac 1110 computer.

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Electrochemical Reductive Alkylation of Quinolines and Isoquinolines

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The reaction between electrochemically generated anion radicals of quinolines and isoquinolines and *t*-butyl halides gives, *via* an electron transfer reaction and formation of *t*-butyl radicals, *t*-butylated heterocyclic compounds. A large fraction of the alkylation takes place in the carbocyclic ring. The mechanism is discussed in light of the product distribution.

Electrochemical generation of anion radicals in an aprotic medium, such as *N*,*N*-dimethylformamide (DMF), in the presence of an alkyl halide may result in a reductive alkylation of the parent compound.[1–3] The coupling has been proposed to occur between an alkyl radical, formed by cleavage during electron transfer from the anion radical to the alkyl halide, and the parent compound or its anion radical.

In liquid ammonia quinoline [4] has been reductively alkylated cathodically with the formation of approximately equal amounts of 1,2- and 1,4-dialkyldihydroquinoline. The reaction was found to proceed *via* an ECEC-mechanism.

The study of the *t*-butylation of quinoline and isoquinoline derivatives is part of a more general investigation of the preparative aspects of the electrochemical alkylation reaction. A knowledge of the substitution pattern may also be of interest for a discussion of the mechanism of the coupling reaction.

In the investigation are included isoquinoline (*1*), 3-methylisoquinoline (*2*), quinoline (*3*), and 2-methylquinoline (*4*).

RESULTS

Voltammetry. The compounds *1* to *4* were investigated by cyclic voltammetry in DMF/0.1 M TBAI (tetrabutylammonium iodide) with suspended alumina using sweep-rates from 10 to 400 mV/s. All of the compounds gave one reversible wave, even at low sweep rate; the shape of the anodic peak was, however, often distorted at potentials less negative than the anodic peak potential due to adsorption phenomena.

On addition of *t*-butyl chloride to *1* the height of the first wave (at −2.18 V *vs.* aq. SCE) increased somewhat and reached a limit with about 50 % increase; at the same time a new wave at −2.55 V (Fig. 1) grew up to a

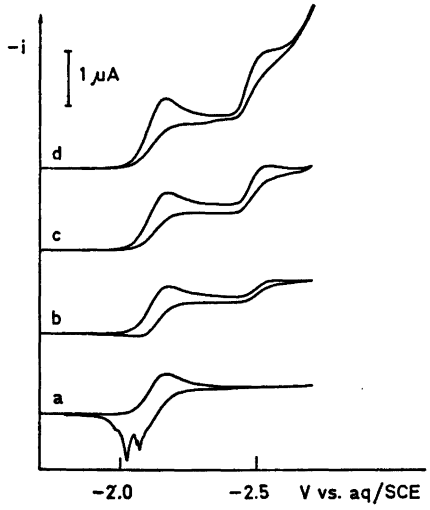


Fig. 1. Cyclic voltammograms of isoquinoline (2.5×10^{-3} M) in DMF/0.1 M TBAI in the presence of *t*-butyl chloride: a, 0; b, 3.2×10^{-3} M; c, 1.3×10^{-2} M; d, 6.5×10^{-2} M. Sweep rate 40 mV/s.

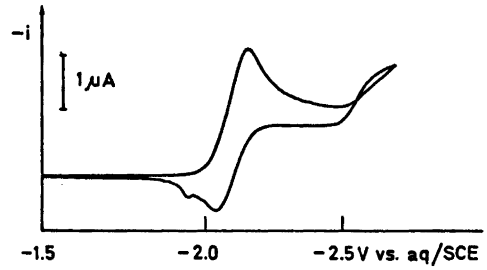


Fig. 2. Cyclic voltammogram of quinoline (2.5×10^{-3} M) in the presence of *t*-butyl chloride (1.3×10^{-2} M). Sweep rate 400 mV/s.

height slightly less than the first one; voltammetry of 6-*t*-butyl-5,6-dihydroisoquinoline showed a peak at −2.55 V. *2*, *3*, and *4* exhibited quite similar behaviour on addition of *t*-butyl chloride.

All the compounds show, at a certain speed rate and in a certain concentration interval of *t*-butyl chloride, a special behaviour illustrated in Fig. 2. At a suitable concentration of *t*-butyl chloride the reverse sweep crosses the forward sweep; a similar phenomenon has been found in cases [5] involving a slow rate-limiting step in the mechanism and has been proposed [6] to be connected with the coupling reaction, in which is formed the electroactive product causing the second wave.

A voltammetric experiment was performed in which naphthalene was added to a solution containing *1* and *t*-butyl chloride in a molar ratio of 1:8. The addition of naphthalene had only a small effect which, if anything, leads to a decrease of the peak height of *1*.

Table 1. Product distribution (isolated yield) in the electrochemical *t*-butylation in DMF containing tetrabutylammonium iodide (TBAI) of *1* (isoquinoline) and *2* (3-methylisoquinoline).

Position of *t*-Bu group	From *1*		From *2*	
	Product	Yield %	Product	Yield %
1	*8*	17	*12*	17
4			*13*	9
5	*7*	19	*10*	3
6	*5*	35	*9*	40
	6	5		
8			*11*	5

Table 2. Product distribution (isolated yield) in the electrochemical *t*-butylation in DMF/TBAI of *3* (quinoline) and *4* (2-methylquinoline).

Position of *t*-Bu group	From *3*		From *4*	
	Product	Yield %	Product	Yield %
2	*14*	16.5		
3/4			*20*	10.5
4	*15*	9.5	*21*	15.5
5	*16*	6	*22*	12
7	*17*	16	*23*	14.5
8			*24*	3.5
Not butylated	*18*	11		
	19	10		

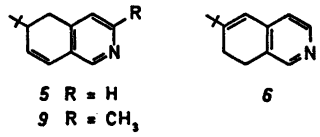
Electrolyses. In Table 1 are given the isolated yields of the products obtained on electrochemical *t*-butylation in DMF of isoquinoline (*1*) and 3-methylisoquinoline (*2*); in Table 2 are given similar data from the reductive alkylation of quinoline (*3*) and 2-methylquinoline (*4*). In Experimental are summarized the main arguments for the proposed structures of the products.

In a competitive alkylation experiment a mixture of 1 g of isoquinoline and 5 g of naphthalene was reduced at −2.2 V (SCE) in the presence of 10 ml of *t*-butyl chloride; extraction of naphthalene and its derivatives from the acidified diluted aqueous DMF with light petroleum separated the neutral fraction from the nitrogen bases. No *t*-butylated naphthalene derivatives were detected by ^{1}H NMR spectroscopy or MS of the neutral fraction.

DISCUSSION

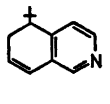
Polarography and cyclic voltammetry of *3* in acetonitrile [7] and liquid ammonia [4] and of *1*, *3*,[8] *2*, and *4*[9] in DMF have been reported. The first reduction leads to the anion radical; $1^{\cdot-}$ in DMF is more stable than $3^{\cdot-}$,[8] which dimerizes faster.

This is in agreement with the preparative results; dimerized products have been isolated (*19*) from the reductive alkylation of *3*, but not from *1*. The dimerization of $3^{\cdot-}$ is, however, not the only reason for its relative instability; on removal of most of proton donor impurities by addition of active alumina a

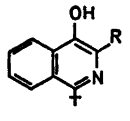


5 R = H
9 R = CH_3

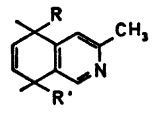
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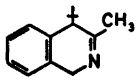
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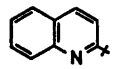
8 R = H
12 R = CH_3



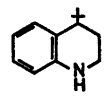
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R' = H
11 R = H
R' = t-Bu



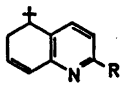
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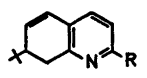
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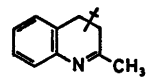
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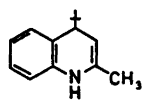
16 R = H
22 R = CH_3



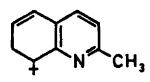
17 R = H
23 R = CH_3



20



21



24

18 1,2,3,4-Tetrahydroquinoline. 19 Dimer of 18.

reversible wave of *3* is found even at slow sweep rates.

In liquid ammonia the alkylation of quinoline anion radical [4] by primary alkyl bromides has been found to follow an ECEC-mechanism, whereas the reductive alkylation in DMF of aromatic and unsaturated compounds has been discussed in terms of coupling between an alkyl radical and an anion radical and/or the substrate.[1–3]

The two most probable reaction routes may be represented by eqns. (1) to (6), in which A is the heteroaromatic compound and BX is *t*-butyl chloride.

$$A + e^- \rightleftharpoons A^{\cdot -} \quad (1)$$

$$A^{\cdot -} + BX \rightleftharpoons A + [BX]^{\cdot -} \quad (2)$$

$$BX^{\cdot -} \rightarrow B^{\cdot} + X^- \quad (3)$$

$$B^{\cdot} + A^{\cdot -} \rightleftharpoons A + B^- \quad (4)$$

$$B^{\cdot} + A^{\cdot -} \rightarrow AB^- \quad (5)$$

$$B^{\cdot} + A \rightarrow AB^{\cdot} \quad (6)$$

The product distribution in reactions involving radical attack on quinoline and isoquinoline depends on several parameters.[10] In acid solution the protonated hetero ring is pref-

erentially attacked by *t*-butyl and other alkyl radicals,[11] quinoline in the 2 and 4 positions, isoquinoline in position 1. Homolytic methylation of quinoline in excess of quinoline with *t*-butylperoxide or lead tetraacetate showed the following[12] order of positional reactivities $8 > 4 > 5 > 2$ and this agrees with the reactivities in the phenylation of quinoline.[13] Isoquinoline was methylated in low yield under these conditions and only 1-methylated products were isolated.[12]

A slightly different positional reactivity was found in the radical methylation of quinoline using Fenton's reagent in DMSO,[14] here the relative yields in positions 2:4:unknown were 3:2:4. In none of these investigations are the observed positional reactivities in agreement with those predicted by free valence numbers [15] or atom localization energies.[16]

A somewhat better agreement between experiment and theory was found [17] in the phenylation of *3* by benzoyl peroxide in excess of *3*. The positional reactivities were found to be $8 > 5 > 4 > 2 > 3 > 6 \geq 7$, which is qualitatively in agreement with that predicted [17] by the ω^*-method, $8 \geq 5 > 4 > 2 > 3 > 6 \geq 7$.

A comparison of the results described above of radical attack on *1* and *3* with those in Tables 1 and 2 does not give support to the assumption that a radical attack on the heterocyclic compound takes place.

Product distribution in a reaction between alkyl radicals and anion radicals of quinoline or isoquinoline is not known; in the reductive *t*-butylation of pyrene [2] the positional reactivity followed the pattern of the hyperfine coupling constants in the ESR spectrum of pyrene anion radicals, but a comparison of the results in Tables 1 and 2 with the ESR spectra [18,19] of quinoline and isoquinoline anion radical in HMPA reveals no apparent connection.

A competition for methyl radicals showed [12] that isoquinoline is 1.5 times as effective as naphthalene in the methylation reaction. The electrochemical *t*-butylation favours isoquinoline with a much higher factor, > 100. At least two explanations are possible. The *t*-butyl radical could be so much more stable than the methyl radical that a much higher selectivity is obtained. This explanation must be confronted with the fact that the relatively unreactive (compared to methyl radicals) phenyl radicals attack *1* or *3* preferentially to the more easily reducible nitrobenzene, but not by a high selectivity factor.[20] Another explanation is that the coupling reaction occurs between the *t*-butyl radical and an anion radical. The difference in reduction potential between isoquinoline and naphthalene (*25*) is about 0.35 V which is enough to ensure that at the reduction potential of *1* the concentration of $25^{\cdot-}$ is very low; the equilibrium constant for the reaction: $1^{\cdot-} + 25 \rightleftharpoons 1 + 25^{\cdot-}$ is about 10^{-3}.

This is in agreement with the voltammetric competition experiment. If the *t*-butyl radicals attacked *25* a smaller part of *1* would have been attacked and thus removed from the catalytic circle. The fact that no increase in the peak height of *1* is observed on addition of *25* is consistent with the results of the preparative competition experiment.

Although not conclusive, the results from this investigation thus mostly favour the mechanism by which the coupling occurs between the alkyl radical and the aromatic anion radical.

Preparatively, the value of the method is that it opens possibilities for a preparation in a simple way of a number of compounds not easily accessible in other ways.

EXPERIMENTAL

General procedure for electrolysis. The substrate (*1*–*4*, 2 g) was reduced at −2.2 V (aq. SCE) in 160 ml of DMF/0.1 M TBAI at a mercury pool electrode in the presence of 10 ml *t*-butyl chloride. After completion of the reduction the catholyte was acidified to pH 2–3 with 4 N aqueous HCl and the major part of the solvent evaporated *in vacuo*. The residue was made alkaline with an aqueous sodium carbonate solution and extracted several times with ether, which after washing with water and drying over potassium carbonate was evaporated. The residue was separated by column chromatography.

Isoquinoline, $n = 2.5$. Isolated was 2.25 g crude product. The residue was separated on silica using diethyl ether as eluent. The compounds were eluted in the order: *8*, *7*, *5*, *6*.

6-t-Butyl-5,6-dihydroisoquinoline, *5*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.92 (9 H, s); 2.0–2.9 (3 H, m); 6.12 (1 H, dd, 10 Hz, 3 Hz); 6.57 (1 H, dd, 10 Hz, 2 Hz); 7.04 [1 H, d(br) 5 Hz]; 8.2–8.5 (2 H, m). UV (96 % ethanol): λ_{max} 261 nm, log ε 4.02, $\lambda_{shoulder}$ 288 nm, log ε 3.63. The choice between a 5,6- or 7,8-dihydroisoquinoline was made from the UV spectrum; during this investigation it has been observed

that 5,6-dihydroisoquinolines and 7,8-dihydroquinolines have $\lambda_{max} = 265 \pm 4$ nm, 5,6-dihydroquinolines at about 295 nm and 7,8-dihydroisoquinolines about 275 nm.

6-t-Butyl-7,8-dihydroisoquinoline, *6*, liquid. ^{1}H NMR ($CDCl_3$): δ 1.12 (9 H, s); 2.0–2.9 (4 H, m); 6.27 [1 H, s(br)]; 6.92 [1 H, d(br) 5 Hz]; 8.22 [1 H, s(br)]; 8.32 [1 H, d(br), 5 Hz]. UV (96 % ethanol): λ_{max} 273 nm, log ε 4.34; λ_{sh} 295 nm, log ε 3.60.

5-t-Butyl-5,6-dihydroisoquinoline, *7*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.84 (9 H, s); 2.4–3.3 (3 H, m); 5.9–6.5 (2 H, m); 6.8–7.1 (1 H, m); 8.2–8.5 (2 H, m). UV (96 % ethanol): λ_{max} 263 nm, log ε 3.94; λ_{sh} 295 nm, log ε 3.30.

1-t-Butyl-4-hydroxyisoquinoline, *8*, m.p. > 300 °C (from chloroform/light petroleum). ^{1}H NMR [$(CD_3)_2CO$]: δ 1.62 (9 H, s); 7.4–7.8 (2 H, m); 8.08 (1 H, s); 8.25–8.65 (2 H, m). IR (KBr), cm^{-1} (intensity): 3300–2300 (s), 1625 (w), 1590 (s), 1335 (s), 1235 (m), 1075 (m), 990 (m), 872 (m), 770 (s). MS [*m/e* (%)]: 201 (12), 200 (17), 186 (28), 159 (63), 145 (13), 111 (13), 97 (28), 95 (22), 83 (39), 81 (30), 69 (70), 57 (74), 55 (74), 43 (100), 41 (91).

8 is probably formed from the initially obtained product, 1-*t*-butyl-1,4-dihydroisoquinoline; this compound reacts as an enamine with oxygen to a hydroperoxide which under influence of base forms *8*.

3-Methylisoquinoline, $n = 2.1$, 2.2 g of product. The residue was separated on silica using diethyl ether as eluent. The compounds were eluted in the order: *12*, *11*, *9*, *10*, *13*.

6-t-Butyl-5,6-dihydro-3-methylisoquinoline, *9*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.92 (9 H, s); 2.48 (3 H, s); 2.0–2.9 (3 H, m); 6.03 (1 H, dd, 10 Hz, 3 Hz); 6.53 (1 H, dd, 10 Hz, 2 Hz); 6.90 [1 H, s(br)]; 8.13 [1 H, s(br)]. UV (96 % ethanol): λ_{max} 262 nm, log ε 3.95; λ_{sh} 290 nm, log ε 3.48. The choice between a 5,6-dihydro- and a 7,8-dihydroisoquinoline came from the ^{13}C NMR spectrum; the signals from the quaternary carbon atoms (C-4a and C-8a) were identified through selective decoupling of H-4, respectively H-1. Long-range couplings from the methylene protons (H-5) and ethylenic proton (H-8) to the quaternary carbon atoms proved the structure.

5 (or 8)-t-Butyl-5,8-dihydro-3-methylisoquinoline, *10*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.87 (9 H, s); 2.50 (3 H, s); 2.4–2.7 (1 H, m); 3.0–3.3 (2 H, m); 6.05–6.15 (2 H, m); 6.92 [1 H, s(br)]; 8.31 [1 H, s(br)].

8 (or 5)-t-Butyl-5,8-dihydro-3-methylisoquinoline, *11*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.88 (9 H, s); 2.51 (3 H, s); 2.4–2.6 (1 H, m); 3.0–3.3 (2 H, m); 6.0–6.2 (2 H, m); 6.93 [1 H, s(br)]; 8.30 [1 H, s(br)]. UV (96 % ethanol): λ_{max} 265 nm, log ε 3.0. MS [*m/e* (%)]: 201 (1), 145 (47), 144 (100), 143 (17), 115 (17), 57 (23), 41 (20).

10 and *11* move differently on TLC, but their spectra are very similar and there is no evidence available to decide whether it is *10* or *11* that is substituted at C-5.

1-t-Butyl-4-hydroxy-3-methylisoquinoline, *12*, ^{1}H NMR ($CDCl_3$): δ 1.57 (9 H, s); 2.53 (3 H, s); 7.0 [1 H, s(br)]; 7.3–7.5 (2 H, m); 8.1–8.4 (2 H, m). IR (KBr) cm^{-1} (intensity): 3600–2200 (s), 1647 (s), 1620 (s), 1495 (m), 1375 (m), 1355 (m), 1295 (m), 1240 (m), 940 (m), 782 (s). UV (96 % ethanol): λ_{max} 332 nm, log ε 3.48; λ_{max} 285 nm, log ε 3.40. Hydrochloride, m.p. 193–195 °C, contained one mol of water of crystallization according to the elementary analysis. MS [*m/e* (%)]: 215 (19), 214 (23), 200 (39), 173 (100), 159 (20), 130 (20), 115 (23), 77 (26), 69 (19), 57 (33), 55 (37), 43 (53), 41 (53).

12 is probably formed in a similar manner from 1-*t*-butyl-1,4-dihydro-3-methylisoquinoline as *8* from its precursor. *12* is not stable in solution and is in nonpolar solution easily transformed under influence of diffuse daylight by uptake of 1 mol oxygen to the 1-hydroperoxide (*26*), which is stable in the crystalline state. ^{1}H NMR of *26* ($CDCl_3$): δ 0.86 (9 H, s); 2.36 (3 H, s); 7.3–8.2 (4 H, m); 12.2 (1 H, br.s). IR (KBr) cm^{-1} (intensity): 3200–2800 (s), 1680 (s), 1645 (m), 1600 (m), 1455 (m), 1255 (m), 1205 (m), 1187 (m), 1050 (m), 932 (m), 748 (s).

4-t-Butyl-1,4-dihydro-3-methylisoquinoline, *13*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.94 (9 H); 2.2–2.3 (3 H, m); 3.30 (1 H, t, 1.5 Hz); 4.6–4.8 (2 H, m); 7.1–7.3 (4 H, m). MS [*m/e* (%)]: 201 (2), 186 (3), 145 (76), 144 (100), 143 (25), 115 (21), 77 (11), 57 (52), 41 (32).

Quinoline, $n = 2.0$. The residue was separated on silica using diethyl ether as eluent and eluted in the order (*14*+*15*), *18*, *19*, *17*, *16*. *14* and *15* were separated on a column of silica using a mixture of 20 % ether and light petrol.

2-t-Butylquinoline, *14*, liquid. ^{1}H NMR ($CDCl_3$): δ 1.45 (9 H, s), 7.3–8.25 (6 H, m); identical with the published spectrum.[21] Probably obtained from the dihydro compound by oxidation during work-up.

4-t-Butyl-1,2,3,4-tetrahydroquinoline, *15*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.93 (9 H, s); 1.4–2.3 (2 H, m); 2.4–2.6 (1 H, dd, 5 Hz, 2.5 Hz); 3.20–3.45 (2 H, m); 3.65 [1 H, s(br)]; 6.30–6.70 (2 H, m); 6.85–7.15 (2 H, m). The tetrahydroderivative is probably obtained because the primarily formed 1,4-dihydroquinoline derivative rearranges into the more easily reducible 3,4-dihydro derivative, which is a cyclic derivative of an aldehyde; if the *t*-butyl group were in the 2-position, the azomethine compound would be a derivative of a ketone and thus less easily reducible.

5-t-Butyl-5,6-dihydroquinoline, *16*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.83 (9 H, s); 2.1–2.95 (3 H, m); 6.1–6.85 (2 H, m); 6.98 (1 H, dd, 7.5 Hz, 5 Hz); 7.35 (1 H, dd, 7.5 Hz, 2 Hz); 8.48 (1 H, dd, 5 Hz, 2 Hz). UV (96 % ethanol): λ_{max} 250 nm, log ε 4.18; λ_{max} 293 nm, log ε 3.95. The assignment of the structure is not definite;

however, the coupling pattern of the ethylenic protons is best accounted for by assuming a methylene group at C-6, but the signals were not well enough resolved to allow an unambiguous assignment.

7-t-Butyl-7,8-dihydroquinoline, *17*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.90 (9 H, s); 2.0–2.7 (1 H, m); 2.8–3.2 (2 H, m); 6.02 (1 H, dd, 10 Hz, 3 Hz); 6.43 (1 H, dd, 10 Hz, 2 Hz); 6.98 (1 H, dd, 7.5 Hz, 4.5 Hz); 7.22 (1 H, dd, 7.5 Hz, 2 Hz); 8.30 (1 H, dd, 4.5 Hz, 2 Hz). UV (96 % ethanol), broad maximum at λ 265 nm, log ε 4.15.

1,2,3,4-Tetrahydroquinoline, *18*, was identified through its NMR spectrum.[22]

Dimer of 1,2,3,4-Tetrahydroquinoline, *19*. ^{1}H NMR ($CDCl_3$): δ 1.3–2.1 (2 H, m); 2.4–2.9 (3 H, m); 3.5–3.95 (1 H, m); 4.25 [1 H, s(br)]; 6.35–7.20 (4 H, m).

Reduction of *2-methylquinoline*, $n = 2.2$. The residue, 2.3 g, was separated on a silica column using acetone (20 %)–light petroleum (80 %) as eluent. Products were eluted in the order: (*24* + *23* + *22*), *20*, *4*, *21*. *22* + *23* + *24* was separated on a similar column using acetone (8 %)–light petroleum (92 %) as eluent.

3 (or 4)-t-Butyl-3,4-dihydro-2-methylquinoline, *20*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.78 (9 H, s); 3.32 (3 H, s); 2.1–2.5 (1 H, m); 2.85–3.0 (2 H, m); 7.0–7.4 (4 H, m). The pattern in the ^{1}H NMR between δ 2 and 3 looks like that of *5*, *9*, *17*, and *23*, which would suggest the *t*-butyl group in the 3-position, but the argument does not carry sufficient weight to prove the structure.

4-t-Butyl-1,4-dihydro-2-methylquinoline, *21*, m.p. 121–123 °C. ^{1}H NMR ($CDCl_3$): δ 0.83 (9 H, s); 2.29 (3 H, s); 2.62 (1 H, d, 1.5 Hz); 4.11 (1 H, d, 1.5 Hz); 7.1–7.4 (4 H, m). IR (KBr) cm^{-1} (intensity): 3250 (s), 2950 (m), 1640 (m), 1475 (m), 1425 (m), 1045 (m), 1003 (m), 991 (m), 778 (m), 758 (s).

The compound is unstable and the real yield may be somewhat higher than the isolated one given in Table 2.

5-t-Butyl-5,6-dihydro-2-methylquinoline, *22*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.82 (9 H, s); 2.48 (3 H, s); 2.3–3.5 (3 H, m); 6.05–6.30 (1 H, m); 6.52 (1 H, dtr, 9 Hz, 1.2 Hz); 6.83 (1 H, d, 8 Hz); 7.23 (1 H, d, 8 Hz). UV (96 % ethanol): λ_{max} 297 nm, log ε 3.95.

7-t-Butyl-7,8-dihydro-2-methylquinoline, *23*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.92 (9, H, s); 2.50 (3 H, s); 2.1–2.6 (1 H, m); 2.85–3.20 (2 H, m); 5.98 (1 H, dd, 10 Hz, 3 Hz); 6.43 (1 H, dd, 10 Hz, 2 Hz); 6.90 (1 H, d, 7.5 Hz); 7.17 (1 H, d, 7.5 Hz). The position of the *t*-butyl group at C-7 rather than at C-6 was found through selective decoupling in the ^{13}C NMR spectrum in a similar manner as described for *9*.

8-t-Butyl-7,8-dihydro-2-methylquinoline, *24*, liquid. ^{1}H NMR ($CDCl_3$): δ 0.89 (9 H, s); 2.50 (3 H, s): 2.60–2.75 (3 H, m): 5.6–6.05 (1 H, m): 6.30 (1 H, dtr, 10 Hz, 1.5 Hz), 6.92 (1 H, d, 8 Hz); 7.18 (1 H, d, 8 Hz).

Competition experiment. Isoquinoline (1 g) and naphthalene (5 g) were reduced at -2.2 V in the presence of 10 ml *t*-butyl chloride, $n = 2.4$ (calculated for the reduction of isoquinoline). After the reduction was completed the catholyte was diluted with water, acidified and extracted with light petroleum, which was washed with water, dried and evaporated. The residue was investigated by NMR, GLC and mass spectroscopy. The aqueous phase was made alkaline, extracted three times with ether, which was washed with water and evaporated. The NMR of the residue resembled that obtained in reductive alkylations in the absence of naphthalene.

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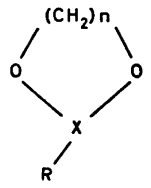
Synthesis and Spectral Characterization of 2-Oxo-1,3,2-dioxarsenanes

ARILD J. DALE [a] and PAUL FRØYEN [b]

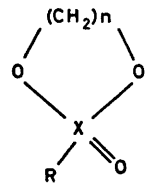
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[b] Department of Chemistry University of Oslo, Blindern, Oslo 3, Norway

This paper reports a synthesis of 2-oxo-1,3,2-dioxarsenanes from acyclic arsonic acid esters and 2,2-dimethyl-1,3-propanediol. Two of the compounds were also obtained by addition of the diol to arsonic acid dichlorides in the presence of pyridine. NMR spectroscopic behaviour of the compounds is consistent with conformational preference of axially oriented As=O. Attempts to prepare 2-oxo-1,3,2-dioxarsolanes were unsuccessful. The high chemical reactivity and thermodynamic instability of these compounds is explained in terms of an arsonale effect.

While tervalent phosphorus and arsenic compounds *1a* (X=P, As) have been known for a long time, the corresponding 2-oxo compounds *1b* have been prepared and studied for X=P only.



1a



1b

The chemistry of the latter compounds shows great variety and has been thoroughly investigated in later years.[1,2]

The preparation of the corresponding arsenic compounds (*1b*, X=As) would be of considerable interest, particularly since these offer a possibility to test whether the models,[2] by which the chemical behaviour of *1b*, X=P has been interpreted, also apply to corresponding arsenic-containing heterocycles. Another aspect which has been given some attention [3] is the conformation which these molecules (*1b*, X=P) adopt, in solution and in the solid state. Conformational studies on tervalent arsenic compounds have lately appeared in the literature.[4] It is clearly of interest to extend these studies to include the conformational behaviour of the pentavalent arsenic compounds *1b*.

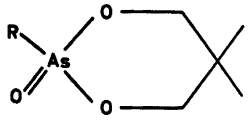
We now report the preparation and spectral characterization of three 2-oxo-1,3,2-dioxarsenanes. We also give the results from attempted preparations of the corresponding 2-oxo-1,3,2-dioxarsolanes.

RESULTS AND DISCUSSION

Possible methods for the preparation of the cyclic esters *1b*, X=As are: (1) Direct esterification of the appropriate arsonic acid with diols, (2) reaction of arsonic acid dihalides with diols in the presence of a suitable base, (3) oxidation of the corresponding tervalent compounds, (4) reaction between dihalides and the disilver salt of an arsonic acid, and (5) transesterification of acyclic arsonates with diols.

From the fact that arsoranes like R—As $(OCH_3)_4$ very readily undergo exchange reactions with 1,2- and 1,3-diols with the formation of bis-ring compounds,[5] we figured that 2-oxo-1,3,2-dioxarsenanes could possibly be prepared by allowing the acyclic arsonates R—AsO $(OCH_3)_2$ to react with a 1,3-diol (method 5).

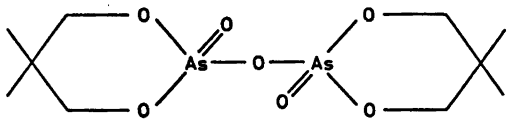
This synthetic route proved to be successful, no formation of the spirocyclic compound being observed. From 2,2-dimethyl-1,3-propanediol and $R-AsO(OCH_3)_2$ ($R=CH_3$ Ph, OCH_3) compounds *2a*, *2b* and *2c* were obtained. While *2a* and *2b* could be distilled at reduced pressure, attempted distillation of *2c* led to the formation of a high boiling substance.



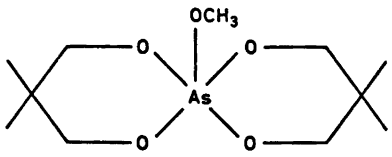
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2a, $R=CH_3$; *2b*, $R=Ph$; *2c*, $R=OCH_3$.

Its ^1H NMR spectrum was in agreement with structure *3*. The tendency of the $As-OCH_3$ grouping to form an

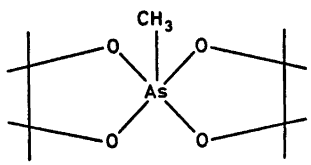


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4

$As-O-As$ bond was recently observed [5] for the oxyarsorane *4*. There is also some evidence that phosphorus compounds akin to *2c* undergo a similar reaction, forming pyrophosphates.[6,5]



5

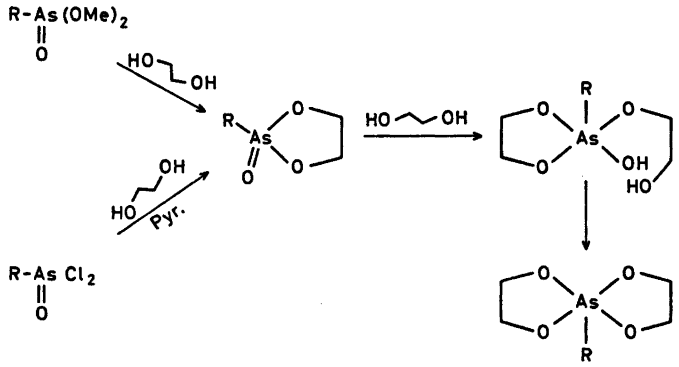
Method 5 was also investigated as a possible route to 2-oxo-1,3,2-dioxarsolanes. Treatment of methyl dimethylarsonate with 2,3-dimethylbutane-2,3-diol led, however, to the spirocyclic compound *5*.

Direct esterification of arsonic acids with 1,2-diols is known to yield the corresponding bis-ring compounds as sole products.[8] Method 3 (oxidation) was lately attempted,[9] but SeO_2 oxidation of 2-methyl-1,3,2-dioxarsolanes led to methylarsonic acid anhydride together with the bis-ring compounds. The reaction took the same course with dioxarsolanes derived from ethane-1,2-diol, butane-2,3-diol, and 2,3-dimethylbutane-2,3-diol. Addition of a 1,2-diol to the reaction mixture led to incorporation of that diol in the resulting spirocyclic compound, and this observation was taken as evidence for the intermediate formation of a cyclic arsonic acid ester.

In another synthetic approach (method 2) we reacted phenylarsonic acid dichloride with 1,2-diols in the presence of pyridine, but once more distillation provided the spirocyclic compounds. Also method 4 failed to give any of the desired 2-oxo-1,3,2-dioxarsolanes.

Some evidence for the formation of a 2-oxo-1,3,2-dioxarsolane was obtained, however, from GLC/MS analysis of the reaction mixture from methyl dimethylarsonate and 2,3-dimethylbutane-2,3-diol in 1:1 molar ratio. Apart from observing the bisring compound, a product giving ions at $m/e=222$ (M^+), 221 (M^+-H), 207 (M^+-CH_3) and 149 ($M^+-CH_3-C_3H_6O$) was observed. The present findings indicate that 2-oxo-1,3,2-dioxarsolanes may be prepared, but that they are unstable both in the chemical and thermodynamic sense. These results were by no means unexpected considering the high chemical reactivity of the corresponding 2-oxo-1,3,2-dioxaphospholanes and the relatively low bond energy for $As=O$ as compared to $P=O$ (390 and 650 kJ mol^{-1}, respectively). It must be concluded that 2-oxo-1,3,2-dioxarsolanes in contrast to 2-oxo-1,3,2-dioxarsenanes are strained compounds. Thus, it may be appropriate to speak of an arsolane effect in analogy with the phospholane effect [8] by which the high chemical reactivity of 2-oxo-1,3,2-dioxaphospholanes is rationalized.

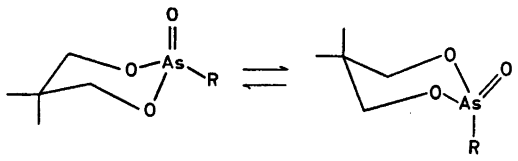
Our failure to isolate 2-oxo-1,3,2-dioxarsolanes is probably due to the reaction sequence shown in Scheme 1.



Scheme 1.

The present findings are also in agreement with the observation that spiroarsoranes containing five-membered rings are chemically more stable than the corresponding compounds containing six-membered rings.[5]

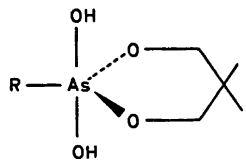
^{1}H NMR parameters for compounds *2a–c* are listed in Table 1. The spectra are consistent with the conformational equilibrium shown in eqn. (1), provided that the interconversion between conformational



states is fast on the NMR time scale. In this respect the spectral results are in analogy with those observed for the corresponding phosphorus compounds.[2] It should, however, be noted that while compounds *2a* and *2b* show nonequivalency for the OCH_2 as well as for the $C-CH_3$ hydrogens, this is not the case for *2c* (although the analogous phosphorus compound does show such nonequivalency).

The above-mentioned effect of the $As-OCH_3$ group seems to be rather general. Thus while the spirocyclic compounds derived from 2,3-dimethylbutane-2,3-diol and 2,2-dimethyl-1,3-propanediol show NMR nonequivalency both in the case of $R = CH_3$ and R = Ph, this is not observed when $R = OCH_3$, even at low temperatures.[5]

The nonequivalency observed for the OCH_2 – and $C-CH_3$ signals for compounds *2a* and *2b* disappear in the presence of traces of water. On the basis of the very low hydrolytic stability of the compounds under study, the effect can be explained in terms of rapid water-catalyzed breaking and formation of As–O bonds. The equivalency of NMR signals can alternatively be explained by postulating the formation of a pentacoordinated intermediate with trigonal bipyramidal geometry, *6*.



6

Table 1. ^{1}H NMR data for compounds *2a–c*. Chemical shifts (δ) are determined for 0.1 M solutions in $CDCl_3$ with internal TMS as standard. J_{H-C-H} 11.5 Hz in compounds *2a–c*.

Compound	$C-CH_3$		OCH_2		$As-CH_3$	Ph	OCH_3
2a	0.85	1.22	3.73	4.38	2.08		
2b	0.88	1.32	3.83	4.52		7.6(*m,p*),7.9(*o*)	
2c	1.05		4.0				3.88

The latter mechanism is similar to the one which has been put forward to explain the difficulties met with when attempting to resolve optically active arsinoxides. In this case, the formation of symmetrical oxide hydrates with TBP geometry, in which the apical positions are occupied by hydroxyl groups, is regarded to be responsible for the observed rapid racemization.[7]

If the conformational preference of 5,5-dimethyl-2-oxo-1,3,2-dioxarsenanes is similar to that of corresponding phosphorus compounds it is reasonable to expect an axially oriented As=O bond for *2a* and *2b*, whereas *2c* would be expected to prefer an equatorial As=O bond.[3,10] Although this assumption cannot be verified by the data so far collected, the large chemical shift difference between the methylene protons in *2a* and *2b* (Table 1) indicates the operation of an anisotropy effect of the As=O bond similar to that observed for corresponding phosphorus compounds with axial P=O bonds. The chemical shift difference between the methylene protons in the corresponding 1,3,2-dioxarsenanes is 0.18 and 0.12 ppm as compared to 0.65 and 0.69 ppm for *2a* and *2b*, respectively.

Additional evidence for the axial orientation of the As=O bond in the case of *2a*, was obtained from an experiment in which increasing amounts of the shift reagent Eu(fod)$_3$ were added to a 0.1 M solution of *2a* in deuteriochloroform. Treatment of the induced chemical shifts as previously described for corresponding phosphorus compounds,[10] led to an S_4/S_3 ratio of 2.0, thus indicating predominance of the conformer with axially oriented As=O.

EXPERIMENTAL

General. NMR spectra were recorded on Varian A60A and HA100-15D spectrometers. Mass spectral data were obtained using an A.E.I. MS 902 mass spectrometer and a Varian MAT 111 for combined GLC-MS.

As the compounds under study are readily hydrolyzed, all reactions were carried out under dry, purified nitrogen.

Trimethyl arsenate was prepared by oxidation of trimethyl arsenite. The procedure was essentially that of Hass.[11] B.p. 65 °C/2 mmHg, lit.[11] 70–75 °C/3–5 mmHg.

Methyl dimethylarsonate and phenyl dimethylarsonate were prepared from the silver salts of the corresponding arsonic acids on heating with methyl iodide. The silver salts were suspended in dry benzene and an equivalent amount of methyl iodide was added. The reaction mixture was vigorously stirred during the addition and was then heated under reflux for 5 h. Silver iodide was separated and the benzene removed on a rotary evaporator. Distillation of the residue through a short Vigreux column afforded the expected products in fair to good yields.

Phenyl dimethylarsonate, b.p. 96 °C/0.01 mmHg, NMR ($CDCl_3$): δ 3.84 (6 H, s) and 7.5–7.9 (5 H) n_D^{20} 1.5436. MS [IP 70 eV; *m/e* (% rel. int.)]: 200 (18, [M–CH_2O]), 182 (12), 170 (26), 169 (32), 153 (28), 152 (26), 137 (15), 123 (33), 109 (32), 108 (14), 107 (17), 106 (11), 91 (25), 78 (65), 77 (48). Methyl dimethylarsonate, b.p. 80 °C/0.3 mmHg, n_D^{20} 1.4699 (lit.[12] 97 °C/10 mmHg, n_D^{20} 1.469) NMR ($CDCl_3$): δ 1.98 (3 H, s) and 3.80 (6 H, s). MS: 153 (3.5, M–CH_3), 138 (9.9, M–CH_2O), 137 (14.9, M–OCH_3), 123 (100), 121 (4.4), 107 (34.0), 93 (21.1), 92 (5.2), 91 (31.5).

The 5,5-dimethyl-2-oxo-1,3,2-dioxarsenanes were prepared from the above-mentioned arsonates. To a stirred solution of 1.04 g (0.01 mol) of dry 2,2-dimethyl-1,3-propanediol in carbon tetrachloride 0.01 mol of the appropriate arsonate was added at ambient temperature. The reaction mixture was stirred for a couple of hours and the solvent evaporated under reduced pressure. The residue was fractionated at the lowest possible pressure. Attempts to distill the 2-methoxy derivative *2c* failed.

The compounds *2a* and *2b* were also prepared from the corresponding arsonic acid dichlorides and 2,2-dimethyl-1,3-propanediol together with an equivalent amount of pyridine. The reaction was monitored by the precipitation of pyridinium hydrochloride and by GLC; the starting materials had been consumed after 1–2 h. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was fractionated as in the above-mentioned procedure. 5,5-Dimethyl-2-oxo-2-methyl-1,3,2-dioxarsenane b.p. 110 °C/0.005 mmHg (accompanied by sublimation) M.p. 85–95 °C. NMR: see Table 1; MS [70 eV; *m/e* (% rel. int.)]: 208 (2, M), 193 (19, [M–CH_3]), 163 (63), 153 (15.5), 145 (62.5), 141 (23.6), 123 (63), 117 (17.2), 109 (23), 107 (39.6), 91 (35.2). Anal. $C_6H_{13}AsO_3$: C, H. 5,5-Dimethyl-2-oxo-2-phenyl-1,3,2-dioxarsenane b.p. 150 °C/0.005 mmHg. For NMR data see Table 1. MS: 270 (10, M), 224 (14.7), 185 (33), 169 (100), 168 (16.3), 152 (97.8), 140 (33.1), 91 (42), 78 (21.1), 77 (41.2), 73 (41), 71 (38), 56 (80), 55 (64.5). Anal. $C_{11}H_{15}AsO_3$: C, H.

5-5-Dimethyl-2-oxo-2-methoxy-1,3,2-dioxarsenane. This compound could not be isolated by distillation. For NMR data see Table 1.

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Pseudomonas Cytochrome *c* Peroxidase. XIII. pH-Denaturation of the Enzyme

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The effect of pH on the oxidized *Pseudomonas* cytochrome *c* peroxidase molecule was studied by measuring the peroxidatic activity, the sedimentation velocity, the circular dichroic spectra in the far UV and Soret regions, and the optical absorption spectra of the enzyme in the pH range 2.5 – 13.0 at a constant ionic strength ($\mu = 0.1$). The enzyme was stable in a narrow pH region, pH 6.0 – 7.4. In the low pH range the gross tertiary structure was observed to change quite simultaneously with the enzymatic activity and secondary structure. The optical absorption spectra indicated that there were no coordinated internal protein ligands in the 6th coordination positions of the heme prosthetic groups at the lowest pH studied. In the high pH range the secondary structure and the protein environment of hemes were observed to remain stable after the tertiary structure had changed and the activity had decreased. According to the optical absorption spectra the 6th internal protein ligands of hemes were retained at the highest pH studied.

Pseudomonas cytochrome *c* peroxidase (cytochrome *c*:H_2O_2-oxidoreductase, EC 1.11.1.5, PaCCP) is a bacterial peroxidase containing two molecules of covalently bound heme *c* as prosthetic groups in a single polypeptide chain of 354 amino acids.[1–4] The molecule contains no disulfide bonds,[3] it has an excess of hydrophobic amino acids,[4] and its isoelectric point is 6.7.[1] The optical absorption spectra of PaCCP show the low spin or hemochrome structure of the prosthetic groups, that is with internal protein ligands at the 5th and 6th coordination positions of the heme iron,[1,5] as in *c*-type cytochromes. The frictional ratio calculated from the hydrodynamic properties of the molecule indicates that PaCCP is quite a symmetric molecule, differing little from a spherical one.[2,6]

PaCCP catalyzes specifically the hydrogen peroxide oxidation of two macromolecular substrates, *Pseudomonas* respiratory chain proteins cytochrome *c*-551 and copper protein azurin.[7]

In the present work the effect of pH on the oxidized Fe(III) – PaCCP molecule was studied in the pH range 2.5 – 13.0 at a constant ionic strength ($\mu = 0.1$).

The pH-stability of the enzyme, determined by measuring the peroxidatic activity, and the effect of high pH on the optical absorption spectra of the enzyme have been studied earlier.[1,5]

MATERIALS AND METHODS

Pseudomonas cytochrome c peroxidase was prepared from the acetone-dried cells of *P. aeruginosa* as previously described.[8] The preparation was homogeneous in disc electrophoresis (pH 8.6, 7 % gel,[9] staining with Coomassie Brilliant Blue G-250 [10]) and in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (10 % gel).[11] After the purification procedure the enzyme was in the oxidized Fe(III) form. The absorbance ratio A_{407}/A_{280} of the preparation was 4.6. The concentration of the enzyme was determined spectrophotometrically using E(1 %, 1 cm) equal to 12.1 at 280 nm.[2] The molar concentration of the enzyme was calculated using a molecular weight of 43 200 based on the iron content of the enzyme.[2,3]

Denaturation of the enzyme. A concentrated stock solution of PaCCP was diluted with buffer and the diluted solution was dialyzed against the same buffer for 3 h at room tem-

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perature. The dialyzed enzyme solution was used in all the measurements described. The pH of the solution was checked after the measurements. The buffers ($\mu = 0.1$) used were: glycine–HCl buffer pH 2.5, sodium acetate buffers pH 3.8–5.3, sodium phosphate buffers pH 6.0–8.0, TRIS-HCl buffer pH 9.0, sodium borate buffers pH 9.3–9.8, and 0.1 M sodium hydroxide pH 13.0.

Peroxidatic activity was assayed spectrophotometrically by measuring the rate of the peroxidatic oxidation of reduced *Pseudomonas* cytochrome *c*-551 as previously described.[5] The reaction was initiated by mixing 2–5 µl of suitably diluted enzyme with 2.0 ml of the reaction mixture. The enzyme was diluted with the buffer used in the denaturation. The activity was expressed in terms of arbitrary units (decrease in absorbance per 10 s per 10 µl of enzyme solution) calculated from the slope of the reaction curve.

Ultracentrifugation analyses. The sedimentation velocities were measured in a Beckman-Spinco Model E analytical ultracentrifuge at a rotor speed of 59 870 rpm at 20 °C as previously described.[2]

Spectropolarimetry. Circular dichroic (CD) spectra were recorded on a Cary 61 spectropolarimeter at 25 °C. Cells with fused quartz windows and path lengths 0.1–1 mm were used. Path lengths were dictated by the absorbance of the solution. The stability of each preparation during the CD measurements was confirmed by recording the optical absorption spectra immediately before and after each CD measurement. Base lines were recorded and subtracted from the dichroic curves. The ellipticities above 250 nm are expressed as molar ellipticities, $[\theta]$, in degrees cm²/dmol. Ellipticities below 250 nm are expressed as mean residual ellipticities, $[\theta]_{MRW} = [\theta]/n$, where n, the number of residues per molecule, is 354 for PaCCP.

Spectrophotometry. Spectrophotometric concentration determinations were performed with a Beckman DU-2 spectrophotometer, peroxidatic activities were measured with a Beckman DK-1 A recording spectrophotometer and optical absorption spectra with a Cary 15 recording spectrophotometer, all at 25 °C.

pH-Measurements were carried out at 25 °C with a Radiometer PHM 3 pH-meter standardized against Beckman buffer pH 7 No. 3581, 0.05 M potassium phthalate buffer pH 4.01 and 0.01 M $Na_2B_4O_7.10H_2O$ pH 9.18.

Chemicals. Sodium dodecyl sulfate (pract., Fluka) and urea (pure, Merck) were recrystallized before use. Other reagents were of analytical grade.

RESULTS

The peroxidatic activity of the enzyme was measured in the pH range 2.5–13.0, and shown in Fig. 1, expressed as the percentage activity of the native PaCCP left after the incubation at different pH values.

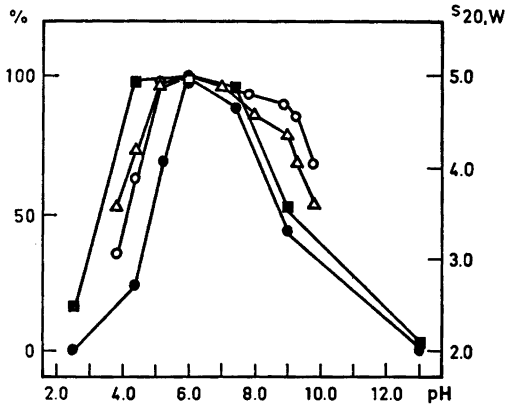


Fig. 1. The pH dependence of (●) the peroxidatic activity of *Pseudomonas* cytochrome *c* peroxidase (PaCCP) as percent of that of the native enzyme; (■) the sedimentation coefficient $s_{20,w}$ of PaCCP; (△) the change of the mean residual ellipticity of PaCCP at 208 nm expressed as percent of the total change caused by 8 M urea at pH 6.0; and (○) the change of the molar ellipticity of PaCCP at 412 nm expressed as percent of the total change caused by 8 M urea at pH 6.0.

The sedimentation coefficient, $s_{20,w}$, of PaCCP was determined in the pH range 2.5–13.0. The value of the sedimentation coefficient decreased from that of the native enzyme, 5.0 S, to 2.5 S at the lowest pH and to 2.0 S at the highest examined (Fig. 1). The protein sedimented as a single boundary at every pH.

Circular dichroic spectra of PaCCP in the far UV (200–250 nm) and the near UV (Soret, 370–450 nm) regions in the pH range 3.8–9.8 as well as in 8 M urea at pH 6.0 are shown in Fig. 2.

The native PaCCP (pH 6.0) has a prominent negative ellipticity band at 208 nm with a shoulder at 223 nm. Characteristic of the spectra in the far UV region in both the low and the high pH regions is the decrease of the negative ellipticity at 208 nm. In 8 M urea the shape of the band has totally changed and the band at 223 nm has disappeared. The change of the mean residual ellipticity at 208 nm expressed as a percentage of the total change caused by 8 M urea (pH 6.0), which describes

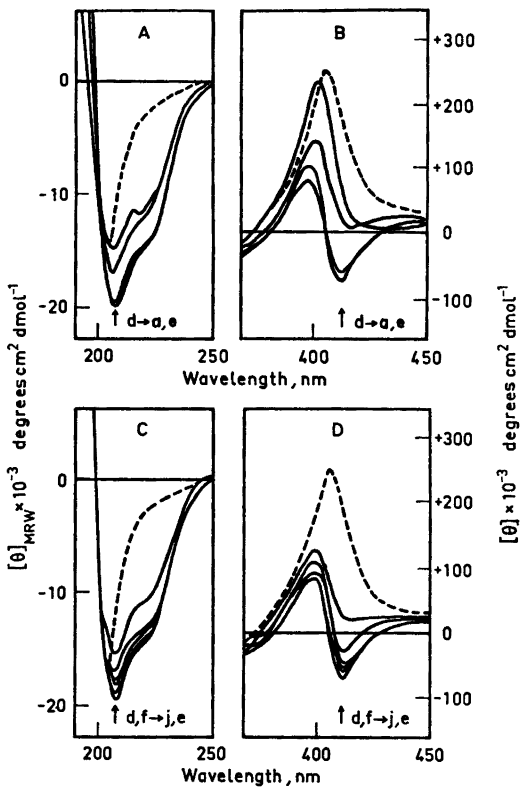


Fig. 2. Circular dichroic spectra of *Pseudomonas* cytochrome *c* peroxidase (47 μM) in the pH region 3.8 – 9.8 in the far ultra-violet (*A*, *C*) and the Soret (*B*, *D*) region. The curves can be distinguished at 208 and 412 nm. (*a*) pH 3.8, (*b*) pH 4.4, (*c*) pH 5.1, (*d*) pH 6.0, (*e*, the broken line) pH 6.0, 8 M urea, (*f*) pH 7.0, (*g*) pH 8.0, (*h*) 9.0, (*i*) pH 9.3, and (*j*) pH 9.8.

the change of the CD spectrum in the far UV region, is shown for the pH range 3.8 – 9.8 in Fig. 1.

At the Soret region the native PaCCP (pH 6.0) has a positive ellipticity band at 400 nm and a negative one at 412 nm. The negative band totally disappears at both low and high pH and the spectrum approaches that of PaCCP in 8 M urea, with one positive band at 407 nm. The change in the molar ellipticity at 412 nm expressed as a percentage of the total change caused by 8 M urea (pH 6.0) which describes the change of the CD spectrum in the Soret region, is shown for the pH range 3.8 – 9.8 in Fig. 1. The change of the CD spectrum in this region is more prominent at low than at high pH. The spectrum of the urea-treated PaCCP was achieved neither by acid (pH 3.8) nor alkaline (pH 9.8) denaturation.

Optical absorption spectra of PaCCP were recorded in the pH range 2.5 – 13.0. Of the visible spectra (350 – 650 nm) measured, the spectrum of the native enzyme at pH 6.0 and the spectra of the denatured enzyme at pH 2.5 and pH 13.0 are shown in Fig. 3.

The characteristic features in the spectrum at low pH are the Soret band at 397 nm with a reduced absorbance compared with that of the native enzyme, reduced absorbance in the 450 – 650 nm region, and new slight maxima around 510 and 625 nm. At high pH a δ-band at 353 nm is observed, the Soret band with increased absorbance has changed to 410 nm, the absorbance at 450 – 650 nm has slightly increased and a shoulder at around 568 nm has appeared.

After denaturation experiments every preparation was checked by *polyacrylamide gel electrophoresis* in the presence of sodium dodecyl sulfate. Only the same polypeptide band as in the untreated PaCCP, with an apparent molec-

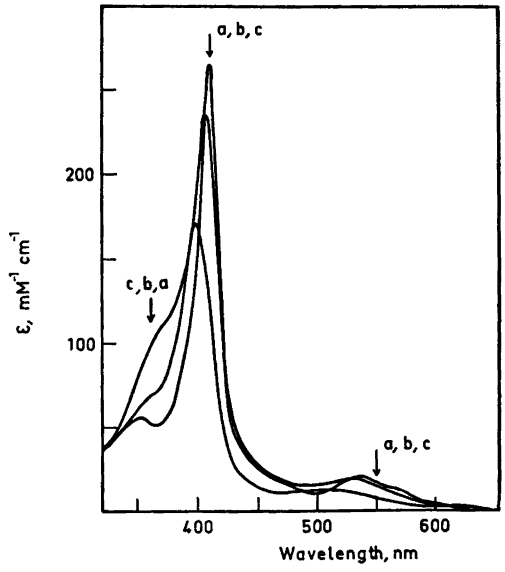


Fig. 3. Optical absorption spectra of oxidized *Pseudomonas* cytochrome *c* peroxidase (47 μM) at (*a*) pH 13.0, (*b*) pH 6.0, and (*c*) pH 2.5 in the visible region. The curves can be distinguished at 360, 407 and 550 nm.

ular weight of 40 000,[3] was observed in each of the preparations.

DISCUSSION

The peroxidatic activity of PaCCP was the most sensitive of the properties measured to changes of pH (Fig. 1). Earlier the enzyme was shown to be stable at pH 6.0,[1] and at this pH it is assumed to be in the native form. The activity decreased rapidly in the acid region and was somewhat more stable in the alkaline region but decreased markedly when the pH was over 7.4. Accordingly to the present study the pH region where the enzymatic activity is stable is quite narrow, pH 6.0 – 7.4. A slightly different pH region, pH 5 – 7, for the stability was observed earlier.[7] Then, however, the activity was measured at pH 7.2 and not at the optimum pH 6.0 as in the present study; this may explain the difference. Peroxidatic activity is lost after only minor changes in the molecule, and before any changes could be observed by the physical methods used in this study. A macromolecular substrate such as *Pseudomonas* cytochrome *c*-551 may demand quite a large area of the enzyme molecule to be unchanged, that is in the native form, for the formation of enzyme-substrate complex.

The sedimentation coefficient obtained from the ultracentrifuge analysis is a measure of the frictional coefficient of the molecule and it depends on the radius of the hydrodynamic particle. The sedimentation coefficient thus describes the folding of the polypeptide chain or the tertiary structure of the protein molecule. It offers a means of following the transition of the molecule from the native to the denaturated state.[12] The sedimentation coefficient of PaCCP remained unchanged to a lower pH than any other property measured, indicating the retention of the gross tertiary structure in the low pH region. Some changes in the molecule have occurred, as seen from the activity and circular dichroic data (Fig. 1). In the high pH region the sedimentation coefficient changed simultaneously with the loss of the enzymatic activity (Fig. 1). PaCCP sedimented as a single boundary at every pH studied. This confirms an earlier report that the molecule consists of one polypeptide chain [3] and has no subunits. The value of the sedimentation coefficient approached 2 S at low as well as at high pH, indicating the increase in the effective hydrodynamic volume caused by the unfolding of the polypeptide chain. The covalent structure of the polypeptide backbone was preserved, as shown by the polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate in which the single protein band had the same apparent molecular weight as the native enzyme.

The circular dichroic spectrum of a protein in the far UV region (180 – 250 nm) offers a means of investigating the conformation of the polypeptide backbone or the secondary structure of the protein molecule, assuming that the contributions of non-peptide chromophores in this region are insignificant.[12,13] In the low pH region the secondary structure of PaCCP, according to the far UV CD spectrum, changed quite simultaneously with the tertiary structure measured by the sedimentation coefficient. It prevailed to some extent after the enzymatic activity had decreased (Fig. 1). In the high pH region the far UV CD spectra show that the secondary structure changed later than the tertiary structure. Theoretical CD curves have been published for the three conformations, helix, β-pleated sheet and unordered, based on the data from five reference proteins.[13] When the spectra of PaCCP are compared qualitatively with the theoretical spectra it seems that PaCCP contains helical as well as β-structure and/or unordered structure. The helical structure decreased at low as well as high pH, as seen from the change of ellipticity at 223 nm (Fig. 2). The CD spectra of PaCCP at low and high pH were also compared with those in 8 M urea at pH 6.0. In 8 M urea PaCCP was mainly in the random coil unordered form, as seen from the spectrum (Fig. 2). The pH-denaturation at either low or high pH did not result in the loss of ordered structure to the same extent as in 8 M urea.

The dichroic band at 370 – 450 nm (the Soret region) is influenced by the effect of the protein environment on the heme transitions, although the heme chromophore itself has no CD band in the Soret region.[14,15] In the low pH region the CD bands of PaCCP in the Soret region changed in a similar way to the far UV CD-band (Fig. 1), that is the heme environment changed simultaneously with the changes

in the secondary structure of the molecule. In the high pH region the heme environment remained unchanged further than the enzymatic activity and the tertiary structure measured by $s_{20,w}$ (Fig. 1). The disappearance of the complex nature of the CD band in the Soret region at low and high pH, as well as in 8 M urea (Fig. 2) indicates that both the two heme groups are in different asymmetric positions in the native molecule and change to identically asymmetric positions in the denaturated molecule. A similar simplification of CD spectra of horse heart cytochrome *c* by urea and other denaturants has been observed and attributed to the opening of the heme crevice and changes in heme ligands.[16,17]

The optical absorption spectra of hemoproteins in the near UV (Soret) and visible wavelength regions depend on the electronic and magnetic structure of heme and reflect the state of the prosthetic group. The optical absorption spectra of Fe(III)-PaCCP at the lowest and highest pH examined are totally different (Fig. 3). At low pH the spectrum shows the characteristics of a high-spin, myoglobin type spectrum,[18] one observed for example in the so-called high-spin cytochromes[19,20] and in horse heart cytochrome *c* below pH 2.5.[21] The "high-spin spectrum" indicates that the internal strong protein ligand is no longer coordinated in the 6th coordination position of the heme iron. At high pH the low-spin type spectrum of Fe(III)-PaCCP is preserved (Fig. 3). The stability of the low-spin structure of the reduced, Fe(II) – PaCCP in the alkaline region has been shown earlier.[5] Thus, in contrast to the acid denaturation, even severe alkaline denaturation does not lead to the loss of the 6th internal strong protein ligands of heme irons. The ligands may have a stabilizing effect on the heme environment in the high pH region observed in CD spectra.

According to the above results Fe(III)-PaCCP is denatured easily at both low and high pH. It may be therefore classified, according to Tanford,[12] with those proteins that are drastically altered when pH is changed.

Acknowledgement. We wish to thank Professor N. Ellfolk for his advice and interest during the investigation.

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Tobacco Chemistry. 41. Structure Determination and Synthesis of 5(13), 7*E*-Megastigmadien-6,9-diol, a New Constituent of Greek Tobacco

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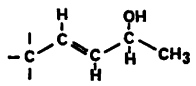
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A new C_{13} nor-carotenoid was isolated from Greek *Nicotiana tabacum* L. and shown by spectroscopic methods and synthesis to be 5(13),7*E*-megastigmadien-6,9-diol.* The additional compounds obtained in the reaction of singlet oxygen with (±)-β-ionol were also characterized.

A large number of C_{13} compounds obviously formed by initial oxidative cleavage of carotenoids and subsequent chemical alterations have been isolated from tobacco.[2] The present paper deals with the structure elucidation and biomimetic synthesis of a new representative of this group of compounds. An account of the additional products obtained in the reaction of singlet oxygen with β-ionol is also given.

RESULTS

The new tobacco constituent, *1*, $C_{13}H_{22}O_2$, was isolated in minute quantity by repeated liquid chromatography of a polar, volatile fraction, B 8,[3] obtained from an ether extract of sun-cured Greek tobacco. It contained a secondary hydroxyl group as shown by IR absorption at 3600 and 3550 – 3120 cm^{-1} and a one-proton quintet at δ 4.39 in the ^1H NMR spectrum. This quintet was converted to a quartet on irradiation at the frequency of an olefinic doublet of doublets at δ 5.85 and to a doublet on irradiation at the frequency of a methyl doublet at δ 1.30. Since, conversely, irradiation at the frequency of the quintet at δ 4.39 converted the doublet of doublets at δ 5.85 to the A part of an AB system, δ_B 6.14, $J_{AB} = 15.5$ Hz, it follows that the new tobacco isolate incorporates partial structure A.



A

Moreover, it was clear from the presence of two three-proton singlets at δ 0.91 and one-proton signals at δ 4.86 and 4.91 in the ^1H NMR spectrum that *1* contains two methyl groups on fully substituted carbon(s) and an exocyclic methylene group (IR band at 1640 cm^{-1}). In view of these results and the elemental composition the nor-carotenoid structure *1* appeared highly probable.

Since the minute quantity available did not allow corroborative ^{13}C NMR studies, the proposed structure of *1* was confirmed by synthesis. This was achieved by subjecting (±)-β-ionol (*2*) to photooxygenation sensitized by rose bengal followed by treatment of the reaction mixture with Na_2SO_3. The main product thus obtained, 5(13),7*E*-megastigmadien-6,9-diol, whose ^{13}C NMR spectrum contained diagnostically important peaks at δ 151.31 (s), 108.86 (t) and at 79.25/79.17 (s) due to the $>C=CH_2$ and $-\overset{|}{C}-OH$ groups, respectively (*cf.* Table 1), gave IR, ^1H NMR and mass spectra identical

* For nomenclature: see Ref. 1.

with those of the new tobacco constituent (*1*). This synthetic product (*1*) is evidently formed in an ene-reaction involving an attack of singlet oxygen on the tetra-substituted 5,6 double bond and loss of a hydrogen from C-13 in (±)-β-ionol (*2*). This attack should occur at either side of the molecule and should furnish two pairs of diastereoisomers, a view corroborated by the fact that the signals ascribed to C-4, and C-6 to C-10 are doublets in the proton noise-decoupled ^{13}C NMR spectrum of the synthetic sample. The stereochemistry and absolute configuration of the natural product (*1*) could not be determined due to shortage of material.

In addition to the major product (*1*) from the reaction of singlet oxygen with (±)-β-ionol (*2*), a few further compounds were isolated and identified. Of these, 4,7-megastigmadien-6,9-diol (*3*) and 6,7-megastigmadien-5,9-diol (*4*) can be viewed as products of ene-reactions, whereas 5,8-epidioxy-6-megastigmen-9-ol (*5*), dihydroactinidiolide (*6*) and 5,6-7,8-diepoxymegastigman-9-ol (*7*) probably arise *via* a 1,4-addition reaction.

The presence of the 4,5 double bond in 4,7-megastigmadien-6,9-diol (*3*) was disclosed by the ^1H NMR spectrum, which contained a broad three-proton signal at δ 1.61 and a one-proton multiplet at δ 5.50 corresponding to a methyl group allylically coupled to an olefinic proton. The ^{13}C NMR spectrum, which in addition to the C-7 and C-8 sp^3 carbon signals displayed resonances due to two sp^2 carbon atoms, a doublet at δ 122.09 and a singlet at δ 136.91, was in accordance with the structure proposed for *3*. Moreover, the mass spectrum of *3* had a prominent peak at *m/e* 154 corresponding to a $C_9H_{14}O_2$ fragment, which arises by retro-Diels-Alder cleavage of the molecular ion induced by the 4,5 double bond (Fig. 1).

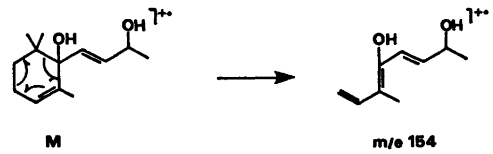
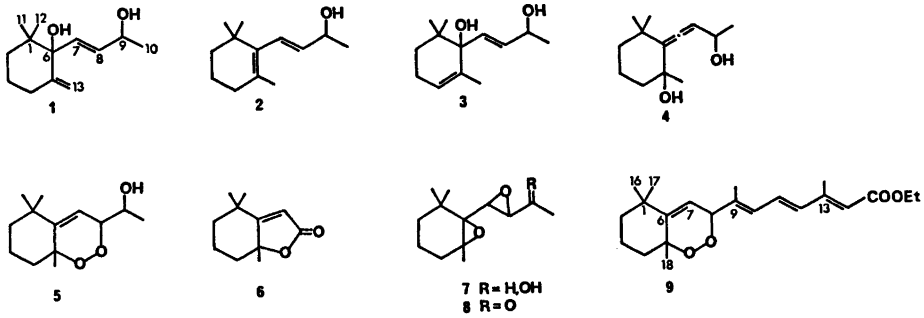


Fig. 1. The retro-Diels-Alder cleavage of the molecular ion.

6,7-Megastigmadien-5,9-diol (*4*) gave a ^{13}C NMR spectrum having signals at δ 117.47, 196.66 and 99.59 corresponding to the carbon atoms of the trisubstituted allenic group and an IR spectrum having a characteristic allene band at 1965 cm^{-1}.

5,8-Epidioxy-6-megastigmen-9-ol (*5*) was isolated as a 1:1 mixture with dihydroactinidiolide (*6*, *vide infra*). Its structure was deduced from the ^{13}C NMR spectrum of this mixture. Thus, subtraction of the signals due to dihydroactinidiolide (*6*) left thirteen signals to be assigned to *5*. Eleven of these had chemical shift values close to those previously published for the C-1 to C-8 and C-16 to C-18 signals for compound *9*.[4] Since the remaining two signals were due to an oxygen-bearing sp^3 methine carbon and a methyl carbon it was reasonable to formulate *5* as 5,8-epidioxy-6-megastigmen-9-ol. The mass spectrum having the base peak at *m/e* 181, which corresponds to an ion formed by a favoured rupture of the 8,9 bond in the molecular ion, provides supporting evidence for the structure proposed.

(±)-Dihydroactinidiolide (*6*) obtained in a pure state from a different fraction, was identified by spectral comparison with an authentic sample.



As shown by GC-MS analysis and by the ^{13}C and ^{1}H NMR spectra, 5,6-7,8-diepoxymegastigman-9-ol (*7*) was obtained as a mixture of isomers. The presence of the 7,8-epoxide group adjacent to the secondary hydroxyl group at C-9 was disclosed by the ^{1}H NMR spectrum of this mixture, which exhibited a one-proton doublet at δ 3.38/3.42 ($J = 2.1$ Hz), a one-proton doublet of doublets at δ 3.04/3.09 ($J =$ 2.1 and 3.3 Hz), a one-proton doublet of quartets at δ 3.76/3.97 ($J = 3.3$ and 6.5 Hz), and a three-proton doublet at δ 1.27/1.30 ($J = 6.5$ Hz). The ^{13}C NMR spectrum not only confirmed the presence of the 7,8-epoxide group, δ 57.26/56.96 (d) and 60.24/59.48 (d), but also suggested that *7* incorporated a tetra-substituted epoxide group, δ 66.10 (s) and 63.80/63.59 (s). This must be attached to C-5 and C-6, a conclusion supported by the fact that the ^{1}H NMR spectrum contained a methyl singlet at δ 1.31/1.36.

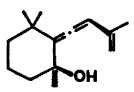
Oxidation using Jones' reagent converted the alcohol (*7*) to 5,6-7,8-diepoxymegastigman-9-one (*8*), whose IR spectrum had an absorption band at 1710 cm^{-1} and whose ^{1}H NMR spectrum contained a three proton singlet at δ 2.09 and an AB quartet centered at δ 3.47 corresponding to the protons of the methyl ketone and 7,8-epoxy groups, respectively. The facts that no doubling of peaks was observed in either the ^{1}H or the ^{13}C NMR spectrum of the ketone (*8*) and that the sample was optically inactive support the view that the mixture of alcohols (*7*) is derived from the two pairs of diastereomeric 5,8-epidioxy-6-megastigmen-9-ols (*5*).

Sensitized photooxygenation of β-ionol (*2*), performed in the presence of catalytic amounts of alkali, has previously been studied by Isoe *et al.*[5] They isolated dihydroactinidiolide (*6*) as the major product and identified one of the minor components as 6,7-megastigmadien-5,9-diol (*4*). The latter compound, which has been patented as a flavour additive to tobacco, and the 7*Z*-derivative of *1* are major products of the photooxidation of *cis*-β-ionol.[6] Compounds *10*–*15* have been obtained on photooxygenation of 3-methyl-1-(2,6,6-trimethylcyclohexen-1-yl)-1,3-butadiene (*16*),[7] a result which is analogous to our findings for the reaction of β-ionol (*2*) with singlet oxygen.

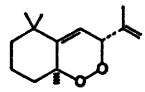
EXPERIMENTAL

IR spectra were measured on Digilab FTS-14 and Perkin-Elmer 257 instruments and mass spectra on an LKB 2091 instrument. Accurate mass measurements were carried out on an Atlas SM 1 instrument at the Laboratory for Mass Spectrometry, the Karolinska Institute, Stockholm. Fourier transform ^{1}H NMR (100 MHz) and ^{13}C NMR (25.16 MHz) spectra were obtained on a Varian XL-100-12 spectrometer equipped with S-124 FT and disk accessories and controlled by a Varian 620/L computer. Gas chromatography was performed on a Varian 1700 instrument using glass capillary columns (50 m × 0.37 mm) coated with HB 5100. High performance liquid chromatography was carried out using a Waters 6000 A solvent delivery system, a U6K injector and an R-401 differential refractometer.

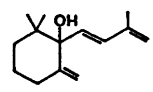
Isolation of 5(13), 7E-megastigmadien-6,9-diol (1) from tobacco. A volatile, neutral fraction (B8)[3] of an extract obtained from 295 kg of sun-cured Greek *Nicotiana tabacum* L. was chromatographed over silica gel using a light petrol/diethyl ether gradient. One of the subfractions obtained was purified further by liquid chromatography using columns packed with Bondapak C_{18}/Porasil (Waters), μ-Bondapak C_{18} (Waters) and μ-Porasil (Waters) to afford 3 mg of *1* as a colourless oil (Found: M 210.1620. Calc. for $C_{13}H_{22}O_2$: 210.1620), which had IR ($CHCl_3$) bands at 3600 (m), 3550–3120 (w) and 1640 (w) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ



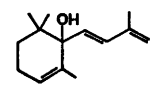
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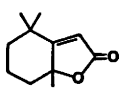
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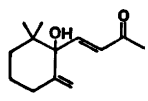
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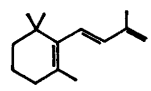
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0.91 (6 H, s), 0.93 (1 H, s), 1.30 (3 H, d, $J=6$ Hz), 4.39 (1 H, quintet, $J=6$ Hz), 4.86 (1 H, m), 4.91 (1 H, m), 5.85 (1 H, dd, $J=15.5$ and 6 Hz) and 6.14 (1 H, broad d, $J=15.5$ Hz) (AB part of an ABX system); MS [*m/e* (composition, %)]: 210 ($C_{13}H_{22}O_2$, 2), 192 ($C_{13}H_{20}O$, 4), 177 ($C_{12}H_{17}O$, 3), 165 ($C_{11}H_{17}O$, 5), 152 ($C_{10}H_{16}O$, 68), 141 ($C_8H_{13}O_2$, 30), 123 (C_9H_{15} and $C_8H_{11}O$, 22), 109 (C_8H_{13} and C_7H_9O, 42), 97 (C_7H_{13} and C_6H_9O, 38), 96 (C_7H_{12} and C_6H_8O, 27), 95 (C_7H_{11} and C_6H_7O, 43), 81 (29), 69 (33), 55 (28) and 43 (100).

Photooxidation of β-ionol. A solution of 1.0 g of (±)-β-ionol and 0.1 g of rose bengal in 25 ml of methanol in a tube cooled by a water jacket was irradiated for 1 h with a 400 W sodium high pressure lamp placed outside the tube while oxygen was bubbled through the reaction mixture. A solution of 1.0 g of Na_2SO_3 in 20 ml of H_2O was added and the reaction mixture was stirred at room temperature for 17 h. Dilution with water, extraction with ether and chromatography on silica gel using a hexane/ethyl acetate gradient eluted in order compounds *5*, *6*, *7*, *1*, *3*, and *4*. Further purification was achieved by high performance liquid chromatography on columns packed with μ-Porasil (Waters) using hexane and ethyl acetate as eluent.

5(13),7E-megastigmadien-6,9-diol (*1*, 134 mg) gave IR, ^{1}H NMR and mass spectra identical to those of the natural product.

4,7-Megastigmadien-6,9-diol (*3*, 9 mg), which was recrystallized from diethyl ether–hexane (1:1) to give white needles, m.p. 108–109 °C. (Found: $[M-H_2O]^+$ 192.1518. Calc. for $C_{13}H_{20}O$: 192.1514). IR bands at 3600 (s), and 3550–3100 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.90 (3 H, s), 0.98 (3 H, s), 1.30 (3 H, d, $J=6$ Hz), 1.61 (3 H, m), 4.39 (1 H, m, $W_{1/2}=14$ Hz), 5.50 (1 H, m, $W_{1/2}=10$ Hz) and 5.6–5.8 (2 H, overlapping signals); MS [*m/e* (composition, %)]: 192 (M–18, $C_{13}H_{20}O$, 17), 154 ($C_9H_{14}O_2$, 68), 136 ($C_9H_{12}O$, 43), 121 (C_9H_{13}, 37), 111 ($C_7H_{11}O$, 100), 109 (C_8H_{13} and C_7H_9O, 63), 96 (C_6H_8O, 44), 93 (C_7H_9, 48), 83 (C_6H_{11} and C_5H_7O 56), 55 (38) and 43 (88).

6,7-Megastigmadien-5,9-diol (*4*, 5 mg), which was recrystallized from ethyl acetate to give white needles, m.p. 140–143 °C. (Found: $[M-H_2O]^+$ 192.1512. Calc. for $C_{13}H_{20}O$: 192.1514). IR (KBr) bands at 3650–3040 (s) and 1965 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.06 (3 H, s), 1.26 (3 H, s), 1.32 (3 H, d, $J=6$ Hz), 1.36 (3 H, s), 4.76 (1 H, quintet, $J=6$ Hz) and 5.45 (1 H, d, $J=6$ Hz); MS [*m/e* (composition, %)]: 210 (M, 1), 192 ($C_{13}H_{20}O$, 10), 177 ($C_{12}H_{17}O$, 28), 149 ($C_{11}H_{17}$ and $C_{10}H_{13}O$, 17), 133 ($C_{10}H_{13}$, 30), 119 (C_9H_{11}, 23), 107 (C_8H_{11} and C_7H_7O, 100), 85 (24) and 43 (88). Acetylation of *4* with acetic anhydride and pyridine at room temperature for 30 min afforded *9-acetoxy-6,7-megastigmadien-5-ol* as a colourless oil, which had IR ($CHCl_3$) bands at 3580 (m), 3560–3300 (m), 1955 (m) and

Table 1. Carbon-13 chemical shifts and assignments for compounds *1*, *3*–*8*.[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11/C-12	C-13
1	39.14	37.24	22.66	33.04	151.31	79.25	131.39	134.21	68.59	23.64	24.16/22.88	108.86
				32.98		79.17	131.27	133.97	68.39	23.54		
3[b]	36.61	33.10	22.47	122.09	131.91	76.76	131.33	134.07	66.61	22.38	24.27/24.52	18.65
4[b]	33.29	40.26[c]	17.96	40.48[c]	68.73	117.47	196.66	99.59	64.08	23.88	31.87[d]/30.83[d]	28.90[d]
5	35.13	40.73	18.75	35.65	79.57	149.00	115.01	82.67	70.21	19.72	25.49[e]/30.48[c]	27.77[c]
6	36.32	40.07	19.53	41.52	86.80	182.26[e]	112.18	172.23[e]			24.05/29.70	24.30
7	33.41	38.43	17.10	32.02	63.80	66.10	57.26	60.24	66.04	20.01	25.27/26.79	20.01
	33.36	38.37		31.90	63.59		56.96	59.48	65.82	19.40		
8	33.39	38.17	17.03	31.80	63.92	65.66	58.94[c]	57.92[c]	*f*	24.86	25.02/26.78	19.69

[a] δ-Values relative to TMS; spectrum recorded in $CDCl_3$. [b] Spectrum recorded in DMSO-d_6. [c,d] Assignment may be reversed. [e] The distinction between the C-6 and C-8 signals was made with the aid of the proton-coupled ^{13}C NMR spectrum; the sharp doublet ($^2J_{CH}=8.3$ Hz) at δ 171.23 was assigned to C-8 and the signal at δ 182.26, broadened due to several three bond C-H couplings, was assigned to C-6. [f] Not visible due to low sample concentration.

1725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.04/1.05 (3 H, s), 1.22/1.24 (3 H, s), 1.32/1.34 (3 H, s), 1.37 (3 H, d, $J = 6$ Hz), 2.04/2.05 (3 H, s) and 5.15–5.55 (2 H, overlapping signals). These data are somewhat at variance with previously published values.[5]

5,8-Epidioxy-6-megastigmen-9-ol (*5*, 7 mg), was present as a 1:1 mixture with dihydroactinidiolide (*6*). Subtraction of the peaks due to *6* from the ^1H NMR spectrum ($CDCl_3$) of the mixture left the following signals for *5*: δ 1.12 (3 H, s), 1.17 (3 H, s), 1.26 (3 H, d, $J = 6$ Hz), 1.61 (3 H, s), 4.15 (2 H, overlapping signals) and 5.67 (1 H, d, $J = 3$ Hz); MS [*m/e* (%)]: 208 (M−18, 1), 193 (2), 181 (100), 163 (13), 135 (22), 121 (18), 107 (18), 95 (18), 81 (11), 69 (17), 55 (13) and 43 (58).

(±)-Dihydroactinidiolide (*6*, 103 mg) obtained in a pure form from a more polar fraction, was identified by comparison of its IR, ^1H NMR and mass spectra with those of an authentic sample.

5,6-7,8-Diepoxymegastigman-9-ol (*7*, 25 mg), which was recrystallized from hexane to give white needles, m.p. 91–93 °C. (Found: $[M-45]^+$ 181.1221. Calc. for $C_{11}H_{17}O_2$: 181.1228). ^1H NMR ($CDCl_3$): δ 1.09 (3 H, s), 1.27/1.30 (3 H, d, $J = 6.5$ Hz), 1.28 (3 H, s), 1.31/1.36 (3 H, s), 3.04/3.09 (1 H, dd, $J = 3.3$ and 2.1 Hz), 3.38/3.42 (1 H, d, $J = 2.1$ Hz), 3.76/3.97 (1 H, dq, $J = 6.5$ and 3.3 Hz); MS [*m/e* (composition, %)]: 208 (M−18, 1), 193 (1), 181 ($C_{11}H_{17}O_2$, 13), 163 ($C_{11}H_{15}O$, 10), 145 ($C_{11}H_{13}$, 6), 123 (C_8H_{15} and $C_8H_{11}O$, 39), 121 (C_9H_{13} and C_8H_9O, 22), 109 (C_8H_{13} and C_7H_9O, 17), 105 (C_8H_9, 15), 95 (C_7H_{11} and C_6H_7O, 22), 85 (C_5H_9O and $C_4H_5O_2$, 29), 71 (15), 69 (18), 67 (12), 55 (17) and 43 (100). Treatment of *7* with Jones' reagent for 1 h at room temperature afforded *5,6-7,8-diepoxymegastigman-9-one* (*8*) which had an IR band at 1710 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.07 (3 H, s), 1.30 (3 H, s), 1.31 (3 H, s), 2.09 (3 H, s), 3.43 (1 H, d, $J = 2$ Hz) and 3.51 (1 H, d, $J = 2$ Hz); MS [*m/e* (%)]: 206 (M−18, 1), 181 (12), 163 (10), 145 (3), 137 (6), 123 (29), 121 (17), 105 (15), 95 (21), 85 (27), 69 (16), 55 (17) and 43 (100).

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Chiral Allenic Alcohols from Addition-Elimination Reactions of Acetylenes with Lithium Aluminium Hydride*

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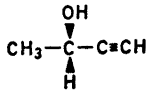
(*R*)-(−)-3,4-Hexadien-1-ol, (−)-2,3-pentadien-1-ol and (−)-2-methyl-3,4-hexadien-2-ol with the maximum enantiomeric purities of 75, 90 − 95 and 90 %, respectively, were formed in lithium aluminium hydride reactions of mono-tetrahydropyranyl derivatives of the appropriate chiral acetylenic diols according to known reactions. (*S*)-(−)-4-Methoxy-2-pentyn-1-ol was also used in the same reaction. The yields varied between 35 and 65 %. Resolved (*S*)-(−)-3-butyn-2-ol was used as the starting material. It was shown that the allene-forming reactions around 0 °C proceed *via* a *trans*-addition of lithium aluminium hydride across the triple bond followed by a highly *anti*-selective 1,2-elimination of metal alkoxide. The overall stereoselectivity increases with the donor properties of the solvent, from diisopropyl ether through diethyl ether to tetrahydrofuran.

Chiral β-allenic alcohols are accessible through Landor's method, in which a 5-alkyl-2-penten-4-yn-1-ol is reduced with a chiral lithium aluminium hydride (LAH) complex,[1,2] and through the LAH reduction of chiral 5-(trialkylammonio)-3-alkyn-1-ols.[3] One chiral α-allenic alcohol (*3c*) was also prepared according to the last mentioned principle.[3]

The above methods give allenic alcohols of low enantiomeric purity (< 33 %) and the overall yields are usually moderate. Clearly, compounds of low enantiomeric purity are of limited usefulness in biochemical-pharmacological experiments in which enantioselective activity is to be determined. We have continued the search for more stereoselective preparations of α- and β-allenic alcohols from a common intermediate and report here that the tetrahydropyranyl (THP) derivative of resolved 3-butyn-2-ol confers high enantiomeric purity to the final allenic products. The procedure gives α-allenic alcohols in good yields and with enantiomeric excess (ee) greater than 90 %; the method is less attractive for β-allenic alcohols although the stereochemical yield of the one prepared is the highest reported (75 % ee).

RESULTS

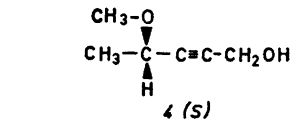
3-Butyn-2-ol was resolved via the (*S*)-(−)-α-methylbenzylaminium salt of the phthalic acid half ester.[4] The enantiomeric purity of the liberated (*S*)-(−)-alcohol (*1*) was determined by NMR on the diasteromeric esters with (−)-α-methoxyphenylacetic acid. Methanol-d_4 as the solvent gave the largest difference in shifts of the diastereotopic terminal acetylenic hydrogens (δ 2.90 and 2.76); acetone-d_6, DMSO-d_6, benzene-d_6, pyridine, $CDCl_3$, CCl_4 and CD_3CN were inferior. The alcohol *1* had > 95 % ee, $[\alpha]_D^{22}$ −51.8° (*c* 3.8, dioxane). Weidman *et al.*[4] reported $[\alpha]_D^{24}$ −40.3° (*c* 3.2, dioxane) for the same alcohol of 100 % ee as determined in NMR with a chiral shift reagent. Our alcohol had α_D^{23} −39.9° (neat), $[\alpha]_D^{23}$ −44.8° (neat, density 0.891).



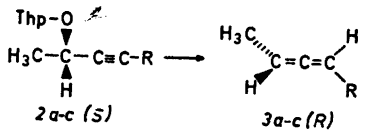
1 (S)

* Allenes and Acetylenes XVI. Part XV: *Acta Chem. Scand. B 31* (1977) 639.

** Correspondence to this author.



4 (S)



2a-c (S) *3a-c (R)*

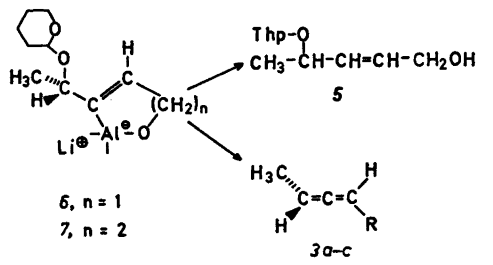
a, R = CH_2CH_2OH
b, R = CH_2OH
c, R = $C(CH_3)_2OH$

The resolved (*S*)-butynol was converted into the derivatives *2a–c* and *4* using standard procedures.[5–7] The latter compounds were treated with LAH according to conditions given in Table 1. The reactions, which are all known from preparations of the racemic allenic compounds,[5,8,9] gave the chiral allenes *3a–c*.

The absolute configuration of the allene *3a* is known.[1] Alcohols *3b* and *3c* can be assigned the (*R*)-configuration according to the Lowe-Brewster empirical rule [10] (*cf.* Discussion).

The enantiomeric purity of the allenic alcohols *3a* and *3c* was determined with $Eu(hfbc)_3$ as described.[3] The α-allenic alcohol *3c* had an enantiomeric excess of 90 % while the β-allenic compound *3a* was less pure, 75 % ee (estimated accuracy ±3 %). The application of the same shift reagent to the (−)-alcohol *3b* was less successful. A ratio of shift reagent to alcohol of 0.24 shifted the α-protons to δ 10.5 (broad multiplet). Increased ratio resulted in considerable peak broadening. Decoupling of the C-2 proton at δ 8.5 simplified the signal to an apparent AB-system (J_{AB} = 11 Hz) while decoupling of the C-4 proton revealed an ABX-system for the C1–C2 protons. In addition to the above pattern for the (−)-alcohol the racemic alcohol *3b* exhibits a narrow multiplet at δ 10.6. However, it might be concluded that the enantiomeric purity of *3b* ($[\alpha]_D^{23}$ −87.6°) prepared from *2b* in diethyl ether ought to be of the same order as that of *3c* (90 % ee) which means that *3b*, $[\alpha]_D^{23}$ −90.7° (run 2) would have around 94 % ee.

Another LAH-reduction of racemic *2b* in ether at −10 °C gave 14 % of the allenol (±)-*3b* and 65 % of compound *5* upon hydrolysis of the reaction mixture after 1 h. Through GLC-comparison with synthetic *cis-5* the isolated product *5* was shown to have the *trans*-configuration (> 97 %). However, when the acetylene *2b* is treated with LAH in refluxing diethyl ether only allenol *3b* can be indicated.



6, n = 1
7, n = 2

5

3a-c

The result indicates that at the lower temperature the reaction proceeds *via* an organometallic

Table 1. Lithium aluminium hydride reductions of compounds *2a–c* and *4* into the chiral allenes *3a–c*.

Run	Cpd	Solvent	Temp /°C	Allene yield %	$[\alpha]_D^{23}$ (*c*, MeOH)
1	*2a*	THF	Reflux	*3a* (35) [a]	−62.5° (7.7)
2	*2b*	THF	24°	*3b* (50) [a]	−90.7° (9.5)
3	*2b*	Et_2O	Reflux	*3b* (65)	−87.6° (9.5)
4	*2b*	$(i\text{-}C_3H_7)_2O$	20°	*3b* (50) [a]	−83.9° (9.1)
5	*2c*	Et_2O	Reflux	*3c* (60)	−87.1° (12.2)
6	*2c*	Et_2O	0°	*3c* (54)	−87.4° (12.3)
7	*4*	Et_2O	Reflux	*3b* (40) [a]	−73.6° (8.4)

[a] Purified further by preparative GLC.

intermediate *6* having the indicated *trans* stereochemistry over the double bond. The reaction of *2b* in diisopropyl ether at 0 °C also takes place *via* the intermediate *6* (> 96 % *trans*).

DISCUSSION

The overall stereochemical course of the reaction (*S*)-*2a*→(*R*)-*3a* is a predominant (to approximately 87 %) attack by hydride on the same side of the propargylic system from which the leaving group departs *i.e.* overall *cis*-substitution. Provided the (*R*)-configurations assigned to *3b* and *3c* are correct the same stereochemistry predominates in their formation from *2b*, *2c* and *4*, for *3c* from *2c* to the extent of more than 95 %. The assignment of absolute configurations to disubstituted allenes according to the Lowe-Brewster rule has so far only failed for 1,2-cyclononadiene.[11] The allenes *3b* and *3c*, unlike 1,2-cyclononadiene, do not present any conformational complications and therefore there is no reason to question the present conclusion based on the Lowe-Brewster rule. Also the reasonable assumption that the same mechanism should be operative in, *e.g.*, *2b*→*3b* as in *2a*→*3a* leads to the same assignment of configurations to the allenes *3b* and *3c*.

We recently have reported that compounds like *2a* upon treatment with LAH in THF are converted into β-allenic alcohols *via* intermediates like *7*. In contrast to *6* these latter intermediates decompose slowly even in refluxing THF.[5] We have chosen to depict the intermediates *6* and *7* as cyclic compounds but this may not be their true nature; indeed, the very different stabilities of *6* and *7* give reason to suspect dissimilar structures.

LAH adds across the acetylenic triple bond of the present derivatives in a *trans* fashion (> 96 %) which is the normal course of this type of reaction;[12] *cis*-addition has been observed with diethyl ether as the solvent but never in THF alone.[12b,c] In our cases this *trans*-addition leading to a transient intermediate may be operative also when no organometallic intermediate can be indicated, *viz.* for compounds *2b*, *2c* and *4* in refluxing ether. The fact that the stereoselectivity of the reaction *2c*→*3c* is the same at 0 and 33 °C (runs 5 and 6) indicates that the mechanism has not changed with temperature. We have observed variation of the stereoselectivity with temperature for other types of leaving groups (to be published).

The addition of LAH to the triple bond is followed by a 1,2-elimination which for the THP-compounds *2b*–*c* follows a nearly exclusive *anti*-mechanism (≥ 95 %). Methoxy as the leaving group does not give the same uniform elimination mechanism as THP-oxy when occupying the same position (*cf.* runs 3 and 7). This difference can possibly be understood as facilitated interaction of the THP-oxy group, with its two oxygens, with the metal atom bound in the vinylic position (*6*). As revealed by studies of models little bridging *via* metal ions (aggregate formation) would be needed for the THP-oxy group to depart in an *anti* mode; the metoxy group clearly has difficulties in this respect leading to slightly increased *syn*-elimination. Research on *E2* reactions in low polar solvents has evidenced the importance of contact between the leaving group and the metal atom of the base.[13,14] The formation of aggregates (of potassium butoxide) was used to explain preferred *anti*-eliminations in toluene.[14]

The β-allenic alcohol *3a* is formed with lower stereoselectivity (75 % ee) than the α-allenic alcohols (90–95 % ee) from similar THP derivatives *i.e.* the *syn*/*anti* ratios of the elimination step are different. This difference might be due to the unknown factors which make the intermediates *6* and *7* differ greatly in stability. Compound *7* is stable for hours in refluxing THF [5] while *6* starts to decompose around 0 °C.

Obviously, the compounds *2a*–*c* are mixtures of diastereomers;[8] however, this has no observable influence on the stereochemical yields.

The influence on stereoselectivity of the donor properties of the ether solvent is seen in the runs 2–4. This nice correlation represents varying *syn*/*anti* ratios in the 1,2-elimination reaction since the addition of LAH to the triple bond is *trans* (> 96 % at 0 °C) in all three cases. Obviously, THF is the solvent of choice for achieving high stereoselectivity. At the same time, however, THF seems to facilitate the LAH reduction of the α-allenic alcohols to conjugated dienes,[24] thus lowering the isolated yields.

The results presented here strongly support the proposed mechanism of the LAH-reduction of a 4,5-epoxy-2-alkyn-1-ol in THF.[15] The authors obtained overall *cis*-substitution over the propargylic system and reasoned in agreement with the herein proven mechanism. Varying results (*cis* or *trans* substitution) have been obtained with other leaving groups (trialkylammonio, tosyloxy and hydroxy) in substitution reactions with LAH in propargylic systems.[3,16] We will comment on these findings in future publications.

EXPERIMENTAL

General. The general IR and NMR instrumentation has been described.[5] These spectra were routinely recorded and are in full agreement with the proposed structures. 100 MHz ^{1}H NMR spectra were taken with a JEOL JNM-FX 100.

The mass spectra were recorded as described.[17] GLC analyses were run on a Varian 1700 instrument, equipped as described.[17] Microanalyses were carried out at the Microanalytical laboratory, Royal Agricultural College, Uppsala. Unless otherwise stated optical rotations were measured in methanol with a Perkin-Elmer 141 spectropolarimeter. All reactions involving $LiAlH_4$ and Grignard reagents were performed under a nitrogen atmosphere.

Materials. (*S*)-(−)-α-Methylbenzylamine was of commercial origin, $[\alpha]_D^{20}$ −36° to −39° (neat). $Eu(hfbc)_3$ was purchased from Aldrich Chemical Co. This reagent was handled under a dry nitrogen atmosphere and NMR-solutions in $CDCl_3$ containing this shift reagent, were centrifuged prior to running the spectra. α-Methoxyphenylacetic acid was resolved *via* the (−)-ephedrine salt[18] to give (*R*)-(−)-α-methoxyphenylacetic acid, $[\alpha]_D^{22}$ −148.7° (*c* 0.6, absolute ethanol). This acid was converted to the acid chloride and 3-butyn-2-ol was acylated according to a method described in the literature.[19]

(S)-(−)-3-Butyn-2-ol-(1) was prepared according to the literature.[4,20] Reaction of this alcohol with (*R*)-(−)-methoxyphenylacetyl chloride (see *Materials*) gave the diastereomeric esters. NMR in methanol-d_4 indicated an enantiomeric purity of the alcohol of > 95 % (see Results).

(S)-(−)-3-Methoxy-1-butyne was prepared as described for similar compounds[6] from *1* and dimethyl sulfate. Yield 46 %. B.p. 68 °C. $[\alpha]_D^{22}$ −130.5° (*c* 7.7). The racemic ether is known.[23]

(S)-(−)-4-Methoxy-2-pentyn-1-ol (4) was prepared according to standard procedures,[8] from the Grignard derivative of (−)-3-methoxy-1-butyne and gaseous formaldehyd. Yield 68 %, b.p. 90 °C/2 kPa. $[\alpha]_D^{22}$ −122.9° (*c* 9.6). Anal. $C_8H_{10}O_2$: C, H.

(−)-3-(2-Tetrahydropyranyloxy)-1-butyne was prepared according to Ref. 7 from *1*. Yield 86 %. $[\alpha]_D^{22}$ −122.3° (*c* 10.8).

(−)-5-(2-Tetrahydropyranyloxy)-3-hexyn-1-ol (2a) was prepared as described.[21] Yield 75 %. $[\alpha]_D^{22}$ −121.0° (*c* 11.3).

(−)-4-(2-Tetrahydropyranyloxy)-2-pentyn-1-ol (2b) was prepared according to literature procedures.[21] Yield 80 %. $[\alpha]_D^{22}$ −118.7° (*c* 11.4).

(−)-2-Methyl-5-(2-Tetrahydropyranyloxy)-3-hexyn-2-ol (2c) was prepared in analogy with similar compounds[8] from (−)-3-(2-tetrahydropyranyloxy)-1-butyne (12.0 g; 0.08 mol) and acetone (4.5 g; 0.08 mol). Yield 79 %, b.p. 93 °C/40 Pa. $[\alpha]_D^{22}$ −106.8° (*c* 9.1). Anal. $C_{12}H_{20}O_3$: C, H.

Reactions of 2a−c *and* 4 *with* $LiAlH_4$.[5,8,9] Approximately 0.03 mol of the acetylenes *2a−c* and *4* in the appropriate solvent (see Table 1) was slowly added to a stirred suspension of $LiAlH_4$ (1.4 equiv. for the THP-oxy derivatives and 1.0 equiv. for the methoxy compound). The mixture was then kept at the indicated temperature and the reactions were followed by GLC. Work-up and distillation yielded the products *3a−c* in Table 1. Some of these were isolated on GLC (20 % Carbowax 20 M) before measuring the rotation (Table 1). Reaction of racemic *2b* with $LiAlH_4$ in ether at −10 °C for 1 h gave 14 % racemic *3b* and 65 % of *trans*-4-(tetrahydro-2-pyranyloxy)-2-penten-1-ol (*trans-5*), which was isolated by preparative GLC (20 % SE-30) and identified by IR, NMR and MS.

cis-*4-(2-Tetrahydropyranyloxy)-2-penten-1-ol (cis-5)* was prepared by hydrogenation[22] of racemic *2b*. The product was isolated and characterized as described for the *trans*-compound. Comparison of *cis*- and *trans-5* was made by GLC on 5 % OV 25; the ratio of retention times *trans*/*cis* at 165 °C was 1.5.

Acknowledgements. We thank Dr. Carin Engdahl for running the 100 MHz ^{1}H NMR spectra. The work was in part supported by the Swedish Natural Science Research Council.

Note added in proof: Dr. G. R. Sullivan of Stanford Magnetic Resonance Laboratory, Stanford University, has obtained resolution of the α-protons of the α-allenic alcohol *3b* using $Eu(dcm)_3$ [Whitesides *et al. J. Am. Chem. Soc. 96* (1974) 1038] in deuteriochloroform (enantiomeric shift difference 24 Hz). The enantiomeric purity of *3b*, $[\alpha]_D$ −83.90, was estimated to be 80 − 90 %, *i.e.* the same alcohol with $[\alpha]$ −90.7° (run 2) should be very close to 100 % optically pure. We are most grateful to Dr. Sullivan for running the spectrum.

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The Crystal Structure of 1,4-Etheno-2,7-dihydroxy-2,4,6,8-tetramethyl-1,2,3,4-tetrahydronaphthalen-3-one

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X-Ray diffraction data from the title compound, $C_{16}H_{18}O_3$, were collected with a computer-controlled Philips PW1100 diffractometer. The unit cell is triclinic, space group $P\bar{1}$, having two independent molecules in the asymmetric unit. Cell dimensions are $a=16.107$, $b=10.649$, $c=8.288$ Å, $\alpha=107.13$, $\beta=90.21$, $\gamma=92.06°$. The structure was solved by the direct method and refined by the full-matrix least-squares technique to a final value of $R=0.058$ for 3479 unique observed reflexions. Bond distances in the crystallographically independent molecules are identical within 3σ. The molecular packing is stabilized by a network of intermolecular hydrogen bonds involving all hydroxyl groups.

The title compound is obtained upon KOH/EtOH treatment of 1,4-etheno-2,8-diacetoxy-2,4,6,8-tetramethyloctahydronaphthal-5-ene-3,7-dione, a Diels-Alder dimer of 6-acetoxy-2,6-dimethyl-2,4-cyclohexadienone, which is formed upon lead tetraacetate oxidation of 2,6-dimethylphenol.

The formation of substituted 1,4-ethenonaphthalenes in Diels-Alder cycloaddition reactions involving oxidation products of methyl homologues of *o*-cresol has been discussed by, *e.g.*, Adler *et al*,[1–6] Holmberg [7,8] and Lindgren *et al*.[9] Within a research program concerning the molecular geometry of this type of Diels-Alder adduct, we have earlier reported the crystal structures of adducts formed upon the action of different oxidation agents on 2,6-dimethylphenol [10–12] and 2,4-dimethylphenol.[13–15] The title compound is obtained upon KOH/EtOH treatment of 1,4-etheno-2,8-diacetoxy-2,4,6,8-tetramethyloctahydronaphthal-5-ene-3,7-dione,[3,7] a Diels-Alder dimer formed upon lead tetraacetate oxidation of 2,6-dimethylphenol.[6,12]

The UV spectrum [3] of the title compound might be interpreted to indicate an interaction between the aromatic system and the etheno part, reflected in an absorption maximum at 311 nm. This structure determination was, in part, performed to elucidate the geometry of molecular regions mentioned.

X-RAY EXPERIMENTAL

A crystal of the approximate volume 0.008 mm³ was selected for the intensity measurements. Lattice parameters were obtained from least-squares treatment of the angular coordinates of 25 reflexions well centered on a Philips PW1100 diffractometer equipped with a graphite monochromator to reflect Cu$K\alpha$ radiation. Crystal data are: space group $P\bar{1}$, $a=16.107(1)$, $b=10.649(4)$, $c=8.228(3)$ Å, $\alpha=107.13(4)$, $\beta=90.21(3)$, $\gamma=92.06(6)°$, $V=1348$ Å³, $Z=4$, $D_{X-ray}=1.27$ g cm^{-3}, $\mu(CuK\alpha)=7.11$ cm^{-1}.

Diffraction intensities within the $+h$-hemisphere of the reciprocal space were collected out to $2\theta=132°$ with the $\theta-2\theta$ scan technique [scan width $1.5°(\theta)$, scan speed $1.32°(\theta)$ min^{-1}]. Background counts were measured half the total scan time at each scan limit.

In order to monitor alignment and possible deterioration of the crystal, three standard reflexions were measured every 90 min. No significant intensity variation was detected for these reflexions. Of the 4690 independent reflexions measured, 3479 were above the threshold value determined by the criterion $I_{net}>4\sigma(I_{net})$, where $\sigma(I_{net})$ is based on counter statistics. Intensities were corrected for Lorentz and polarization effects, but not for absorption.

STRUCTURE DETERMINATION AND REFINEMENT

The overall temperature factor and scale factor were calculated from a least-squares fitted Wilson curve,[16] and normalized structure factors, $|E|$, were generated. Phases for the 353 $|E|$ values greater than 1.80 were computed for 128 sets by a modified version of the MULTAN[17] direct phase determination procedure. The E map computed with the set showing the second best reliability index, neglecting the trivial set, revealed all 38 non-hydrogen atoms. The structural parameters, assuming first isotropic and then anisotropic thermal vibrations, were refined according to the full-matrix least-squares technique. The positions of 26 hydrogen atoms were deduced from a difference Fourier synthesis, and of the remaining 10 from chemical reasoning. The hydrogens were included with fixed coordinates and isotropic temperature factors in the final refinement, involving one molecule at a time. The R-value converged to 0.058 for the 3479 unique observed reflexions. Hughes'[18] weighting scheme with $F_{o,min} = 2.75$ was used throughout the refinement. The atomic scattering factors for carbon and oxygen were taken from the *International Tables for X-Ray Crystallography*,[19] and that of hydrogen from Stewart *et al.*[20] The final parameters for the non-hydrogen atoms are listed in Table 1, and those for the hydrogens in Table 2. A list of the observed and calculated structure factors is available from the authors on request.

RESULTS AND DISCUSSION

The molecular geometries of the two crystallographically independent molecules, henceforth called unprimed and primed, respectively, are shown in Fig. 1. Bond lengths and angles involving the non-hydrogen atoms are given in Fig. 2 and Table 3, respectively. The standard deviations are estimated to be ≤ 0.004 and ≤ 0.005 Å for the C–C and C–O bonds in the respective molecules. The esd's for the angles are $\leq 0.3°$ in both molecules. The mean C–H bond lengths are 0.94 (range 0.75 – 1.04 Å) and 0.97 Å (range 0.82 – 1.10 Å), unprimed and primed respectively, in good agreement with the value of 0.94 Å given by Stewart *et al.*[20] The average bond distances for the C(sp^2) – C(sp^3)

Table 1. Positional and thermal parameters (× 10^4) for the non-hydrogen atoms in the two molecules. Primed atoms belong to the primed molecule. The B_{ij} values refer to the temperature factor expression $\exp[-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}hk + B_{13}hl + B_{23}kl)]$. Estimated standard deviations are given in parentheses.

ATOM	X	Y	Z	B11	B22	B33	B12	B13	B23
C(1)	1613(2)	3299(3)	7124(4)	23(1)	73(3)	115(5)	13(3)	3(3)	63(6)
C(2)	716(2)	2871(3)	7436(4)	20(1)	65(3)	133(5)	12(3)	-7(3)	24(6)
C(3)	668(2)	2927(3)	9338(4)	23(1)	71(3)	148(5)	13(3)	14(4)	41(6)
C(4)	1488(2)	3364(3)	10335(4)	22(1)	74(3)	108(5)	15(3)	9(3)	22(6)
C(4A)	2130(2)	2413(3)	9332(3)	18(1)	63(3)	107(5)	4(3)	5(3)	33(5)
C(5)	2644(2)	1684(3)	10010(4)	25(1)	84(3)	102(4)	8(3)	3(4)	64(6)
C(6)	3248(2)	921(3)	9019(4)	21(1)	71(3)	138(5)	6(3)	0(4)	72(6)
C(7)	3302(2)	884(3)	7317(3)	17(1)	61(3)	118(5)	4(3)	8(3)	27(5)
C(8)	2784(2)	1602(3)	6571(3)	18(1)	66(3)	106(4)	-5(3)	2(3)	37(5)
C(8A)	2197(2)	2372(3)	7627(3)	17(1)	64(3)	113(5)	5(3)	0(3)	51(5)
C(9)	1751(2)	4654(3)	8399(4)	25(1)	65(3)	182(6)	6(3)	-2(4)	73(6)
C(10)	1679(2)	4691(3)	10004(4)	24(1)	62(3)	164(6)	6(3)	-8(4)	6(6)
C(11)	75(2)	3743(3)	7026(4)	29(1)	100(4)	184(6)	35(3)	-24(4)	36(7)
O(12)	557(1)	1538(2)	6443(3)	21(1)	79(2)	176(4)	15(2)	-10(3)	-17(5)
O(13)	39(2)	2663(3)	9958(3)	28(1)	195(4)	209(5)	-27(3)	30(3)	123(7)
C(14)	1412(2)	3429(4)	12192(4)	33(2)	122(4)	123(5)	43(4)	27(4)	42(7)
C(15)	3850(2)	199(4)	9774(4)	30(1)	117(4)	193(6)	38(4)	2(5)	130(8)
O(16)	3939(1)	168(2)	6424(3)	24(1)	81(2)	146(4)	22(2)	13(3)	16(4)
C(17)	2885(2)	1553(3)	4737(4)	26(1)	99(3)	116(5)	12(3)	14(4)	66(6)
C'(1)	6463(2)	3319(3)	7974(4)	21(1)	79(3)	112(5)	9(3)	10(3)	32(6)
C'(2)	5617(2)	2682(3)	7207(4)	19(1)	63(3)	129(5)	5(3)	17(4)	25(6)
C'(3)	5518(2)	2959(3)	5477(4)	19(1)	76(3)	158(5)	19(3)	7(4)	56(6)
C'(4)	6249(2)	3776(3)	5043(4)	23(1)	84(3)	178(6)	21(3)	16(4)	113(7)
C'(4A)	7003(2)	2947(3)	5108(4)	19(1)	67(3)	142(5)	3(3)	-2(4)	78(6)
C'(5)	7535(2)	2447(3)	3791(4)	23(1)	79(3)	127(5)	-1(3)	5(4)	86(6)
C'(6)	8198(2)	1692(3)	3997(4)	21(1)	70(3)	115(5)	0(3)	11(3)	49(6)
C'(7)	8280(2)	1427(3)	5543(3)	17(1)	59(3)	113(4)	1(3)	-8(3)	26(5)
C'(8)	7758(2)	1942(3)	6927(3)	19(1)	60(3)	104(4)	-6(3)	-3(3)	30(5)
C'(8A)	7117(2)	2709(3)	6679(3)	16(1)	63(3)	110(4)	-3(3)	1(3)	31(5)
C'(9)	6424(2)	4763(3)	8061(5)	21(1)	68(3)	213(7)	2(3)	12(4)	-8(7)
C'(10)	6305(2)	4988(3)	6588(5)	24(1)	71(3)	251(8)	12(3)	17(5)	81(8)
C'(11)	4898(2)	3220(3)	8375(4)	23(1)	94(3)	152(6)	8(3)	28(4)	12(7)
O'(12)	5648(1)	1287(2)	6907(3)	26(1)	70(2)	152(4)	-1(2)	10(3)	50(4)
O'(13)	4916(2)	2585(3)	4577(3)	30(1)	152(3)	209(5)	-11(3)	-38(4)	137(6)
C'(14)	6111(2)	4064(4)	3364(5)	33(2)	148(5)	252(8)	40(4)	17(5)	261(10)
C'(15)	8800(2)	1186(3)	2571(4)	33(2)	109(4)	140(5)	33(4)	44(4)	82(7)
O'(16)	8906(1)	652(2)	5817(3)	24(1)	73(2)	135(4)	22(2)	-6(3)	24(4)
C'(17)	7916(2)	1656(3)	8587(4)	27(1)	90(3)	116(5)	10(3)	-2(4)	65(6)

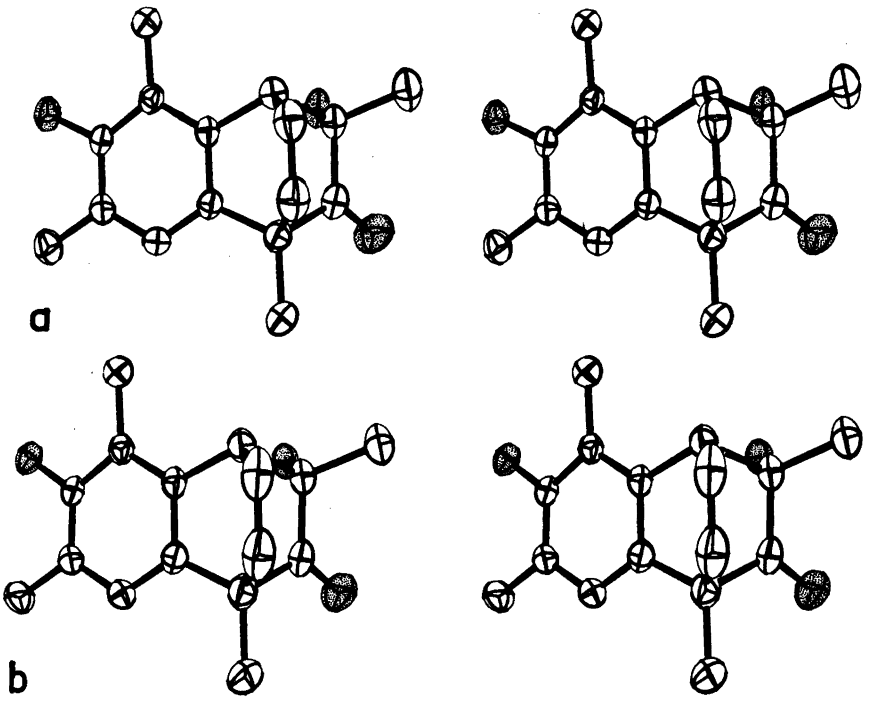


Fig. 1. Stereoscopic views of the two molecules in the asymmetric unit. *a*, Unprimed and *b*, primed molecule. The oxygen atoms are represented with shaded ellipsoids.

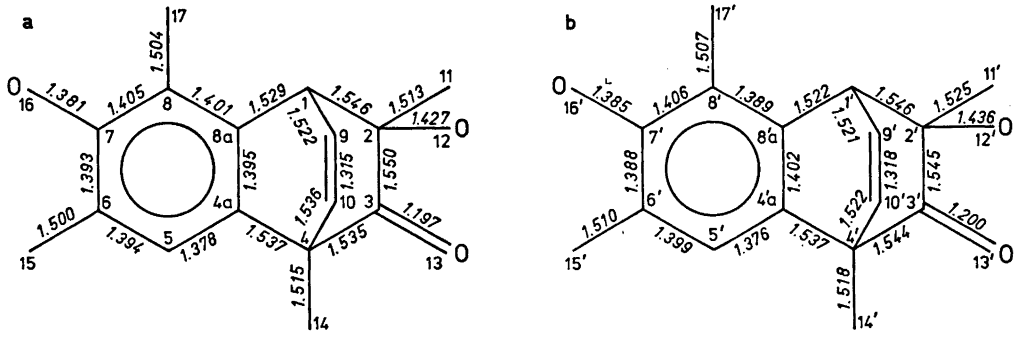


Fig. 2. Bond distances (Å) involving the non-hydrogen atoms. *a*, Unprimed and *b*, primed molecule.

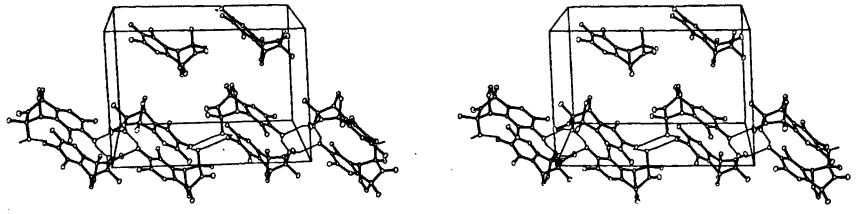


Fig. 3. A stereoscopic view of the molecular packing. O – H and O···H interactions in the hydrogen bonds are indicated with weak lines. The origin is at the bottom left corner, *a* is directed to the right, *b* upwards and *c* towards the reader.

Table 2. Positional ($\times 10^3$) and thermal ($\times 10^2$) parameters for the hydrogen atoms. Primed atoms belong to the primed molecule.

ATOM	X	Y	Z	B
H(C1)	164	313	594	254
H(C5)	262	176	1114	235
H(C9)	183	546	810	281
H(C10)	179	549	1091	289
H1(C11)	19	461	754	374
H2(C11)	10	358	608	374
H3(C11)	-44	351	690	374
H(O12)	2	149	617	309
H1(C14)	129	250	1227	352
H2(C14)	197	377	1276	352
H3(C14)	104	399	1256	352
H1(C15)	384	-70	910	389
H2(C15)	434	64	1017	389
H3(C15)	357	16	1086	389
H(O16)	375	-35	547	291
H1(C17)	238	100	406	284
H2(C17)	285	240	452	284
H3(C17)	342	114	430	284
H(C'1)	649	313	902	251
H(C'5)	752	267	267	253
H(C'9)	655	555	902	323
H(C'10)	619	585	645	341
H1(C'11)	439	290	786	321
H2(C'11)	486	417	877	321
H3(C'11)	500	309	929	321
H(O'12)	511	98	672	275
H1(C'14)	557	443	330	431
H2(C'14)	598	313	238	431
H3(C'14)	655	463	308	431
H1(C'15)	938	112	302	342
H2(C'15)	878	30	199	342
H3(C'15)	880	180	180	342
H(O'16)	890	-11	499	263
H1(C'17)	845	207	908	272
H2(C'17)	800	77	835	272
H3(C'17)	737	175	913	272

bonds are 1.526 Å in both molecules, which is $\geq 5\sigma$ longer than the standard value of 1.501 Å given by Lide;[21] this is presumably due to the fact that many of the long bonds involve highly substituted atoms.

The two independent molecules are quite similar; all individual bonds differ $\leq 3\sigma$ and most angles $\leq 1°$. Corresponding angles involving O(16) and O'(16) differ by about 5°, however, indicating that these hydroxyl groups have sligthly differing orientations, possibly due to their participation in the hydrogen bonding system.

The aromatic rings, C(4a)–C(8a), *cf.* Fig. 2, and C'(4a)–C'(8a), show mean values for the C–C bond lengths of 1.394 and 1.395 Å, respectively; the bonds C(4a)–C(5), 1.378 and 1.376 Å, unprimed and primed respectively, and C(7)–C(8), 1.405 and 1.406 Å, likewise, are the ones that differ most from these means. The distances of the ring atoms from the L.S. planes for the aromatic rings, A and A', are given in Table 4. It can be seen that A is somewhat more planar (±0.009 Å) than A' (±0.017 Å). Table 4 also summerizes the deviations from planarity for the L.S. planes in ring system B and B', each of which consists of the three boat-shaped rings. The dihedral angle between the A plane and the plane defined by the atoms C(4a), C(8a), C(9) and C(10) is 148.6°. The corresponding angle in the primed molecule is 154.1°. The torsion angles C(1)–C(8a)–C(4a)–C(5) are −177 and −179°, primed and unprimed respectively; and those of C(4)–C(4a)–C(8a)–C(8) are 177 and 178°, likewise. These conformation angles exclude the above mentioned possibility of an interac-

Table 3. Bond angles (°) involving the non-hydrogen atoms.

	Unprimed molecule	Primed molecule
C(2)–C(1)–C(8a)	107.4	106.4
C(2)–C(1)–C(9)	104.6	105.5
C(8a)–C(1)–C(9)	107.4	107.8
C(1)–C(2)–C(3)	107.0	106.8
C(1)–C(2)–C(11)	112.5	112.0
C(1)–C(2)–O(12)	109.1	108.3
C(3)–C(2)–C(11)	110.1	111.1
C(3)–C(2)–O(12)	107.8	108.5
C(11)–C(2)–O(12)	110.1	110.0
C(2)–C(3)–C(4)	114.1	114.0
C(2)–C(3)–O(13)	122.1	122.3
C(4)–C(3)–O(13)	123.8	123.7
C(3)–C(4)–C(4a)	104.9	103.0
C(3)–C(4)–C(10)	101.4	102.9
C(3)–C(4)–C(14)	112.5	112.2
C(4a)–C(4)–C(10)	106.4	107.7
C(4a)–C(4)–C(14)	115.4	115.2
C(10)–C(4)–C(14)	115.0	114.6
C(4)–C(4a)–C(5)	125.2	125.7
C(4)–C(4a)–C(8a)	114.7	113.8
C(5)–C(4a)–C(8a)	120.1	120.5
C(4a)–C(5)–C(6)	120.7	120.6
C(5)–C(6)–C(7)	118.4	117.9
C(5)–C(6)–C(15)	121.1	120.5
C(7)–C(6)–C(15)	120.4	121.7
C(6)–C(7)–C(8)	122.7	123.1
C(6)–C(7)–O(16)	115.7	120.9
C(8)–C(7)–O(16)	121.5	116.0
C(7)–C(8)–C(8a)	116.8	117.2
C(7)–C(8)–C(17)	120.4	119.6
C(8a)–C(8)–C(17)	122.8	123.3
C(1)–C(8a)–C(4a)	112.9	113.2
C(1)–C(8a)–C(8)	125.7	126.0
C(4a)–C(8a)–C(8)	121.3	120.8
C(1)–C(9)–C(10)	115.1	115.0
C(4)–C(10)–C(9)	115.9	115.7

Table 4. Least-squares planes and deviations. The planes are described in terms of normalized equations in the orthogonal coordinate system (m,n,p), having $p||c$, n in the bc plane and m in the abc octant. The primed planes belong to the primed molecule. Atoms marked with asterisks were omitted from the calculations of the least-squares planes.

Plane A	0.6614 m +	0.7496 n +	0.0265 p =	4.1883
B1	0.2501 m +	0.9680 n −	0.0194 p =	2.9769
B2	−0.7222 m +	0.6879 n −	0.0722 p =	0.7638
B3	0.9526 m +	0.2961 n +	0.0695 p =	4.4300
A′	0.5963 m +	0.7989 n +	0.0787 p =	9.0310
B1′	0.0630 m +	0.9823 n +	0.1762 p =	3.8006
B2′	0.8365 m −	0.5102 n −	0.1999 p =	5.3484
B3′	0.8804 m +	0.4727 n −	0.0379 p =	11.0076

Planes A,A′

Atom	Deviation (A)	(A′)
C(4a)	0.003 Å	−0.009 Å
C(5)	−0.009	−0.004
C(6)	0.008	0.017
C(7)	−0.002	−0.017
C(8)	−0.004	0.004
C(8a)	0.004	0.009

Planes B1,B1′

Atom	Deviation (B1)	(B1′)
C(1)*	0.732 Å	0.742 Å
C(2)	−0.005	−0.003
C(3)	0.005	0.003
C(4)*	0.702	0.741
C(4a)	−0.005	−0.004
C(8a)	0.005	0.004

Planes B2,B2′

Atom	Deviation (B2)	(B2′)
C(1)*	−0.746 Å	0.740 Å
C(2)	0.007	−0.010
C(3)	−0.008	0.010
C(4)*	−0.739	0.717
C(9)	−0.009	0.012
C(10)	0.009	−0.012

Planes B3,B3′

Atom	Deviation (B3)	(B3′)
C(1)*	−0.657 Å	−0.651 Å
C(4)*	−0.645	−0.632
C(4a)	0.003	0.004
C(8a)	−0.003	−0.004
C(9)	0.003	0.004
C(10)	−0.003	−0.004

Table 5. Distances (Å), angles (°) and symmetry codes for the atoms involved in the hydrogen bonds.

	$X^i-H^i\cdots Y^{ii}$	X···Y	X−H	H···Y	∠X−H···Y	i	ii
I:	O(12)−H(O12)···O′(16)	2.784	0.881	1.962	154.5	$x+1,y,z$	x,y,z
II:	O′(16)−H(O′16)···O(12)	2.692	0.889	1.874	152.1	x,y,z	$1-x,-y,1-z$
III:	O(16)−H(O16)···O′(12)	2.819	0.860	2.171	131.9	x,y,z	$1-x,-y,1-z$
IV:	O′(12)−H(O′12)···O(16)	2.939	0.907	2.034	174.9	x,y,z	x,y,z

tion between the aromatic system and the etheno bridge.

The molecular packing pattern is illustrated in Fig. 3. It consists of strings, parallel to the a axis, of hydrogen bonded molecules. Distances, angles and symmetry codes for the atoms involved are listed in Table 5. Two pairs of independent molecules are connected through octagons consisting of one O−H group from each molecule. Two different octagons exist: the bonds I and II, *cf.* Table 5, and their centric equivalents across $1,0,\frac{1}{2}$ form one; the bonds III and IV and their equivalents across the center of symmetry $\frac{1}{2},0,\frac{1}{2}$, the other. Each string binds neighbouring strings by van der Waals forces. There is, however, one such contact shorter than 3.5 Å; $C(10)^i-C'(9)^{ii}$ = 3.39 Å ($i\equiv x,y,z$; $ii\equiv 1-x,\ 1-y,\ 2-z$).

A further discussion of this structure and of related ones will be given in a forthcoming article.

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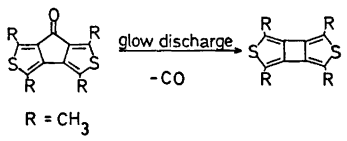
Reactions of Phenylthiophenes in Glow Discharges

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Naphthalene is the major product when phenylthiophenes are treated in the low-temperature plasma of glow discharges. The possibility of intervention of phenylcyclobutadiene is discussed.

The reactions of organic compounds in the low-temperature plasma of glow discharges have attracted much interest in recent years and the subject has been extensively reviewed.[1-3] Our interest in this field originated some time ago from an unsuccessful attempt to decarbonylate a thiophene analogue of fluorenone in a glow discharge [eqn. (1)]. The corresponding reaction with fluorenone is reported to give an excellent yield of biphenylene.[4]



(1)

However, in our case the reaction produced a complex mixture of material with high molecular weight which was not further characterised. We then decided to make a systematic study of the reactions of thiophenes in glow discharges and here report our results for the phenylthiophenes.

There were several reasons for our choice of substrates. First of all, phenylthiophenes have been extensively studied by mass spectrometry [5-7] and also photochemically,[8] both methods more or less related to plasma reactions.[1] Secondly, the benzene moiety has proved much more stable during electron impact than thiophenes, thereby increasing the possibility to isolate identifiable products. Finally, the glow discharge reaction of thiophene itself has quite recently been reported from this Laboratory and the experimental details of the reactor are found in that report.[9]

RESULTS AND DISCUSSION

Only the ether-soluble products, amounting to nearly 80 % of the material passing the plasma zone, were analysed. The rest were polymeric deposits on the reactor walls, insoluble in both ether and chloroform, and gaseous products escaping the traps.

Table 1. Ether soluble products (excluding unchanged starting material) from reactions of phenylthiophenes in a glow discharge. The total yield of the sum of these products was in the range of 20 – 30 %.

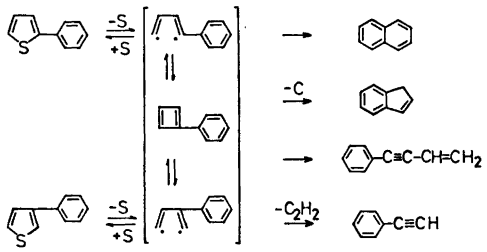
Products	Starting material 2-Phenylthiophene	3-Phenylthiophene	2-Phenylthiophene + acetylene [a]
2-Phenylthiophene	–	28 ± 5	–
3-Phenylthiophene	26 ± 5	–	–
Naphthalene	62 ± 10	44 ± 10	23 ± 5
Indene	5 ± 2	6 ± 2	15 ± 3
Biphenyl	0	0	Trace
1-Phenyl-3-buten-1-yne	2 ± 2	7 ± 3	15 ± 3
Phenylacetylene	5 ± 2	15 ± 3	23 ± 5
3 Comps. with M = 184	0	0	8 ± 2
Others	Trace	Trace	16 ± 3

[a] Here the yields are calculated by excluding both unchanged and isomerised starting material.

Unchanged starting material constituted the major part (up to 60 %) of the ether-soluble products. The minor part had the composition shown in Table 1.

An immediate feature of these results is that 2-phenylthiophene isomerises to 3-phenylthiophene and *vice versa*. This reaction is probably not a mere fission/recombination of phenyl and thienyl radicals, but rather an *intra*-molecular rearrangement indicated by the absence of biphenyl, bithienyl, thiophene or benzene among the reaction products.

The major reaction product from both 2- and 3-phenylthiophene is naphthalene. Excluding starting material and isomerised starting material, the amount of naphthalene approaches 80 – 90 % of the ether soluble products. It is interesting that both isomers give naphthalene, indicating again a deep-seated rearrangement. Smaller amounts of indene, 1-phenyl-3-buten-1-yne and phenylacetylene were also produced. On the basis of this product distribution, the sequences shown in Scheme 1 are postulated.

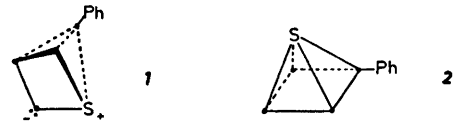


Scheme 1.

The intermediates are formulated as two phenylbutadiene diradicals in rapid equilibrium through phenylcyclobutadiene, a hypothesis that is supported by recent work of Suhr and Rosskamp.[10] They studied the reaction of phenylacetylene with acetylene in a low-temperature plasma and reported naphthalene as the major product and styrene, indene, benzene and toluene as the other liquid products. The formation of naphthalene was rationalised by postulating phenylcyclobutadiene to be the primary product which subsequently ring-opened to 1-phenylbutadiene diradical and cyclised to naphthalene. Also the formation of 1-phenyl-3-buten-1-yne through phenylcyclobutadiene finds support in this work, where the analogous vinylacetylene is reported to be formed from cyclobutadiene *via* the corresponding diradical.

It is also interesting to note that Bowie *et al.*[5] postulated the phenylcyclobutadiene structure for the ion with $m/e = 128$ produced by loss of sulfur from the molecular ion of 2-phenylthiophene during electron impact in the mass spectrometer. In the opinion of the present authors the m/e 128 ion could, however, as well be attributed to a naphthalene structure.

The isomerisation of 2-phenylthiophene and 3-phenylthiophene is also explained by this reaction scheme. It is pertinent that 2-phenylthiophene has been found to undergo an irreversible photo-isomerisation to 3-phenylthiophene, also without breaking the aryl-aryl bond.[8] The current hypothesis is that this isomerisation proceeds through the excited intermediate/transition state *1* which is stabilised by Möbius-like overlap.[8]



Although *1* would also explain the plasma induced isomerisations, it does seem neither necessary nor probable in explaining the formation of naphthalene or 1-phenyl-3-buten-1-yne.

In order to explain the fragmentation of ^{13}C-labelled phenylthiophenes during electron impact in the mass spectrometer, Weringa *et al.*[7] postulated the intermediate Ladenburg structure *2*. It is very difficult to rule out such a possibility for the plasma isomerisation reactions, but again it is certainly not necessary for the naphthalene formation.

With the intention of checking whether phenylcyclobutadiene is an intermediate or merely a transition state, the plasma reaction of 2-phenylthiophene was performed also in the presence of acetylene (Table 1). One important result of this experiment is that biphenyl was detected in trace amounts only. Considering the high Diels-Alder reactivity of cyclobutadienes,[11] it is reasonable to regard phenylcyclobutadiene as nothing more than a transient species in these reactions. It is also worth mentioning

that the three isomeric products with molecular weight 184 (Table 1) correspond to starting material substituted by one ethynyl group. This indicates the existence of ethynyl radicals capable of attacking the thienyl and/or the phenyl ring.

The results presented provide an explanation for the failure to produce a thiophene analogue of biphenylene by a glow discharge reaction as mentioned initially (eqn. 1). The apparent reason being that sulfur is lost from the ketone before decarbonylation (or from the decarbonylated product) and that no simple route to stable products is open with the consequent formation of a very complex mixture of products.

EXPERIMENTAL

The substrates 2- and 3-phenylthiophene, were prepared according to literature procedures.[12–14] The glow discharge reactor [9] was operated at an input effect of 30 – 50 W (as measured by the wattmeter between the transmitter and the generator plates). The rate of distillation per applied energy units was in the range of 10 – 60 g/kWh (0.1 – 0.3 mol/kWh) and was regulated by changing the temperature of the distillation flask.

The ether soluble products, accounting for 70 – 80 % of the material passing the plasma zone, were collected in the cooling trap at – 78 °C and were analysed by GLC, performed on a Hewlett-Packard 5700 A with 10 % OV 17 or 10 % PEG 4000 columns (*l* 2.5 m, i.d. 0.02 m) using a flame ionisation detector. The identity of the products was also established by GLC/MS with the use of a Perkin Elmer 990 gas chromatograph coupled with a Hitachi-Perkin Elmer RMU-GL mass spectrometer. The preparative separation of naphthalene was performed on a Varian Aerograph 200 gas chromotograph. The NMR analyses were run on Varian HA-60A and HA-100.

All recorded products (Table 1) were identified by comparison with authentic samples. 1-Phenyl-3-buten-1-yne was prepared by treatment of 1-phenyl-3-tosyloxy-1-butyne with *t*-BuOK in THF. The tosylate was kindly supplied by Dr. J. L. Derocque, University of Saarland, to whom we extend our acknowledgements.

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Short Communications

Stereochemistry of the Methylcitric Acids Formed in the Citrate Synthase Reaction with Propionyl-CoA

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Examination of the methylcitric acid present in the urine from patients with propionic acidaemia shows two chromatographic peaks of approximately equal intensity.[1] By correlation with hydroxycitric acids of known stereochemistry, the absolute configurations of the acids corresponding to these peaks have been shown to be 2*S*, 3*S*† (*1*) and 2*R*, 3*S* (*2*).[2]

Radioactive propionate was found to be incorporated *in vivo* into *1* and *2*, suggesting that their biosyntheses involve condensation of propionyl-CoA with oxaloacetate,[1] a reaction similar to that occurring in the biosynthesis of citric acid. Stern had previously noted that oxaloacetate was consumed when mixed with propionyl-CoA and citrate synthase, and this lends support to the hypothesis that citrate synthase is the enzyme responsible for the biosynthesis of methylcitrate. The V_{max} for the reaction involving propionyl-CoA was, however, found to be only 10^{-4} times that for acetyl-CoA.[3] Later, methylcitric acid of unknown stereochemistry was detected in a reaction catalysed by pig heart citrate synthase.[4]

We have now found that *1* and *2*, in a total yield of 10 – 15 % calculated from propionyl-CoA, are the products from the pig heart citrate synthase mediated reaction between propionyl-CoA and oxaloacetate. It is therefore likely that the acids *1* and *2* produced by propionic acidaemia patients are biosynthesised in this side reaction of citrate synthase. The time course of the reaction is shown in Fig. 2, from which it can be seen that the rate of reaction is, under the experimental conditions, about one order of magnitude lower than reported.[3] The optical purities of *1* and *2* obtained in the *in vitro* experiments are high but, due to the small amounts investigated, no accurate figures can be given. The ratio of *1* to *2* was found to vary between approximately 0.3 and 3.

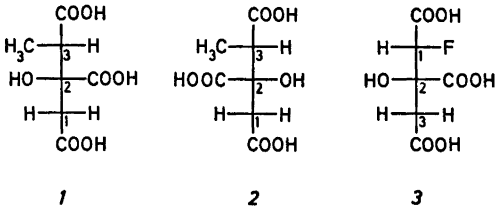


Fig. 1. Stereostructures of the methylcitric acids (*1*, *2*) formed in the citrate synthase reaction with propionyl-CoA. Proposed [15] stereostructure of the fluorocitric acid (*3*) formed in the citrate synthase reaction with fluoroacetyl-CoA.

† The numbering is that of 2-hydroxy-1,2,3-alkanetricarboxylic acids.

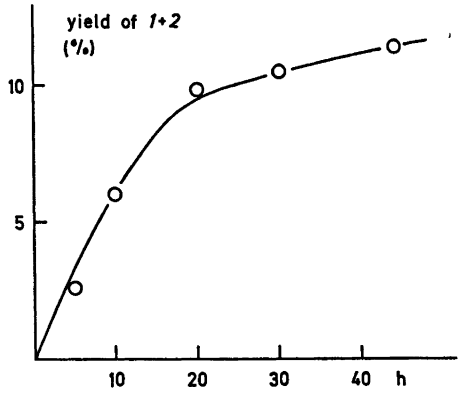


Fig. 2. Time course of the formation of methylcitric acids in the citrate synthase mediated reaction of propionyl-CoA with oxaloacetate. The ratio of acids *1*/*2* was approximately 3 at all determined points. The yield is calculated from propionyl-CoA.

The stereostructures of the methylcitric acids formed show that in this case citrate synthase is apparently unspecific with respect to *re* or *si* attack on oxaloacetate, but stereospecific with respect to reaction with the propionyl-CoA moiety. A specific methylcitrate synthase which only synthesises *1* occurs in a yeast.[2,5] The biosynthesis of *1*, but not that of *2*, is stereochemically analogous to the biosynthesis of citric acid, known to proceed in a *si* fashion [6] except in the case of a few anaerobic bacteria.[7,8] One explanation of the lack of stereospecificity in the present case is that the steric effects of the extra methylene group in propionyl-CoA inhibit the proper binding of oxaloacetate to the enzyme which occurs in the synthesis of citric acid from acetyl-CoA; thus the reaction involving propionyl-CoA leads to both *re* and *si* attack on the keto carbonyl group of oxaloacetate.

Methylcitrate formation could arise either from a minor contaminant in the enzyme preparation or by involvement of citrate synthase itself at a site different from, or identical with, the normal active site. Evidence in favour of the latter interpretation comes from the observation that propionyl-CoA has a high affinity for citrate synthase and competes with acetyl-CoA for its binding site.[4,9] A site different from the normal active site seems less likely as binding studies indicate that only one site per subunit is available for the substrates.[10–13] After treatment with butanedione,[14] which modifies arginine, the modified enzyme shows a significant difference ($p < 0.001$) in susceptibility to the two reactants (Fig. 3). This means at least that the reaction mechanisms are different, as is also obvious from the stereostructures of the products. The methylcitrate formation is less inhibited by butanedione modification than is citrate formation and this can be explained if it is assumed that arginine is necessary for the close binding of oxaloacetate to the enzyme which occurs only during citrate formation.

Since only one orientation of the methyl group in the propionyl moiety seems to be possible during the condensation to methylcitric acid, one would expect the fluorine atom in fluoroacetyl-CoA to be similarly oriented during the condensation leading to fluorocitric acid, but this is possibly not the case. Only one stereoisomer of the latter acid has been detected in the reaction mixture and the 1*R*,2*R* configuration has been ascribed to this isomer (*3*).[15] As seen in Fig. 1, the structure of *3* does not correspond to the structures of either *1* nor *2*. The absolute configuration of *3* has not been rigorously proved, however. In the choice between two enantiomers, the one demanding a *si* attack on oxaloacetate was regarded as the most probable.

Experimental. Pig heart citrate synthase (Boehringer) was used either as received or after removal of salts by gel filtration. Acetyl-CoA and propionyl-CoA were prepared from the corresponding acid anhydrides and CoA (Sigma).[16] Oxaloacetic acid (Sigma) was dissolved in 1 % $NaHCO_3$, stored at 0 °C and used within 1 day. The incubation mixtures consisted of 10 μN citrate synthase (1 mg), 8 mM propionyl-CoA and 15 mM oxaloacetate in 2 ml of 30 mM potassium phosphate buffer and had final pH values 7.3 – 7.7. Reaction mixtures were left at 22 °C for 20 h and lyophilised either directly or after gel filtration. A control experiment was performed with heat denatured enzyme. The methylcitric acids formed were isolated and characterized as in previous work.[2] For the quantitative determinations a simplified GLC internal standard procedure was used; after the methylcitric acids had been converted to trimethyl esters by reaction with diazomethane in ether, a sufficient amount of a standard solution (containing trimethyl citrate) was added so that the ratio sample/standard was close to unity. The mixture was then analysed by GLC.

Kinetic investigation. Using parallel mixtures, reactions were interrupted at various times by addition of 0.5 M NaOH and the samples were lyophilised and methylated before determination of the extent of reaction by GLC as described above.

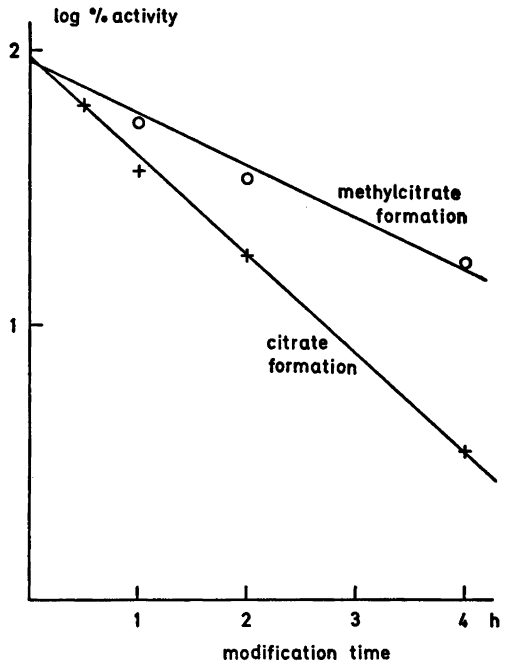


Fig. 3. Effect of butanedione modification on the activity of citrate synthase.

Arginine modification. Citrate synthase at 20 μN was reacted with 60 mM butanedione in 1 ml 0.1 M borate buffer pH 7.5.[14] The modification was stopped by gel filtration of the mixture on a column of Sephadex G-15 (Pharmacia), using 0.125 M borate pH 7.5 as eluent. An aliquot was treated similarly but without butanedione. The enzyme was assayed for residual citrate synthase activity [17] and methylcitrate synthase activity by incubation for 20 h as above. To prevent restoration of essential arginine groups, the latter reaction was performed in 0.1 M borate pH 7.5 instead of phosphate buffer.[18]

Acknowledgement. This work was supported by the Swedish Natural Science Research Council.

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Reduction of 3,3,5,5-Tetramethylcyclopentane-1,2-dione and its Reaction with Methylmagnesium Bromide

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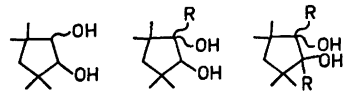
In connection with our interest in reactions [1] of 3,3,5,5-tetramethylcyclopentane-1,2-dione [2] and especially the equilibria [3] in alkaline solutions we found it desirable to synthesize a series of cyclopentane-1,2-diols (Scheme 1).

We report here the preparations of *cis*- and *trans*-3,3,5,5-tetramethylcyclopentane-1,2-diols by reduction of 3,3,5,5-tetramethylcyclopentane-1,2-dione and the spectrometric [4–11] determination of configurations of these new diols. We also describe the reaction of the title compound with methylmagnesium bromide.

Preparation of 3,3,5,5-tetramethylcyclopentane-1,2-diols. When 3,3,5,5-tetramethylcyclopentane-1,2-dione was reduced with sodium borohydride in methanol at room temperature, the only isolated product was *trans*-3,3,5,5-tetramethylcyclopentane-1,2-diol (*trans-1*). The crystallized product showed only one peak analyzed by glass capillary gas chromatography.

The diketone was hydrogenated at about 100 atm. and 60 – 70 °C to give exclusively the diol *cis-1*. Hydrogenation at lower temperatures was incomplete.

Determination of configurations. In some cases it is possible to assign the configurations by comparing the IR spectra of the isomers in the OH stretching region.[5,6,12,13] In the case of cyclopentane-1,2-diols only the *cis*-isomer is capable of forming an intramolecular hydrogen bond thus showing both the absorptions of free and bonded OH groups in dilute (< 0.005 M) solutions.



1 *2* *3*

The IR spectra of *cis-1* and *trans-1* were measured by using 0.005 and 0.0025 M CCl_4 solutions. The *cis*-isomer shows two OH bands at 3643 (free) and 3576 cm^{-1} (bonded) the intensity ratios being equal in both concentrations.

The spectrum of *trans-1* shows only one OH band (3630 cm^{-1}). When the concentration is 0.005 M, the *trans*-isomer also shows a band at *ca.* 3400 cm^{-1} due to an intermolecular hydrogen bond, which does not exist at higher dilution (0.0025 M).

Arginine modification. Citrate synthase at 20 μN was reacted with 60 mM butanedione in 1 ml 0.1 M borate buffer pH 7.5.[14] The modification was stopped by gel filtration of the mixture on a column of Sephadex G-15 (Pharmacia), using 0.125 M borate pH 7.5 as eluent. An aliquot was treated similarly but without butanedione. The enzyme was assayed for residual citrate synthase activity [17] and methylcitrate synthase activity by incubation for 20 h as above. To prevent restoration of essential arginine groups, the latter reaction was performed in 0.1 M borate pH 7.5 instead of phosphate buffer.[18]

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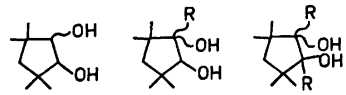
In connection with our interest in reactions [1] of 3,3,5,5-tetramethylcyclopentane-1,2-dione [2] and especially the equilibria [3] in alkaline solutions we found it desirable to synthesize a series of cyclopentane-1,2-diols (Scheme 1).

We report here the preparations of *cis*- and *trans*-3,3,5,5-tetramethylcyclopentane-1,2-diols by reduction of 3,3,5,5-tetramethylcyclopentane-1,2-dione and the spectrometric [4–11] determination of configurations of these new diols. We also describe the reaction of the title compound with methylmagnesium bromide.

Preparation of 3,3,5,5-tetramethylcyclopentane-1,2-diols. When 3,3,5,5-tetramethylcyclopentane-1,2-dione was reduced with sodium borohydride in methanol at room temperature, the only isolated product was *trans*-3,3,5,5-tetramethylcyclopentane-1,2-diol (*trans-1*). The crystallized product showed only one peak analyzed by glass capillary gas chromatography.

The diketone was hydrogenated at about 100 atm. and 60 – 70 °C to give exclusively the diol *cis-1*. Hydrogenation at lower temperatures was incomplete.

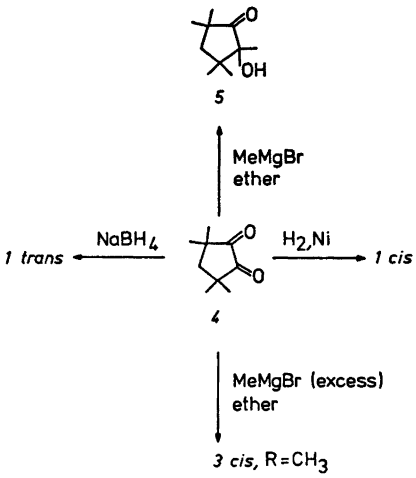
Determination of configurations. In some cases it is possible to assign the configurations by comparing the IR spectra of the isomers in the OH stretching region.[5,6,12,13] In the case of cyclopentane-1,2-diols only the *cis*-isomer is capable of forming an intramolecular hydrogen bond thus showing both the absorptions of free and bonded OH groups in dilute (< 0.005 M) solutions.



1 *2* *3*

The IR spectra of *cis-1* and *trans-1* were measured by using 0.005 and 0.0025 M CCl_4 solutions. The *cis*-isomer shows two OH bands at 3643 (free) and 3576 cm^{-1} (bonded) the intensity ratios being equal in both concentrations.

The spectrum of *trans-1* shows only one OH band (3630 cm^{-1}). When the concentration is 0.005 M, the *trans*-isomer also shows a band at *ca.* 3400 cm^{-1} due to an intermolecular hydrogen bond, which does not exist at higher dilution (0.0025 M).



The ^1H NMR spectra of the diols show characteristic differences. The methylene protons of *cis-1* and *trans-1* give an AB and an A_2 spectrum, respectively. The methine proton in the *cis*-isomer absorbs at a lower field than in the *trans*-isomer. This is in accordance with earlier findings.[9] The absorption signals of all the 12 methyl protons in *cis-1* are observed as one singlet, but the ^{13}C shielding differences of the corresponding carbons are clear (see Experimental). We found an analogous result in the case of *cis-3*.

Finally, *cis-1* and *trans-1* can be distinguished by studying ^{13}C NMR spectra. The differences in ^{13}C shieldings are most striking in the cyclopentane ring itself and especially at the nonprotonated carbons.

The results show that in the case of symmetrically 3,3,5,5-substituted cyclopentane-1,2-diols, the ^1H NMR method presented is applicable for assignment of the configuration even if only one of the isomers is available.

Reaction of 3,3,5,5-tetramethylcyclopentane-1,2-dione with methylmagnesium bromide. The title compound reacts with an equimolar amount of the Grignard reagent yielding after hydrolysis 2-hydroxy-2,3,3,5,5-pentamethylcyclopentanone. The other carbonyl group remains unattacked when using a small excess of magnesium (17 %). No impurities were observed by glass capillary GLC. The structure was established with ^{13}C NMR, ^1H NMR, mass, IR and UV spectra.

We found that 2,2,4,4-tetramethylcyclopentanone is much less reactive toward the same Grignard reagent than the corresponding diketone. Under the same reaction conditions (17 % excess of magnesium) only about 50 % of the starting monoketone reacted. When the product from this preparation was treated with a 3-fold excess of the Grignard reagent, the reaction was still incomplete.

The greater reactivity of one carbonyl group of the diketone compared with the corresponding monoketone may be accounted for by the planar conformation [14] of the ring and nearly *cis*-coplanar carbonyl groups [15] of the former compound. The monoketone and the product *5* have energetically more favoured half-chair conformations.[16]

cis-1,2,3,3,5,5-Hexamethylcyclopentane-1,2-diol was synthesized starting from the title compound and using the 2-step method described above. The configuration was found to be *cis* by using the spectrometric method described in this paper (see Experimental). Analogous results have been observed in the reaction of 3,3,4,4-tetramethylcyclobutane-1,2-dione with methylmagnesium iodide.[17]

Experimental. ^1H NMR spectra were obtained with a JEOL JNM-PMX60 NMR spectrometer and ^{13}C NMR spectra with a JEOL JNM PFT100 spectrometer. IR spectra were recorded with a Perkin-Elmer 125 Grating Spectrometer in 10 % CCl_4 solutions and (NIR) in 0.005 and 0.0025 M solutions using the cell length of 1.0 cm. UV spectra were measured with a Shimadzu UV-200 Double Beam Spectrometer. Mass spectra were recorded with a JEOL JMS-D 100 instrument.

Melting points, determined in open capillary tubes with an electrothermal apparatus, are uncorrected.

3,3,5,5-Tetramethylcyclopentane-1,2-dione (*4*) was synthesized and *methylmagnesium bromide* was prepared as described in Ref. 2.

cis-*3,3,5,5-Tetramethylcyclopentane-1,2-diol* (*cis-1*). The diketone *4* (1.54 g) was hydrogenated in ethanol (70 ml) over Raney nickel at 70 °C using a hydrogen pressure of about 100 atm. When the reaction time was *ca.* 10 h, the orange colour of the diketone had disappeared. The solvent was evaporated in vacuum. The remaining crude product was sublimed (10 mmHg/water bath). The yield of the twice sublimed colourless crystals was 1.05 g (66 %), m.p. 58 °C. ^1H NMR (60 MHz, $CDCl_3$): δ 1.03 (12 H, s), 1.50 (centre of AB q, 2 H, J 14 Hz), *ca.* 3.0 (2 H, s, depending on conc.), 3.76 (2 H, s). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 25.2 (off-res. q), 30.3 (q), 39.5 (s), 53.1 (t), 81.7 (d). NIR (0.0025 M CCl_4): 3643 (free OH), 3576 cm^{-1} (intramolecularly bonded OH). MS (70 eV, *m/e*): 158 (M).

trans-*3,3,5,5-Tetramethylcyclopentane-1,2-diol* (*trans-1*). The diketone *4* (1.0 g) was dissolved in methanol (15 ml). Sodium borohydride (0.34 g) was added in portions in the course of 20 min at room temperature. The mixture was stirred further for 30 min. Most of the solvent was removed by distillation. The remaining mixture was poured into water (30 ml) containing some diluted hydrochloric acid. The aqueous solution was saturated with sodium chloride, extracted with diethyl ether and the ether solution was dried with magnesium sulfate. After removing the solvent the crude

product was recrystallized from aqueous propanol. The yield of the colourless crystals was 0.72 g (70 %), m.p. 102–103 °C. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.93 (6 H, s), 1.08 (6 H, s), 1.48 (2 H, s), ca. 3.0 (2 H, s, depending on conc.), 3.58 (2 H, s). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 24.8 (off-res. q), 29.9 (q), 35.3 (s), 52.1 (t), 83.5 (d). NIR (0.0025 M CCl_4): 3630 cm^{-1}. MS (70 eV, *m/e*): 158 (M).

2-Hydroxy-2,3,3,5,5-pentamethylcyclopentanone (5). The diketone *4*, (1.54 g) dissolved in dry diethyl ether, was added to a diethyl ether solution of methylmagnesium bromide, prepared from 0.28 g of magnesium turnings, during half an hour. The temperature of the stirred mixture was kept at 15 °C. The addition complex was hydrolysed with cold ammonium chloride solution, and the aqueous solution was then extracted with diethyl ether. The ether solution was dried with calcium sulfate. After removing the solvent the crude product was sublimed under vacuum. The yield of the colourless product was 0.92 g (54 %), m.p. 45–47 °C. ^{1}H NMR (60 MHz, CCl_4): δ 0.93 (3 H, s), 1.02 (3 H, s), 1.12 (3 H, s), 1.14 (3 H, s), 1.17 (3 H, s), 1.70 (centre of AB q, 2 H, J 13 Hz), *ca.* 2.5 (1 H, s, depending on concentration). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 20.7 (off-res. q), 23.7 (q), 24.1 (q), 27.9 (q), 28.3 (q), 39.7 (s), 40.7 (s), 49.2 (t), 82.0 (s), 225.4 (s). IR (10 % CCl_4): 3500 (OH), 1738 cm^{-1} (C=O). UV [abs. ethanol (ε)]: 307 (41) nm. MS (70 eV, *m/e*): 170 (M).

cis-*1,2,3,3,5,5-Hexamethylcyclopentane-1,2-diol (cis-3).* The diketone *4* (1.54 g) dissolved in dry diethyl ether (7 ml) was added to the Grignard reagent solution prepared from 0.58 g of magnesium turnings during one hour at room temperature. After the addition the reaction mixture was refluxed for 3 h. After a work-up similar to that described above the crude product was dried and was then treated with a 3-fold excess of methylmagnesium bromide. The reaction product was isolated as described above. After removing the solvent the white mass solidified. The crude diol was purified by vacuum sublimation. The yield of the colourless crystals was 0.25 g (13 %), m.p. 58–62 °C. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.97 (6 H, s), 1.12 (12 H, s), 1.52 (centre of AB q, 2 H, J 14 Hz), *ca.* 2.5 (2 H, s, depending on concentration). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 14.7 (off-res. q), 21.4 (q), 26.8 (q), 41.3 (s), 54.8 (t), 83.7 (s). IR (10 % CCl_4): 3625, 3533, 3435 cm^{-1} (OH). NIR (0.0025 and 0.005 M CCl_4): 3627 (free), 3560 cm^{-1} (intramolecularly bonded), $\Delta\nu$ 67 cm^{-1}. MS (70 eV, *m/e*): 186 (M).

Each of the new compounds (*cis-1*, *trans-1*, *cis-3*, *5*) showed only one peak by glass capillary GLC using Carlo Erba Fractovap Mod. G I and FFAP stationary phase in capillary column (20 m × 0.3 mm ID).

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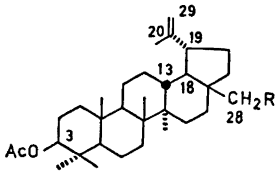
Received March 28, 1977.

Triterpenes. The HBr Catalysed Rearrangement of Lupenyl Acetate and Stereochemistry of the Epoxidation of the Products

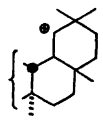
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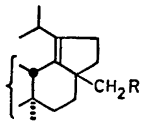
The acid catalysed rearrangement of lupenyl acetate (*1a*) is known [1] to proceed *via* the skeletally rearranged carbocation *2*. We reported previously [2] an HBr catalysed rearrangement of betulin diacetate (*1b*), giving *3b*, where the carbon skeleton is not altered. It was recently claimed [3] that lupenyl acetate (*1a*) does not



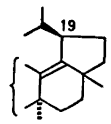
1a R = H
b R = OAc



2



3a R = H
b R = OAc

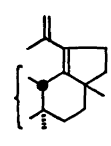


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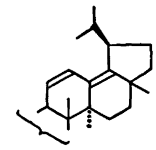
give *3a* on HBr treatment. However, we have found that *3a* is formed, when *1a* is treated with HBr in Ac_2O-AcOH-benzene solution and can be isolated, but is also readily isomerised to *4*.*

Compound *3a* has been reported previously [4] as a component of the preparatively unseparable mixture of products from the PtO_2 catalysed hydrogenation of *5*. We have now isolated and characterised *3a* along with the tetrahydro derivative *12* from this mixture. Compound *3a* has also been reported [5] to occur in Nature but the reported physical data differ from those now obtained for *3a*. The natural product is probably correctly formulated *3a* but impure, according to the close similarity of the mass spectra.

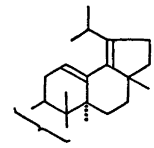
When the diene *5* is first isomerised [6] to the mixture of compounds *6** and *7*, and these products hydrogenated,[6] the same dihydro compound, identical with *4*, is obtained in both cases. Compound *4* from these reactions gave the epoxide *8*. X-Ray crystallographic measurements [7] gave the 13α,18α-epoxy-19α(H)-structure *8* for this epoxide. This establishes the 19α(H)-structures for *4* and *6*.



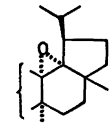
5



6

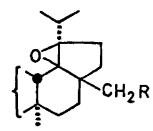


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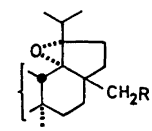


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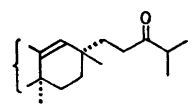
The epoxidation of *3a* with *m*-chloroperbenzoic acid gives the β-epoxide *9a*, also obtained from the corresponding betulin derivative *9b* of known [8] β-epoxy structure. Thus the isomeric epoxide obtained [8,9] from *3a* with ozone has α-epoxy structure *10a*.



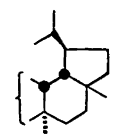
9a R = H
b R = OAc
c R = OH
d R = OTos



10a R = H
b R = OAc



11



12

Both epoxides *9a* and *10a* give the baccharane derivative *11* on a BF_3 treatment in analogy with the corresponding betulin epoxides *9b* and *10b*.[8]

Experimental. Melting points are uncorrected. ^1H NMR spectra were recorded on a Jeol JNM-PMX 60 spectrometer, IR spectra on a Perkin-Elmer 125 spectrometer using KBr pellets, mass spectra on a Perkin-Elmer 270 B mass spectrometer, specific rotations in $CHCl_3$ solution on a Perkin-Elmer 141 polarimeter.

* For the stereostructure at C-19 see below.

Isomerisation of lupenyl acetate (1a). (a) Lupenyl acetate (*1a*) (0.5 g) in benzene (17 g), Ac_2O (7 g), and 36 % HBr in AcOH (13 g) was allowed to stand at ambient temperature for 50 h. Work up and chromatography on 10 % $AgNO_3$ impregnated silica plates (eluent: $CHCl_3$ – light petroleum b.p. 60 – 80 °C, 2:1) gave 3β-acetoxy-19α(H)-lup)-13(18)-ene (*4*) (0.2 g), m.p. (EtOH) 177 °C, $[\alpha]_D$ −21° (*c* 0.82). (Ref. 6, m.p. 184 – 185 °C *in vacuo*, $[\alpha]_D$ −20.7°). *ν* 1730, 1235. *δ* (CCl_4) 4.4 (1 H, m), 3.7 – 2.1 (3 H, m), 1.97 (3 H, s), 1.1 – 0.83 (8 methyls). *m/e* 468 (M^+), 205 (100 %).

(b) Lupenyl acetate (*1a*) (0.5 g) in benzene (17 g), Ac_2O (7 g), and 36 % HBr in AcOH (11 g) was allowed to stand at room temperature for ten days. The reaction is sensitive to the concentration of HBr. Work up and chromatography on 10 % $AgNO_3$ impregnated silica plates gave 3β-acetoxy-19α(H)-lup-13(18)-ene (*4*) (0.1 g) as above and 3β-acetoxy-lup-18-ene (*3a*) (0.1 g), m.p. (EtOH) 238 °C, $[\alpha]_D$ +13° (*c* 1.0). (Ref. 5, m.p. 210 – 212 °C, $[\alpha]_D$ +6.8°.) *ν* 1730, 1245. *δ* (CCl_4) 4.4 (1 H, m), 3.05 (1 H, sept. *J* 7 Hz), 2.6 – 2.0 (4 H, m), 1.96 (3 H, s), 1.1 – 0.8 (8 methyls). *m/e* (% rel. int.) 468 (32, M^+), 204 (94), 189 (100), 177 (64). This compound was identical (m.p., mixed m.p., $[\alpha]_D$, TLC, MS, IR, ^{1}H NMR) with the compound obtained from the hydrogenation of diene *5* (see below).

Isomerisation of 3β-acetoxy-lup-18-ene (3a). 3β-Acetoxy-lup-18-ene (*3a*) was treated as lupenyl acetate (*1a*) above with an HBr – Ac_2O – AcOH – benzene mixture. Work up gave 3β-acetoxy-19α(H)-lup-13(18)-ene (*4*), identical with the compound obtained above.

Hydrogenation of 3β-acetoxy-lupa-18,20(29)-diene (5). 3β-Acetoxy-lupa-18,20(29)-diene (*5*) (1 g) was hydrogenated over PtO_2 according to Ref. 4. Chromatography on 10 % $AgNO_3$ impregnated silica plates (eluent: $CHCl_3$ – light petroleum b.p. 60 – 80 °C, 3:1) gave 3β-acetoxy-18β(H),19α(H)-lupane [6,10] (*12*) (0.25 g), m.p. (EtOH) 271 °C. $[\alpha]_D$ +21° (*c* 1.0) (Ref. 6, m.p. 272 – 274 °C, $[\alpha]_D$ +24°) and 3β-acetoxy-lup-18-ene (*3a*) (0.63 g) identical with the compound from the isomerisation of lupenyl acetate (*1a*).

Epoxidation of 3β-acetoxy-19α(H)-lup-13(18)-ene (4). 3β-Acetoxy-19α(H)-lup-13(18)-ene (*4*) (0.2 g) from the isomerisation of *1a*, or from the hydrogenation of the dienes *6* and *7* was stirred with $NaHCO_3$ (0.5 g) and *m*-chloroperbenzoic acid (0.15 g) in CH_2Cl_2 (10 ml) for 30 min. The mixtures were washed with water and Na_2SO_3 solution, dried and chromatographed on silica plates ($CHCl_3$ eluent). Each reaction gave the same main product 3β-acetoxy-13α,18α-epoxy-19α(H)-lupane (*8*) (80 – 100 mg), m.p. (EtOH) 194 °C, $[\alpha]_D$ +25° (*c* 0.92). *ν* 1730, 1245, 1095, 1010, 980, 870. *δ* (CCl_4) 4.45 (1 H, m), 1.98 (3 H, s), 1.16 – 0.84 (8 methyls). *m/e* 484 (M^+), 140 (100 %).

3β-Acetoxy-18β,19β-epoxylupane (9a). (a) 3β-Acetoxy-lup-18-ene (*3a*) was epoxidised with *m*-chloroperbenzoic acid as *4* above to give 3β-acetoxy-18β,19β-epoxylupane (*9a*), m.p. (EtOH) 235 °C, $[\alpha]_D$ +37° (*c* 1.0). (Ref. 6, m.p. 236 – 238 °C, $[\alpha]_D$ +32°.)

(b) 3β,28-Diacetoxy-18β,19β-epoxylupane [8] (*9b*) (1.0 g) and KOH (0.12 g) in EtOH (100 ml) were refluxed for 1 h. Work up and chromatography gave as the main product 3β-acetoxy-28-hydroxy-18β,19β-epoxylupane (*9c*) (0.52 g), m.p. (EtOH) 246 °C, $[\alpha]_D$ +38° (*c* 1.0).

Hydroxyacetate *9c* (0.2 g), *p*-toluenesulfonylchloride (0.4 g), and pyridine (3 g) were stirred overnight and worked up. Chromatography on a silica plate gave 3β-acetoxy-28-tosyloxy-18β,19β-epoxylupane (*9d*) (0.14 g), m.p. (EtOH) 160 °C (decomp.), $[\alpha]_D$ +12° (*c* 1.1). *δ* (CCl_4) 7.78 and 7.30 (à 2 H, d, *J* 8 Hz), 4.4 (1 H, m), 4.3 and 3.77 (à 1 H, d, *J* 9 Hz), 2.45 (3 H, s), 2.05 (3 H, s), 1.1 – 0.8 (8 methyls).

Tosylate *9d* (100 mg), NaI (220 mg), Zn (300 mg), $(Me_2N)_3PO$ (2 ml), and $MeOCH_2CH_2OMe$ (2 ml) were kept at 100 °C overnight (*cf.* Ref. 11). Work up and crystallisation from EtOH gave 3β-acetoxy-18β,19β-epoxylupane (*9a*), identical (m.p., mixed m.p., $[\alpha]_D$, IR, ^{1}H NMR, TLC) with the compound above.

3β-Acetoxy-18α,19α-epoxylupane (10a). 3β-Acetoxy-lup-18-ene (*3a*) (200 mg) was ozonised in CH_2Cl_2 (20 ml) at −75 °C until the solution remained faintly blue. Excess ozone was driven off with a nitrogen stream and the solution allowed to reach room temperature. Chromatography on a silica plate ($CHCl_3$ eluent) gave 3β-acetoxy-18α,19α-epoxylupane (*10a*) (130 mg), m.p. (MeOH) 242 °C, $[\alpha]_D$ +40° (*c* 0.8). (Ref. 9, m.p. 238 °C, $[\alpha]_D$ +40°). *ν* 1730, 1240, 1130, 1100, 1023, 1000, 980. *δ* ($CDCl_3$) 4.5 (1 H, m), 2.3 (2 H, m), 2.05 (3 H, s), 1.1 – 0.85 (8 methyls). M^+ 484.

Rearrangement of 3β-acetoxy-18,19-epoxylupanes (9a) *and* (10a). Both 3β-acetoxy-18α,19α-epoxylupane (*10a*) and 18β,19β-epoxy isomer (*9a*) (0.2 g) were treated with BF_3-etherate (5 drops) in dry benzene (3 ml) for 5 min. Work up and chromatography on silica plates ($CHCl_3$ eluent) gave 3β-acetoxy-18,19-secolup-13(18)-en-19-one (*11*) (yields 80 – 95 %), m.p. (EtOH) 130 °C, $[\alpha]_D$ −5° (*c* 0.72). *ν* 1725, 1710, 1245. *δ* ($CDCl_3$) 4.96 (1 H, br. s), 4.53 (1 H, m), 2.9 – 2.0 (7 H, m), 2.04 (3 H, s), 1.15 – 0.85 (8 methyls). M^+ 484.

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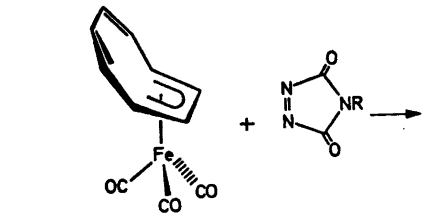
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The Formation of Barbaralone Derivatives from the Reaction between Triazolinediones and Cyclooctatetraene-iron Tricarbonyl

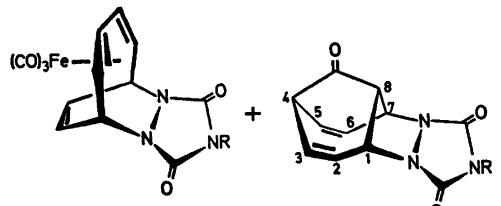
HENRIK OLSEN

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Coordination of a polyene to a metal center can lead to drastic modification of its reactivity. Thus cyclooctatetraene (COT) cycloadds to tetracyanoethylene (TCNE) *via* its [4.2.0]-bicyclic valence isomer in classic Diels-Alder fashion,[1] while the iron tricarbonyl complex *1* yields a 1,3-addition product containing an Fe–C σ bond.[2] The even more powerful dienophile 4-phenyl-1,2,4-triazoline-3,5-dione (*2*, R = Ph, PTAD) is reported to combine with *1* in low yield at 25 °C to give the 1,4-adduct *3*.[3] Apart from the $Fe(CO)_3$ moiety the reaction appears to be completely analogous to that between PTAD and COT under ambient conditions.[4] We have reinvestigated the transformation with both MTAD and PTAD (*2*, R = CH_3, Ph) and have found that the barbaralone derivative *4* is formed along with complex *3*. The result implies carbonyl insertion into an intermediate 1,3-addition product.



1 *2*



3 *4*

Mixing of equimolar quantities of MTAD and *1* (in CH_2Cl_2, 25 °C, 30 min) followed by chromatography (silica gel, ethyl acetate) leads to the isolation of three compounds. Besides unreacted *1* (22 %) and *3* (R = CH_3, 34 %, m.p. 185 °C, dec.) the barbaralone derivative *4* was obtained (R = CH_3, 16 %, m.p. 214–215 °C). The latter gave a satisfactory elemental analysis and molecular weight (MS). The presence of the keto group was substantiated by derivatization (2,4-DNP) and IR ($CHCl_3$): 1750 (m), 1772 (s) and 1714 (s) cm^{-1} (C=O and N–CO–N). ^1H NMR spectrometry (90 MHz, $CDCl_3$) established the symmetry and structure of *4* by revealing six signals readily analyzable by spin decoupling. The absorption at lowest field is an AB quartet showing additional fine structure [δ 6.41 (2 H, H-3 and H-5) 5.99 (2 H, H-2 and H-6); $J_{23} = J_{56} = 9$ Hz; $J_{12} = J_{67} = 4$ Hz; $J_{34} = J_{54} = 6.5$ Hz; $J_{31} = J_{57} = 1$ Hz]. The bridgehead protons α to nitrogen resonate at δ 5.11 (2 H, H-1 and H-7, dd, $J_{18} = J_{78} = 8$ Hz). At high field the remaining absorption consists of two doublets of triplets centered at δ 3.68 (1 H, H-8, $J_{48} = 2$ Hz) and 3.39 (1 H, H-4) and a singlet at δ 3.08 (N-CH_3). The broad band decoupled ^{13}C NMR spectrum (22.63 MHz, $CDCl_3$) is in complete agreement with structure *4* exhibiting only eight peaks: δ 201.1, 156.2, 134.5, 126.2, 61.7, 50.9, 45.3 and 25.8.

The corresponding reaction with PTAD yielded *1* (22 %), the previously characterized *3* (R = Ph, 33 %, m.p. 156–157 °C; lit.[3] m.p. 155 °C) and the barbaralone system *4* (R = Ph, 14 %, m.p. 225–226 °C). The N-phenylbarbaralone was characterized as above, its spectro-

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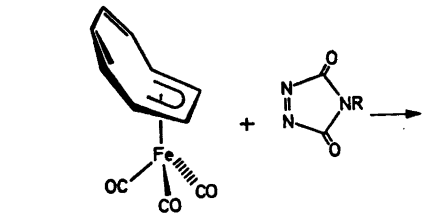
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The Formation of Barbaralone Derivatives from the Reaction between Triazolinediones and Cyclooctatetraene-iron Tricarbonyl

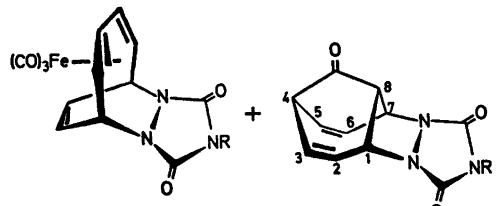
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1 *2*



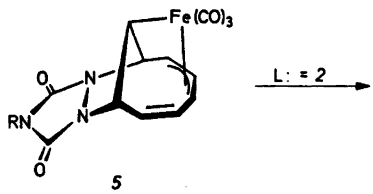
3 *4*

Mixing of equimolar quantities of MTAD and *1* (in CH_2Cl_2, 25 °C, 30 min) followed by chromatography (silica gel, ethyl acetate) leads to the isolation of three compounds. Besides unreacted *1* (22 %) and *3* (R = CH_3, 34 %, m.p. 185 °C, dec.) the barbaralone derivative *4* was obtained (R = CH_3, 16 %, m.p. 214–215 °C). The latter gave a satisfactory elemental analysis and molecular weight (MS). The presence of the keto group was substantiated by derivatization (2,4-DNP) and IR ($CHCl_3$): 1750 (m), 1772 (s) and 1714 (s) cm^{-1} (C=O and N–CO–N). ^1H NMR spectrometry (90 MHz, $CDCl_3$) established the symmetry and structure of *4* by revealing six signals readily analyzable by spin decoupling. The absorption at lowest field is an AB quartet showing additional fine structure [δ 6.41 (2 H, H-3 and H-5) 5.99 (2 H, H-2 and H-6); $J_{23} = J_{56} = 9$ Hz; $J_{12} = J_{67} = 4$ Hz; $J_{34} = J_{54} = 6.5$ Hz; $J_{31} = J_{57} = 1$ Hz]. The bridgehead protons α to nitrogen resonate at δ 5.11 (2 H, H-1 and H-7, dd, $J_{18} = J_{78} = 8$ Hz). At high field the remaining absorption consists of two doublets of triplets centered at δ 3.68 (1 H, H-8, $J_{48} = 2$ Hz) and 3.39 (1 H, H-4) and a singlet at δ 3.08 (N-CH_3). The broad band decoupled ^{13}C NMR spectrum (22.63 MHz, $CDCl_3$) is in complete agreement with structure *4* exhibiting only eight peaks: δ 201.1, 156.2, 134.5, 126.2, 61.7, 50.9, 45.3 and 25.8.

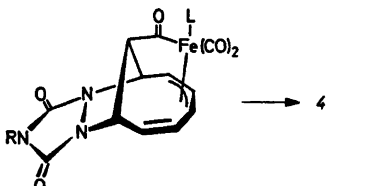
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scopic properties being analogous with those of the *N*-methyl derivative.

The formation of tetracycle *4* is strongly suggestive that PTAD and MTAD, similar to TCNE, interact with complex *1* to give a 1,3-addition product *5*. Further transformation may proceed by migration of C-8 to CO (*6*) followed by extrusion of the metal component to give *4*. Other polycyclic σ-bonded iron complexes undergo carbonyl insertion in the presence of suitable ligands (*e.g.* CO,[2b,3,5] NO,[2b,3] Ph_3P [5])* or under the influence of Ce(IV).[2c] Since cyclic azo compounds react with iron carbonyls to form complexes in which the azo group behaves as a two electron ligand,[6] it seems plausible that the N=N bond in triazolinedione *2* can function as a ligand in the present instance. Were this the case, less than one equivalent of *2* should be available for combination with *1*. Accordingly we isolate comparable amounts of barbaralone *4* and unreacted *1*.



5



6

The combination of triazolinediones with complex *1* is comparable with the corresponding reaction between TCNE and methoxy-COT iron tricarbonyl, where both a 1,3- and a 1,4-addition product have been reported.[2c] Based on a series of product structure studies the proposal has been advanced that the reaction pathway involves a common zwitterionic intermediate which closes either at the 3- or the 4-position.[2] It is noteworthy, however, that there is a parallel between the response of complexed and uncomplexed cyclooctatetraenes to treatment with powerful dienophiles. Whereas neither COT-$Fe(CO)_3$ [2] nor COT [4] combine with TCNE to give a 1,4-cycloadduct (*i.e.*, structure *3*), both CH_3O-COT-$Fe(CO)_3$ [2c] and CH_3O-COT [7] do so. Likewise MTAD/PTAD add 1,4 to COT-$Fe(CO)_3$ (*1*), to COT [4] and to CH_3O-COT.[8,**] Charge separated intermediates have been presumed in the uncomplexed COT adductions as well,[4,7,8] but it has now been demonstrated by rate studies in various solvents that the proposition was premature.[9] Consequently the question as to the nature of the preliminary steps in the formation of adducts derived from cyclopolyolefin-$Fe(CO)_3$ complexes and dienophiles must be regarded as still open.

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* Green and Wood [3] have depicted products derived from *1* and the dienophiles hexafluoroacetone and 1,1-dicyano-2,2-*bis*(trifluoromethyl)-ethylene as tricyclo[6.2.1.$0^{4,7}$]undeca-2,9-dienes containing a four-membered ring. The reported structural data are consistent with the barbaralone formulation as well. Indirect support favoring the latter is found in the Ce(IV) oxidation of the TCNE/*1* adduct to a *symmetrical* triquinacene derivative.[2a,c]

** The reaction between MTAD/PTAD and CH_3O-COT-$Fe(CO)_3$ has to our knowledge not been reported.

Purity and pH-Dependence of *Streptomyces griseus* Protease 1

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Pronase, the extracellular enzyme preparation from *Streptomyces griseus*, is a very complex mixture of various proteases.[1-3] Among these microbial enzymes are at least three serine proteases homologous to the mammalian pancreatic proteases.[4] The finding of homologous proteases from species of so widely differing phylogenetic origin was of considerable interest and prompted further investigations into the structure, mechanism and specificity of the microbial enzymes in relation to the pancreatic serine proteases. The complete primary structures of all the three Pronase serine enzymes have now been determined,[5-6] and the tertiary structure of one of these enzymes, SGP1 (identical with SGPB of Ref. 5), was recently published.[7] Studies on the mechanism and specificity of one of the other serine enzymes, SGP3 (identical with SGPA of Ref. 5), have also been reported (see for examples Refs. 8 – 10).

We have begun a kinetic investigation of the active site of SGP1, with a number of peptide substrates, with the ultimate goal of correlating some of the results from that study with the tertiary structure of the enzyme. As a prerequisite for the above-mentioned studies, we here present data on the purity and pH-dependence of a preparation of SGP1.

Purity. Although some data on the purity of SGP1 have been reported earlier,[11] we now provide further evidence for the purity, necessary prior to a detailed specificity study.

To remove inactive material from a preparation of SGP1 (a kind gift from Dr. L. B. Smillie), the enzyme was chromatographed on a 2.5 × 87 cm Sephadex G75 Superfine bed.[3] SGP1 was eluted with a K_{av} of 0.538, which is in good agreement with previously reported values of 0.557 and 0.560 for the "F-peak", which contains SGP1 to ~90 %.[3] Most of the SGP1 eluate was concentrated by ultrafiltration, the buffer was changed to 0.25 M NH_4HCO_3, pH 7.7, by chromatography on a Sephadex G-25 column, and the enzyme solution was frozen and lyophilized.

The purity of the enzyme was studied by polyacrylamide gel electrophoresis at pH 6.8 and 8.5 as described earlier.[2,12] Application of 20 μg of freshly chromatographed or lyophilized SGP1 to analytical gels (*cf.* Ref. 12) resulted in both cases in a single stainable band moving towards the cathode with exactly the same rate as band 1 (=SGP1) in Pronase. To obtain further evidence for the purity of the preparation, a sample of 100 μg of lyophilized SGP1 was run on a small preparative polyacrylamide gel.[2] The enzymatic activities associated with this preparation of SGP1 can be seen from Fig. 1. There is one main peak (fractions 28 – 31) which is active towards both casein and GPNA. These two activities appear to have the same relative intensities from fraction to fraction, thus providing strong evidence for the homogeneity of the peak. On the trailing edge of the peak are a few fractions (24 – 26) with faint activity towards casein and GPNA, constituting about 3 % of the total activity towards these substrates. These fractions, which show approximately the same relative activities towards casein/GPNA as fractions 28 – 31, may either simply be due to "tailing" of the main peak or, alternatively, may constitute a modified form of SGP1 (corresponding to band 2 of Ref. 2) with a slightly different charge, but with essentially the same relative activities towards casein/GPNA as SGP1. Such microheterogeneities have been found to be formed during certain preparation methods for the closely related enzyme SGP3.[13] No activity towards L-Leu-β-naphthylamide could be demonstrated in any of the 50 fractions, which indicates that the preparation is free from the aminopeptidases present in Pronase.[2,3] A very small activity towards BANA was found in fractions 17 – 19, which corresponds well with the

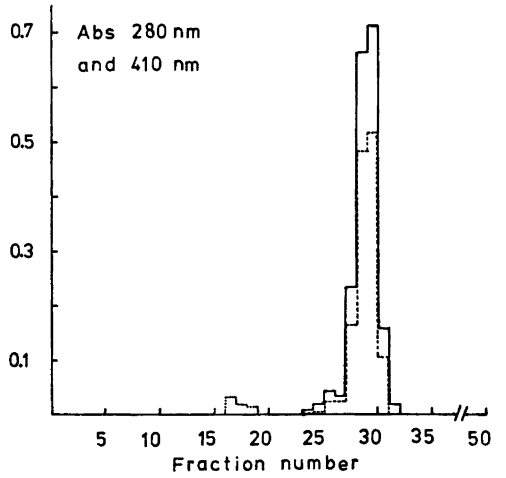


Fig. 1. Histogram showing enzymatic activities of a preparation of SGP1, found in the different gel fractions of a small preparative polyacrylamide gel after 3 1/3 h electrophoresis (cathodic migration) in 0.38 M borate – 0.01 M Ca^{2+} buffer, pH 6.8. The gel fractions (1.5 mm thick) were mashed and extracted with a 0.03 M borate buffer pH 7.5, 0.03 M in $CaCl_2$. Activity towards casein (–), glutaryl-L-Phe-*p*-nitroanilide = GPNA (- - -), and benzoyl-DL-Arg-*p*-nitroanilide = BANA (· · ·) at pH 7.5.

position of SGP4 [2] or *Streptomyces griseus* trypsin.[6] By comparing the activity of fractions 17–19 with that of a known amount of SGP4, it has been possible to estimate the amount of enzyme present in the fractions to be less than 0.1 % of the total amount of enzyme (100 μg) applied to the gel. Since the BANA activity is completely separated from the SGP1 fractions 28–31, it is clear that there is no intrinsic "trypsin-like" activity in SGP1.

In conclusion, the results provide evidence that this preparation of SGP1 is at least 99.9 % pure with respect to its activity towards casein/GPNA. A small amount (about 3 %) of this activity was found in a peak closely associated with the main (97 %) peak (=SGP1) of the preparation. However, because the relative activities towards casein/GPNA of the small peak are similar to those of the main peak, this would seem unlikely to affect significantly the kinetic properties of the preparation.

pH-Dependence. Besides providing information about the pK_a of catalytically important residues, knowledge of the pH-dependence of an enzyme makes it possible to chose optimal conditions for kinetic studies. The pH-dependence of k_{cat} for the SGP1-catalyzed hydrolysis of the uncharged substrate Ac-Pro-Leu-*p*-nitroanilide [14] in the pH-interval 4.1–10.8 can be seen from Fig. 2. k_{cat} increases with increasing pH up to about pH 8, being constant from pH 8 up to at least pH 10.8. K_m, on the other hand, is virtually constant ($K_m = 3 \pm 1$ mM) within the investigated interval, and the pH-dependence of SGP1 therefore shows close similarity with those of SGP3,[15] α-lytic protease [16] and elastase,[17] but differs from that of chymotrypsin,[18] where K_m increases quite rapidly above pH 8. Fig. 2 suggests that the pH-dependence of SGP1 is dependent on an ionizing group with an apparent pK_a of about 6.7. This value is almost identical with the pK_a value (6.6) previously determined for the pH-dependence of SGP3,[15] and is very similar to the pK_a's of other homologous serine proteases.[16,18] In α-lytic protease [19] and trypsin,[20] there is now evidence that the group responsible for this pK_a is Asp-102 and not, as earlier believed, His-57.[16,18] It might be expected that Asp-102 is responsible for the pK_a around 6.7 of the pH-dependencies of other homologous serine proteases as well, including SGP1 and SGP3.

Gertler has reported $pK_{a1} = 6.6$ and $pK_{a2} = 9.5$ for the pH-dependence of the second order rate constant of inhibition of SGP1 by Ac-Leu-Phe chloromethyl ketone.[21] While $pK_{a1} = 6.6$ is in excellent agreement with the value obtained in the present investigation, Fig. 2 does not provide any support for a pK_{a2} around 9.5, but indicates that a pK_{a2} value must be greater than 10.8. A possible explanation for the low pK_{a2} of 9.5 might be that chloromethyl ketones are unstable in water solutions of higher pH's, and will decompose quite rapidly in alkaline media.[22,23] Consequently, the concentration of chloromethyl ketone in an inhibition mixture at alkaline pH will decrease more or less rapidly depending on the pH. The result will be an apparent slower inhibition of SGP1 at higher pH's, and this effect might well account for the low pK_{a2} of 9.5.

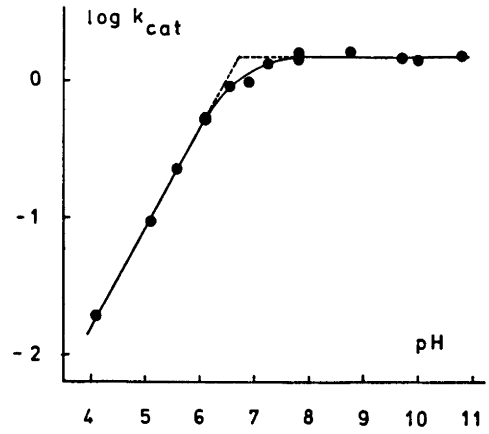


Fig. 2. pH-Dependence of k_{cat} for the SGP1-catalyzed hydrolysis of Ac-Pro-Leu-*p*-nitroanilide at 37 °C followed at 410 nm. The reaction-solutions contained 0.2–1 mM substrate in 1.1 ml 50 mM buffer (acetate, maleate, tris or glycine-buffer), 10 mM $CaCl_2$ with 2.5 % acetone and 0.17–3 μM enzyme. Enzyme-free blanks, otherwise identical in composition to the reaction mixtures, were used throughout the measurements.

Acknowledgements. I would like to thank Dr. L. B. Smillie for his generous gift of SGP1. I am indebted to Dr. Bo Löfqvist and Dr. L. B. Smillie for valuable criticism of the manuscript, to Dr. D. Burton for linguistic advice and to Mrs. G. Norén for secretarial assistance.

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Formation of α-Allenic Dithioketals in a [2,3] Sigmatropic Rearrangement of Intermediates Derived from *S*-Propargylic Dithioesters*

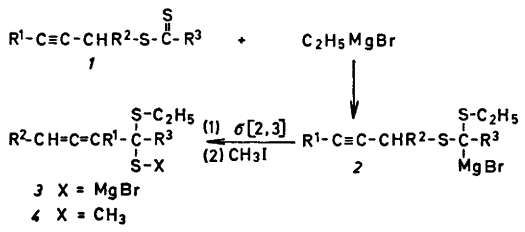
LARS-INGE OLSSON

Department of Organic Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-721 23 Uppsala, Sweden

Alkyl Grignard reagents may react with thiocarbonyl compounds, such as thioketones and dithioesters, under thiophilic addition.[1] Recently it was reported [2] that thiophilic addition of Grignard reagents to *S*-allylic dithioesters is followed by a [2,3] sigmatropic shift of the intermediate carbanion, which gives rise to dithioketals of β-unsaturated ketones. Closely related [2,3] sigmatropic rearrangements of α-metallated allylic thioethers have also been observed.[3]

This communication reports that the thiophilic addition of ethylmagnesium bromide to the double bond of the thiocarbonyl group of *S*-propargylic dithioesters in THF at −30 °C is spontaneously followed by a [2,3] sigmatropic rearrangement of the intermediate carbanion. Subsequent methylation of the so formed allenic thiolate gives α-allenic dithioketals in 58 − 74 % yield, (see Scheme 1). These dithioketals represent a hitherto unknown type of allenic compound.

Scheme 1. α-Allenic dithioketals *4a − d* from the reaction of *S*-propargylic dithioesters *1a − e* with ethylmagnesium bromide.



Compound	R^1	R^2	R^3	Yield [a] of *4* (%)
1a [5b]	H	H	C_2H_5	64
1b [7]	CH_3	H	C_2H_5	69
1c	C_6H_5	H	C_2H_5	58
1d	CH_3	H	C_6H_{13}	74
1e	CH_3	CH_3	C_2H_5	–

[a] Isolated by column chromatography.

The allene-forming [2,3] sigmatropic rearrangement in a propargylic system described here is closely related to several analogous reactions known from the literature, especially those in propargylic sulfonium ylides,[4] which afford allenic sulfides.

The propargylic dithioesters *1a − e* were prepared according to the literature.[5] A mixture of THF and HMPT was used as the solvent for the alkylation of the alkyldithiomagnesium bromide with the appropriate propargylic bromide. The dithioesters were heat sensitive and could not be distilled at 10 Pa without decomposition but had to be purified by column chromatography.

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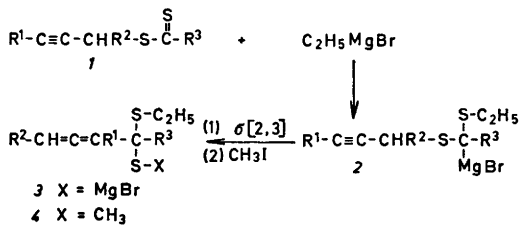
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as it is known to favour thiophilic addition.[6] The progress of the reaction can be observed by the disappearance of the characteristic yellow colour of the dithioesters. The so formed carbanions *2a–d* rearrange spontaneously through a [2,3] sigmatropic shift and *in situ* methylation of the intermediate *3* by methyl iodide gives the α-allenic dithioketals *4* in the indicated yields. *In situ* alkylation is used to prevent decomposition of *3* which is otherwise likely to occur.[2] Elimination of ethylthiomagnesium bromide would give an α-allenic thioketone which could react with ethylmagnesium bromide in a second thiophilic addition or could be reduced by the Grignard reagent.

The *S*-propargylic dithioester *1e* obviously reacts with ethylmagnesium bromide in a thiophilic addition, indicated by the discoloration of the reaction mixture, but fails to give an allenic rearrangement product under the above reaction conditions. Reaction of *1e* with isopropylmagnesium bromide, which is known to be more reactive in thiophilic additions,[6] also gave a negative result.

Hydrolysis of the intermediate *3* from a reaction run in the absence of CH_3I gives a variety of products, among which no allenic ketone can be found.

Interestingly, the possibility of a thermal [3,3] sigmatropic rearrangement in *S*-propargylic dithioesters affording *S*-allenylic dithioesters has been pointed out in the literature.[7]

Experimental. The general IR and NMR instrumentation has been described.[8] The spectra were routinely recorded and are in full agreement with the proposed structures. Mass spectra were run on an LKB 9000 instrument using the GLC inlet. Correct mass spectral data, run at an ionizing energy of 70 and 15 eV, were obtained for all products.

Preparative column chromatography was performed on silica gel 60 (230–400 mesh), using light petroleum as eluent.

Reactions involving Grignard reagents were performed under nitrogen.

General procedure for the preparation of dithioesters 1a–e. Dithioesters *1a–e* were prepared according to Ref. 5. The appropriate Grignard reagent in THF was added dropwise to a solution of CS_2 in THF at −15 °C. After stirring for 1 h at this temperature, the alkyldithiomagnesium bromide was alkylated with the appropriate propargylic bromide, dissolved in a mixture of THF and HMPT (20 % v/v). The reaction mixture was then allowed to reach room temperature during 2 h. The dithioesters were isolated by column chromatography, in the following yields: *1a* [5b] 44 %, *1b* [7] 56 %, *1c* 53 %, *1d* 70 % and *1e* 70 %.

Reaction of dithioesters 1a–e *with a Grignard reagent.* These reactions were run in analogy with the procedure described in Ref. 2. To 0.045 mol of ethylmagnesium bromide in THF, cooled to −30 °C, a mixture of 0.045 mol of methyl iodide and 0.015 mol of the dithioester was added dropwise. The reaction mixture was stirred at this temperature until the characteristic yellow colour of the dithioester had disappeared (1–2 h). Water was then added and the mixture was extracted with ether. The organic phase was washed with saturated NH_4Cl and dried over $MgSO_4$. The allenic products were purified by preparative column chromatography. They have spectroscopic properties characteristic of allenic compounds which are here exemplified for *4a*: IR: 1950 (m), 845 (m, terminal allene) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.03 (3 H, t), 1.22 (3 H, t), 1.82 (2 H, q), 2.05 (3 H, s), 2.57 (2 H, q), 4.83–5.50 (3 H, m). MS [IP 15 eV; *m/e* (% rel. int.)]: 188 (4, M), 173 (95), 160 (11), 159 (100), 158 (93), 141 (31), 127 (45), 126 (12), 117 (20), 111 (10), 79 (10).

Acknowledgements. I am indebted to Dr. Alf Claesson for his kind interest in this work and for valuable discussions. The work was in part supported by the Swedish Natural Science Research Council.

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Cyclic Tetra- and Octapeptides of Sarcosine in Combination with Alanine or Glycine. Syntheses and Conformation

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A series of cyclic tetrapeptides, c-L-MeAla-Sar$_3$, c-L-Ala-Sar$_3$, c-D-Ala-Sar-L-Ala-Sar, c-D-Ala-Sar-D-Ala-Sar, c-L-Ala$_2$-Sar$_2$, c-Ala$_3$-Sar, c-Ala$_4$, c-Gly-Sar$_3$, c-Gly-Sar-Gly-Sar, c-Gly$_2$-Sar$_2$, c-Gly$_3$-Sar, c-Gly$_4$ have been synthesised. The yield in the cyclisation step depended on the amino acid sequence in the linear peptide. Cyclic dipeptides, formed by cleavage of the peptide chain during the cyclisation reaction, partially racemised tetrapeptides and octapeptides were also isolated. c-D-Ala-Sar-D-Ala-Sar could be obtained only from peptides which contained both D- and L-alanine residues. Most of the cyclic tetrapeptides adopted the conformation of the parent compound, cyclotetrasarcosyl which is centrosymmetric with the amide sequence *cis,trans,cis,trans*. The same conformation was also found even when an NH amide bond must thereby become *cis*. When α-substituents make this conformation impossible and in trifluoroacetic acid other conformers are observed, but these have the same *cis,trans* sequence.

The barriers to inversion are very high when one or two N-CH$_3$ amide groups are *trans*, but considerably lower when the two *trans* positions are occupied by NH amide groups, due to the ability of the latter to rotate through the ring.

When dissolved at − 60 °C, the isolated cyclic octapeptides, c-(L-Ala-Sar$_3$)$_2$, c-(L-Ala$_2$-Sar$_2$)$_2$, c-(Gly-Sar$_3$)$_2$, c-(Gly$_2$-Sar$_2$)$_2$, except c-Gly$_8$, showed the same conformation as cyclooctasarcosyl with the amide sequence *cis,cis,trans,trans, cis,cis,trans,trans*. On heating the solutions, conformational changes took place analogous to those observed in a series of cyclic pentapeptides.

Only a few isolated investigations on cyclic tetrapeptides have been reported. The simplest representative, cyclo-tetraglycyl, was the first to be synthesised by Schwyzer in 1956 [1] and cyclo-glycyltri-L-alanyl and cyclo-tetra-L-alanyl were prepared on an insoluble polymer.[2]

A partially N-methylated tetrapeptide, tentoxin, cyclo-N-methyl-L-alanyl-L-leucyl-N-methyl-*trans*-dehydrophenylalanylglycyl has been isolated [3] from a pathogenic fungus and studied by X-ray methods [4] and NMR spectroscopy.[5] NMR studies have also been carried out on three cyclic tetrapeptides of proline combined with either glycine or sarcosine.[6]

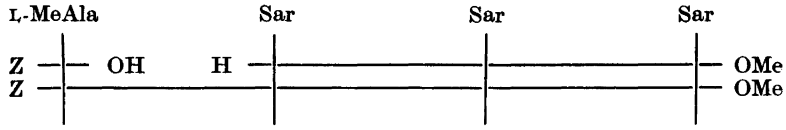
The simplest fully N-methylated cyclic tetrapeptide, cyclotetrasarcosyl, synthesised in this laboratory, shows a high degree of conformational homogeneity. The same centrosymmetric conformation with the amide sequence *cis,trans,cis,trans* (Fig. 6 A), was found both in solution [7,8] and in the crystal.[9] For c-Gly$_4$, a conformation with the four amide groups *trans* [1] has been suggested. Theoretical calculations on c-L-Ala$_4$ concluded that with all amide bonds *trans* an acceptable conformation could be obtained only if these were distorted from planarity.[10] It was therefore of interest to examine whether the unique conformation of c-Sar$_4$ might be adopted by other cyclic tetrapeptides and to study favoured conformations when *trans* preferred NH amides are present.

A series of cyclic tetrapeptides have been synthesised, replacing successively all four sarcosine units by either alanine (D and L) or glycine residues. (Scheme 1). Some results from the conformational study have already been communicated.[11,12] This paper describes the syntheses and gives additional results from the conformational investigations.

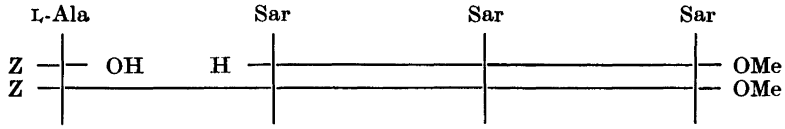
From some of the cyclisation reactions were also isolated cyclic octapeptides (Table 1) which represent an interesting extension of our investigations, and will be discussed.

Scheme. 1. Routes to the different linear tetrapeptides.

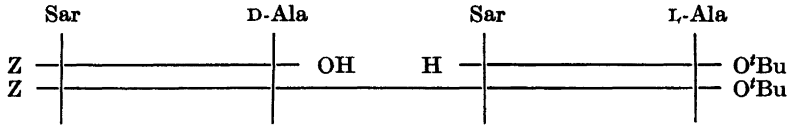
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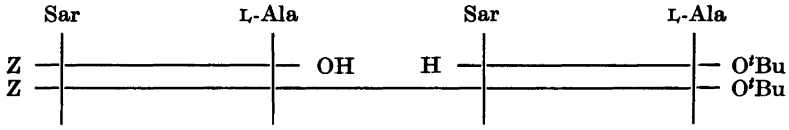


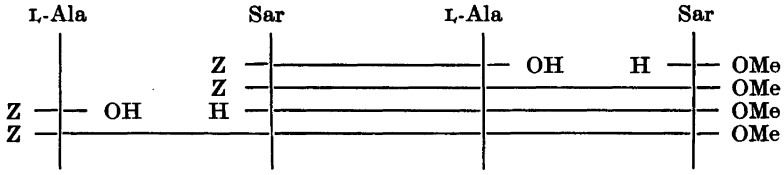
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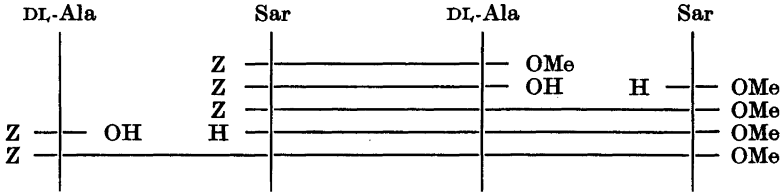


For cylco-D-alanylsarcosyl-L-alanylsarcosyl, c-D-Ala-Sar-L-Ala-Sar and cyclo-D-alanylsarcosyl-D-alanylsarcosyl, c-D-Ala-Sar-D-Ala-Sar

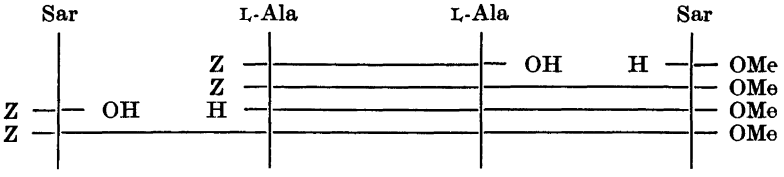




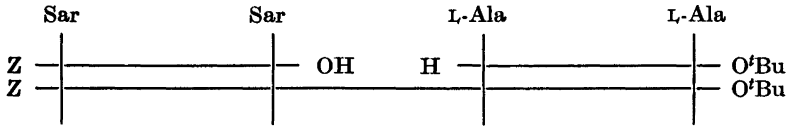




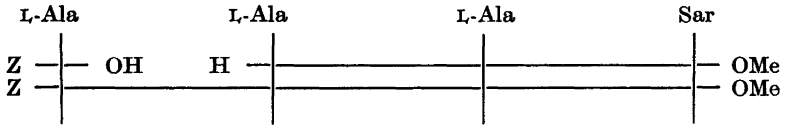
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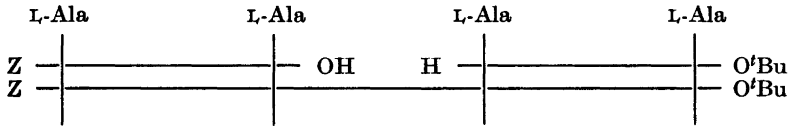
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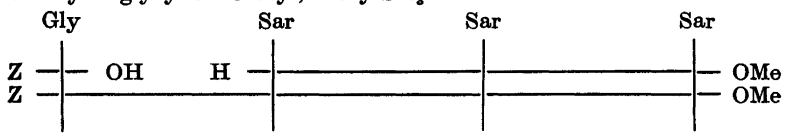
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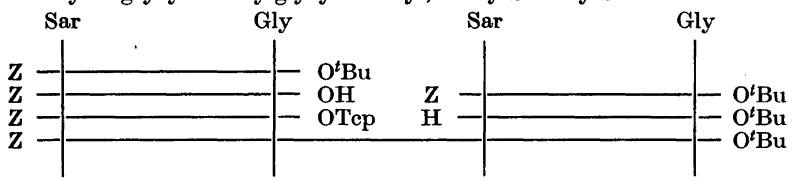
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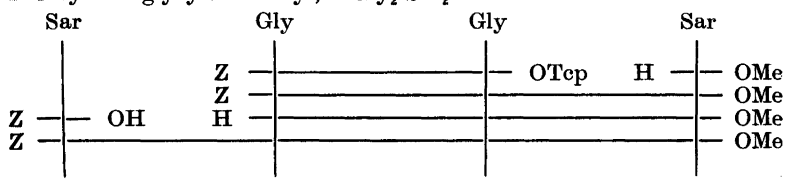
For cyclo-glycyltrisarcosyl, c-Gly-Sar$_3$

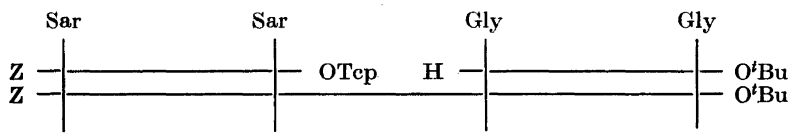


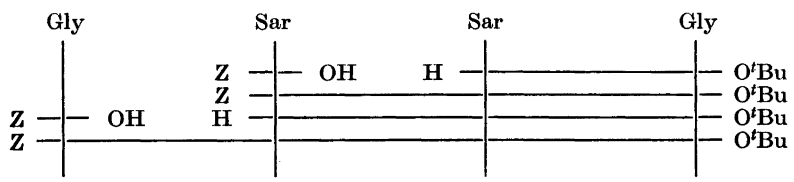
For cyclo-glycylsarcosylglycylsarcosyl, c-Gly-Sar-Gly-Sar



For cyclo-diglycyldisarcosyl, c-Gly$_2$-Sar$_2$

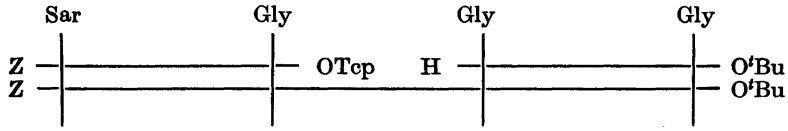






Scheme 1. Continued.

For cyclo-triglycylsarcosyl, c-Gly_3-Sar



For cyclo-tetraglycyl, c-Gly_4

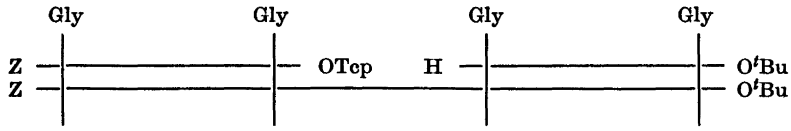


Table 1. Yields (%) of cyclic peptides from tetrapeptide trichlorophenyl ester.

Starting material	Dipeptides		Tetrapeptides		Octapeptides	
HCl.L-MeAla-Sar_3-OTcp			c-L-MeAla-Sar_3	(20)	c-(L-MeAla-Sar_3)$_2$	(traces)
HCl.L-Ala-Sar_3-OTcp	c-Sar_2	(33)	c-Ala-Sar_3	(25)	c-(Ala-Sar_3)$_2$	(traces)
	c-Ala-Sar	(16)				
HCl.Sar-D-Ala-Sar-L-Ala-OTcp			c-D-Ala-Sar-L-Ala-Sar	(27)		
			c-D-Ala-Sar-D-Ala-Sar	(1)		
HCl.Sar-L-Ala-Sar-L-Ala-OTcp			c-D-Ala-Sar-L-Ala-Sar	(1)		
			c-L-Ala-Sar-L-Ala-Sar	(traces)		
HCl.L-Ala-Sar-L-Ala-Sar-OTcp	c-Ala-Sar	(64)	c-D-Ala-Sar-L-Ala-Sar	(<1)		
HCl.DL-Ala-Sar-DL-Ala-Sar-OTcp	c-Ala-Sar	(52)	c-D-Ala-Sar-L-Ala-Sar	(5)		
			c-D-Ala-Sar-L-Ala-Sar	(4)		
			c-L-Ala-Sar-L-Ala-Sar			
HCl.Sar-L-Ala_2-Sar-OTcp			c-L-Ala_2-Sar_2	(2.5)	c-(L-Ala_2-Sar_2)$_2$	(3)
HCl.Sar_2-L-Ala_2-OTcp	c-Sar_2	(30)				
	c-Ala_2	(13)				
HCl.L-Ala_3-Sar-OTcp			c-Ala_3-Sar	(4.5)		
HCl.L-Ala_4-OTcp			c-Ala_4	(5)		
HCl.Gly-Sar_3-OTcp	c-Gly-Sar	(6)	c-Gly-Sar_3	(23)	c-(Gly-Sar_3)$_2$	(3)
	c-Sar_2	(4)				
HCl.Sar-Gly-Sar-Gly-OTcp			c-Gly-Sar-Gly-Sar	(42)		
HCl.Sar-Gly_2-Sar-OTcp			c-Gly_2-Sar_2	(3)	c-(Gly_2-Sar_2)$_2$	(13)
HCl.Sar_2-Gly_2-OTcp	c-Sar_2	(25)	c-Gly_2-Sar_2	(traces)	c-(Gly_2-Sar_2)$_2$	(3)
	c-Gly_2	(10)				
HCl.Gly-Sar_2-Gly-OTcp	c-Gly-Sar	(57)				
HCl.Sar-Gly_3-OTcp			c-Gly_3-Sar	(3)		
HBr.Gly_4-SCH_2COOH			c-Gly_4	(4.5)	c-Gly_8	(3)

SYNTHESES

The linear peptides were synthesised as shown in Scheme 1. The benzyloxycarbonyl group was used for protection of the amino function, the methyl ester or *t*-butyl ester for the carboxyl group and *N*,*N*′-dicyclohexylcarbodiimide as the coupling reagent. For cyclisation the methyl (*t*-butyl) ester was first hydrolysed, the carboxyl group then converted to the 2,4,5-trichlorophenyl ester, and the

peptide active ester after removal of the benzyloxycarbonyl group cyclised in pyridine at 115 °C. The cyclic peptides were isolated either by fractional crystallisation, sublimation, or chromatography on silica gel.

Some tetrapeptides gave the corresponding cyclic peptides in high yield (40 %), while closely related peptides produced the cyclic tetrapeptides either in low yield or not at all; instead cyclic dipeptides, octapeptides and racemised tetrapeptides were formed (Table 1). The product distribution clearly demonstrated that the sequence of amino acids in the linear tetrapeptide is important. The yield of cyclic tetrapeptide being high when the linear peptide has a high probability of being folded in the same way as the strongly preferred conformation of c-Sar_4 (Fig. 6A) which has alternating *cis* and *trans* amide bonds and requires the configuration DDLL (or LLDD) of the amino acids. This means that a suitable sequence consists of alternating *N*-methylated amino acids, such as sarcosine, which easily form *cis* amide bonds. In addition, it was found preferable to have an NH amino acid as the second residue in the chain (see below).

The major byproduct was formation of cyclic dipeptides and the product distribution (Table 1) corresponds to cleavage of the central amide bond and shows that the reaction takes place in the linear peptide. If the second amino acid is *N*-methylated the free amino end can easily move close to the second carbonyl carbon and form an unstable cyclol.[13,14] This splits up to give a cyclic dipeptide and a dipeptide active ester which subsequently may cyclise to a second molecule of cyclic dipeptide. This reaction is dominating when the fourth amino acid is of the *trans*-preferred NH-type (alanine or glycine), whereas if the fourth amino acid is *N*-methylated formation of the cyclic tetrapeptide is preferred to formation of the unstable cyclol. Hardly any cyclic dipeptides are formed when the second amino acid is alanine or glycine, which was necessary to have to obtain c-L-Ala_2-Sar_2 and c-Gly_2-Sar_2 (Table 1).

Doubling reactions, well-known from cyclisation of tri- and pentapeptides [15] and also encountered in tetradepsipeptides,[16,17] was found to take place, but to little extent, in the cyclic homologues of sarcosine,[8] and the cyclic pentapeptides of sarcosine combined with either alanine or glycine.[18] In the present tetrapeptides, a few of the reactions led to isolable quantities of cyclic octapeptides (Table 1). Highest yield (13 %) was obtained on cyclising Sar-Gly_2-Sar-OTcp, which gave a low yield of cyclic tetrapeptide and cannot easily form cyclic dipeptides.

Racemisation has been shown to occur during cyclisation of pentapeptides.[18] Also from the cyclisation of tetrapeptides, pairs of cyclic tetrapeptides were isolated (Table 1). Sar-D-Ala-Sar-L-Ala-OTcp gave two cyclic tetrapeptides which were isolated either by fractional crystallisation or by sublimation. They had different IR and NMR spectra, but identical mass spectra (*m/e* 284). The NMR spectrum of the major product clearly showed this to be c-D-Ala-Sar-L-Ala-Sar with the same conformation as c-Sar_4, centrosymmetric and with the alanine residue in the *trans* positions. The other compound showed the presence of two conformers (in the NMR spectrum) and had a high optical rotation ($[\alpha]_D + 92°$) and must be c-D-Ala-Sar-D-Ala-Sar, formed by racemisation of the C-terminal L-alanine. The peptide chain has a high probability of being folded in the same way as the favoured conformation of the major product and the free amino end can easily approach the active ester and form an intermediate as shown in Fig. 1. Cleavage of the active ester may occur by elimination of phenol removing either H from the amino group which will result in the major product or by elimination of the α-H of

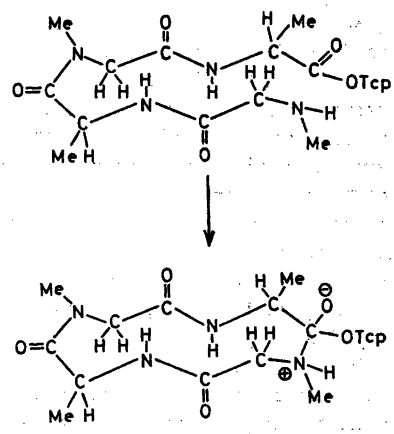


Fig. 1.

Table 2. Properties of the

	Sub-lim. temp. °C/0.01 mmHg	M.p.,°C	IR (cm^{-1}) bands in KBr C=O stretch	*trans* CONH	Low temp. NMR data (100 MHz) NMR coal.-temp. °C	Chem. shift, ppm (gem. coupl. C-CH_3 *trans*	*cis*	N-CH_3 *trans*	*cis*
c-L-MeAla-Sar_3	180	318	1670,1640			1.26		2.66,2.87	3.04
c-L-Ala-Sar_3	190	315(subl.)	1690,1680,1650	1550		1.28		2.83	3.03,3.07
c-D-Ala-Sar-L-Ala-Sar	290	>350	1670,1650	1570		1.41			3.23
c-D-Ala-Sar-D-Ala-Sar	220	340(subl.)	1670,1650,1640	1550	30	1.33,1.42			3.03,3.08
c-L-Ala_2-Sar_2	240	310(subl.)	1670,1640	1570		1.42	1.62	3.09	3.21
c-Ala_3-Sar	250	>350	1660	1560		1.39,1.40	1.57		3.25
c-Ala_4	210	315(subl.)	1650	1540		complex			
c-Gly-Sar_3	230	322	1685,1650	1550	150			2.85	3.02,3.08
c-Gly-Sar-Gly-Sar	260	>350	1660	1560	30				3.17,3.20
c-Gly_2-Sar_2	260	310	1690,1670,1650	1560	100			2.89,3.02	3.09
c-Gly_3-Sar	270	>350	1670,1650,1640	1540					3.26
c-Gly_4(subl.)	270	340(subl.)	1690,1640	1540					
c-Gly_4 (cryst.H_2O)		340(subl.)	1680,1660	1550					

the L-alanine residue which subsequently will give the two obtained cyclic tetrapeptides. When Sar-L-Ala-Sar-L-Ala-OTcp and L-Ala-Sar-L-Ala-Sar-OTcp which cannot easily fold in the same way as c-Sar_4 were cyclised only traces of cyclic tetrapeptides (LL and DL-isomers) were isolated, and c-Ala-Sar was the major product (64 %) from the latter peptide. When DL-Ala-Sar-DL-Ala-Sar-OTcp was cyclised, besides some cyclic dipeptides, both c-D-Ala-Sar-L-Ala-Sar (5 %) and the DD + LL isomer (4 %) were isolated. These experiments show that the DD- and LL-isomers of c-Ala-Sar-Ala-Sar are obtained only through racemisation of peptides containing both D- and L-alanine residues. The c-Ala_3-Sar was isolated in low yield and adopted the c-Sar_4 conformation, as shown by NMR spectroscopy. The expected c-L-Ala_3-Sar (Table 1) cannot adopt this conformation, and some racemisation must have taken place possibly in the coupling reactions since DCC was used. Similarly the X-ray analysis of the expected c-L-Ala_3-Sar_3 showed that the crystal contained the racemate.[19]

This demonstrates that favoured conformations of the peptide chains play an important role both for ring size and yield of the resulting cyclic peptides.

Physical and spectral properties of the cyclic tetrapeptides

The cyclic tetrapeptides are well defined solids, sublimable and with sharp melting points (Table 2). Their solubility in organic solvents is generally low, lower than that of the corresponding cyclic octapeptides and the closely related cyclic pentapeptides.[18] A few are even only slightly soluble in water. The mass spectra show the molecular ions as intense peaks. The fragmentation patterns are specific for each cyclic peptide indicating that they split up differently depending on the amino acids present. This is in contrast to the

cyclic tetrapeptides.

const., Hz) of CH_2: C-2 & C-8 outer	inner	C-5 & C-11 outer	inner	CH	NH	Solvent
3.23	5.3 (14.5)	3.54,3.58	4.33,4.41(18)	5.4		$CDCl_3$
3.24	5.39(14.5)	3.54,3.58	4.14,4.38(18)	4.86	6.88(10)	$CDCl_3$
		4.03	4.39 (18)	5.13	7.62(10)	TFA
		3.75,3.85,3.90	3.99,4.31,4.55(18)	4.95	7.49(10),7.68(7),8.01(10)	$CDCl_3$ trace TFA
3.6	5.6 (15)	4.08	4.68(18)	4.34,5.12	7.78(10),7.92(8)	TFA
		4.02	4.38(18)	4.24,5.11,5.11	7.63(10),7.81(10),7.83(7)	TFA
3.44,3.53	5.25,4.84(15)	3.69,3.70	4.14,4.32(18)		6.86(10)	$CDCl_3$ trace TFA
3.83	5.13(15)	4.10	4.48(18)		7.61(10),7.94	TFA
3.47,3.54	4.85,5.28(15)	3.81	4.18(18)			$CDCl_3$ trace TFA
3.92	5.30(15)	not resolved			8.22 8.78	TFA
		4.07(7)	4.19(9)		7.91	TFA

behaviour of the cyclic pentapeptides [18] which showed identical fragmentation patterns. The infrared spectra show from two to four carbonyl absorptions (Table 2) but it was not possible to assign these to *cis* and *trans* amide bonds or to particular locations in the molecules. The cyclic tetrapeptides which contain one or more NH-amide bonds always show the diagnostic *trans*-CO – NH band at 1550 cm^{-1}. An indication of the number of *trans* amide bonds could be obtained by comparing the ratio of the area below this NH-absorption and the CO-absorptions. The NMR spectra which mostly could be recorded below the coalescence of the rings showed that only one conformation is present in $CDCl_3$, while additional conformers were observed in TFA or mixtures of $CDCl_3$ and TFA. Dropwise addition of C_6D_6 was useful to resolve overlapping signals and also to determine conformations due to the different upfield shifts of the lines.

THE INDIVIDUAL CYCLIC TETRAPEPTIDES

A few crystal structures, c-Ala-Sar_3, c-Gly-Sar_3 and c-Gly-Sar-Gly-Sar have been determined by X-ray methods [19] and a conformation identical to that of c-Sar_4 [9] is adopted with the same dihedral angles and transannular distances and with the NH amide groups in the *trans* positions.

In solution ($CDCl_3$) the NMR spectra (Table 2) of c-L-MeAla-Sar_3, c-L-Ala-Sar_3 [11] and c-Gly-Sar_3 are closely related to that of c-Sar_4 which has two N-CH_3 singlets *cis* and *trans* (at highest field) and two methylene quartets with J of 18 and 14 Hz (most widely separated). The same conformation is adopted and the NH amide group of the two latter occupies a *trans* position shown in IR by the *trans*-CO-NH band at 1550 cm^{-1}. The α-CH_3 groups are situated in the 2-(or 8-)position (Fig. 6 A), necessarily pointing out of the ring. This position in

c-Sar$_4$ is bent more out of the ring than the corresponding 5-(or 11-) position and explains why the α-CH$_3$ group of c-L-MeAla-Sar$_3$ prefers the 2-position. The NMR signals may now be correlated with the different protons in the molecules. The CH$_2$ quartet with $J = 14$ Hz belongs to the 2(8)-position with the inner H shifted to low field. The N-CH$_3$ singlets at lowest field belong to the *cis* amide bonds. This is opposite to what has been found in linear peptides such as polysarcosine [20] and poly-N-methyl-L-alanine.[21] The dihedral angles of NHCH correspond well with the observed coupling constants, the inner H having a $J = 10$ Hz and the high-field H (the outer proton) a small coupling constant. According to the Bystrov curve,[22] large values are expected for angles of 0 and 180° and small values for angles around 60°. The geminal coupling constants (18 and 14 Hz) fit the reported dependence [23] on the orientation of the methylene group to an adjacent carbonyl group, being largest when the dihedral angle of $R-C_{CO}-C_\alpha-R'$ is 0 or 180° and smallest for 60 and 120°. In TFA, the NMR spectra of c-L-Ala-Sar$_3$ and c-Gly-Sar$_3$ (Fig. 2) show that two conformers are present, the major is identical to that in $CDCl_3$, the minor, 20 and 30 % (Fig. 2a) respectively, has the same large (10 Hz) and small NHCH coupling and the same CH$_2$-quartets ($J = 18$ and 15 Hz). A noteworthy difference is that the lowest shifted CH$_2$-proton (Fig. 2) (at δ 5.4) is now situated at δ 4.6. The N-CH$_3$ lines appear unresolved in the *cis* N-CH$_3$ region, but on dropwise addition of benzene one moves faster

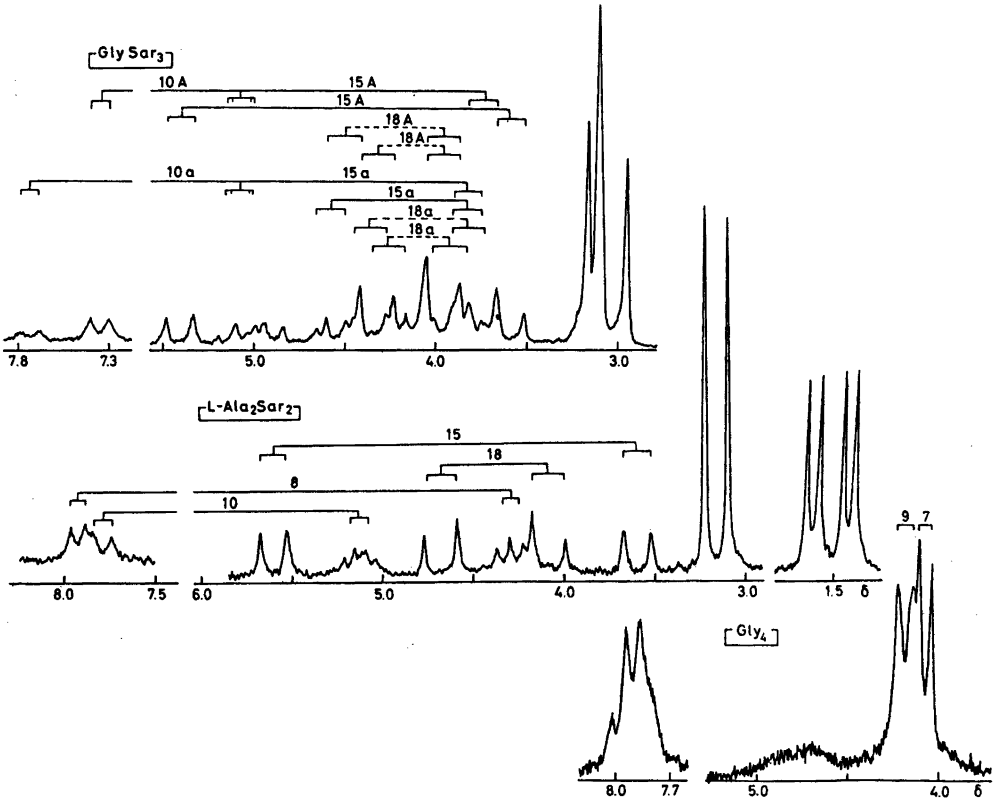


Fig. 2. The 100 MHz NMR spectrum at 35 °C of c-Gly-Sar$_3$ in TFA:$CDCl_3$ = 4:1 (upper spectrum). A represents the major conformer and a the minor conformer, c-L-Ala$_2$-Sar$_2$ in TFA and c-Gly$_4$ in TFA (lower spectrum). The splitting pattern was partially deduced from spin-decoupling and the quartets shown by dotted lines are presumed from the C_6D_6 addition.

to higher field which indicates that this belongs to a *trans* amide bond.[18] This conformation will be considered in the discussion.

c-D-Ala-Sar-L-Ala-Sar shows in TFA (the only solvent which could be used) an NMR spectrum [12] (Table 2) which fits the c-Sar_4 conformation with the NH-amide bonds *trans*. The rate of exchange of the amide protons is particularly slow in this molecule, full exchange in TFA-*d* takes more than 12 h at 25 °C, whereas in both c-Ala-Sar_3 and c-Gly-Sar_3 full exchange take less than 5 min. This may suggest that a second conformer is not present in c-D-Ala-Sar-L-Ala-Sar and that the rate of exchange of the *trans* amide protons is generally slow in the c-Sar_4 conformation.

c-Gly-Sar-Gly-Sar shows in TFA (the only solvent which could be used) an NMR spectrum at −20 °C with two conformers present in equal concentrations whereof one is the crystal conformation. The second conformer is unsymmetric with two hardly split N-CH_3 singlets (seen when C_6D_6 is added), a broadened NH line and the CH_2-protons are broad and unresolved and did not sharpen on further cooling to −30 °C. Addition of benzene moved the N-CH_3 lines only slightly to higher field indicating that they belong to *cis* amide bonds. At about −10 °C the two N-CH_3 singlets of the unsymmetric conformer coalesced to a single line and the CH_2-protons appeared as one broad line. No change took place in the conformer adopting the c-Sar_4 skeleton until 20 °C, then the CH_2-quartets started to broaden and at 70 °C, the NMR spectrum shows only two sharp lines of the CH_2-protons and one N-CH_3 singlet. The conformational situation will be considered in the discussion.

c-L-Ala_2-Sar_2 (Fig. 2) shows with its two α-CH_3 doublets, two N-CH_3 singlets, two CH_2-quartets, $J = 18$ and 15 Hz two CH bands and two NH doublets, $J = 10$ Hz (slowly exchanged with D) and 8 Hz (rapidly exchanged with D), that the conformation of c-Sar_4 is adopted having one N-CH_3- and one NH-amide group *trans* and the second N-CH_3- and NH-amide group *cis*. This clearly demonstrates that the conformation with alternating *cis* and *trans* amide bonds is retained even when a *trans* preferred NH-amide bond has to be *cis*. Any additional lines due to a second conformer was not visible in the NMR spectrum.

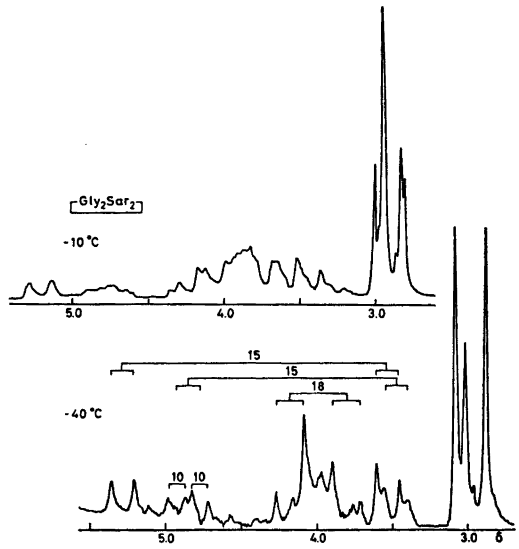


Fig. 3. The 100 MHz spectrum of c-Gly_2-Sar_2 at −40 °C and at −10 °C (upper spectrum). Crystals were dissolved in $CHFCl_2$ with a trace of TFA at −50 °C and allowed to warm up in the probe (NH region left out).

The closely related c-Gly_2-Sar_2 shows when dissolved in $CHFCl_2$ (added traces of TFA) at −40 °C (Fig. 3) one major conformer (*ca.* 80 %) with two N-CH_3 singlets, one not resolved CH_2-quartet with $J = 18$ Hz and two with $J = 15$ Hz whereof one exhibits a large ($J = 10$ Hz) and a small vicinal coupling. The other CH_2 group is not well resolved, but the quartet is narrow. The NH protons could not be observed in this solvent. The NMR spectrum of the major conformer shows that the c-Sar_4 conformation is adopted with one N-CH_3- and one NH-amide *cis* and the other N-CH_3- and NH-amide *trans*. Two possibilities exist for arranging the groups around the ring (Fig. 6 A); the two NH groups can be either in positions 1 and 4 or in 4 and 7, only the former is allowed for c-L-Ala_2-Sar_2 due to α-substituents. The NMR data do not decide which of the two is adopted by c-Gly_2-Sar_2 at low temperature and thus in the solid. The additional conformer increases in concentration on raising the temperature and at the thermodynamic equilibrium (*ca.* −10 °C) (Fig. 3) this conformer is dominating, about 20 % of the original one remains and a few extra N-CH_3 lines may belong to a third conformer possibly the other allowed

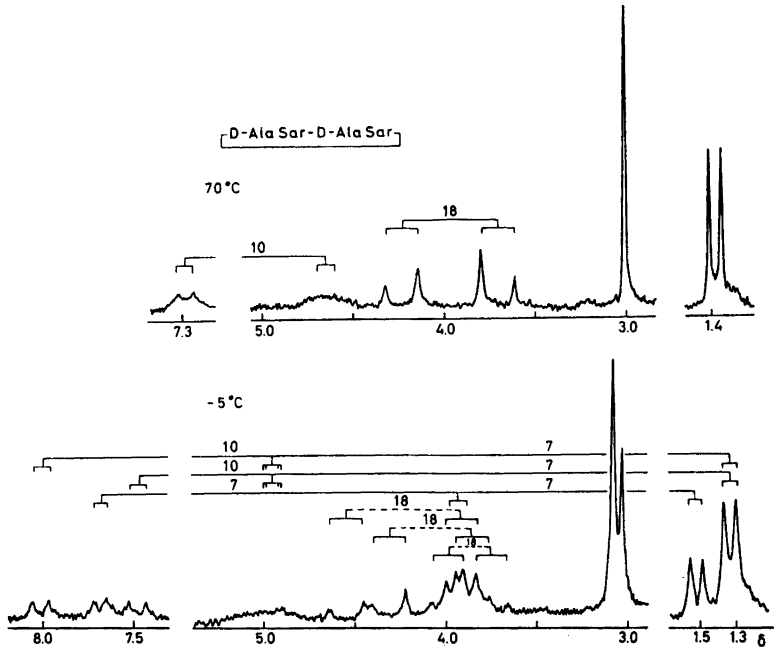


Fig. 4. The 100 MHz NMR spectrum of c-D-Ala-Sar-D-Ala-Sar in $CDCl_3$ plus a trace of TFA at 70 and −5 °C (lower spectrum). The splitting pattern was partially deduced from spin-decoupling and the quartets shown by dotted lines are presumed from the C_6D_6 addition.

on the c-Sar_4 skeleton. The second conformer has the N-CH_3 lines unresolved in the *cis* N-CH_3 region and one moves fast to higher field on addition of C_6D_6 (to a $CDCl_3$ solution). The signals of the methylene protons are unresolved and broad ($CDCl_3$ solution) but sharpen on cooling to −20 °C suggesting a coalescence phenomenon for this conformer. The CH_2-quartets of the original conformer do not coalesce below 80 °C, a similar phenomenon as observed in c-Gly-Sar-Gly-Sar where the conformer which adopts the c-Sar_4 conformation has a high barrier to inversion whereas the second exhibits a much lower barrier to inversion (see the discussion).

c-D-Ala-Sar-D-Ala-Sar shows at 70 °C a simple NMR spectrum (Fig. 4) (one N-CH_3 singlet, one CH_2-quartet, $J = 18$ Hz, and one NH doublet, $J = 10$ Hz). On cooling, the quartet began to broaden and at 5 °C (Fig. 4) two species (2:1) are present, the minor has the same lines as the spectrum recorded at 70 °C. The major conformer is unsymmetric with two N-CH_3 lines, two not well resolved CH_2-quartets which both seem to have $J = 18$ Hz and two NH doublets, $J = 10$ and 7 Hz. The ratio of the conformers did not change on changing the solvent from TFA to $CDCl_3$ (with a trace of TFA). This molecule cannot adopt the c-Sar_4 conformation due to the configuration of the alanine residues and alternative conformations will be considered in the discussion.

c-Ala_3-Sar was isolated in low yield and shows an NMR spectrum (in TFA) (Table 2) which by comparison with the NMR spectrum of c-L-Ala_2-Sar_2 fits exactly this conformation having two NH-amide groups *trans* and one *cis*. The expected c-L-Ala_3-Sar cannot adopt the c-Sar_4 conformation due to the α-substituents and some racemisation must have taken place. The isolated peptide has to be either c-L-Ala_2-D-Ala-Sar or c-D-Ala_2-L-Ala-Sar the only possible on the c-Sar_4 skeleton.

c-Gly_3-Sar shows at −25 °C (in TFA with a trace of $CDCl_3$) one N-CH_3 singlet, one CH_2-quartet ($J = 15$ Hz) which exhibits a large (10 Hz) and a small CHNH coupling. The

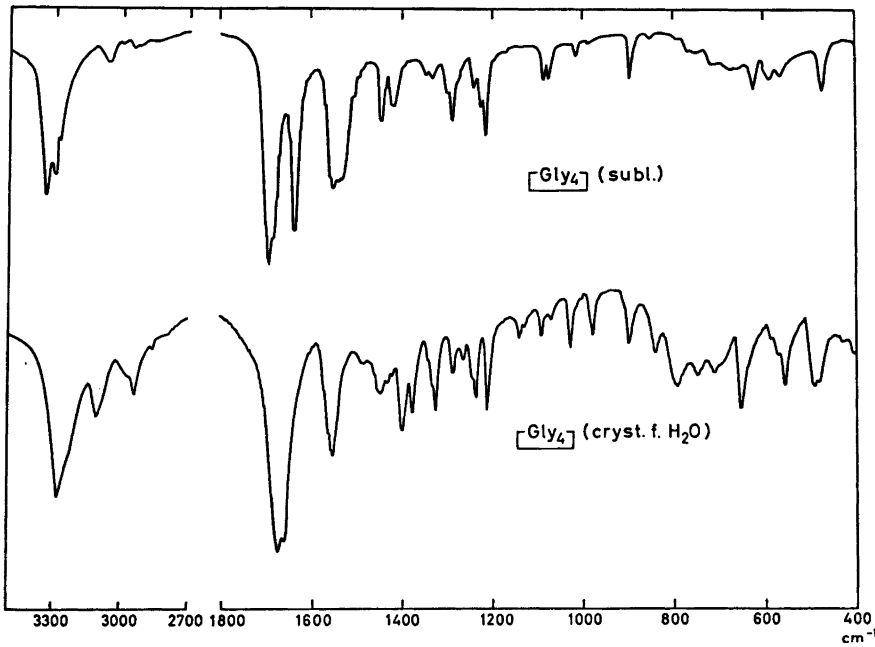


Fig. 5. The IR spectrum of c-Gly$_4$ as a KBr-disc, crystallised from water (lower curve) and sublimed.

remaining CH_2 protons are unresolved (at δ 4.35) and the NH protons are grouped at δ 8.2 (broadest) and 8.7. On warming, the CH_2 lines start to coalesce at -15 °C, and addition of benzene moves the N-CH_3 group comparatively little upfield which indicates that this is part of a *cis* amide bond. No certain conclusion can be drawn for this molecule.

c-L-Ala$_4$ was obtained in low yield and shows a complex NMR spectrum (in TFA) with more than eight α-CH_3 doublets indicating the presence of either several conformers or isomers containing both D- and L-alanine residues due to racemisation during the synthesis. c-L-Ala$_4$ with all amide groups *trans* should have only one α-CH_3 doublet.

c-Gly$_4$ crystallised from water as very small crystals which were insoluble in organic solvents and only slightly soluble in water and TFA and it was difficult to dissolve enough for the NMR measurements. The crystallised product could be sublimed (at 270 °C/0.01 mmHg) and thereby became a little more soluble in TFA. The sublimed product could on the other hand not easily be resublimed as a higher temperature was now required. The IR spectra (in KBr) (Fig. 5) of the crystallised and sublimed c-Gly$_4$ are different. Among several dissimilarities is the ratio of the area below the NH-absorption at 1550 cm^{-1} and the CO-absorptions at 1640–1690 cm^{-1} (NH/CO), being smallest for the crystallised product. Comparison with this NH/CO ratio of the other cyclic tetrapeptides of known conformation suggests that the crystallised c-Gly$_4$ contains two *trans* amide bonds while the sublimed product contains three or four *trans* amide bonds. This ratio may vary with alternation of the conformation even without change in the number of *trans* amide bonds, although it is true, c-Gly$_6$ and c-Gly$_3$ which have all amide bonds *trans* showed a higher ratio. Only one of the other cyclic tetrapeptides, c-Ala$_4$, isolated by sublimation only, showed the same high NH/CO ratio as the sublimed c-Gly$_4$ and could possible have an analogous behaviour. Such a difference was not observed for the sarcosine containing cyclic tetrapeptides. c-Gly-Sar-Gly-Sar, for instance, was crystallised from water and also sublimed but showed no spectral (IR and NMR) differences. A slow conversion of the sublimed c-Gly$_4$ to the crystallised c-Gly$_4$ was observed as a KBr-disc of sublimed product after one

year gave an IR spectrum showing the presence of both conformations. A fast conversion took place on dissolution (in TFA) of the sublimed product as the IR spectrum of the evaporated sample was identical to that of the crystallised product. The NMR spectra (in TFA) of crystallised and sublimed (Fig. 2) c-Gly_4 are identical showing two partly resolved NH triplets and two CH_2 doublets, one, ($J = 9$ Hz) is broader than the other ($J = 7$ Hz). Addition of benzene moved the broadest doublet most upfield, 84 Hz and 65 Hz (1 ml C_6D_6 added to 0.5 ml TFA). By cooling to -20 °C the peaks became broad and unresolved but did not change on further cooling to -50 °C (traces of $CDCl_3$ was added). This NMR spectrum can only fit conformations with alternating *cis* and *trans* amide bonds and with the methylene groups rapidly flipping. If the four amide bonds were *trans* as originally suggested by Schwyzer [1] only one CH_2 doublet should be present. A recent reinvestigation using NMR (^1H and ^{13}C) spectroscopy [25] also led to the conclusion that this molecule has four *trans* but not planar, amide bonds. This is in disagreement with our findings, but may perhaps be explained by postulating that these authors have studied the doubling product, c-Gly_8, which may sometimes be obtained in their synthesis (see Experimental part).

DISCUSSION

The conformation of c-Sar_4 (Fig. 6 A) is obviously very favoured in cyclic tetrapeptides as it is chosen by most of the cyclic tetrapeptides in this series both in the solid and in solution and also by cyclic peptides where NH amide bonds have to be *cis*. A crystal structure of dehydrotentoxin [4] showed the same conformation even with the two NH amide bonds *cis* and the *N*-CH_3 amide bonds *trans*, the only possibility on this skeleton due to the α-substituents. The crystal structure of three closely related tetradepsipeptides showed that one, c-D-MeVal-L-Hyv-L-MeVal-D-Hyv,[26] adopts an almost identical conformation to c-Sar_4 with the ester groups in the *trans* positions, whereas the DDDL-isomer and the c-L-MeLeu-D-Hyv-L-MeIle-D-Hyv [27] which cannot adopt this conformation because of the relative configuration of the α-substituents, choose a conformation having also alternating *cis* amide and *trans* ester bonds but with the four carbonyl oxygens on the same side of the molecule. A difference exists, however, between the depsipeptide and the peptide structures, the ester oxygens are bent towards the transannular carbonyl carbon while in the peptides both the *trans* *N*-CH_3 (NH) and the CO groups are more perpendicular to the ring. No crysta

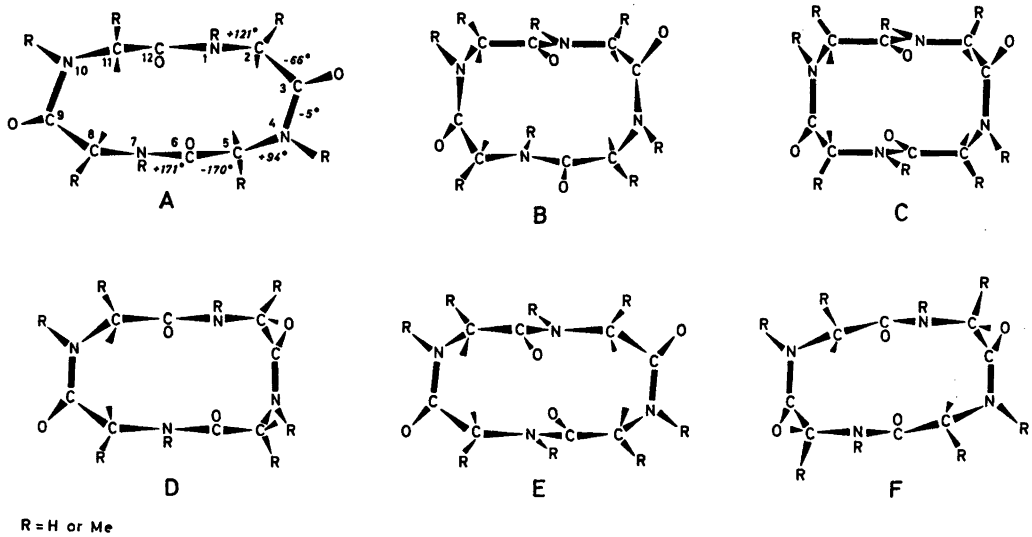


Fig. 6. Possible conformations.

structures have yet been determined of cyclic tetrapeptides which do not adopt the c-Sar_4 conformation. In solution a few proline containing cyclic tetrapeptides have been studied by NMR (^1H and ^{13}C) spectroscopy,[6] and the alternating *cis,trans* amide sequence is evidenced. In our series, c-D-Ala-Sar-D-Ala-Sar does not adopt the c-Sar_4 conformation and additional conformers are observed in TFA for some of the cyclic tetrapeptides. Since only the amide sequence *cis,trans,cis,trans* is observed with certainty in such twelve membered rings, conformations with this sequence only will be considered here.

The five possible conformations (Fig. 6 B–F) can be derived from the c-Sar_4 conformation (Fig. 6 A) by the following operations.* Rotation by 180° of one *trans* amide group leads to conformer B and rotation of both to C. Flipping of one and both *cis* amide groups also turn the adjacent methylene groups and lead to D and C, respectively. Conformer E is deduced from c-Sar_4 by flipping of one *cis* and rotation of one *trans* amide group and conformer F by flipping of the same *cis* amide group and rotation of the other *trans* amide group. Both possess two-fold axial symmetry and require the LLLL (DDDD) configuration of the amino acids. Conformer A and C are centrosymmetric and require the configuration DDLL while B and D are unsymmetric and require the configurations DDLL and DDDD (LLLL), respectively. When the ring contains two *trans* NH-amide groups, rotation of these through the ring exhibit a low barrier; thus, *ca.* 14 kcal/mol ($T_c = 30$ °C) is found for c-Gly-Sar-Gly-Sar.** With one or two *trans* N-CH_3 amide bonds present, as in c-Gly-Sar_3 and c-Sar_4, the coalescence temperatures are high 150 °C (Table 2) and 180 °C,[8] respectively and correspond to inversion barriers of *ca.* 21 and 24 kcal/mol. Rotation of a *trans* amide group through the ring is then so hindered that full inversion may be obtained through a different process.[24] Flipping of the *cis* amide groups should require low energy since no group has to rotate through the ring.

c-D-Ala-Sar-D-Ala-Sar shows one major unsymmetric and one minor symmetric conformer (Fig. 4) whereof the unsymmetric conformer with its coupling constants (10, 7 and 16–18 Hz) fits the conformer D (Fig. 6) with the NH-amide groups *trans*. The symmetric one may either be conformer E or F (Fig. 6), both can be derived from D by rotation of one *trans* NH-amide bond which requires relatively low energy and corresponds to the observed coalescence (30 °C). The coupling constants ($J = 10$ and 18 Hz) do not decide whether conformer E or F is preferred, but may indicate that it is F. In the crystal structures of the cyclic tetradepsipeptides [26,27] conformer F is found. Of the three cyclic tetrapeptides containing proline,[6] c-L-Pro-Gly-L-Pro-Gly showed one major unsymmetric and one minor symmetric conformer and a low barrier to inversion (*ca.* 14 kcal/mol). The major conformer is suggested to be D again with the NH-amide bonds *trans*, and the minor species may adopt either conformer E or F. The c-L-Pro-Sar-L-Pro-Sar [6] was found to have the proline nitrogen as part of the *cis* amide bonds and showed no coalescence of the ring measured up to 70 °C and this is expected with N-CH_3 amide bonds *trans*.

The additional species observed in TFA solution for c-Ala-Sar_3, c-Gly-Sar_3, c-Gly-Sar-Gly-Sar and c-Gly_2-Sar_2 were in an earlier communication [12] attributed to protonation of one amide group. c-D-Ala-Sar-L-Ala-Sar, c-L-Ala_2-Sar_2 and c-Sar_4 which showed only one conformation in TFA were suggested to be rapidly protonated and deprotonated. No change in the NMR spectra were observed on changing the temperature, however, in c-Gly-Sar-Gly-Sar and c-Gly_2-Sar_2 the additional CH_2 lines coalesced at ca −15 °C. This indicates the presence of a second conformer stabilised in the polar solvent, and is now the preferred explanation of the observed spectra. The low observed coalescence temperature makes it unlikely that rotation of a *trans* amide group takes place while flipping of the *cis* amide groups is more reasonable. At temperatures below −15 °C the molecules adopt in addition to the c-Sar_4 conformation, conformers with one (Fig. 6 D) or both (Fig. 6 C) *cis* amide groups flipped. This also explains the additional conformer of c-Ala-Sar_3 and c-Gly-Sar_3 with one *cis* amide bond flipped (Fig. 6 D).

* R in Fig. 6 does not refer to a certain configuration of the amino acids but indicates the least hindered positions in the molecules.

** 1 cal = 4.184 J.

This conformer should have approximately the same NHCH- and CH_2-coupling constants as the c-Sar_4 conformer and corresponds to the observed NMR spectra. The only visible difference (Fig. 2) is that the CH_2 proton at lowest field in the major conformer has moved to higher field in the minor conformer and indicates that the adjacent *cis* amide bond has flipped. In c-D-Ala-Sar-L-Ala-Sar where no additional conformer is observed, flipping of the *cis* amide bonds is prohibited by the α-substituents.

For c-Gly_3-Sar and c-Gly_4 the conformational situation has not been finally elucidated. They may adopt, in solution, partly or entirely the c-Sar_4 conformation or one of the conformations discussed, but no indication is found for another amide sequence than alternating *cis* and *trans* amide bonds.

CYCLIC OCTAPEPTIDES

The cyclic octapeptides were studied by NMR spectroscopy, the method which gives the most informative picture of the conformational situation. By dissolving the peptides at low temperature (−50 °C), it should be possible to observe the conformation before any *cis,trans* isomerisation (needs *ca.* 20 kcal/mol) takes place. Slow warming of the sample in the NMR-tube and recording the NMR spectra should reveal any conformational changes.

c-(L-Ala-Sar_3)$_2$ (Fig. 7) when dissolved at −50 °C, shows one major and one minor (*ca.*

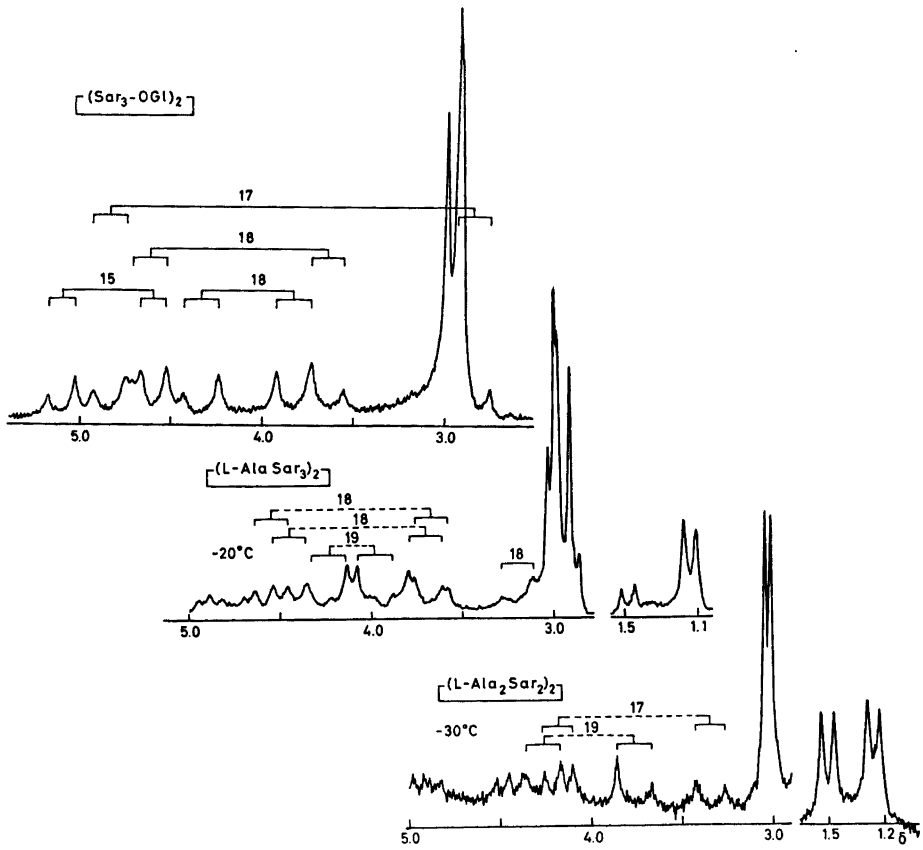


Fig. 7. The 100 MHz NMR spectrum of c-(L-Ala_2-Sar_2)$_2$ at −30 °C (lower spectrum) and c-(L-Ala-Sar_3)$_2$ at −20 °C (middle spectrum). Both were dissolved in $CHFCl_2$ with a trace of TFA at −50 °C and allowed to warm up in the probe (NH-region left out). The upper spectrum is of c-(Sar_3-OGl)$_2$ in $CDCl_3$ at −20 °C. OGl stands for $-OCH_2CO-$.

20 %) conformer both with one C-CH_3 doublet, three N-CH_3 singlets (1:1:1) and three CH_2 quartets (major). At about −15 °C the minor conformer starts to disappear, then the major conformer also disappears and new lines appear. At 5 °C the NMR spectrum consists of many N-CH_3 singlets and the CH_2 lines are broad and unresolved indicating that more than one conformer is present.

The NMR spectrum of the initially isolated c-(Gly-Sar_3)$_2$ shows at −50 °C only one conformer with two N-CH_3 lines (2:1) and with the CH_2 protons partially resolved. At about −20 °C the CH_2 protons start to coalesce and additional lines due to new conformers appear at −10 °C. Another sample which had been used for NMR measurements and subsequently isolated by evaporation of the solvent and treated with ether showed, however, at −50 °C a similar spectrum to that of c-(L-Ala-Sar_3)$_2$ with two conformers present.

c-(L-Ala_2-Sar_2)$_2$ (Fig. 7) is present in one conformer at −50 °C with two C-CH_3 doublets, two N-CH_3 singlets and two CH_2 quartets. At about −10 °C new conformers appear, and at 0 °C hardly any of the original lines can be seen and the lines in the CH_2 region are broad and unresolved.

c-(Gly_2-Sar_2)$_2$ shows at −70 °C two N-CH_3 singlets (1:1) and broad CH_2 lines suggesting that one conformer is present. The CH_2 protons started to coalesce already at about −50 °C, but the N-CH_3 protons did not coalesce until 0 °C. New conformers appeared at *ca.* −15 °C and hardly any of the initial conformer was present at 0 °C.

By comparing the NMR spectra of these four cyclic octapeptides, their conformations are shown to be closely related, all have centrosymmetry or two-fold axial symmetry and resemble the conformation of cyclooctasarcosyl [8,28] (Fig. in Ref. 8) and of the cyclic octadepsipeptide, c-(Sar_3-OGl)$_2$ [28] (Fig. 7) (OGl stands for the glycolic acid residue, $-OCH_2CO-$). The two latter take the same conformation both in the crystal and solution and undergo no conformational changes on changing the temperature. The X-ray structure of c-Sar_8 [29] showed approximately two-fold axial symmetry with the amide sequence *cis,cis,trans,trans,cis,cis, trans,trans*. The four cyclic octapeptides mentioned can easily adopt this skeleton by placing the *trans* preferred NH groups in the *trans* positions. For c-(L-Ala_2-Sar_2)$_2$ and c-(Gly_2-Sar_2)$_2$ only one possibility exists for arranging the amide groups on the c-Sar_8 skeleton, the one with the four *trans* amide positions occupied by the NH amides. In the case of c-(L-Ala-Sar_3)$_2$ and c-(Gly-Sar_3)$_2$ with two possibilities for the NH amide groups it is likely that the two conformations which are present in solution at low temperature differ only in having the NH amide groups in different *trans* positions. In c-Sar_4 and c-Sar_5 the most widely split CH_2-quartets belong to CH_2 groups adjacent to nitrogens which are part of *trans* amide bonds.[8] Applying this to the cyclic octapeptides and to the depsipeptide, the two most widely split CH_2-quartets in c-Sar_8 should belong to CH_2 groups adjacent to *trans* bonded nitrogen. In the depsipeptide one of these is shifted downfield and belongs to the CH_2 group adjacent to the ester oxygen which is part of a *trans* ester bond, while the other which corresponds to the most widely split quartet is present. In c-(L-Ala-Sar_3)$_2$ the most widely split quartet seems to be present in the minor conformation but is lacking in the major one, concluding that the NH group and the ester oxygen prefer different *trans* positions. Which one belongs to which cannot be decided.

Only c-Sar_8 and c-(Sar_3-OGl)$_2$ do not undergo any conformational changes while the cyclic octapeptides which contain NH amino acids are, on warming from −50 °C, transformed to more stable conformations in solution. This phenomenon was very marked in a series of cyclic pentapeptides [18] where several conformational changes took place, and the ability of the NH amides to form hydrogen bonds was proposed as the driving force.[24] Also here it is proposed that external hydrogen bonds stabilise the crystal conformers while internal hydrogen bonds stabilise the conformers in solution. The behaviour of the cyclic tetrapeptides which do not show the same phenomenon support this proposal as these rings are too small to form intramolecular hydrogen bonds.

c-Gly_8 which with its eight *trans*-preferred NH amide groups forms the other extreme of this series of cyclic octapeptides cannot adopt the c-Sar_8 skeleton. This molecule shows a simple NMR spectrum in TFA solution with one CH_2-doublet and one NH-triplet. No

coalescence phenomenon was observed on cooling the solution to −50 °C. (The solubility was to low when the peptide was added to cooled solvent). This shows that c-Gly_8 possesses a lower barrier to inversion than the corresponding sarcosine containing molecules.

EXPERIMENTAL

The general methods used for preparation of benzyloxycarbonyl-peptide methyl (*t*-butyl) esters, acids, 2,4,5-trichlorophenyl esters and of cyclic peptides are given in Ref. 8 (Z-Sar_2-OMe, Z-Sar_2-OH, Z-Sar_3-OTcp and c-Sar_3). Solvents used in the reactions were of analytical grade, the light petroleum had b.p. 40 – 60 °C. The monobedded ion-exchange resin, Amberlite, MB-1, analytical grade was used, and the eluent was methanol (water in the case of c-Gly_4). The fully protected linear peptides were prepared in methylene chloride, otherwise stated. After removal of the Z-group the residual peptide ester (confirmed by NMR) was used immediately on isolation.

Many of the peptide derivatives were either viscous oils or non-crystalline solids. Their identity and purity were confirmed by NMR spectroscopy (Varian 60A) and thin-layer chromatography performed on silica gel G in various solvent systems: 2 % methanol-chloroform (A), 5 % methanol-chloroform (B), 10 % methanol-chloroform (C), 15 % methanol-chloroform (D), 20 % methanol-chloroform (E), methanol-chloroform-water 70:20:10 (F), ethanol-water-acetic acid 85:10:5 (G).

Samples for elemental analysis of the Z-peptide esters were purified on a silica gel column, eluting with chloroform followed by chloroform added increasing amount of methanol 2 – 5 %. Abbreviations: Et_3N = triethylamine, DCC = dicyclohexylcarbodiimide, HOTcp = 2,4,5-trichlorophenol, DMF = *N*,*N*-dimethylformamide, TFA = trifluoroacetic acid, AcOH = glacial acetic acid.

For the NMR studies (Varian HA 100) where the peptides were dissolved at low temperature, the peptide was placed on a layer of glass wool in a glass tube inside the NMR-tube and held above the solvent by an O-ring. The whole arrangement was cooled to the desired temperature and the peptide pushed into the solvent by a glass rod which during the cooling was held inside the glass tube by another O-ring.

*Cyclo-*N*-methyl-*L*-alanyltrisarcosyl.* Z-L-MeAla-OH[30] (1.8 g = 7.6 mmol) and Sar_3-OMe[8] (1.8 g = 7.3 mmol) [obtained by hydrogenation of Z-Sar_3-OMe[8] (3.5 g = 9.2 mmol)] were dissolved in CH_2Cl_2 (50 ml) cooled to 0 °C and DCC (1.7 g = 8.2 mmol) added in portions. The reaction mixture was allowed to attain room temperature during 15 h and worked up as described.[8] Treatment with ether and light petroleum gave the Z-L-MeAla-Sar_3-OMe as a viscous oil (2.9 g = 86 %), homogeneous by TLC, R_F 0.45 (B). To Z-L-MeAla-Sar_3-OMe (2.5 g = 5.4 mmol) in methanol (20 ml) was added 1 N NaOH (5.6 ml), stirred at room temperature for 1.5 h and worked up as described.[8] The resulting Z-L-MeAla-Sar_3-OH (1.8 g) was purified by extraction from a CH_2Cl_2 solution into an alkaline layer, acidification with 2 N HCl and again extraction into CH_2Cl_2 or $CHCl_3$, followed by drying and evaporation gave a non crystalline viscous oil (1.6 g = 67 %), TLC, R_F 0.65 (G). Anal. $C_{31}H_{36}N_4O_7$: C, H. The acid (1 g = 2.2 mmol), HOTcp (0.6 g = 3 mmol) in CH_2Cl_2 (30 ml) and DCC (0.5 g = 2.4 mmol) added at 0 °C gave after being worked up as described,[8] the Z-L-MeAla-Sar_3-OTcp as a foamy solid (1.2 g = 86 %), TLC, R_F 0.45 (B). Anal. $C_{27}H_{31}N_4O_7Cl_3$: C, H. This (1 g = 1.6 mmol) was dissolved in methanol (50 ml) containing conc. HCl (0.1 ml) and 5 % Pd-C (0.2 g), hydrogenated for 50 min and worked up as described.[8] The resulting semi solid (0.7 g = 1.3 mmol = 83 %) of HCl. L-MeAla-Sar_3-OTcp was dissolved in DMF (50 ml) and added dropwise to stirred pyridine (500 ml) at 115 °C over a period of 1 h and stirred for another 2 h. After evaporation, the residue was taken into methanol (5 ml) and undissolved material filtered off (58 mg). This appeared to be pure cyclic tetrapeptide. The filtrate was diluted with methanol (60 ml) and passed through an ion-exchange column, eluting with methanol. The evaporated residue was taken into acetone (5 ml) and filtered. The precipitate contained cyclic tetrapeptide and some DC-urea which was removed by sublimation at reduced pressure. The filtrate was evaporated and the residue which contained more cyclic tetrapeptide, some DC-urea and traces of cyclic octapeptide (TLC) was purified on a small silica gel column, eluting with chloroform followed by chloroform added increasing amount of methanol (2 – 20 %) and sublimed. Yield of c-L-MeAla-Sar_3 (20 %), subl. temp. 180 °C/0.01 mmHg, m.p. 315 °C, TLC, R_F 0.5 (C), *m/e* 298, $[\alpha]_D^{22}$ + 16° (*c* 0.5, AcOH). Anal. $C_{13}H_{22}N_4O_4$: C, H, N.

Cyclo-L-alanyltrisarcosyl. Z-L-Ala-OH[31] (1.8 g = 8.1 mmol), Sar_3-OMe (1.9 g = 7.8 mmol) and DCC (1.8 g = 8.7 mmol) in CH_2Cl_2 (50 ml) gave the Z-L-Ala-Sar_3-OMe as a viscous foam (3.1 g = 88 %), TLC, R_F 0.4 (B), R_F 0.7 (C). Anal. $C_{21}H_{30}N_4O_7$: C, H, N. The ester (3 g = 6.7 mmol) in 1 N NaOH (7 ml) (1.5 h) resulted in the Z-L-Ala-Sar_3-OH (2.5 g = 86 %), softened at 80 °C, TLC, R_F 0.65 (G). Anal. $C_{20}H_{28}N_4O_7$: C, H, N. The acid (1.9 g = 4.3 mmol), HOTcp (1.1 g = 5.6 mmol) and DCC (0.9 g = 4.4 mmol) gave the Z-L-Ala-Sar_3-OTcp as a viscous foam (2.3 g = 88 %), TLC, R_F 0.7 (C), which (1.3 g = 2.1 mmol) was hydrogenated and the HCl.L-Ala-Sar_3-OTcp (1 g = 1.9 mmol = 90 %) cyclised and worked up as described earlier. The residue

after passage through an ion-exchange column was taken into methanol (5 ml) and undissolved DC-urea filtered off. The evaporated residue was taken into acetone (5 ml) and undissolved DC-urea and cyclic tetrapeptide filtered off, these were separated by sublimation. The filtrate was evaporated, treated with ether and sublimed, some more cyclic tetrapeptide and traces of cyclic octapeptide were isolated. Yields of cyclic compounds: c-L-Ala-Sar_3 (25 %), subl. temp. 190 °C/0.01 mmHg, m.p. 315 °C (subl.), TLC, R_F 0.45 (C), *m/e* 284, $[\alpha]_D^{22}+70.6°$ (*c* 0.5, AcOH). Anal. $C_{12}H_{20}N_4O_4$: C,H,N. c-Sar_2 (33 %), c-Ala-Sar (16 %) and c-(Ala-Sar_3)$_2$ (2 %).

*Cyclo-*L*-alanyltrisarcosyl-*L*-alanyltrisarcosyl.* Z-L-Ala-Sar_3-OMe (5.2 g = 11.5 mmol) in methanol (200 ml) was hydrogenated over Pd-C (1 g). The resulting oil (4.3 g = 10.4 mmol) of L-Ala-Sar_3-OMe, Z-L-Ala-Sar_3-OH (4.7 g = 10.8 mmol) and DCC (2.4 g = 11.6 mmol) added at −15 °C gave the Z-(L-Ala-Sar_3)$_2$-OMe (6.6 g = 86 %), softened at 85 °C, TLC, R_F 0.5 (D). This (4.2 g = 5.7 mmol) in NaOH (6 ml) (2 h) gave the Z-(L-Ala-Sar_3)$_2$-OH as a viscous foam (3.2 g = 78 %), TLC, R_F 0.8 (G) and the acid (3 g = 4.1 mmol), HOTcp (1.1 g = 5.5 mmol) and DCC (0.95 g = 4.6 mmol) resulted in the Z-(L-Ala-Sar_3)$_2$-OTcp (3 g = 83 %), softened at 65 °C, TLC, R_F 0.55 (D), which was hydrogenated and the HCl.(L-Ala-Sar_3)$_2$-OTcp (0.9 g = 1.1 mmol = 78 %) cyclised. The residue after passage through an ion-exchange column was taken into methanol (5 ml) and some DC-urea filtered off. The cyclic octapeptide crystallised out from acetone (5 ml) at 0 °C and traces of DC-urea was removed by sublimation (heated to 200 °C/0.01 mmHg). The remaining powder was pure c-(L-Ala-Sar_3)$_2$ (12 %), m.p. 345 °C (subl.) TLC, R_F 0.25 (F), *m/e* 568. Anal. $C_{24}H_{40}N_8O_8$: C, H, N.

*Cyclo-*D*-alanylsarcosyl-*L*-alanylsarcosyl and cyclo-*D*-alanylsarcosyl-*D*-alanylsarcosyl (or cyclo-*L*-alanylsarcosyl-*L*-alanylsarcosyl).*

From HCl.Sar-D-Ala-Sar-L-Ala-OTcp.

Z-Sar-OH [32] (23 g = 103 mmol), L-Ala-O^{t}Bu[33] (15 g = 103 mmol) and DCC (23 g = 111 mmol) resulted in an oil (28 g = 78 %) of Z-Sar-L-Ala-O^{t}Bu, TLC, R_F 0.6 (A). This (11 g = 31.4 mmol) was hydrogenated and the Sar-L-Ala-O^{t}Bu (6.3 g = 29.1 mmol) together with Z-Sar-D-Ala-OH [18] (8.7 g = 29.6 mmol) and DCC (6.6 g = 32 mmol) gave the Z-Sar-D-Ala-Sar-L-Ala-O^{t}Bu as a viscous foam (11 g = 77 %), TLC, R_F 0.4 (B), R_F 0.7 (C). Anal. $C_{24}H_{36}N_4O_7$: C, H, N. The ester (5 g = 10.1 mmol) was dissolved in TFA (30 ml) and kept at 20 °C for 1.5 h, evaporated and the residual oil added dropwise to ether (800 ml). The resulting solid was further purified, in the usual way, by extraction from a chloroform solution into an alkaline aqueous layer, acidification and extraction into chloroform and gave the Z-Sar-D-Ala-Sar-L-Ala-OH (3.8 g, 76 %) softened at 70 °C, TLC, R_F 0.7 (G). Anal. $C_{20}H_{28}N_4O_7$: C, H, N. This (3.5 g = 8 mmol), HOTcp (1.9 g = 9.6 mmol) and DCC (1.8 g = 8.7 mmol) resulted in Z-Sar-D-Ala-Sar-L-Ala-OTcp (4.4 g = 89 %) as a foamy solid, TLC, R_F 0.8 (C), which (3.5 g = 5.7 mmol) was hydrogenated and the HCl.Sar-D-Ala-Sar-L-Ala-OTcp (2.7 g = 5.2 mmol = 93 %) cyclised. After evaporation of the pyridine, the residue was taken into methanol (10 ml) and pure c-D-Ala-Sar-L-Ala-Sar (270 mg) filtered off. The filtrate was diluted with methanol (70 ml), passed through an ion-exchange column and the residue sublimed. Two cyclic tetrapeptides were isolated, at 220 and 290 °C (0.01 mmHg) with identical mass spectra and *m/e* 284 which is the required molecular weight for the cyclic tetrapeptide and its isomers. The IR spectra are different and the NMR spectra showed that the major product is c-D-Ala-Sar-L-Ala-Sar. The minor product is suggested to be c-D-Ala-Sar-D-Ala-Sar originated by racemisation. Yields of cyclic compounds: c-D-Ala-Sar-L-Ala-Sar (27 %), subl. temp. 290 °C/0.01 mmHg, m.p. > 350 °C. Anal. $C_{12}H_{20}N_4O_4$: C, H, N. c-D-Ala-Sar-D-Ala-Sar (1 %), subl. temp. 220 °C/0.01 mmHg, m.p. 340 °C (subl.), TLC, R_F 0.75 (E), $[\alpha]_D^{22}+92$ (*c* 0.3, AcOH).

From HCl.Sar-L-Ala-Sar-L-Ala-OTcp.

Z-Sar-L-Ala-O^{t}Bu (12 g = 34 mmol) in TFA (50 ml) (1 h) gave the Z-Sar-L-Ala-OH (8 g = 80 %), m.p. 145 °C, TLC, R_F 0.75 (F). Anal. $C_{14}H_{18}N_2O_5$: C, H, which (5.9 g = 20 mmol), together with Sar-L-Ala-O^{t}Bu (4.1 g = 19 mmol) and DCC (4.5 g = 21.8 mmol) gave the Z-Sar-L-Ala-Sar-L-Ala-O^{t}Bu, a foamy solid (8.6 g = 92 %), TLC, R_F 0.4 (B), R_F 0.7 (C). Anal. $C_{24}H_{36}N_4O_7$: C, H, N. This (4.5 g = 9.1 mmol) in TFA (40 ml) (1 h) gave the Z-Sar-L-Ala-Sar-L-Ala-OH as a solid (3.1 g = 77 %), which (2.5 g = 5.7 mmol) together with HOTcp (1.3 g = 6.5 mmol) and DCC (1.3 g = 6.3 mmol) resulted in the Z-Sar-L-Ala-Sar-L-Ala-OTcp as a solid (2.9 g = 83 %), which (2.5 g = 4 mmol) was hydrogenated and the HCl.Sar-L-Ala-Sar-L-Ala-OTcp (1.8 g = 3.5 mmol = 86 %) cyclised. Only 8 mg of c-D-Ala-Sar-L-Ala-Sar precipitated from methanol (10 ml), the filtrate was diluted with methanol (50 ml) and passed through an ion-exchange column and the residue after evaporation sublimed. Only traces of c-L-Ala-Sar-L-Ala-Sar (or c-D-Ala-Sar-D-Ala-Sar) and about 10 mg (1 %) of c-D-Ala-Sar-L-Ala-Sar were isolated altogether.

From HCl.L-Ala-Sar-L-Ala-Sar-OTcp.

Z-Sar-L-Ala-OH (7 g = 23 mmol), HCl.Sar-OMe (3.2 g = 23 mmol), Et_3N (2.3 g = 23 mmol) and DCC (5.2 g = 25 mmol) resulted in Z-Sar-L-Ala-Sar-OMe, a viscous oil (7.5 g = 86 %), TLC, R_F 0.35 (A), R_F 0.65 (B). This (7 g = 18.5 mmol) was hydrogenated and the Sar-L-Ala-Sar-OMe (4.2 g = 17.1 mmol) together with Z-L-Ala-OH (4 g = 17.9 mmol) and DCC (3.9 g = 18.9 mmol) gave the Z-L-Ala-Sar-L-Ala-Sar-OMe as a solid (6.8 g = 88 %). The ester (6 g = 13.3 mmol) in NaOH (14 ml) (2 h) gave the Z-L-Ala-Sar-L-Ala-Sar-OH as a solid (5.1 g =

88 %), TLC, R_F 0.7 (G). Anal. $C_{20}H_{28}N_4O_7$: C, H, which (3.2 g = 7.3 mmol), HOTcp (1.7 g = 8.6 mmol) and DCC (1.6 g = 7.8 mmol), resulted in the Z-L-Ala-Sar-L-Ala-Sar-OTcp as a foam (4 g = 88 %), TLC, R_F 0.6 (C) and this (3 g = 4.9 mmol) was hydrogenated and the HCl.L-Ala-Sar-L-Ala-Sar-OTcp (2.1 g = 4 mmol = 84 %) cyclised. Only traces of c-D-Ala-Sar-L-Ala-Sar precipitated from methanol (70 ml), the filtrate was passed through an ion-exchange column and the residue after evaporation sublimed. The major product was c-Ala-Sar (64 %, calc. from two cyclisations per chain) and only traces (< 1 %) of c-D-Ala-Sar-L-Ala-Sar were isolated.

From HCl.DL-Ala-Sar-DL-Ala-Sar-OTcp.

HCl.DL-Ala-OMe[34] (42 g = 302 mmol), Et_3N (31 g = 307 mmol), Z-Sar-OH[32] (69 g = 309 mmol) and DCC (68 g = 330 mmol) gave a viscous oil (84 g = 90 %) of Z-Sar-DL-Ala-OMe, TLC, R_F 0.65 (B). The ester (80 g = 259 mmol), in NaOH (262 ml) (2 h) resulted in Z-Sar-DL-Ala-OH (66 g = 85 %), TLC, R_F 0.75 (F), recrystallised from acetone, m.p. 120 °C. Anal. $C_{14}H_{18}N_2O_5$: C, H, N. The acid (45 g = 153 mmol), HCl.Sar-OMe[35] (21 g = 151 mmol), Et_3N (15.5 g = 153 mmol) and DCC (34 g = 165 mmol) resulted in Z-Sar-DL-Ala-Sar-OMe, a viscous oil (51 g = 88 %), TLC, R_F 0.4 (A), R_F 0.65 (B). Anal. $C_{18}H_{25}N_3O_6$: C, H. This (10 g = 26.3 mmol) was hydrogenated and the Sar-DL-Ala-Sar-OMe (6.1 g = 24.9 mmol), Z-DL-Ala-OH[31] (5.6 g = 25.1 mmol) and DCC (5.6 g = 27.2 mmol) resulted in the Z-DL-Ala-Sar-DL-Ala-Sar-OMe, a non crystalline solid (10 g = 89 %), TLC, R_F 0.45 (B). Anal. $C_{21}H_{30}N_4O_7$: C, H, N, which (9 g = 20 mmol) in NaOH (22 ml) (1.5 h) gave the Z-DL-Ala-Sar-DL-Ala-Sar-OH (7.7 g = 88 %), softened at 75 °C, TLC, R_F 0.7 (G). The acid (4 g = 9.1 mmol), HOTcp (2.1 g = 10.6 mmol) and DCC (2.1 g = 10.2 mmol) gave the Z-DL-Ala-Sar-DL-Ala-Sar-OTcp (5 g = 89 %), TLC, R_F 0.7 (C) which (3.5 g = 5.7 mmol) was hydrogenated and the HCl.DL-Ala-Sar-DL-Ala-Sar-OTcp (2.6 g = 5 mmol = 89 %) cyclised. The precipitate from methanol (10 ml) was sublimed and consisted of c-D-Ala-Sar-L-Ala-Sar (60 mg) and c-D-Ala-Sar-D-Ala-Sar together with c-L-Ala-Sar-L-Ala-Sar (18 mg). The filtrate was concentrated on a rotary evaporator without heating and a second crop of the DD- and LL-isomers (31 mg) together with only traces of c-D-Ala-Sar-L-Ala-Sar precipitated. The solution was kept in a refrigerator overnight and some more of the DD and LL isomers plus traces of DC-urea were isolated. The filtrate was diluted with methanol (100 ml), passed through an ion-exchange column, evaporated and the residue treated with ether and sublimed and gave mostly c-Ala-Sar (74 mg). Yields of cyclic compounds: c-D-Ala-Sar-L-Ala-Sar (5 %), c-D-Ala-Sar-D-Ala-Sar together with c-L-Ala-Sar-L-Ala-Sar (4 %), $[\alpha]_D = 0$ (c 0.8, AcOH), c-Ala-Sar (52 %, calc. two cyclisations per chain).

Cyclo-di-L-alanyldisarcosyl.

From HCl.Sar-L-Ala_2-Sar-OTcp.

Z-L-Ala_2-OH[36] (15 g = 51 mmol) [prepared from Z-L-Ala-OH,[31] HCl.L-Ala-OMe[37] using DCC and the Z-L-Ala_2-OMe hydrolysed in NaOH], HCl.Sar-OMe (7.1 g = 51 mmol), Et_3N (5.2 g = 51 mmol) and DCC (11.5 g = 55 mmol) resulted in Z-L-Ala_2-Sar-OMe, a viscous oil (15.5 g = 80 %), TLC, R_F 0.4 (A), R_F 0.65 (B). This (20 g = 52.8 mmol) was hydrogenated and the L-Ala_2-Sar-OMe (12.2 g = 49.8 mmol) gave together with Z-Sar-OH (12 g = 53.8 mmol) and DCC (11.3 g = 54.8 mmol) the Z-Sar-L-Ala_2-Sar-OMe, a viscous oil (19.5 g = 87 %), TLC, R_F 0.4 (B). Anal. $C_{21}H_{30}N_4O_7$: C, H, N, which (11 g = 24 mmol) in NaOH (25 ml) (2.5 h) gave the Z-Sar-L-Ala_2-Sar-OH, a viscous oil (8.8 g = 83 %), TLC, R_F 0.7 (F). This (7 g = 16 mmol), HOTcp (3.7 g = 18.8 mmol) and DCC (3.7 g = 17.9 mmol) resulted in a solid (9.2 g = 93 %) of Z-Sar-L-Ala_2-Sar-OTcp, TLC R_F 0.7 (C), which (2.3 g = 3.7 mmol) was hydrogenated and the HCl.Sar-L-Ala_2-Sar-OTcp (1.8 g = 3.5 mmol = 94 %) cyclised. The residue after passage through an ion exchange column was taken into acetone (10 ml) and undissolved material filtered off. This was mostly DC-urea, some cyclic octapeptide and traces of the cyclic tetrapeptide (TLC, E) and could be separated by sublimation. The filtrate was evaporated and a new portion of acetone (5 ml) added, the precipitation was now pure cyclic tetrapeptide. The residue after evaporation consisted of cyclic tetrapeptide and some oily impurities. Sublimation sometimes led to decomposition of the cyclic tetrapeptide into cyclic dipeptides. A safer method for isolation was purification on a small silica gel column eluting with chloroform followed by chloroform containing methanol (2 – 5 %). Yields of cyclic compounds; c-L-Ala_2-Sar_2 (2.5 %), subl. temp. 240 °C/0.01 mmHg, m.p. 310 °C (subl.), TLC, R_F 0.2 (C), R_F 0.6 (E), *m/e* 284, $[\alpha]_D^{22} - 69°$ (*c* 0.5, AcOH), c-(L-Ala_2-Sar_2)$_2$ (3 %), subl. temp. 280 °C/0.01 mmHg, m.p. 315 °C, TLC, R_F 0.3 (F), R_F 0.15 (E), *m/e* 568.

From HCl.Sar_2-L-Ala_2-OTcp.

Z-Sar_2-L-Ala_2-O^{t}Bu[18] (6.9 g = 14 mmol) in TFA (40 ml) (1 h) gave the Z-Sar_2-L-Ala_2-OH as a solid (4.7 g = 77 %). Anal. $C_{20}H_{28}N_4O_7$: C, H. This (4.7 g = 10.8 mmol), HOTcp (2.6 g = 13.2 mmol) and DCC (2.5 g = 12 mmol) resulted in a solid (5.2 g = 78 %) of Z-Sar_2-L-Ala_2-OTcp, which (1.3 g = 2.1 mmol) was hydrogenated and the HCl·Sar_2-L-Ala_2-OTcp (1 g = 1.9 mmol = 91 %) cyclised. A white solid was left after passage through an ion-exchange column and evaporation, this was sublimed, and only c-Sar_2 (30 %) and c-Ala_2 (13 %) isolated.

Cyclo-tri-L-alanylsarcosyl. Z-L-Ala-OH (3 g = 13.4 mmol), L-Ala_2-Sar-OMe (3.2 g = 13 mmol) and DCC (3 g = 14.5 mmol) resulted in a solid (4.2 g = 71 %) of Z-L-Ala_3-Sar-OMe, TLC, R_F 0.6 (C) which (3 g = 6.6 mmol) in NaOH (7 ml) (2 h) gave the Z-L-Ala_3-Sar-OH as a

solid (2 g = 69 %), TLC, R_F 0.65 (G). Anal. $C_{20}H_{26}N_4O_7$: C, H, N. The acid (1.6 g = 3.6 mmol), HOTcp (0.9 g = 4.5 mmol) and DCC (0.84 g = 4 mmol) gave the Z-L-Ala_3-Sar-OTcp as a foamy solid (1.8 g = 80 %), which (1.5 g = 2.4 mmol) was hydrogenated and the HCl.L-Ala_3-Sar-OTcp (1 g = 1.9 mmol = 79 %) cyclised. The precipitate from methanol (10 ml) was sublimed and DC-urea and some (20 mg) cyclic tetrapeptide isolated. The filtrate was passed through an ion-exchange column and the evaporated residue which was small gave by sublimation a few mg more of cyclic tetrapeptide. Yield of c-Ala_3-Sar (4.5 %), subl. temp. 250 °C/0.01 mmHg, m.p. > 350 °C, *m/e* 284. The NMR spectrum showed that the isolated cyclic tetrapeptide was either c-L-Ala_2-D-Ala-Sar or c-D-Ala_2-L-Ala-Sar and racemisation of one or two L-alanine residues had occurred.

Cyclo-tetra-L-alanyl.[2] Z-L-Ala_2-OH[36] (12 g = 40.8 mmol), L-Ala_2-O^{t}Bu[18] (8.7 g = 40.2 mmol) and DCC (9.1 g = 44.1 mmol) gave the Z-L-Ala_4-O^{t}Bu (13.7 g = 69 %), which (10 g = 20 mmol) in TFA (40 ml) (1 h) gave the Z-L-Ala_4-OH[36] (7.6 g = 85 %). The acid (3.9 g = 8.9 mmol), HOTcp (2.1 g = 10.6 mmol) and DCC (2 g = 9.7 mmol) in DMF (20 ml) resulted in the Z-L-Ala_4-OTcp as a white powder (4.6 g = 85 %), which (1.5 g = 2.4 mmol) was hydrogenated and the HCl.L-Ala_4-OTcp (1.1 g = 2.1 mmol = 87 %) cyclised. The precipitate from methanol (25 ml) was sublimed, and appeared to be pure cyclic tetrapeptide (25 mg). The filtrate was passed through an ion-exchange column, the evaporated residue sublimed and some cyclic tetramer was isolated. Yield of c-Ala_4 (5 %), subl. temp. 200 – 240 °C/0.01 mmHg, m.p. 315 °C (subl.), *m/e* 284.

Cyclo-glycyltrisarcosyl. Z-Gly-OH[31] (7 g = 33.4 mmol), Sar_3-OMe[8] (8 g = 32.6 mmol) and DCC (7.5 g = 36 mmol) resulted in a viscous foam (10.5 g = 74 %) of Z-Gly-Sar_3-OMe, TLC, R_F 0.4 (B), R_F 0.6 (C). Anal. $C_{20}H_{26}N_4O_7$: C, H, N, which (9 g = 20.6 mmol) in NaOH (22 ml) (2 h) gave the Z-Gly-Sar_3-OH (7.8 g = 89 %), TLC, R_F 0.6 (G), m.p. 165 °C. The acid (4 g = 9.4 mmol), HOTcp (2.2 g = 11.1 mmol) and DCC (2.2 g = 10.6 mmol) resulted in the Z-Gly-Sar_3-OTcp as a foamy solid (4.6 g = 81 %), TLC, R_F 0.6 (C), which (2.4 g = 4 mmol) was hydrogenated and the HCl.Gly-Sar_3-OTcp (1.8 g = 35 mmol = 90 %) cyclised. The precipitate from methanol (15 ml) was pure cyclic tetrapeptide (175 mg). The filtrate was passed through an ion-exchange column and the evaporated residue taken into methanol (10 ml). The precipitate consisted of cyclic tetrapeptide, DC-urea and some cyclic octapeptide (TLC, E,F) whereof the tetrapeptide and the DC-urea were isolated by sublimation (to 210 °C/0.01 mmHg) and the octapeptide remained as a white powder. The filtrate was after evaporation taken into acetone (10 ml) and DC-urea and cyclic octapeptide filtered off. The evaporated residue was treated with ether and sublimed. Small amounts of cyclic tetrapeptide and the two possible cyclic dipeptides were isolated. Yields of cyclic compounds: c-Gly-Sar_3 (23 %), subl. temp. 230 °C/0.01 mmHg, m.p. 322 °C, *m/e* 270, TLC, R_F 0.6 (E). Anal. $C_{11}H_{18}N_4O_4$: C, H, N. c-(Gly-Sar_3)$_2$ (3 %), subl. temp. 300 °C/0.01 mmHg (partly decomp.), m.p. 316 °C, *m/e* 540, TLC, R_F 0.3 (F), c-Gly-Sar (6 %), c-Sar_2 (4 %).

Cyclo-glycylsarcosylglycylsarcosyl. Z-Sar-OH (22.3 g = 100 mmol), Gly-O^{t}Bu (13.1 g = 100 mmol) and DCC (21 g = 102 mmol) resulted in Z-Sar-Gly-O^{t}Bu (28.5 g = 84 %), softened at 65 °C, TLC, R_F 0.5 (A). Anal. $C_{17}H_{24}N_2O_5$: C, H. This (20 g = 59.5 mmol) in TFA (80 ml) (1 h) gave the Z-Sar-Gly-OH (14.4 g = 86 %), m.p. 85 °C, TLC, R_F 0.7 (F), and the acid (13.5 g = 48.2 mmol), HOTcp (12 g = 61 mmol) and DCC (11 g = 53.3 mmol) gave a foamy solid (19.6 g = 89 %) of Z-Sar-Gly-OTcp which (10 g = 22 mmol) together with Sar-Gly-O^{t}Bu (4.5 g = 22 mmol) [obtained by hydrogenation of Z-Sar-Gly-O^{t}Bu (8 g = 23.8 mmol)] were dissolved in CH_2Cl_2 (100 ml) and kept at 25 °C (4 days). The reaction mixture was diluted with $CHCl_3$, washed and worked up in the usual way to give the Z-Sar-Gly-Sar-Gly-O^{t}Bu (8.6 g = 86 %), softened at 75 °C, TLC, R_F 0.55 (C). Anal. $C_{22}H_{32}N_4O_7$: C, H, N.

This (3 g = 6.4 mmol) in TFA (40 ml) (1 h) gave the Z-Sar-Gly-Sar-Gly-OH (2.2 g = 84 %), softened at 65 °C, TLC, R_F 0.7 (G), and the acid (2 g = 4.9 mmol) and HOTcp (1.3 g = 6.6 mmol) were dissolved in DMF (10 ml), diluted with CH_2Cl_2 (50 ml) and DCC (1.2 g = 5.8 mmol) added at 0 °C. The mixture was allowed to attain room temperature during 15 h and the DC-urea filtered off together with some Z-Sar-Gly-Sar-Gly-OTcp which had precipitated out. The filtrate was worked up as usual and resulted in a solid (2 g = 70 %) of Z-Sar-Gly-Sar-Gly-OTcp, TLC, R_F 0.65 (C), which (1.8 g = 3 mmol) was hydrogenated and the HCl.Sar-Gly-Sar-Gly-OTcp (1.3 g = 2.6 mmol = 86 %) cyclised. The precipitate from methanol (20 ml) was cyclic tetrapeptide which crystallised from water as needles (280 mg). The methanol solution was passed through an ion-exchange column, but hardly any residue was left after evaporation. Yield of c-Gly-Sar-Gly-Sar (42 %), subl. temp. 260 °C/0.01 mmHg, m.p. > 350 °C, *m/e* 256, TLC, R_F 0.5 (E). Anal. $C_{10}H_{16}N_4O_4$: C, H, N.

Cyclo-diglycyldisarcosyl

From HCl·Sar-Gly_2-Sar-OTcp.

Z-Gly_2-OH[38] (30 g = 112 mmol) [prepared from H-Gly_2-OH, Z-Cl and NaOH in the usual way, recrystallised from water], HOTcp (28 g = 142 mmol) and DCC (26 g = 126 mmol) were dissolved in DMF (150 ml) and stirred at 25 °C for 24 h. The precipitated DC-urea was filtered off and the evaporated residue washed with ether to give a white solid (47 g = 94 %) of Z-Gly_2-OTcp. This (45 g = 101 mmol) together

with HCl.Sar-OMe (14 g=100 mmol) and Et_3N (10.5 g=103 mmol) were suspended in DMF (20 ml) and CH_2Cl_2 (100 ml), kept at 25 °C for 3 days and the now clear solution evaporated. The residue which was dissolved in $CHCl_3$ (400 ml) and washed as usual gave the Z-Gly_2-Sar-OMe (31 g=88 %), TLC, R_F 0.45 (B). An analytical sample melted at 92 °C. Anal. $C_{16}H_{21}N_3O_6$: C, H, N. The ester (27 g=76.9 mmol) was hydrogenated and the Gly_2-Sar-OMe (15.2 g=70 mmol) together with Z-Sar-OH (16 g=71.7 mmol) dissolved in CH_2Cl_2 (500 ml) and DCC (16 g=77 mmol) added at −10 °C. When the reaction mixture attained room temperature (15 h), the precipitate was filtered off. This consisted of DC-urea and some tetrapeptide ester which could be isolated by suspending the precipitate in $CHCl_3$ and filtration of insoluble DC-urea. The CH_2Cl_2-solution was diluted with $CHCl_3$ (500 ml) and worked up in the usual way to give the Z-Sar-Gly_2-Sar-OMe (25.8 g=87 %). A sample (0.3 g) was further purified. TLC, R_F 0.5 (C), m.p. 162 °C. Anal. $C_{19}H_{26}N_4O_7$: C, H, N.

The ester (10 g=23.7 mmol) in NaOH (25 ml) (1 h) gave the Z-Sar-Gly_2-Sar-OH (7.3 g=75 %), TLC, R_F 0.7 (F). A sample was recrystallised from methanol with added traces of water, m.p. 147 °C. Anal. $C_{18}H_{24}N_4O_7$: C, H, N, and the acid (4.5 g=11 mmol), HOTcp (2.6 g=13.2 mmol) and DCC (2.5 g=12.1 mmol) resulted in the Z-Sar-Gly_2-Sar-OTcp (5 g= 77 %), TLC, R_F 0.55 (C), m.p. 165 °C, which (2 g=3.4 mmol) was hydrogenated and the HCl.Sar-Gly_2-Sar-OTcp (1.3 g=2.6 mmol= 77 %) cyclised. After evaporation of the pyridine, the residue was dissolved in methanol (10 ml) and passed through a small silica gel column eluting with methanol. A yellow oil was first eluted, then a white powder which appeared to be the cyclic octapeptide. The oil was dissolved in methanol (80 ml), passed through an ion-exchange column and the evaporated residue taken into methanol (5 ml). The undissolved material (15 mg) appeared to be cyclic tetrapeptide and some more could be isolated when the filtrate was evaporated, treated with ether and sublimed. If the residue is oily, sublimation of it tends to decompose the cyclic tetrapeptide into cyclic dipeptides, and it is recommended to purify on a small silica gel column, eluting with chloroform, followed by chloroform containing methanol (2−5 %). Yields of cyclic compounds: c-Gly_2-Sar_2 (3 %), subl.temp. 260 °C/0.01 mmHg, m.p. 310 °C, TLC, R_F 0.4 (E), *m/e* 256. c-(Gly_2-Sar_2)$_2$ (13 %), m.p. >350 °C, TLC, R_F 0.3 (F). Anal. $C_{20}H_{32}N_8O_8$: C, H, N.

From HCl.Sar_2-Gly_2-OTcp.

Z-Sar_2-OTcp (9 g=19 mmol) [prepared from Z-Sar_2-OH, HOTcp and DCC] and H-Gly_2-O^{t}Bu (3.6 g=19.1 mmol) [obtained by hydrogenation of Z-Gly_2-O^{t}Bu [39] (6.5 g=20.1 mmol), prepared from Z-Gly-OH, H-Gly-O^{t}Bu using DCC] were dissolved in CH_2Cl_2 (150 ml), kept for 3 days at 25 °C and worked up in the usual way to give the Z-Sar_2-Gly_2-O^{t}Bu (7.8 g=88 %), TLC, R_F 0.5 (C). An analytical sample melted at 141 °C. Anal. $C_{22}H_{32}N_4O_7$: C, H, N.

The ester (4 g=8.6 mmol) in TFA (25 ml) (1 h) gave the Z-Sar_2-Gly_2-OH which was suspended in acetone and filtered (3 g=85 %), m.p. 135 °C, TLC, R_F 0.7 (G). The acid (2.7 g=6.6 mmol), HOTcp (1.6 g=8.1 mmol) and DCC (1.5 g=7.3 mmol) gave the Z-Sar_2-Gly_2-OTcp as a foamy solid (3.2 g=84 %), TLC, R_F 0.75 (C), which (1.5 g=2.5 mmol) was hydrogenated and the HCl.Sar_2-Gly_2-OTcp (1 g=2 mmol=83 %) cyclised. After passage through an ion-exchange column, the evaporated residue was taken into methanol (10 ml) and undissolved material which consisted of cyclic octapeptide (15 mg) and traces of cyclic tetrapeptide (TLC) filtered off. The evaporated filtrate was treated with ether and sublimed and gave mainly cyclic dipeptides. Yields of cyclic compounds: c-Gly_2-Sar_2 (traces), c-(Gly_2-Sar_2)$_2$ (3 %), c-Sar_2 (25 %), c-Gly_2 (10 %).

From HCl.Gly-Sar_2-Gly-OTcp.

Z-Sar-OH (3.5 g=15.7 mmol), Sar-Gly-O^{t}Bu (3.2 g=15.8 mmol) and DCC (3.7 g=17.9 mmol) gave the Z-Sar_2-Gly-O^{t}Bu as a viscous oil (5.5 g=13.5 mmol=86 %), TLC, R_F 0.4 (A), R_F 0.6 (B). This was hydrogenated and the Sar_2-Gly-O^{t}Bu (3.4 g=12.4 mmol) together with Z-Gly-OH (2.7 g=12.9 mmol) and DCC (2.8 g=13.6 mmol) resulted in the Z-Gly-Sar_2-Gly-O^{t}Bu as a foamy solid (4.8 g=84 %), which (4 g=8.6 mmol) in TFA (50 ml) (1.2 h) gave the Z-Gly-Sar_2-Gly-OH (2.9 g=83 %). This (2.5 g=6.1 mmol), HOTcp (1.5 g=7.6 mmol) and DCC (1.4 g=6.8 mmol) in DMF (20 ml) and CH_2Cl_2 (100 ml) gave the Z-Gly-Sar_2-Gly-OTcp as a solid (3.1 g=87 %), which (1.3 g=2.2 mmol) was hydrogenated and the HCl·Gly-Sar_2-Gly-OTcp (1 g=2 mmol=92 %) cyclised. The residue after passage through an ion-exchange column consisted of pure c-Gly-Sar (TLC) and sublimation gave 57 % (calc. two cyclisations per chain). No cyclic tetrapeptide nor cyclic octapeptide were observed.

Cyclo-triglycylsarcosyl. Z-Sar-Gly-OTcp (8.4 g=18.3 mmol) [prepared from Z-Sar-Gly-OH, HOTcp and DCC] and Gly_2-O^{t}Bu (3.5 g=18.6 mmol) in CH_2Cl_2 (100 ml) at 25 °C (3 days) resulted in Z-Sar-Gly_3-O^{t}Bu (7.3 g=87 %), TLC, R_F 0.7 (E). An analytical sample melted at 155 °C. Anal. $C_{21}H_{30}N_4O_7$: C, H, N. This (4.5 g=10 mmol) in TFA (25 ml) (1 h) gave the Z-Sar-Gly_3-OH which was suspended in acetone and filtered (3.2 g=82 %), m.p. 197 °C, TLC, R_F 0.75 (G) and the acid (2.5 g=6.3 mmol), HOTcp (1.6 g=8.1 mmol) and DCC (1.5 g=7.2 mmol) in DMF (25 ml) gave the Z-Sar-Gly_3-OTcp as a white powder which was suspended in acetone and filtered (2.4 g=66 %) and (1.8 g=3.1 mmol) hydrogenated and the HCl.Sar-Gly_3-OTcp (1.3 g=2.7 mmol=87 %) cyclised. After evaporation of the pyridine,

the residue was dissolved in methanol-water 1:1 (80 ml) and the precipitated DC-urea filtered off. The filtrate was passed through an ion-exchange column, eluting with methanol-water 1:1 and the evaporated residue dissolved in a small amount of water from which c-Gly_3-Sar crystallised as needles (3 %), subl.temp. 270 °C/0.01 mmHg, m.p. > 350 °C, *m/e* 242, TLC, R_F 0.3 (E), R_F 0.7 (F).

Cyclo-tetraglycyl.[1] Z-Gly_4-O^{t}Bu was prepared from Z-Gly_3-OTcp and H-Gly_2-O^{t}Bu in pyridine and hydrolysed in TFA to Z-Gly_4-OH which together with ethyl chloroformate and thioglycollic acid followed by treatment with HBr in acetic acid gave the HBr.Gly_4-SCH_2COOH.[1] This (0.5 g = 1.2 mmol) in DMF (100 ml) was added to stirred pyridine (600 ml) at 115 °C over a period of 4 h and stirred for another 4 h. Some precipitated polymer was filtered off and the evaporated residue dissolved in water (50 ml), passed through an ion-exchange column, eluting with water and by dissolving the evaporated residue in water (5 ml) a "white powder" which appeared to be c-Gly_4 (12 mg, 4.4 %) crystallised out, subl.temp. 270 °C/0.01 mmHg, m.p. 340 °C (subl.), *m/e* 228. The filtrate was evaporated to *ca.* 1 ml and ethanol (*ca.* 2 ml) added, small needles of c-Gly_8 (8 mg, 3 %) crystallised out, m.p. > 350 °C, *m/e* 456.

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Studies on Anodic Substitution Reactions. XI.* The Anodic Acetoxylation of Triptycene and Fluorene and its Implications for the Side-chain Substitution Mechanism

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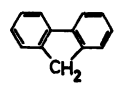
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The anodic acetoxylation of triptycene (*1*) and fluorene (*2*) exhibits a high nuclear to α substitution ratio (250 and 35, respectively) relative to structurally similar substrates. This behaviour is in accordance with the ECEC mechanism generally postulated for anodic α substitution.

Triptycene (*1*, 9,10-*o*-benzeno-9,10-dihydroanthracene, abbreviated Tr-H where appropriate) and fluorene (*2*, abbreviated Fl-H where appropriate) possess structural features which make them critically interesting substrates for anodic substitution. Due to the rigid structure of the triptycene system, its



1



2

bridge-head radical (9-Tr·) and carbocation (9-Tr+) are highly strained species,[1] and since radicals and carbocations are postulated as intermediates in anodic side-chain (α) substitution reactions [2] (eqns. 1 – 3) one would qualitatively predict that Tr-H would either not at all undergo α substitution or at least give a high ratio of nuclear to α substitution product compared to other, structurally related compounds.

$$ArCHR_2 \xrightarrow{-e} (ArCHR_2)^{+\cdot} \quad (1)$$

$$(ArCHR_2)^{+\cdot} + B: \longrightarrow Ar\dot{C}R_2 + BH^+ \quad (2)$$

$$Ar\dot{C}R_2 \xrightarrow{-e} Ar\overset{+}{C}R_2 \xrightarrow{Nu^-} ArC(Nu)R_2 \quad (3)$$

A second critical feature of Tr-H is its inability to orient any of its three aromatic faces parallel to the electrode surface close enough to form a π-donor-acceptor type adsorption complex. The existence of such complexes has been postulated on the basis of adsorption studies on aromatic hydrocarbons.[3] Stereochemical studies [4] of anodic α substitution have, however, largely failed to detect any unambiguous consequences of the substrate-electrode interaction in the later stages of the reaction sequence (eqn. 3).

Also fluorene (*2*) would be expected to undergo α substitution to a low or negligible extent, since one of the corresponding intermediates of the ECE mechanism, 9-Fl+, formally is an antiaromatic species, making its formation energetically less favored. Thus it is predicted that fluorene, like *1*, should exhibit a relatively high nuclear to α acetoxylation ratio.

This paper reports a study of the anodic acetoxylation of Tr-H and Fl-H, showing that α acetoxylation is indeed less favored than for relevant reference compounds (triphenylmethane for *1* and diphenylmethane for *2*).

* Part X, see Blum, Z., Cedheim, L. and Nyberg, K. *Acta Chem. Scand. B 29* (1975) 715.

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Table 1. Oxidation half-wave potentials of *1*–*4* and related compounds.

Compound	$E_{1/2}$/V [a]	Ref.
Triptycene (*1*)	1.48 [b]	This work
Triphenylmethane (*3*)	1.62 [b]	This work
9,10-Dihydroanthracene	1.53 [c]	6
Anthracene	0.84 [c]	6
9,10-Diphenylanthracene	0.86 [d]	6
Fluorene (*2*)	1.32 [b]; 1.25 [c]	This work; 6
Diphenylmethane (*4*)	1.84 [b]	This work

[a] *Vs.* the Ag/Ag^+ (0.1 M) reference electrode. [b] In acetonitrile/tetrabutylammonium tetrafluoroborate (0.1 M) with 3 % trifluoroacetic anhydride added. [c] In acetonitrile/sodium perchlorate. [d] In acetonitrile/tetraethylammonium perchlorate.

RESULTS

Voltammetry. Tr-H, Fl-H, triphenylmethane (*3*) and diphenylmethane (*4*) displayed irreversible two-electron behaviour when studied by cyclic voltammetry in acetonitrile/0.1 M tetrabutylammonium tetrafluoroborate with 3 % trifluoroacetic anhydride added to keep nucleophilic impurities under control.[5] Half-wave potentials for *1*–*4* are given in Table 1, together with those of some structurally related hydrocarbons.

Preparative studies. Anodic oxidation of Tr-H at a carbon anode in the system acetonitrile-acetic acid (2.7:1)/tetrabutylammonium acetate (0.15 M) primarily gave monoacetoxylation products which were further oxidized to di-, tri-, and higher acetoxylation products during the run. Only the monoacetoxylation products were rigorously identified in view of the rather complex mixture of diacetates obtained (in principle, fifteen diacetate isomers are possible) and the low priority of this particular problem. After passage of 3.5 F/mol of Tr-H at constant current (constant potential conditions gave the same result) a 47 % current yield of a mixture of monoacetates (54 %), diacetates (35 %) and higher acetates (11 %) was obtained. Analysis of the monoacetate fraction showed it to consist of predominantly 1- and 2-TrOAc, 31 and 68.5 %, respectively, but also with a small and well reproducible percentage, 0.5 %, of the α substitution product, 9-TrOAc, present. A similar product distribution (1-/2-/9-isomer: 38/62/0.5) was obtained with platinum as the anode material in a constant potential run.

A few runs were performed using tetrabutylammonium tetrafluoroborate as the supporting electrolyte in order to see if the known tendency of α acetoxylation being favored in the absence of acetate ion [7] would hold for Tr-H too. None of the products discussed above were, however, detectable in these experiments, conducted with both carbon and platinum as the anode material.

When *3* was oxidized at a carbon anode at 1.6 V *vs.* SCE in acetonitrile-acetic acid (1:1)/potassium acetate (0.6 M) a 50 % current yield of a mixture of monoacetates (98 %) and higher acetates (2 %) was obtained after passage of 0.9 F/mol of *3*. The α/nuclear substitution ratio in the monoacetate fraction was 66:34 and the *o:m:p* proportion 44:24:32.

Fluorene (*2*) was similarly oxidized at platinum (c.p.e. at 1.6 V *vs.* SCE) in acetic acid/sodium acetate (1.0 M). After passage of 0.2 F/mol of *2* a 15 % current yield of monoacetates was detectable. The isomer ratio, 2-FlOAc/1-, 3-, 4-FlOAc/9-FlOAc, was 93:3.5:3.5. With tetrabutylammonium tetrafluoroborate as a supporting electrolyte, a 0.5 % current yield of 9-FlOAc was detectable; no nuclear substitution products were formed.

DISCUSSION

To put these results into a wider perspective, a number of relevant isomer distribution ratios for anodic acetoxylation reaction have been collected in Table 2.

The results are reported as the nuclear to α substitution ratios, both the experimental values and those corrected for the differing ratios between the number of nuclear and α hydrogen atoms in different compounds. It should be noted that the experimental value for *1* probably is too small, since it has been obtained in a high-conversion (3.5 F/mol of *1*) run in which a large amount of di- and higher acetates were formed. Since the presence of one nuclear acetoxy group facilitates further nuclear acetoxylation in the same ring, it is reasonable to assume that selective removal of the initially formed monoacetates with respect to 9-TrOAc occurs (9-TrOAc would, if anything, be oxidized

Table 2. Isomer distributions in the anodic acetoxylation of certain aromatic compounds.

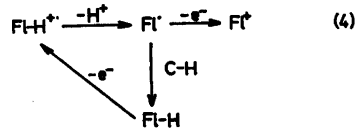
Compound	Ratio of nuclear to α substitution		Ref.
	Exp.	Per α-H [a]	
Triptycene (*1*)	200	250	This work
Triphenylmethane (*3*)	0.52	0.17	This work
Fluorene (*2*)	28	35	This work
Diphenylmethane (*4*)	4.3	4.3	7
Toluene	2.4	7.2	7
Ethylbenzene	1.0	2.0	7
Isopropylbenzene	1.1	1.1	7
Indan	0.27	1.4	8
2-Methylindan	0.19	1.0	9
2,2-Dimethylindan	0.020	0.1	9

[a] Corrected for differing ratios of the number of nuclear and α hydrogen atoms; scale arbitrary.

at a higher potential than *1*). The *maximal* effect of this phenomenon would bring up the experimental nuclear to α substitution ratio for *1* to 200/0.54≈370 (assuming that all higher acetates are nuclear and have the acetoxy groups in one ring only). Unfortunately, low-conversion runs could not be performed with *1*, since the analytical method, based on NMR analysis of the monoacetates, was too insensitive under such conditions.

The *first* crucial step in determining the nuclear to α substitution ratio is the attack of the base/nucleophile upon the radical cation, either at an α hydrogen atom (eqn. 2) or at a nuclear carbon atom. Any factor that destabilizes the transition state for proton abstraction relative to that of nuclear attack will increase the nuclear/α substitution ratio. Triptycene, the corresponding bridgehead radical of which is destabilized due to deviation from planarity of the radical center [1] and due to lack of overlap between the aromatic rings and the odd-electron orbital (also manifested [11] in the lack of hyperfine splitting by the bridgehead hydrogens in the ESR spectrum of Tr-H$^{+\cdot}$), does indeed exhibit an anomalously high nuclear/α substitution ratio relative to that of a structurally similar compound, triphenylmethane.

The *second* crucial step in determining the nuclear to α substitution ratio is the further oxidation of the radical to the carbocation (eqn. 3). Any factor which destabilizes the carbocation should lead to an increase of the nuclear/α substitution ratio, provided one can assume that competing processes, such as coupling or hydrogen atom abstraction from other electrolyte components, are unaffected by the very same factor. Fluorene, which can be converted to an antiaromatic species, Fl$^+$, in the course of the reaction sequence of eqns. 1 – 3, gives a relatively high nuclear/α substitution ratio relative to that shown by diphenylmethane. For fluorene, it is also pertinent to note that the current yield of product(s) is low or very low compared to other substrates of similar structure but with higher oxidation potential (*e.g.*, triptycene, triphenylmethane, several alkylbenzenes [12]). If concurrent Kolbe oxidation of acetate ion is the only process competing with Fl-H oxidation, we feel that the yield of substitution product(s) of Fl-H should be considerably higher. One possibility to explain this anomaly is that fluorene is effectively regenerated from Fl$^\cdot$ (eqn. 4).



(4)

We have previously suggested [2b] that the nuclear to α substitution ratio in anodic substitution reactions appears to be governed by the ratio between the positive charge density at a free nuclear position and that of a carbon atom adjacent to the carbon atom carrying the removable hydrogen atom. In view of the results shown in Table 2 this hypothesis is clearly an oversimplification which only covers the gross features of the reaction. Substrates like Tr-H and Fl-H which possess unusual structural elements cannot be treated in this simple manner.

An interesting analogy can be drawn between the radical cation/anion reaction and the corresponding neutral molecule/neutral radical reaction (eqns. 5 and 6) leading to proton abstraction. Here one can note that (a) the

(5)

$$\mathrm{Ar-\overset{|}{\underset{|}{C}}-H+B\cdot \longrightarrow [Ar-C\cdots H\cdots B]^{\ddagger} \longrightarrow Ar-\overset{|}{\underset{|}{C}}\cdot + BH} \qquad (6)$$

initial states are different, (b) the final states are identical, and (c) the transition states in all likelihood must be identical assuming identical reaction conditions in the two cases. It then follows that the energy difference between the two initial states should give an idea of the difference in selectivity between the two types of reactions. Data for the attack by free radicals at the α position of alkylbenzenes are scarce [13] and not entirely compatible with those available for anodic substitution processes, but it is at least known for attack by phenyl radicals that the experimental nuclear to α substitution ratio is 6.7, 0.82, and 0.67 for toluene, ethylbenzene, and isopropylbenzene, respectively.[13a] This should be compared to the corresponding values for the radical cation/acetate reaction, 2.4, 1.0, and 1.1. While these systems of course are not strictly comparable due to the different composition of the reagents (Ph and CH_3COO) we think it would be worthwhile to explore this analogy with a more suitable reagent. One possible candidate would be the benzoate system where both reactions of eqns. 5 and 6 should be feasible.[2a,14]

Finally, the half-wave potentials of Table 1 are worth commenting on. We [4] and others [3] have earlier assumed that, for effective electron transfer to occur, the substrate must be adsorbed to the anode surface with its aromatic face parallel to the surface. Although stereochemical consequences of this hypothesis are so far largely lacking,[4] we have ascribed this to the less than ideal nature of the substrates and reactions studied until now and never questioned this basic assumption. It is then disturbing to find that triptycene, which cannot possibly align any of its aromatic rings parallel to the anode surface and still be close enough to it, is easier to oxidize than, *e.g.*, triphenylmethane and 9,10-dihydroanthracene, both substrates with no problem in this respect. This might indicate that the requirement for overlap between electrode and substrate orbitals is not as stringent as previously assumed.

EXPERIMENTAL

Materials. 9-Acetoxytriptycene was prepared according to known methods.[15–17] The 2-acetoxy isomer was prepared from 2-aminotriptycene [18] analogously to that used for the 9-acetoxy compound.[17] The nuclear acetoxy derivatives of *2* were synthesized according to known methods,[19] as were also 2- and 9-acetoxyfluorene.[20]

Anodic oxidation of triptycene (*1*). A solution of triptycene (1.0 g) and Bu_4NOAc, HOAc (2.0 g) in acetonitrile (27 ml)–HOAc (10 ml) was oxidized at a carbon anode (9 cm^2) at a constant current density of 28 mA cm^{-2} until 3.54 F mol^{-1} of substrate has passed through the solution. The electrolyte solution was poured into water (200 ml) and the mixture extracted with ether (3 × 100 ml). The combined ether extracts were washed with water (3 × 100 ml), saturated sodium hydrogen carbonate solution (100 ml) and water (100 ml). After addition of acetone (50 ml) the solvent was evaporated until a solution, suitable for GLC analysis, was obtained. GLC analysis (2 m × 0.3 mm 3 % OV-101 on Chromosorb W column, 130–300 °C/10 °C min^{-1}) with 2,2′,4,4′,6,6′-hexamethylbiphenyl as an internal standard showed that the amount of recovered triptycene was 37 %, the combined yield of 9-TrOAc + 1-TrOAc + 2-TrOAc 34 %, and the residual components 29 %. The amount of diacetates was estimated by GLC/MS to be 22 %.

The isomer distribution of the monoacetate fraction was determined in the following way: The above-mentioned extract was evaporated to dryness and the solid material subjected to column chromatography on acidic alumina. Elution with carbon tetrachloride separated unreacted TrH, whereafter the monoacetate fraction was eluted by dichloromethane. The procedure was repeated once with the monoacetate fraction (as checked by GLC/MS) in order to obtain material pure enough for further analysis. The material then obtained consisted of the monoacetates to more than 95 % and was subjected to GLC and NMR spectroscopic analysis. ^1H NMR spectrum of mixture in $CDCl_3$: δ 2.09 (3 H, s, CH_3CO), 4.28 (2 H, d, bridge-head H) and 6.60–7.46 (11 H, *m*, aromatic H).

By addition of $Eu(fod)_3$ the acetoxy signal was split into a doublet (integrated areas 69:31) and the δ 4.28 signal into a quartet. By addition of authentic 2-TrOAc the 69 % component is shown to correspond to this compound. Since 9-TrOAc can be analyzed by GLC (0.5 % of the monoacetate mixture) it is evident that the 31 % component must be 1-TrOAc.

Anodic oxidation of triphenylmethane (*2*). A solution of triphenylmethane (3.0 g) and potassium acetate (2.0 g) in acetonitrile (20 ml)–acetic acid (20 ml) was oxidized anodically at a carbon anode (anode potential 1.6 V *vs.* SCE) until 0.9 F mol^{-1} had been passed through the solution. After work-up as described above the

product mixture was analyzed by GLC/MS, comparing with authentic specimens of all possible monoacetates. GLC analysis (2 m × 0.3 mm 3 % OV-101 on Chromosorb W column, 139 – 270 °C/6 °C min^{-1}) established the *o:m:p* proportion to be 66:15:8:11. The current yield was 50 % (2,2',4,4',6,6'-hexamethylbiphenyl as internal standard) and the monoacetate/higher acetates ratio 98:2.

Anodic oxidation of fluorene (3). A solution of fluorene (1.66 g) in HOAc/1 M NaOAc (50 ml) was oxidized anodically at a platinum anode at 1.6 V *vs.* SCE until 0.2 F mol^{-1} of *3* had been passed through the solution. After the usual work-up the product mixture was chromatographed on a silica gel column, using (1) pentane and (2) pentane-diethyl ether (1:1) as eluents. The separation process was followed by GLC analysis (2 m × 0.3 mm 5 % neopentylglycol succinate on Chromosorb W column at 200 °C; pentamethylbenzyl acetate as internal standard). The current yields of 2-acetoxyfluorene, 9-acetoxyfluorene, and 1-/3-/4-acetoxyfluorene were 14, 0.5 and 0.5 %, respectively. With tetrabutylammonium tetrafluoroborate as supporting electrolyte only the 9-isomer was obtained (0.5 % current yield).

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Conjugate Addition of Lithium Dimethylcuprate, Lithium Diphenylcuprate and a Chiral Lithium Phenyl(aryl)cuprate Reagent to Methyl 3-(2-Furyl)acrylate

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Addition of $LiMe_2Cu$ or $LiPh_2Cu$ containing lithium halides to methyl *trans*-3-(2-furyl)-acrylate gave methyl 3-(2-furyl)-butanoate and methyl 3-(2-furyl)-3-phenylpropanoate, respectively. Addition of the cuprates essentially free from lithium halides required longer reaction times, higher temperature, or a large excess of the cuprate. Addition of the mixed chiral cuprate, lithium phenyl[2-(1-dimethylaminoethyl)-phenyl]cuprate, prepared from (−)*N*,*N*-dimethyl-1-phenylethylamine, to methyl 3-(2-furyl)acrylate gave methyl 3-(2-furyl)-3-phenylpropanoate with a small negative rotation.

In the search for a stable, chiral ligand to copper we have chosen to study the (−)-2-(1-dimethylaminoethyl)phenyl group. Addition of an organolithium reagent to such an organocopper compound should give a chiral cuprate which could transfer one ligand in 1,4-addition reactions with α,β-unsaturated carbonyl compounds and induce optical activity at the β carbon.

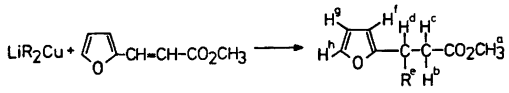
Addition of such mixed, racemic lithium diorganocuprates, LiRR*Cu, to methyl *trans*-cinnamate [R being methyl, butyl and phenyl, R* being 2-(1-dimethylaminoethyl)phenyl] gives good yields of the 1,4-addition product when diethyl ether is used as a solvent.[1] When tetrahydrofuran is used as a solvent, other reactions compete with the conjugate addition.[2]

We have now investigated the conjugate addition to methyl *trans*-3-(2-furyl)acrylate of some common organocuprates and of the chiral lithium phenyl [2-(1-dimethylaminoethyl)phenyl]cuprate prepared from (−)*N*,*N*-dimethyl-1-phenylethylamine. We have also studied the effect of lithium salts on the reactivity of the cuprates. It has previously been shown that some Grignard and lithium reagents undergo conjugate addition to 3-(2-furyl)acrolein.[3]

RESULTS

The influence of the method of preparation of the cuprates, the temperature and the solvent on the conjugate addition of lithium dimethylcuprate and lithium diphenylcuprate to methyl 3-(2-furyl)-acrylate are summarised in Table 1.

Methyl and phenyl groups are transferred selectively and in high yields by the cuprates to the β carbon of the enoate.



1 R=CH_3 *2* R=C_6H_5

We have observed a substantial difference in reactivity of cuprates from different preparations. When care was taken to exclude the lithium halides in the preparation of lithium diphenylcuprate, the relatively salt-free cuprate was less reactive towards the methyl furylacrylate than the "ordinary" cuprate reagent containing stoichiometric amounts of lithium bromide and iodide (see experiments 4 and 5 in Table 1).

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Table 1. 1,4-Addition of lithium diorganocuprates, LiRR'Cu, to methyl *trans*-3-(2-furyl)acrylate. Reactions were run in diethyl ether at 0 °C unless indicated otherwise. R* = (−)2-(dimethylaminoethyl)phenyl.

Exp. No.	LiRR'Cu	Method of preparation	Cuprate: substrate molar ratio	Reaction time/h	Main product	GLC yield/ %	Isolated yield/ %
1	$LiMe_2Cu$	MeLi(LiBr) + CuI	1.1:1	1.5	*1* [a]	90	63.5
2	$LiMe_2Cu$	MeLi(com)[b] + CuI	2.0:1	12 [c]	*1*	90	
3	$LiMe_2Cu$	MeLi(com) + CuI	12.0:1	1	*1*	80	
4	$LiPh_2Cu$	PhLi(LiBr) + CuI	1.3:1	1	*2* [d]	97	64.5
5	$LiPh_2Cu$	PhLi(LiBr) + PhCu	1.5:1	1	*2*	10	
6	LiPhR*Cu	R*Li + PhCu(LiBr + LiI)	1.4:1	1	*2* [e]	97	64.5

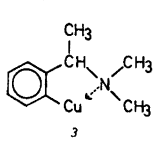
[a] *1* is methyl 3-(2-furyl)butanoate. [b] Commercial methyllithium (Fluka). [c] Reaction temp. 20 °C. [d] *2* is methyl 3-(2-furyl)-3-phenylpropanoate. [e] Specific rotation of isolated ester: $[\alpha]_{578}^{20} = -0.67°$ (*c* 0.159, chloroform).

When methyllithium, prepared from bromomethane and lithium, was used to prepare the cuprate, the resulting solution contained $Li(CH_3)_2Cu$, LiBr and LiI in molar ratios of 1:2:1. This reagent was very reactive.

Commercial methyllithium (Fluka) and copper(I) iodide gave a cuprate, which required longer reaction times and higher temperatures or alternatively a large excess of the cuprate (see expts. 1, 2, and 3 in Table 1). With cuprates containing stoichiometric amounts of lithium halides only a small excess of the cuprate was necessary to give high yields of 1,4-addition products within one hour (experiments 1 and 4 in Table 1).

When tetrahydrofuran, THF, was used as a solvent, the reaction rate drastically decreased, and most of the substrate (*ca.* 80 %) remained unchanged after 48 h.

Reaction of a small excess of chiral lithium phenyl[2-(1-dimethylaminoethyl)phenyl]cuprate with methyl 3-(2-furyl)acrylate in ether (in the presence of lithium halides) gave the 1,4-addition product at a rate comparable to that of lithium diphenylcuprate. The phenyl group was selectively transferred with the simultaneous formation of chiral 2-(1-dimethylaminoethyl)phenylcopper, *3*. The specific rotation of the product was small but reproducible. The optical yield did, however, not exceed 2.2 %.



3

DISCUSSION

The asymmetric induction obtained so far is small. Further work on other systems is under way.

It has been suggested that copper in a lithium diorganocuprate is the attacking nucleophile in reactions with organic halides,[6] and it is reasonable to assume that this could be the case in conjugate additions to enones and enoates as well. If copper were the attacking nucleophile there would have to be vacant sites to allow attack on the enoate. Exchange of ether for the more strongly complexing THF would lead to the observed solvent effect. A similar effect of solvent change on the reactivity of cuprates has been observed previously [2] and is opposite to what is observed for organolithium reagents.[5]

The salt effect has mostly been discussed for the reactions of lithium diorganocuprates with organic halides.[7] The observed difference in reaction rates (compare expts. 1, 2, 3, 4 and 5 in Table 1) probably is the result of different concentrations of lithium halides. It could be assumed that more reactive species than clusters of lithium diorganocuprates are formed in the presence of lithium halides.[8]

The effect of lithium salts on the addition of $CH_3Li - Li(CH_3)_2Cu$ to 4-*t*-butylcyclohexanone has recently been discussed.[9] It was suggested that lithium forms a complex with the carbonyl oxygen when ether is used as a solvent, while no complex is formed in THF. It can be assumed that a similar complexation of

lithium to the carbonyl oxygen of the α,β-unsaturated ester would increase the reactivity of the β-carbon. This would account for both the salt effect and the solvent effect observed.

EXPERIMENTAL

All handling and reactions of organometallic reagents were carried out under dry, oxygen-free nitrogen. Dry diethyl ether and tetrahydrofuran, THF, were distilled from sodium benzophenone ketyl. Commercial butyllithium (Merck) in hexane and methyllithium (Fluka) in ether were used after their strength had been determined by titration.

Lithium dimethylcuprate and methyl 3-(2-furyl)acrylate.[9] Lithium dimethylcuprate was prepared by addition of 55.5 ml of a 1.42 M solution of methyllithium (79 mmol, prepared from bromomethane and lithium) to copper(I) iodide (7.6 g, 40 mmol) in 20 ml of ether at 0 °C. After stirring for 30 min, Gilman Test I [10] was negative, and methyl 3-(2-furyl)acrylate (4.56 g, 30 mmol), in 30 ml of ether was added. The mixture was stirred for 1.5 h at 0 °C and then poured into aqueous NH_4Cl, extracted with ether, washed with water, and dried over $MgSO_4$. GLC analysis showed methyl 3-(2-furyl)butanoate as the major component (> 90 %) and a small amount of a high-boiling product. After evaporation of the ether and distillation, 3.2 g of methyl 3-(2-furyl)butanoate [63.5 %, based on methyl 3-(2-furyl)acrylate] was isolated, b.p. 85 °C/0.05 mmHg. ^1H NMR (270 MHz, $CDCl_3$): δ 3.63 (3 H, s) H_a, 2.43 (1 H, dd, J_{bc} 15.3 Hz) H_b, 2.70 (1 H, dd, J_{cd} 6.4 Hz) H_c, 3.34 (1 H, qdd, J_{bd} 8.1 Hz) H_d, 1.28 (3 H, d, J_{de} 7.0 Hz) $R_e=CH_3$, 5.98 (1 H, ddd, J_{fg} 3.2 Hz, J_{df} 0.5 Hz) H_f, 6.23 (1 H, dd, J_{gh} 1.8 Hz) H_g, 7.27 (1 H, dd, J_{fh} 0.8 Hz) H_h.

When this experiment was repeated with *commercial* methyllithium for the preparation of lithium dimethylcuprate, it was necessary to raise the reaction temperature to 20 °C and leave the reaction with stirring overnight to get the same high yield of the 1,4-addition product. Lithium dimethylcuprate was present in 100 % excess. When a 12-fold excess of this cuprate was used at 0 °C, 80 % of the methyl furylacrylate was consumed after 1 h (GLC).

With THF as a solvent instead of diethyl ether for the addition of lithium dimethylcuprate (methyllithium prepared from bromomethane and lithium), most of the methyl furylacrylate (~80 %) remained unchanged after 48 h.

Lithium diphenylcuprate and methyl 3-(2-furyl)acrylate. Lithium diphenylcuprate was prepared by addition of 89 ml of a 0.89 M ether solution of phenyllithium (79 mmol, prepared from bromobenzene and lithium in ether) to copper(I) iodide (7.6 g, 40 mmol) in 20 ml of ether. After stirring for 30 min, methyl 3-(2-furyl)acrylate (4.56 g, 30 mmol) in 30 ml of ether was added dropwise. After stirring for 1 h at 0 °C, the reaction mixture was poured into aqueous NH_4Cl, extracted with ether, washed with water, and dried. GLC analysis indicated two products, methyl 3-(2-furyl)-3-phenylpropanoate, *ca.* 97 %, and a small amount of a high-boiling product. After evaporation of the ether and distillation, 4.45 g of methyl 3-(2-furyl)-3-phenylpropanoate, 64.5 %, was isolated, b.p. 92 °C/0.03 mmHg. ^1H NMR (270 MHz, $CDCl_3$): δ 3.47 (3 H, s) H_a, 2.86 (1 H, dd, J_{bc} 15.6 Hz, J_{bd} 7.9 Hz) H_b, 3.05 (1 H, dd, J_{cd} 7.6 Hz) H_c, 4.55 (1 H, dd, J_{df} 0.9 Hz) H_d, 7.11 – 7.21 (5 H, m) $R_e=C_6H_5$, 5.99 (1 H, ddd, J_{fg} 3.2 Hz) H_f, 6.18 (1 H, dd, J_{gh} 1.9 Hz) H_g, 7.21 (1 H, dd, J_{fh} 0.9 Hz) H_h.

Hydrolysis (ethanol/KOH) of the ester afforded 3-(2-furyl)-3-phenylpropanoic acid, m.p. 104.5 – 105 °C. Anal. $C_{13}H_{12}O_3$: C, H. ^1H NMR (270 MHz, $CDCl_3$): δ 2.90 (1 H, dd, J_{bc} 16.0 Hz) H_b, 3.00 (1 H, dd, J_{cd} 7.9 Hz) H_c, 4.51 (1 H, dd, J_{bd} 7.5 Hz) H_d, 6.01 (1 H, ddd, J_{df} 0.5 Hz, J_{fh} 0.5 Hz) H_f, 6.23 (1 H, dd, J_{fg} 3.3 Hz) H_g, 7.27 (1 H, dd, J_{gh} 1.7 Hz) H_h, 7.19 – 7.26 (5 H, m) $R_e=C_6H_5$, 11.5 (1 H, s).

(+)- and (−)-3(2-furyl)-3-phenylpropanoic acid. Racemic 3-(2-furyl)-3-phenylpropanoic acid (4.0 g) was dissolved by heating in 40 ml of a ethanol-water solution (3:1). (−)1-Phenylethylamine (1.8 g) was added. Needle shaped crystals precipitated. After recrystallisation and hydrolysis (+)3-(2-furyl)-3-phenylpropanoic acid was obtained, $[\alpha]_{578}^{20} = +41.51°$ (*c* 0.01573; $CHCl_3$). When the procedure was repeated with (+)1-phenylethylamine and racemic acid (−)3-(2-furyl)-3-phenylpropanoic acid was obtained, $[\alpha]_{578}^{20} = -39.5°$ (*c* = 0.00710; $CHCl_3$).

(−)Methyl 3-(2-furyl)butanoate. (−)3-(2-Furyl)-3-phenylpropanoic acid (132 mg) was treated with an excess of CH_3I (800 mg) in CH_2Cl_2 (30 ml) (ion-pair alkylation with tetrabutylammonium hydrogensulfate, 510 mg, dissolved in 0.1 M NaOH, 30 ml). After boiling for 30 min conventional workup resulted in (−)methyl 3-(2-furyl)butanoate (122 mg) $[\alpha]_{578}^{20} = -30.5°$ (*c* 0.0244; $CHCl_3$).

Preparation of salt-free lithium diphenylcuprate and its addition to methyl 3-(2-furyl)-acrylate. Phenylcopper was prepared by addition of an ether solution of phenyllithium (10.7 mmol, 0.92 M, prepared from bromobenzene and lithium) to copper(I) iodide (2.09 g, 11 mmol) in 10 ml of ether at 0 °C. A white precipitate of phenylcopper appeared. The precipitate was allowed to settle. The solids were washed three times with ether so as to remove the soluble lithium halides. Phenyllithium (9.3 ml) was then added. The white colour of phenylcopper disappeared, and a black solution of lithium diphenylcuprate was obtained. Methyl 3-(2-furyl)acrylate (1.0 g, 6.6 mmol) was added, and the reaction mixture was stirred for 1 h at 0 °C. Less than 10 % of

methyl 3-(2-furyl)-3-phenylpropanoate was formed, and most of the methyl furylacrylate remained unchanged (GLC).

Chiral lithium phenyl[2-(1-dimethylaminoethyl)phenyl]cuprate and methyl 3-(2-furyl)acrylate. Lithium phenyl[2-(1-dimethylaminoethyl)-phenyl]cuprate was prepared by addition of an ether solution of chiral 2-(1-dimethylaminoethyl)phenyllithium (prepared by stirring (−)*N*,*N*-dimethyl-1-phenylethylamine, 16.2 mmol, with butyllithium, 14.6 mmol, in ether, 20 ml, at room temperature for 48 h [11]) to a suspension of phenylcopper in ether (prepared by adding phenyllithium, 0.89 M, 14.6 mmol, in ether to copper(I) iodide, 14.6 mmol, in ether, 20 ml, at 0 °C). After stirring for 30 min, a solution of methyl 3-(2-furyl)acrylate (1.65 g, 10.8 mmol) in 30 ml of ether was added dropwise and the mixture was left with stirring for 1 h. After the reaction mixture had been poured into aqueous NH_4Cl, the organic components were extracted in ether and the basic compounds were removed by extraction with 2 × 30 ml of dilute HCl. The ether fraction was washed with a saturated solution of $NaHCO_3$, and water and then dried. According to GLC there was no *N*,*N*-dimethyl-1-phenylethylamine left. Methyl 3-(2-furyl)-3-phenylpropanoate was present in *ca.* 97 %. The product was isolated after evaporation of the ether and distillation. The specific rotation was $[\alpha]_{578}^{20} = -0.67°$ (*c* 0.159, chloroform) and the optical yield correspondingly 2.2 %.

Acknowledgement. This work has been supported by grants to B. G. from the Finnish Oil Industry, Neste Oy, and the Research Institute of the Åbo Akademi Foundation, and by a grant from the Swedish Board of Technical Development.

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The Electron Impact Mass Spectra of 4-Hydroxy and 4-Methoxy Derivatives of 5,6-Dihydro-2-pyrones

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Dedicated to Professor K. A. Jensen on his 70th birthday

The electron impact mass spectra of twenty 6-substituted 5,6-dihydro-2-pyrones carrying either a 4-methoxy or a 4-hydroxy group have been recorded and discussed. The main fragmentation modes, inferred from metastable ion analyses (metastable refocusing and DADI technique) as well as from high resolution mass measurements, have been rationalized and compared. The decomposition depends strongly on the 6-substituent (alkyl, phenetyl, alkenyl, or cycloalkanespiro), and in most cases the 4-hydroxy substituted compounds give rise to more complex fragmentation patterns than the corresponding 4-methoxy derivatives. This is ascribed to contribution of the 4-oxo tautomers of the 4-hydroxy compounds.

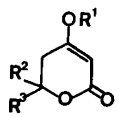
This paper reports on the electron-impact-induced fragmentation of a number of 6-substituted 4-hydroxy-5,6-dihydro-2-pyrones and their corresponding 4-methyl ethers. The behaviour of such compounds are of interest due to their structural similarity of the Kawa-lactones as well as to 2-pyrones and tetrahydro-2-pyrones (δ-lactones).

The mass spectra of substituted monocyclic 2-pyrones have been reported and reviewed.[1] An initial loss of the C^6-substituent together with expulsion of CO and the formation of substituted cyclopropenyl ions appear to be processes of general importance. Similarly, loss of the side chain from the molecular ions is a characteristic behaviour of tetrahydro-2-pyrones. No systematic study of the fragmentation of simple dihydro-2-pyrones has been reported so far, although a series of Kawa-lactones, some being dihydro-2-pyrones and others 2-pyrones, has been investigated in some detail.[2] It appears that the fragmentation upon electron impact depends highly on the presence of a styrylic double bond.

This investigation is centered around C^6-substituted 5,6-dihydro-2-pyrones, in which the C^6-substituents are characterized as being either alkyl/aralkyl, cycloalkanespiro, or *trans*-1-alkenyl.

6-Alkyl/aralkyl derivatives of 5,6-dihydro-2-pyrones. The compounds given in Table 1 have been investigated. Representative mass spectra are given in Fig. 1 together with the major decomposition modes. These are all verified by exact mass measurements and by metastable ions (metastable refocusing as well as the DADI technique have been applied).

Table 1. 6-Alkyl-aralkyl derivatives.



	R^1	R^2	R^3
1a	H	H	C_5H_{11}
1b	CH_3	H	C_5H_{11}
2a	H	C_2H_5	C_2H_5
2b	CH_3	C_2H_5	C_2H_5
3a	H	H	$CH_2CH_2C_6H_5$
3b	CH_3	H	$CH_2CH_2C_6H_5$

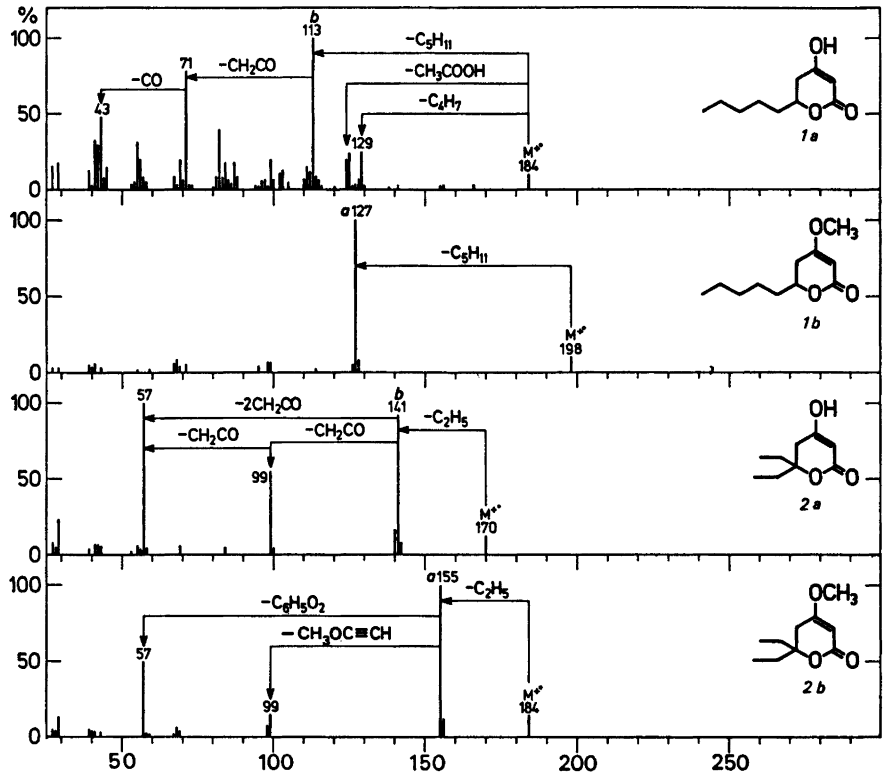
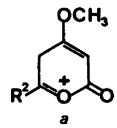


Fig. 1. Mass spectra of *1a*, *1b*, *2a* and *2b* recorded at 80, 15, 50, and 15 °C, respectively.

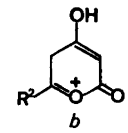
The typical α-cleavage reaction of 2-pyrones and tetrahydro-2-pyrones with loss of the C^6 side chain is also found to be an important process for the 6-mono- and 6,6-disubstituted dihydro-2-pyrones. For the 4-methoxy-substituted compounds, type b, this reaction is predominant and the corresponding $[M-R^3]^+$ ion,

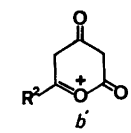


$R^3=H$: *m/e* 127; $R^3=C_2H_5$: *m/e* 155

a, possesses a considerable stability. It is carrying 42 % of the total ion current ($\sum_{25}$) of *1b* and in the case of *3b* it has a higher abundance than the tropylium ion (*m/e* 91).

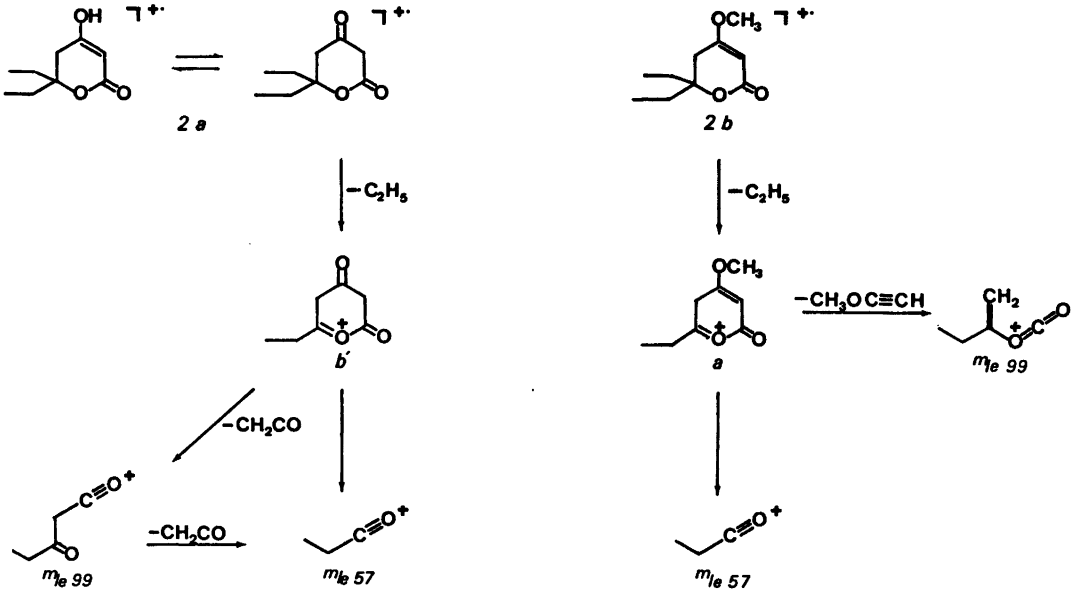
The corresponding ion, *b*, formed from the 4-hydroxy-substituted compounds (*1a*, *2b*, and *3a*) appears to be less stable than *a*. It has an





$R^3=H$: *m/e* 113; $R^3=C_2H_5$: *m/e* 141

abundance of 12.3 % of $\sum_{25}$ in *1a*. In the spectrum of *3a* the tropylium ion is the base peak and ion *a* amounts to a relative intensity of 7 % only. One reason for the observed decrease in stability of the $[M-R^3]^+$ ion may be that its structure preferentially corresponds to *b'*, as the further decomposition is elimination of $CH_2=CO$. In *1a* and *3a* this process is followed by a CO loss, in *2a* by another CH_2CO loss. In *2a* the $[b'-CH_2CO]^+$ ion appears at *m/e* 99. The spectrum of *2b* also exhibit a *m/e* 99 ion which corresponds to the loss of $CH_3OC{\equiv}CH$ from *a*, but in this case further loss of ketene cannot be detected. The $[C_2H_5CO]^+$ ion at *m/e* 57 is found only to have ion *a* as precursor.



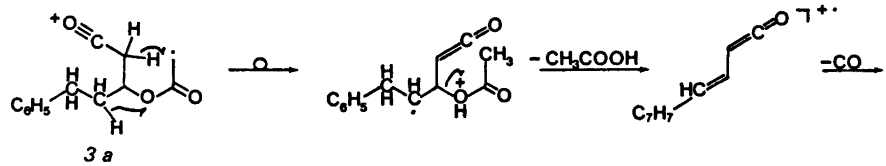
Scheme 1.

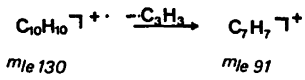
This latter process corresponds to a *retro*-Diels-Alder decomposition with charge retention on the C_2H_5CO species. Reversed charge retention leads to formation of the *m/e* 98 ion being an important key-fragment in the fragmentation of the 6-cycloalkanespiro and the 6-(*trans*-1-alkenyl) derivatives discussed below.

Possible structures for the various ions in question are shown in the rationale, Scheme 1.

The type a derivatives here discussed have been shown by NMR to exist more or less enolized depending on the solvent. In dimethyl sulfoxide they are fully enolized, but in less polar solvents the enolization is diminished.[3] A keto-form of *1a* and *3a* is supported by the formation of the $[M-C_2H_4O_2]^{+\cdot}$ ions. Such ions may according to the suggested rationale, Scheme 2 for *3a*, be generated by a double hydrogen rearrangement in the ring-opened molecular ion of the keto form with subsequent elimination of a molecule of acetic acid. In addition a stepwise formation of the *m/e* 158 ion is indicated to take place by elimination of H_2O and CH_2CO.

6-Cycloalkanespiro derivatives of 5,6-dihydro-2-pyrones. The compounds given in Table 2 have been investigated. As representative for this series the mass spectra of *6a* and *6b* are shown in Fig. 2.





Scheme 2.

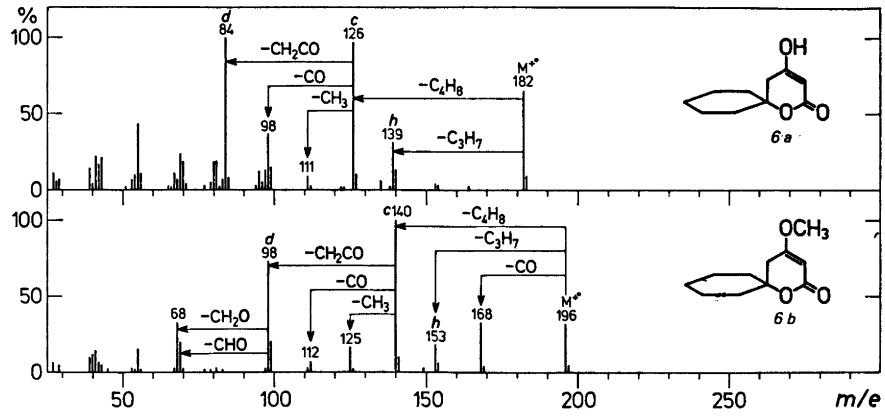


Fig. 2. Mass spectra of *6a* and *6b* recorded at 70 and 40 °C, respectively.

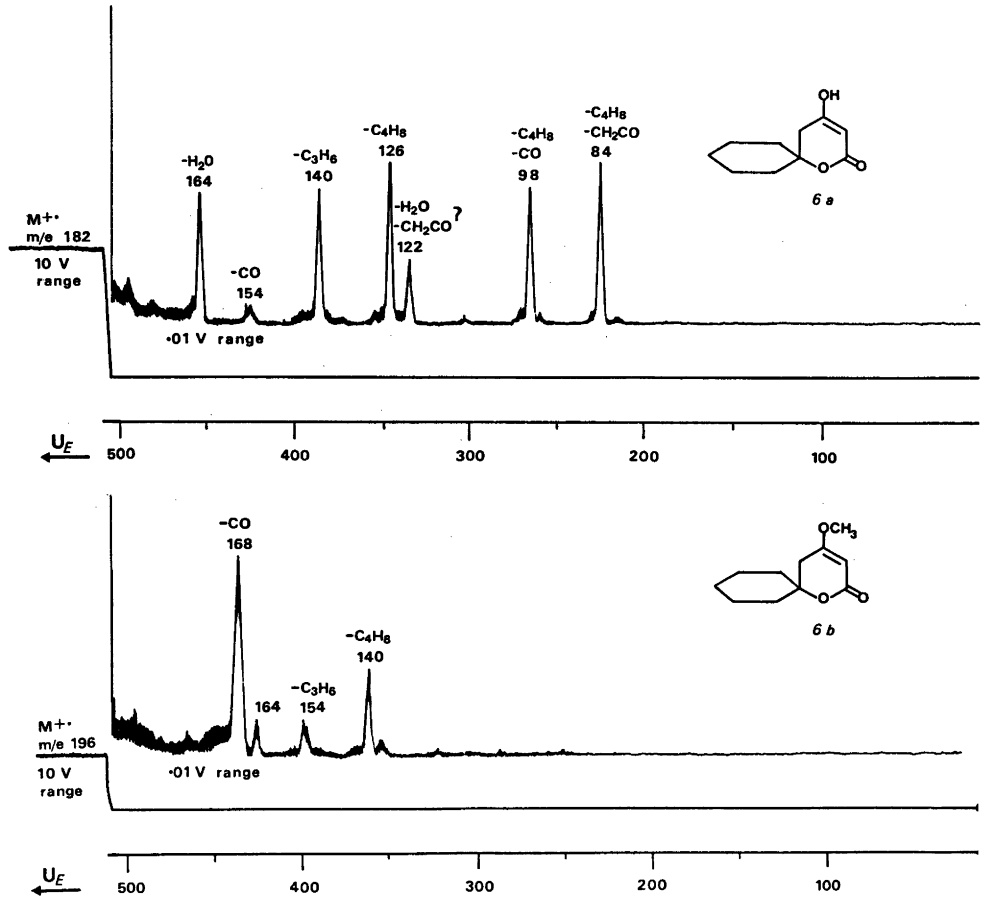
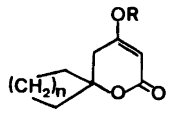


Fig. 3. DADI spectra [4] of *6a* and *6b*, showing the metastable transitions of the molecular ions. The calculated masses of the daughter ions – all formed directly from the molecular ions – as well as the compositions of the eliminated neutrals are indicated.

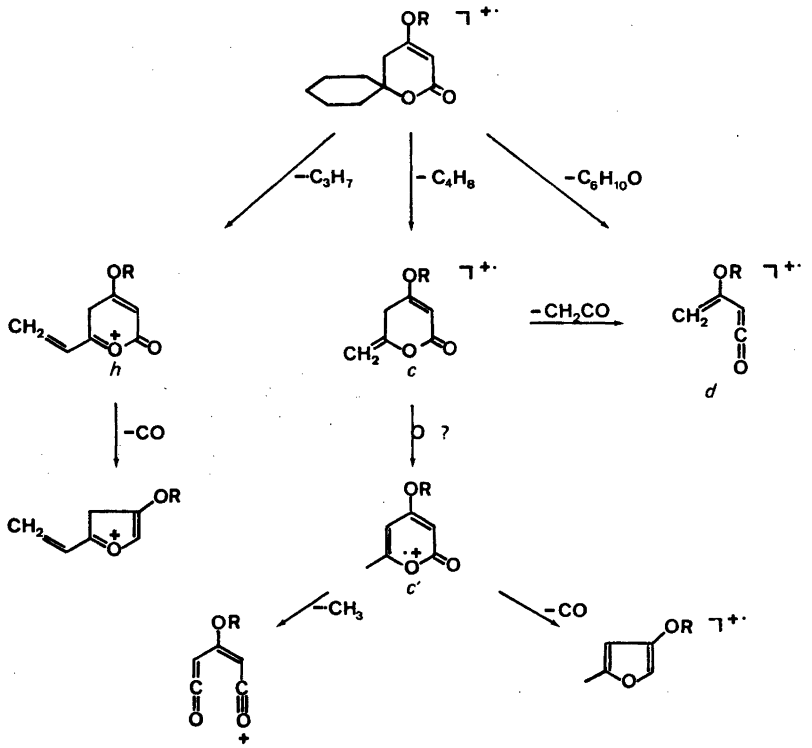
Table 2. 6-Cycloalkanespiro derivatives.



	R	*n*
4a	H	1
4b	CH_3	1
5a	H	2
5b	CH_3	2
6a	H	3
6b	CH_3	3
7a	H	4
7b	CH_3	4

The fragmentation patterns of this series are dominated by odd electron ions most of which are formed in several competing decompositions as demonstrated by the presence of numerous metastable ions. The DADI spectra of the molecular ions of *6a* and *6b* are given in Fig. 3, suggesting concerted losses of stable molecules such as cycloalkanes, CO, and CH_2CO. The varying abundances of the metastable ions in the DADI spectra [4] obtained from the molecular ions in this series suggest that the degree to which these processes are followed changes considerably. However, it appears that only few of these reactions contribute significantly to the ion abundances since the general appearance of the mass spectra is very much alike.

An important ion in all cases is brought forth by decomposition of the cycloalkanespiro ring, probably giving rise to elimination of a cycloalkane (ethene in *4a* and *4b*) as shown in the rationale, Scheme 3, for *6a* and *6b*. The resulting ion is formulated as *c*, from which the observed loss of CH_2CO, yielding *d*, would be a likely process. However, it is possible that the rearrangement $c \rightarrow c'$ occurs also to some extent. The mass spectrum of 4-methoxy-6-methyl-2-pyrone [5] exhibits abundant $[M-CH_3]^+$ and $[M-CO]^{+\cdot}$ ions, whereas elimination of CH_2CO from the molecular ion does not take place.



Scheme 3.

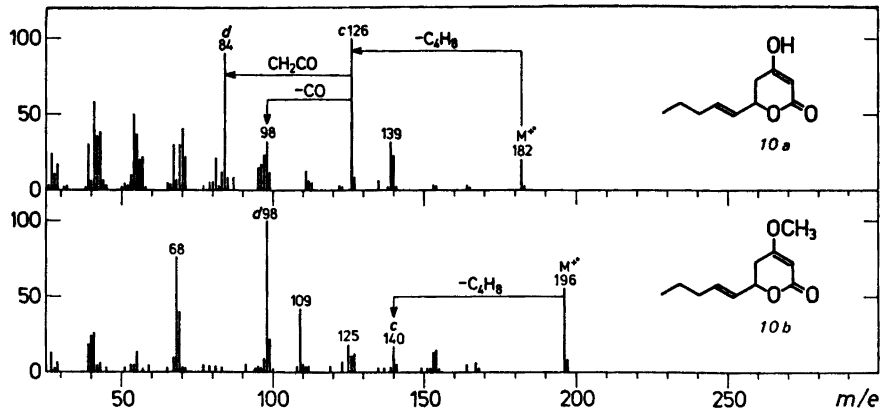


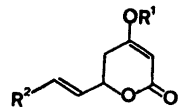
Fig. 4. Mass spectra of *10a* and *10b* recorded at 20 and 25 °C, respectively.

Ion *d* is additionally formed directly from the molecular ion, either by elimination of cyclohexanone (a *retro*-aldol reaction) or by concerted losses of cyclobutane and ketene. In the type b compounds ion *d* (*m/e* 98) eliminates CHO and CH_2O yielding *m/e* 69 and *m/e* 68.

The direct elimination of an alkyl group (C_3H_7 in Scheme 3), requiring a hydrogen rearrangement of the molecular ion, appears to be a general process (except for compounds *4a* and *4b*), yielding an even electron ion of relative high abundance, formulated as *h*. Also the initial CO loss is an often occurring process. However, the corresponding $[M-CO]^{+\cdot}$ peak appears with varying abundances and is insignificant in several spectra.

6-(trans-1-Alkenyl) derivatives of 5,6-dihydro-2-pyrones. The compounds given in Table 3 have been investigated. The mass spectra of *10a* and *10b* are given in Fig. 4.

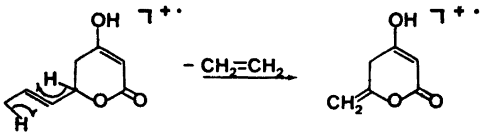
Table 3. 6-(*trans*-1-Alkenyl) derivatives.



	R^1	R^2
8a	H	CH_3
8b	CH_3	CH_3
9a	H	C_2H_5
9b	CH_3	C_2H_5
10a	H	C_3H_7
10b	CH_3	C_3H_7

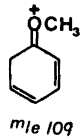
Introduction of the double bond has a marked effect upon the electron-impact-induced fragmentation, and inhibits completely formation of the pyronium ion by elimination of the C^6 side chain which is the most characteristic process of the compounds with saturated substituents. Instead a formal breakage of the double bond takes place with charge retention preferentially at the pyrone ring. This process may be rationalized if it is anticipated that the double bond introduces a new site for charge localization initiating a hydrogen rearrangement. This is illustrated in the mass spectrum of kawain having a styryl substituent at the C^6 position. In this case fission of the double bond accompanied by rearrangement of one hydrogen atom has been reported.[3] The origin of this hydrogen is C^6 and C^5 (1:1), as demonstrated by labelling experiments,[6] and the resulting ion is the highly stable tropylium ion. Formation of similar stable ions is not possible in the decomposition of compounds *8* to *10* all having aliphatic substituents. However, it is anticipated that the double bond also in these cases initiates hydrogen rearrangement, and subsequent rupture of that bond may then lead to the formation of stable neutral molecules (ethene, propene, and butene, respectively). The resulting ion, which constitutes the base peak (at *m/e* 126) in *8a*, *9a*, and *10a* is probably identical to the corresponding ion, *c*, in the spectra of the cycloalkanespiro compounds (the DADI spectra of *m/e* 126 in *10a*

and *6a*, showing degradation to *m/e* 98 and *m/e* 84, are virtually identical). To obtain this structure, rearrangement of two hydrogen atoms is required. Although other modes are possible and random rearrangements cannot be excluded a possible origin of these hydrogen atoms may be:



In *8b*, *9b*, and *10b* the alkene elimination is less prevalent and the corresponding ion (*m/e* 140) appears with significantly lower abundance. This may suggest that the double bond is a less favoured site for charge localization and thus restrains the possibility for hydrogen rearrangements. Such changes in charge distribution in the molecular ions can hardly be attributed to differences induced by the methoxy group as compared to the hydroxy group, but may be rationalized if the keto-form of the type a compounds is anticipated.

Characteristic for the type b compounds is the presence of the *m/e* 109 ion (C_7H_9O). Metastable data have revealed that besides a direct formation from the molecular ions this ion is also generated in a two-step process *via* the $[M-CO_2]^{+\cdot}$ ion. This indicates that loss of CO_2 takes place in all three cases although the abundance of the $[M-CO_2]^{+\cdot}$ ion is insignificant in *9b* and *10b*. A possible structure for the *m/e* 109 ion is



m/e 109

The presence of this ion as well as the relative low abundance of *m/e* 140 constitutes the major general difference between the spectra of type b compounds and those of the corresponding cycloalkanespiro isomers. Similar diagnostic differences are not encountered for corresponding 4-hydroxy substituted compounds.

SUMMARY

The fragmentation upon electron impact of 5,6-dihydro-2-pyrones is found to depend strongly on the nature of the 6-substituent. In most cases the fragmentation pattern for the 4-hydroxy derivatives (a) is more differentiated than for the corresponding 4-methoxy analogues (b). This is ascribed to the contribution of a 4-oxo form of a.

(a) 6-Alkyl derivatives show the general loss of the 6-substituent yielding the important pyronium ion *a* (with 4-methoxy) or *b* (with 4-hydroxy) in agreement with the behaviour of similarly substituted 2-pyrones and tetrahydro-2-pyrones. The 4-hydroxy group diminishes considerably the relative stability of *b* as compared to *a*.

(b) 6-Cycloalkanespiro derivatives exhibit a prevalent pyronium ion *c*, including a CH_2 group from the cycloalkanespiro ring, probably formed by elimination of a cycloalkane.

(c) 6-(1-Alkenyl) derivatives exhibit spectra resembling considerably those of the isomeric cycloalkanespiro compounds. This makes diagnostic differentiation between type a compounds of the two series ambigous. The formation of ion *c*, corresponding to formal rupture of the double bond in the side chain, is attributed to hydrogen rearrangements to the double bond and subsequent elimination of an alkene.

EXPERIMENTAL

Mass spectra were obtained on a Varian MAT 311A mass spectrometer using the direct sample insertion system with the lowest feasible sample temperature and ionization by electron impact (70 eV). Peaks corresponding to doubly charged ions appearing at half mass numbers as well as peaks of abundance lower than 2 % were omitted from the spectra shown. All the decompositions given are supported by appropriate metastable transitions detected by metastable refocusing or by the DADI technique.

Microanalyses were carried out at the Microanalytical Department of the University of Copenhagen by Mr. Preben Hansen. Melting points were determined on a Büchi melting point apparatus. ^1H NMR spectra were recorded on a Jeol JNM-PMX60 NMR spectrometer; chemical shifts are given in ppm relative to TMS.

The 4-hydroxy-5,6-dihydro-2-pyrones investigated were all prepared according to a

procedure earlier described.[7] In the following the aldehyde or ketone used for preparation is mentioned, and data for the primarily obtained compounds are given in parenthesis. For purification all compounds were triturated with ether and were all shown to be analytically pure by elemental analysis (C, H).

4-Hydroxy-6-pentyl-5,6-dihydro-2-pyrone (1a). Hexanal (yield 86 %, m.p. 65–70 °C), m.p. 72–74 °C. NMR (DMSO-d_6): 0.87 (t, 3 H), 1.33 (m, 8 H), 2.30 (s, 1 H), 2.46 (d, 1 H, J 3 Hz), *ca.* 4.2 (m, 1 H), 5.28 (s, 1 H).

4-Hydroxy-6,6-diethyl-5,6-dihydro-2-pyrone (2a). 3-Pentanone (yield 70 %, m.p. 82–93 °C), m.p. 98–100 °C. NMR (DMSO-d_6): 0.85 (d of t, 6 H), 1.65 (d of q, 4 H), 2.42 (s, 2 H), 4.92 (s, 1 H).

4-Hydroxy-6-cyclobutanespiro-5,6-dihydro-2-pyrone (4a). Cyclobutanone (yield 63 %, m.p. 110–116 °C), m.p. 125–129 °C. NMR (DMSO-d_6): 1.4–2.4 (complex, 6 H), 2.64 (s, 2 H), 4.94 (s, 1 H).

4-Hydroxy-6-cyclopentanespiro-5,6-dihydro-2-pyrone (5a). Cyclopentanone (yield 60 %, m.p. 95–125 °C), m.p. 131–133 °C. NMR (DMSO-d_6): 1.73 (s broad, 8 H), 2.56 (s, 2 H), 4.93 (s, 1 H).

4-Hydroxy-6-cyclohexanespiro-5,6-dihydro-2-pyrone (6a). Cyclohexanone (yield 60 %, m.p. 98–105 °C), m.p. 117–121 °C. NMR (DMSO-d_6): 1.2–2.0 (complex, 10 H), 2.45 (s, 2 H), 4.97 (s, 1 H).

4-Hydroxy-6-cyclooctanespiro-5,6-dihydro-2-pyrone (7a). Cyclooctanone (yield 45 % m.p. 128–130 °C), m.p. 129–130.5 °C. NMR (DMSO-d_6): 1.1–2.1 (broad, 14 H), 2.43 (s, 2 H), 4.42 (s, 1 H).

*4-Hydroxy-6-(*trans*-1-butenyl)-5,6-dihydro-2-pyrone* (9a). *trans*-2-Pentenal (yield 52 %, m.p. 90–92 °C), NMR (DMSO-d_6): 0.97 (t, 3 H J Hz), 2.04 (q, 2 H J 1 and 7 Hz), 2.40 (s, 1 H), 2.54 (d, 1 H J 2 Hz), 4.6–5.1 (m, 1 H), 4.95 (s, 1 H), 5.58 (q, 1 H J 5 and 15 Hz), 5.90 (q, 1 H J 5 and 15 Hz).

The preparation of compounds *3a*, *8a*, and *10a* has been described earlier.[7]

From the compounds *1a* to *10a* the corresponding 4-methoxy derivatives were prepared.

On reaction with dimethyl sulfate and potassium carbonate in acetone at room temperature [8] followed by purification on preparative TLC (silica gel, ether as eluent), the following compounds, all showing satisfactory NMR spectra, were obtained.

4-Methoxy-6-pentyl-5,6-dihydro-2-pyrone (1b). R_F 0.56, oil (Found for M+: 198.1250. Calc. for $C_{11}H_{16}O_3$: 198.1256).

4-Methoxy-6,6-diethyl-5,6-dihydro-2-pyrone (2b). R_F 0.62, oil (Found for M+: 184.1100. Calc. for $C_{10}H_{16}O_3$: 184.1099).

4-Methoxy-6-cyclobutanespiro-5,6-dihydro-2-pyrone (4b). R_F 0.50, crystals of m.p. 53–55 °C (Found for M+: 168.0782. Calc. for $C_9H_{12}O_3$: 168.0786).

4-Methoxy-6-cyclopentanespiro-5,6-dihydro-2-pyrone (5b). R_F 0.70, oil (Found for M+: 182.0943. Calc. for $C_{10}H_{14}O_3$: 182.0943).

4-Methoxy-6-cyclohexanespiro-5,6-dihydro-2-pyrone (6b). R_F 0.53, crystals of m.p. 74–77 °C (Found for M+: 196.1082. Calc. for $C_{11}H_{16}O_3$: 196.1099).

4-Methoxy-6-cyclooctanespiro-5,6-dihydro-2-pyrone (7b). R_F 0.70, viscous oil (Found for M+: 224.1410. Calc. for $C_{13}H_{20}O_3$: 224.1412).

The preparation of compounds *3b* and *8b* has been described earlier.[7]

4-Hydroxy-5,6-dihydro-2-pyrones gave when reacted with ethereal diazomethane the 4-methoxy-5,6-dihydro-2-pyrones as well as the 2-methoxy-5,6-dihydro-4-pyrones. The isomeric pyrones were easily separated on preparative TLC (silica gel, ether as eluent), the 5,6-dihydro-4-pyrones having the lower R_F-value. In this manner *9b* and *10b*, both giving satisfactory NMR spectra, were obtained.

*4-Methoxy-6-(*trans*-1-butenyl)-5,6-dihydro-2-pyrone* (9b). R_F 0.59, oil (Found for M+: 182.0941. Calc. for $C_{10}H_{14}O_3$: 182.0943).

*4-Methoxy-6-(*trans*-1-pentenyl)-5,6-dihydro-2-pyrone* (10b). R_F 0.62, oil (Found for M+: 196.1101. Calc. for $C_{11}H_{16}O_3$: 196.1099).

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On the Thermal Decomposition of Thiobenzophenone S-Oxide[1]

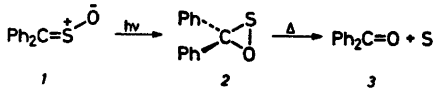
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Dedicated to Professor K. A. Jensen on his 70th birthday

Thiobenzophenone *S*-oxide (*1*) undergoes thermal decomposition in solution around 130 °C in the absence of oxygen to give benzophenone (*3*) and sulfur together with small amounts of thiobenzophenone (*4*) and sulfur dioxide. The reaction was studied by means of differential thermal analysis (DTA) and our results lead us to propose that two initial processes, a ring closure to give 3,3-diphenyloxathiirane and a "dimerization", are operating. Some evidence for a consecutive step, partially involving the starting material, was found. Decomposition of thiobenzophenone *S*-oxide in an oxygen atmosphere affords benzophenone and sulfur dioxide in high yields. In agreement with DT-analyses this reaction is formulated as an oxidation of thiobenzophenone *S*-oxide by molecular oxygen. Mass spectrometric evidence for carbene formation in the gas phase at temperatures above 230 °C has been obtained.

A study of the photolytic conversion of thiobenzophenone *S*-oxide (*1*) into benzophenone (*3*) and sulfur was reported recently, and evidence for the intermediacy of the thermally labile 3,3-diphenyloxathiirane (*2*) was presented.[2]

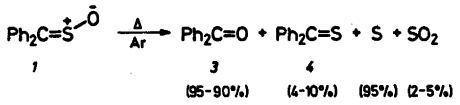


1 *2* *3*

The results of CNDO/B calculations have been interpreted as showing that the electrocyclic ring closure of sulfine giving the three-membered oxathiirane is photochemically as well as thermally allowed.[3] The intermediacy of oxathiiranes has also been proposed for the thermolysis of thiophosgene *S*-oxide [4] and thiopropanal *S*-oxide.[5] Furthermore, oxathiirane *S*-oxides have been postulated as intermediates in the thermal decomposition of thiocarbonyl *S*-dioxides (sulfenes).[6]

We report here a study of the thermal decomposition of thiobenzophenone *S*-oxide (*1*) by means of differential thermal analysis (DTA).[7] The analyses were performed and evaluated as described in the experimental section.

The sulfine *1* is stable at room temperature in the absence of light but decomposes in solution (0.15 M) at around 130 °C under an argon atmosphere to give benzophenone (*3*) (95 %), sulfur (95 %), thiobenzophenone (*4*) (4 %) and sulfur dioxide (2 %). At higher concentrations (0.5 M) the yields were 90, 95, 10 and 5 %, respectively. Yields were not altered by addition of sulfur or sulfur dioxide, or by continuous removal of sulfur dioxide by means of a stream of argon gas.



The DTA curves show only one peak over a wide range of concentrations and heating rates, excluding consecutive reactions with comparable rates.[8]

In diphenylmethane under argon, variation of the initial activation data and the mechanistic coordinates [8-10] was found by increasing the concentration of *1* from 0.12 to 0.52 M (*cf.* Table 1).

Table 1. Kinetic and mechanistic data derived from DTA experiments (argon atmosphere) with different concentrations of *1*. Heating rate $m = 1.58 \pm 0.11$ K/min.

Conc. (M)	Reaction enthalpy $-\Delta H$ (kJ/mol)	Activation energy E_a (kJ/mol)	log k (k in min^{-1})	Shape index S_{corr}	Reaction type index M(J/mol K)	Specific temperature difference *um* (K)
0.115	69.7	310.6	35.9	0.63	8.03	5.2
0.180	69.9	263.3	30.7	0.84	13.81	5.9
0.310	59.7	250.7	29.5	0.87	12.97	6.2
0.430	74.1	146.1	16.6	0.97	21.42	11.0
0.520	81.3	140.2	15.8	0.94	29.46	11.4

From these results (Table 1) it appears that competing first and second order reactions take place, as indicated by the increase in the shape index from 0.63 to 0.97 on increasing the initial concentration of *1*.[10,11] The shape indices for pure first and second order reactions are 0.55 [12] and 1.1,[13] whereas the corresponding reaction type indices are 20.7 [12] and 13.0 [13] (J/mol K), respectively. However, the pronounced increase in the reaction type index from 8.03 to 29.46 (J/mol K) shows that the initial first and second order reactions are followed by secondary reactions, including reaction of the intermediates with the starting materials.[10]

Two pathways are feasible for the initial unimolecular reaction: (a) Ring closure giving the oxathiirane *2*, or (b) CS bond rupture leading to diphenylcarbene and sulfur monoxide, followed by attack of sulfur monoxide on the carbene giving benzophenone and sulfur.

Attempts to trap diphenylcarbene by thermolyzing *1* in 1,1-diphenylethene, which has been found to be an excellent trapping agent for diphenylcarbene,[14] at 150 °C (argon), afforded less than 1 % of 1,1,2,2-tetraphenylcyclopropane (for comparison, 1,1,2,2-tetraphenylcyclopropane could be isolated in 32 % yield when diphenyldiazomethane was thermolyzed in 1,1-diphenylethene [14]). We have also been unable to detect "carbene products", *e.g.* tetraphenylethene, in the reaction mixtures in which diphenylmethane was used as solvent.

Further evidence against carbene formation was obtained from the thermolysis of *1* in gas phase in the ion source of a mass spectrometer. The intensity of the peak at $m/e = 166$ ($M^+ - SO$) relative to that of the peak at $m/e = 214$ (M^+) was almost constant in the temperature range from 50 to 150 °C. However, carbene formation at higher temperature (t = temperature of ion-source; sample temperature *ca.* 20 °C) is indicated by increase in the 166:214 ratio (r):

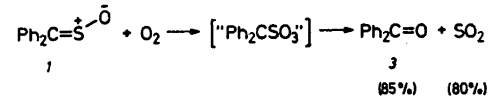
t (°C)	50	120	150	230	250
10 r	1.5	1.6	1.8	3.2	6.5

On the basis of the above results we formulate the initial unimolecular reaction as a ring closure of the sulfine *1* to give the oxathiirane *2*.

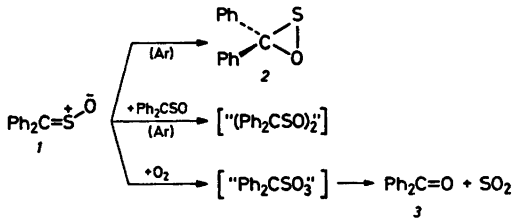
The bimolecular reaction apparently leads to a dimer ("$(Ph_2CSO)_2$") which may decompose further. Walter and Bauer have recently reported a related study of the thermal decomposition of tertiary thioamide *S*-oxides, in which they observed the formation of amide, thioamide and sulfur monoxide.[15] However, independent information concerning the decomposition of the dimer is not available.

Thermolysis of *1* under oxygen was found to proceed *ca.* 4 times faster than under argon. Benzophenone and sulfur dioxide were detected in 85 and 80 % yield, respectively and DT-analyses revealed nearly first order kinetics [$E_a = 181.3 \pm 5.4$ kJ/mol, $\Delta H = -69.5 \pm 9.2$ kJ/mol, S_{corr}: 0.52 – 0.61 and M: 19.50 – 17.91 (J/mol K)].

The reaction is therefore formulated as an oxidation of the sulfine *1* by molecular oxygen, analogous to the reaction between oxygen and thiobenzophenone.[16]



The initial reactions occurring in the thermal decomposition of thiobenzophenone *S*-oxide in solution are thus summarized in Scheme 1.



Scheme 1.

At higher temperatures in the gas phase CS bond rupture leading to diphenylcarbene and sulfur monoxide is probably dominant.

The above mentioned consecutive reactions may involve either the starting material or secondary products such as sulfur allotropes, but it is not possible to further elucidate this reaction sequence on the basis of present data.

EXPERIMENTAL

All reactions were carried out in the absence of light to prevent the known photolytic decomposition [2] of *1*.

Thermolysis of thiobenzophenone S-*oxide (1).* Argon or oxygen was bubbled through 10 ml solutions of *1* (0.15 – 0.50 M) in diphenylmethane for 20 min at 30 °C. The solution was then heated to 150 °C (while maintaining the gas flow) for 30 min. After cooling to room temperature, 10 ml of dichloromethane containing octadecanol (as internal GLC standard) was added and the mixture was submitted to GLC analysis (Pye Unicam 104 chromatograph with dual flame-ionization detector, in conjunction with a Varian Aerograph 477 electronic integrator; 2 m × 6 mm column of 3 % OV1 on Gaschrom Q (100 – 120 mesh); nitrogen as carrier gas). From the chromatograms the amounts of unconverted *1*, benzophenone (*3*), and thiobenzophenone (*4*) could be calculated (calibration curves). The yields of sulfur dioxide were determined by iodine/thiosulfate titration [17] (average of several experiments). The sulfur formed was isolated by filtration of the final mixture, washed with ether and dried *in vacuo*.

The relative rates of benzophenone formation during the thermolyses under argon or oxygen atmosphere, respectively (equal concentrations of *1*) were calculated on the basis of the benzophenone yields found by GLC.

Differential thermal analysis (DTA). (a) *Equipment.* A modified version of the solution DTA apparatus [18] developed in Mülheim/Ruhr [19] was employed. The instrument has Teflon stirring disks, copper/constantan thermocouples and 5 ml test tubes installed in an alumina heating block for the sample and reference solutions. Temperatures and temperature differences were monitored with a two-channel recorder (model BD 6, Kipp and Zonen, Delft, The Netherlands; voltage measurement range 0.02 – 5 mV).

(b) *Procedure.* A solution of thiobenzophenone *S*-oxide (*1*) and the pure solvent (diphenylmethane, reference glass) were thermostated at 350 K. Constant heating rates between 0.75 and 1.5 K/min could be employed, and 1.5 K/min was adopted as standard heating rate.

(c) *Evaluation.* The DTA curves were analyzed using a FORTRAN IV program and a PDP 10 computer (Digital Equipment Corp., Maynard, Mass., U.S.A.).[13] The algorithm is based on the Borchardt-Daniels equation [7] and the Arrhenius law, and yields both initial and overall activation parameters. The program takes account of temperature dependence of the cell constant, c, and the decreasing significance of the rate constants as a function of increasing extent of reaction. Furthermore, the half-width, h, and the shape index, S, of the DTA curves are extrapolated to $c \to \infty$ and zero temperature change, corresponding to a reaction without temperature feedback and with the same activation parameters.[12]

The reaction type index, M, is calculated from eqn. 1:

$$M = \frac{mRu\ (\ln 10)^2}{h_\infty n^{0.52}} \qquad (1)$$

where n = reaction order, h_∞ = corrected half width, m = heating rate, and u = specific time.[12,13]

Non-compatibility of a reaction with an elementary first- or second order process is indicated:

1. By the mechanistic data, since $S = 0.55$ and $M = 20.7$ J/mol K for a first and $S = 1.10$ and $M = 13.0$ J/mol K for a second order curve.
2. By correlation coefficients for the straight lines over the entire range of < 0.97. For elementary reactions a value of 0.99 – 1.00 is usual.
3. By a dependence of the specific time, u, on the reactant concentration.
4. By differences in the u values for the initial and the complete reaction range.

For complex processes the interpretation is based on comparison with the results of model calculations.

The enthalpies were obtained from the integrated curve areas:

$$\Delta H = \frac{cC_p}{n_0} \int_0^\infty \theta\, dt \qquad (2)$$

ΔH = reaction enthalpy, C_p = heat capacity, n_0 = amount of reactant, θ = measured temperature difference.

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Photochemistry of Sulfur Compounds Related to the 1,2-Dithiole System. Part VII. Photoisomerization of α-(1,2-Dithiol-3-ylidene)-cycloalkanones

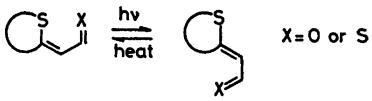
CHRISTIAN LOHSE and CARL TH. PEDERSEN *

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Dedicated to Professor K. A. Jensen on his 70th birthday

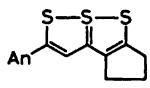
A series of α-(1,2-dithiol-3-ylidene)cycloalkanones are transformed upon irradiation into photoproducts which possess a *trans*-1,2-dithiolylidene ketone structure. The photoproducts revert to starting materials by a dark process obeying first order kinetics. The stability of the *trans* form has been shown to be controlled mainly by steric factors; it is strongly dependent on the ability of the carbonyl group to be coplanar with the dithiole ring. A parallel was observed between the variation of the rate constant for the back reaction with ring size, and the variation of the ^{13}C chemical shift of the carbonyl carbon in the *cis* form with ring size.

It has been shown in several cases that compounds of the general type *1* upon irradiation are transformed into photoproducts, which possess the *trans* configuration *2*.[1]



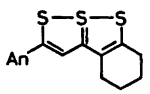
1 *2*

During a study of the photochemistry of 1,6,6aλ^4-trithiapentalenes [4] a great difference was observed in the rate constants for the thermal back reaction of the *trans* forms of the two compounds *3* and *4*.



3

k = 5.17 S^{-1}

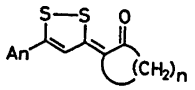


4

k = 10500 S^{-1}

This difference can be explained by steric strain in the *trans* forms introduced by the condensed cycloalkane rings.

It was found of interest to study whether the same effect was operative in 1,2-dithiol-3-ylidene cycloalkanones *5*, and to study the influence of the ring size on the rate constants



An = 4-$CH_3OC_6H_4$

5

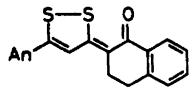
for the dark reaction. Therefore, a series of α-(1,2-dithiol-3-ylidene)cycloalkanones *5a* — *5k* with *n* ranging from 3 to 13 were irradiated, and the rate constants for the thermal back reaction were measured.

RESULTS AND DISCUSSION

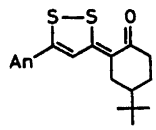
All compounds upon irradiation in ethanolic solution were transformed into photoproducts, which decayed to starting materials by a dark process obeying first-order kinetics. The rate

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Table 1. Rate constants for thermal *cis-trans*-isomerization and ^{13}C chemical shift of carbonyl carbon of *5*.



5j



5k

Compound	*n*	k_{EtOH}/ $10^{-5}s^{-1}$ [a]	δ-$^{13}C(>CO)$/ ppm	M.p. [b]/ °C
5a	3	4.00	201.74	Ref. 9
5b	4	233	189.39	Ref. 8
5c	5	27.2	195.05	Ref. 9
5d	6	75.9	193.55	Ref. 9
5e	7	140	192.77	130–131
5f	8	173	191.35	137–138
5g	9	146	191.47	151–152
5h	10	149	191.61	153–154
5i	13	61.0	191.80	132–133
5j		42.8	179.85	172–173
5k		118	189.39	156–157

[a] Rate constants for the thermal back reaction obtained from 10^{-5} M solutions in 96 % ethanol. [b] All compounds show satisfactory elemental analyses (C, H, S).

constants for this reaction are given in Table 1.

When irradiations were carried out in polymethacrylate matrices it was observed that the photoproducts formed were stable and reverted to starting materials by prolonged heating only. This supports the *trans* structure of the photoproducts in the same way as discussed for simple 1,2-dithiolylidene ketones.[2]

The difference in rate constants for *5a* and *5b* is substantial.

Models of the *trans* forms of the two compounds show that the carbonyl group in *5a*, Fig. 1, is forced by the cyclopentane ring to be coplanar with the dithiole ring. If the rate constant for *5a*, 4×10^{-5} s^{-1} is compared to the

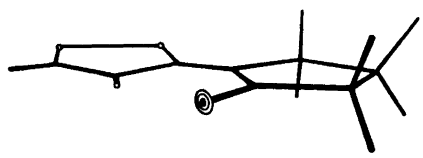


Fig. 1. Model of the *trans* form of *5a*.

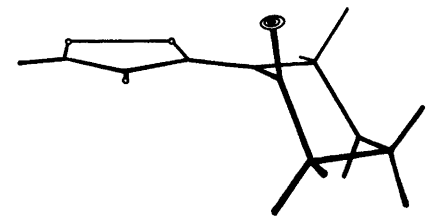
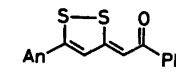


Fig. 2. Model of the *trans* form of *5b*.

rate constant of *6*,[2] 9.6×10^{-3} s^{-1} it is obvious that the coplanarity forced by the cyclopentane



6

ring stabilizes the *trans* form. It has been shown that electronic factors only play a minor role in determining the rate constants of the back reaction.[3]

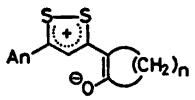
If we look at a model of the *trans* form of *5b* with the cyclohexane ring in a chair conformation, Fig. 2, it is seen that the carbonyl group in the *trans* form is forced out of the dithiole plane at an angle of *ca.* 60° which means that the *trans* form in this case loses resonance stabilization from the dithiole ring. This is reflected in the higher rate constant for the dark reaction.

With larger rings the system is less rigid and it is again possible for the carbonyl to come close to coplanarity with the dithiole ring, which is equivalent to a gain in stability of the *trans* form. However, with these larger rings a new destabilizing factor is introduced, *i.e.* the hydrogen-hydrogen interaction in the rings which may counteract the possibility of coplanarity of the carbonyl group. This is observed as an increase in the rate constant of the series *5c* to *5f*. The strain introduced by the hydrogen-hydrogen interaction reaches its maximum for the ten-membered ring and then decreases.

It is not possible to make a detailed analysis of the influence of this hydrogen-hydrogen interaction since, as far as we know, no studies of the conformation of such α-methylene substituted cyclic ketones have been published.

The variation in rate constants in the series *5c* to *5i* could be the result of small variations

in the polarization of the carbonyl group in the *trans* forms. This will give rise to forms such as *7* which lower the double bond character of



7

the bond between the dithiole ring and the side chain and thereby give a more free rotation round this bond.

If one considers the ^{13}C chemical shift of the carbonyl carbon atom in the *cis* α-(1,2-dithiol-3-ylidene)cycloalkanones,[3] it is observed that the ^{13}C chemical shift varies in the same manner with ring size as do the life times of the *trans* dithiolylidene cycloalkanones (*cf.* Fig. 3). On the other hand this variation finds a parallel in the variation of the ^{13}C chemical shift of the carbonyl carbon atom in the simple cycloalkanones,[4,5] where it is assumed that the variation is due to sterical interactions in the ring.

The polarization in the carbonyl group o the *cis* form is probably very different from the polarization of the *trans* form due to the strong sulfur-oxygen interaction in the *cis* form.[6,7] We will therefore assume that the variation in rate constant for the dark reaction in the series *5c* to *5i* is caused by variations in the sterical interactions in the rings.

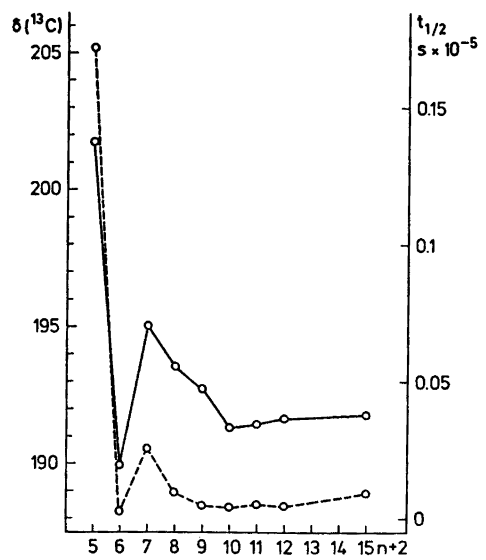


Fig. 3. Variation of half-life time of the *trans* form of *5* with ring size – – – and variation of ^{13}C chemical shift of carbonyl carbon of *5* with ring size ———.

The low rate constant of *5j* (of the same magnitude as that of *6*) compared to *5b* can be explained by the stabilizing effect of the condensed benzene ring, which in the *trans* form is still planar with the carbonyl group.

For $n = 8$ and 9 it is observed that the decay of the photoproduct does not exactly obey first order kinetics. For these two compounds we are probably observing two decays, the decay mentioned in the table and a decay which is somewhat faster. The fast decay is for *5f ca.* 900×10^{-5} s^{-1} and for *5g ca.* 1000×10^{-5} s^{-1}. We have no explanation for these two fast decays, but it can be ruled out that they are caused by the presence of impurities.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded on a Jeol FX60 Fourier Transform NMR spectrometer operating at 15.03 MHz with deuterium internal lock. Proton decoupled spectra were obtained using noise decoupling and 8K data points. The pulse length used was 6 μs corresponding to a flip angle of 60°. The chemical shifts are given an accuracy of 0.07 ppm. $CDCl_3$ was used as solvent with TMS as internal standard.

The rate constants for the *trans-cis*-isomerization were obtained from 10^{-5} M ethanolic solutions by a flash photolytic equipment described previously.[2] The rate constants were reproducible within 10 % except for *5f* and *5g* where the uncertainty was 20 %.

α-(1,2-Dithiol-3-ylidene)cycloalkanones were prepared from 3-(*p*-methoxyphenyl)-1,2-dithiolylium hydrogen sulfate and cycloalkanones as described.[8–10] All compounds were recrystallized from 2-methoxyethanol.

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On the Formation and Beckmann Rearrangement of *N*-Cyanatoimines. Crystal Structure of 2-Phenyl-4(3*H*)-quinazolinone

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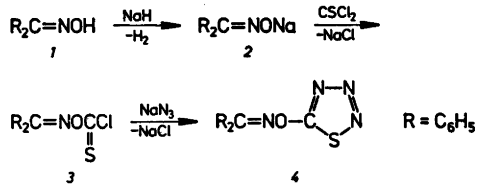
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Dedicated to Professor K. A. Jensen on his 70th birthday

N-Cyanato(diphenylmethanimine) (*5*) has been generated in carbon tetrachloride solution from the corresponding thiatriazole (*4*). It is thermally unstable isomerizing rapidly in a Beckmann type rearrangement to *N*-phenylbenzimidoyl isocyanate (*6*). This is also thermally unstable but was characterized by infrared spectroscopy and the reaction with ethanol. *N*-Phenylbenzimidoyl isocyanate undergoes intramolecular electrophilic substitution in carbon tetrachloride with formation of *7*. A three-dimensional single-crystal X-ray structure determination at 85 K has proved *7* to be 2-phenyl-4(3*H*)-quinazolinone, thus settling the discussion on the exact structure of this compound. The crystals are monoclinic, space group $C2/c$ with cell dimensions at 85 K: $a = 30.095(13)$ Å, $b = 4.964(2)$ Å, $c = 16.630(4)$ Å, $\beta = 121.50°(3)$, with eight molecules per unit cell. The positional parameters and isotropic temperature factors for all atoms have been refined to a conventional *R* index of 9.7 % for 1322 reflections.

In continuation of studies on the chemistry of thiatriazoles and cyanates we have investigated the thermal decomposition of the hitherto unknown 5-(diphenylmethaniminoxy)-1,2,3,4-thiatriazole (*4*) with the intention of obtaining an *N*-cyanatoimine (*5*). This type of compound has barely been studied although Grigat and Pütter assumed that the product obtained by treating *p*-quinonedioxime with cyanogen chloride in the presence of base was the corresponding quinone di(*N*-cyanatoimine).[1] However, the product was not characterized or investigated because of its highly explosive nature.

Compound *3* was prepared *in situ* according to the method of Cross *et al.*[2] Upon reaction with sodium azide compound *4* was obtained (39 %) (Scheme 1). It is a thermally unstable

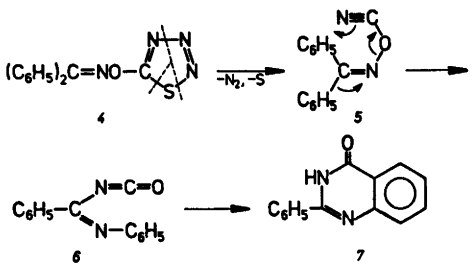


Scheme 1.

compound, melting and decomposing almost explosively around 130 °C. The infrared spectrum is devoid of an azide band in the 2100—2200 cm^{-1} region [3] which unambiguously proves it to be a thiatriazole and not a thioacyl azide.[3,4] Thermal degradation in boiling CCl_4 results in precipitation of a white solid which is identified as 2-phenyl-4(3*H*)-quinazolinone (*7*) (Scheme 2).

Although 2-substituted 4-quinazolinones have been known for a long time, considerable doubt has existed as to which of the three possible tautomers is actually the dominant. Recently, these structures have been formulated as 2-substituted 4-hydroxyquinazoles,[5] 2-substituted 4(1H)-quinazolinones [6] and 2-substituted 4(3*H*)-

* On leave from Department of Pharmacy, University of Oslo, Oslo 3, Norway.



Scheme 2.

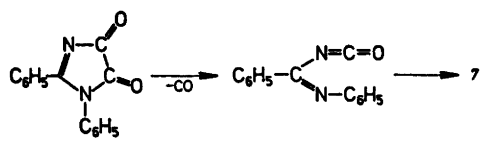
quinazolinones.[7,8] The assignment of the latter structure to the phenyl derivative (*7*) is based on energetic considerations, since 1-methyl-2-phenyl-4(1*H*)-quinazolinone was shown to undergo intramolecular rearrangement to 3-methyl-2-phenyl-4(3*H*)-quinazolinone at 260–305 °C.[7] This assignment was supported by IR, UV and ^{1}H NMR studies of *7*. As such studies, however convincing, can rarely be considered proof, we have undertaken a three-dimensional single-crystal X-ray structure determination at 85 K to settle the question once and for all (*vide infra*).

Transients in the formation of *7* from *4* was studied by monitoring the degradation of *4* in CCl_4 by infrared spectroscopy (Scheme 2). On boiling the rise and subsequent disappearance of a strong doublet at 2256 and 2220 cm^{-1} is observed. The first step in the sequence leading to *7* is expected to be loss of nitrogen and sulfur with formation of *5*, the extrusion reaction being common to all hitherto known thiatriazoles.[9] Formation of *5* may be succeeded by isomerization of the cyanate group in analogy with alkyl cyanates which are known to undergo isomerization a reaction which has never been demonstrated for aryl cyanates.[10] Group frequencies for both the cyanate (compound *5*) and the isocyanate group (compound *6*) are reported to be observed in the 2200–2300 cm^{-1} region,[11] thus excluding distinction between compound *5* and *6*. However, significant observations are made in the 700 cm^{-1} region. Generally two very strong single bands appear between 700 and 800 cm^{-1} in the spectra of monosubstituted benzene derivatives.[12]* Accordingly compound *1* as well as *4* exhibit a strong single band at 695 cm^{-1} since the phenyl groups in these compounds are identically attached. During the degradation reaction a doublet of strong intensity is observed at 695 and 690 cm^{-1} indicating the presence of two differently attached phenyl groups. We thus favor structure *6* over structure *5* for the transient since *5* would be expected to exhibit a single band in this region. The separation of *5* cm^{-1} between the two bands compares favorably with published data for related molecules.[13] Obviously the primarily formed cyanate *5* is unstable isomerizing to *6* in a reaction recognized as a Beckmann type rearrangement.[14] Migration of the phenyl group (*5*→*6*) is accompanied by migration and rearrangement of the cyanate group. It is not known whether an electron-deficient moiety (=N$^+$) is intermediate or whether rearrangement of –OCN to –NCO is a cyclic process. On the other hand allylic cyanates isomerise very rapidly to isocyanates by a cyclic mechanism[15] while simple alkyl cyanates are reasonably stable isomerizing *via* an ion-pair mechanism.[16] In fact the rapid isomerization of allylic cyanates prevents their isolation; only rearranged products are obtained on attempted preparation.[15] In a likewise manner a cyclic transition state of relatively low charge separation (*8*) may explain the rapid isomerization of *5* to *6* in boiling non-polar CCl_4.



8

Isocyanate *6* exhibits a remarkable reactivity resulting in an intramolecular electrophilic attack on one of the phenyl groups. The same reaction was observed earlier by Goerdeler and Sappelt in the thermal degradation of imidazolindiones (*9*) strongly supporting the present results (eqn. 1).[17]

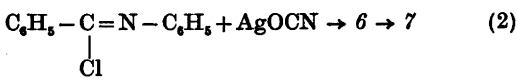


(1)

Compound *6* was not isolated or characterized by the latter investigators.

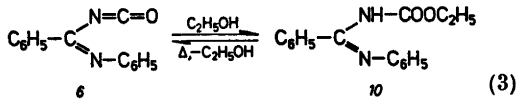
* In the present case only the 700 cm^{-1} band is observed because of the solvent (CCl_4) employed.

Further evidence for the reactivity of *6* is obtained from the reaction between *N*-phenylbenzimidoyl chloride and silver cyanate in acetonitrile. Obviously the first step will be formation of *6* but as expected compound *7* is the end product isolated in 13 % yield (eqn. 2).



$$C_6H_5-C(Cl)=N-C_6H_5 + AgOCN \rightarrow 6 \rightarrow 7 \qquad (2)$$

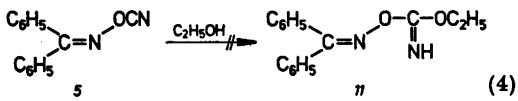
Support for the structure assignments of the intermediates was also obtained from thermal degradation of *4* in ethanol. The product obtained (*10*) is characterized as a reaction product between *6* and ethanol by elemental and mass spectrometric (M^+ 268 *m/e*, calc. 268) analysis (eqn. 3).



$$C_6H_5-C(N=C=O)=N-C_6H_5 \underset{\Delta,-C_2H_5OH}{\overset{C_2H_5OH}{\rightleftharpoons}} C_6H_5-C(NH-COOC_2H_5)=N-C_6H_5 \qquad (3)$$

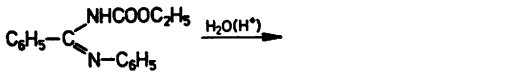
6 *10*

The alternative structure *11* (eqn. 4) is ruled out since the product on hydrolysis in boiling N hydrochloric acid for 1.5 h gives rise to benzoic acid (yield 90 %, eqn. 5). Oxime *11* would afford benzophenone in analogy with benzophenone oxime, which is hydrolyzed quantitatively to benzophenone in aqueous acid.[18]

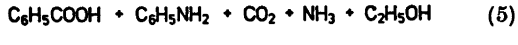


$$(C_6H_5)_2C(OCN)=N \xrightarrow{C_2H_5OH} (C_6H_5)_2C(O-C(=NH)-OC_2H_5)=N \qquad (4)$$

5 *11*



$$C_6H_5-C(NHCOOC_2H_5)=N-C_6H_5 \xrightarrow{H_2O(H^+)}$$



$$C_6H_5COOH + C_6H_5NH_2 + CO_2 + NH_3 + C_2H_5OH \qquad (5)$$

Finally compound *10* splits off ethanol on heating above the m.p. (101 – 102 °C) (eqn. 3) with formation of *7* (see Scheme 2) in agreement with the structure assignment.

Ghadiali and Shah has described compound *10* as an oil obtained from *N*-phenylbenzimidoyl chloride and sodium ethylcarbamate.[19] We have, however, not been able to reproduce their results.

CRYSTALLOGRAPHIC SECTION

Experimental and structure determination. Film diagrams indicated monoclinic symmetry, and the cell volume implied eight molecules in the unit cell. The systematic absences are those of space groups $C2/c$ and Cc. The later structure determination and refinement showed the space group to be the centrosymmetric $C2/c$.

An automatic Picker four-circle diffractometer with graphite-monochromatized Cu$K\alpha$ radiation, equipped with a modified Enraf-Nonius low-temperature device, (liquid N_2) was utilized for preliminary experiments and for the recording of intensity data. The temperature was kept constant within 0.5° at 85 K. A flat, needle-shaped crystal of approximate dimensions 0.3 × 0.05 × 0.02 mm was used for the data collection. The unit cell parameters were determined by a least-squares treatment of the 2θ-values of 17 reflections (2θ from 36 to 103°).

Three-dimensional intensity data were recorded with short (0.083°) ω-scans near the peak positions using a scan speed of 0.25° min^{-1}. Backgrounds were estimated on the basis of a series of background measurements for varying 2θ values. This method of data collection was chosen in order to improve the counting statistics. The intensities of two standard reflections which were remeasured after every 200 reflections showed no significant variation.

Estimated standard deviations for the intensities were obtained from the expression $\sigma(I) = [I + (0.02\ I)^2]^{\frac{1}{2}}$. Of the 1693 unique reflections measured ($2\theta_{max} = 130°$) 1322 had intensities greater than $2\sigma(I)$. These were regarded as observed reflections and the remaining 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 [20] for carbon, nitrogen and oxygen, and of Stewart *et al.*[21] for hydrogen.

The phase problem was solved by direct methods utilizing the MULTAN program assembly.[22] When the structure model had been refined to R-factor = 0.12 the hydrogen atoms were placed in calculated positions and included in the refinement. Fullmatrix least squares refinement of all positional parameters and isotropic temperature factors for all atoms resulted in a conventional R of 0.097 and a weighted R_w of 0.084. The function minimized was $S = (\Delta F)^2/\sigma^2(F_o)$.

A difference electron density map showed only spurious peaks of maximum height 0.6 e Å^{-3}. Atomic parameters are given in Table 1. A listing of calculated and observed reflections is available from Department of Chemistry, University of Oslo, Oslo 3, Norway. (Also available from the authors upon request.)

Table 1. Fractional atom coordinates and thermal parameters with estimated standard deviations.

Atom	*x*	*y*	*z*	*B*
N(1)	.1524(2)	.1598(9)	.1765(3)	2.47(9)
C(2)	.1240(2)	.3291(12)	.1123(4)	2.80(11)
N(3)	.0706(1)	.3556(9)	.0701(3)	1.89(8)
C(4)	.0422(2)	.2034(12)	.0957(4)	2.97(11)
C(5)	.0718(2)	−.0021(12)	.1678(4)	2.66(11)
C(6)	.1248(2)	−.0138(11)	.2054(4)	2.69(11)
C(7)	.1538(2)	−.2068(11)	.2743(4)	2.47(10)
C(8)	.1304(2)	−.3775(11)	.3059(4)	2.46(10)
C(9)	.0764(2)	−.3642(12)	.2687(4)	2.81(11)
C(10)	.0468(2)	−.1722(12)	.1995(4)	2.83(11)
C(11)	−.0063(1)	.2418(8)	.0585(3)	3.10(8)
C(12)	.1503(2)	.5232(11)	.0814(3)	2.38(10)
C(13)	.2052(2)	.5456(12)	.1363(4)	2.61(11)
C(14)	.2297(2)	.7317(11)	.1088(4)	2.40(10)
C(15)	.2004(2)	.8871(12)	.0295(4)	2.74(11)
C(16)	.1471(2)	.8642(12)	−.0242(4)	3.03(12)
C(17)	.1223(2)	.6817(11)	.0018(4)	2.74(11)
H(3)	.0574(18)	.532(10)	.0095(32)	3.3(12)
H(7)	.1899(19)	−.210(11)	.2976(34)	3.9(13)
H(8)	.1539(18)	−.494(11)	.3580(33)	3.2(12)
H(9)	.0623(21)	−.465(12)	.2935(39)	4.9(15)
H(10)	.0129(24)	−.180(13)	.1710(43)	7.1(19)
H(13)	.2278(17)	.4531(100)	.1975(32)	2.8(11)
H(14)	.2687(19)	.751(10)	.1524(34)	3.5(12)
H(15)	.2171(20)	.993(12)	.0117(35)	4.6(15)
H(16)	.1244(21)	.958(12)	−.0871(40)	6.2(17)
H(17)	.0788(22)	.669(12)	−.0479(40)	6.0(16)

Crystal data

2-Phenyl-4(3*H*)-quinazolinone $C_{14}H_{10}N_2O$, monoclinic, space group: $C2/c$. Cell dimensions at 85 K: $a=30.095(13)$ Å, $b=4.964(2)$ Å, $c=16.630(4)$ Å, $\beta=121.50°(3)$, $V=2118.3$ Å^3. $M=222.2$, $Z=8$, $D_{calc}=1.394$ g cm^{-3}, $F(000)=928$. Systematically absent reflections: hkl for $h+k$ odd, $h0l$ for l odd.

Description of the structure. A view of the molecule is given in Fig. 1, where the atomic numbering also is indicated. Bond lengths and bond angles are listed in Table 2. The bond lengths and angles do not deviate significantly from those found for similar compounds.[23]

The heterodicycle is planar (see Table 3) with the plane of the phenyl ring (C(12)–C(17)) tilted 14.4° relative to this plane. The dihedral angles N(1)–C(2)–C(12) C(13) and N(3)–C(2)–C(12)–C(13) are −12.1 and 165.9°, respectively.

The intermolecular N(3)–O(11) hydrogen bond of 2.827(12) Å is of the type and length usually found for this type of molecules.[24]

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer instrument Model 21 calibrated against CO_2. Elemental analyses were found in agreement with calculated values for all stable compounds.

Preparation of 5-(diphenylmethaniminoxy)-1,2,3,4-thiatriazole (4). A solution of *3*[2] was prepared by adding the sodium salt of benzophenone oxime (0.12 mol prepared from the oxime and sodium hydride) to a stirred solution of thiophosgene (0.12 mol) in tetrahydrofuran (250 ml) initially cooled to 0 °C at such a rate that the temperature did not exceed 10 °C. After stirring at 10 °C for an additional 15 min, a solution of sodium azide (18 g) in ice-water (250 ml) was added at a rate which kept the temperature below 10 °C. Following this addition the stirring was continued for 30 min at 10–15 °C and water (150 ml) and ether (150 ml) were added. After washing with ice-water (3 × 50 ml) the ether layer was dried over $MgSO_4$ at 0 °C and evaporated *in vacuo* to give a semisolid material. A crystalline material (13.1 g, 39 % based on *2*) was obtained after digestion with hexane:ether (2:1, 50 ml) followed by hexane:ether (4:1, 50 ml). Further purification was achieved by digestion with

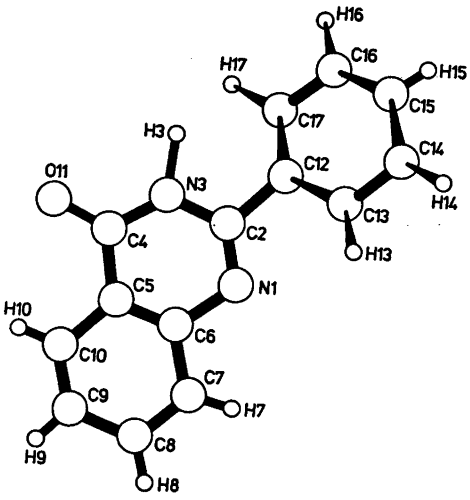


Fig. 1. The molecule projected on the plane of the dicycle, with the atomic numbering indicated.

cold ethanol to give an almost colorless material which was used for the subsequent experiments. An analytically pure sample was obtained by recrystallization from ether at −80 °C. M.p. *ca.* 130 °C (decomp.). IR(CCl_4): 3070 w, 1495 d,w, 1458 vs, 1445 s, 1330 m, 1310 m, 1288 s, 1229 w, 1056 w, 978 m, 930 m, 870 s, 695 s, 632 w, 604 w.

Thermal decomposition of 4 *in boiling* CCl_4. (a) Decomposition monitored by infrared spectroscopy. Thiatriazole *4* (1.0 g) in CCl_4 (10 ml) was kept at reflux temperature and samples (0.5 ml) for infrared analysis were taken at intervals. Nitrogen evolution was immediately observed but had diminished considerably after 10 min and practically stopped after 15 min. The infrared spectra exhibit a weak doublet at 2256 and 2220 cm^{-1} which had developed to a strong band in 10 min. However, the band at 1485 cm^{-1} arising from *4* is still observable. The latter band is absent after 25 min, but the bands at 2256 and 2220 cm^{-1} had decreased slightly and crystalline *7* had begun to separate from the solution. This compound is insoluble in cold CCl_4 as evidenced by lack of the strong $\nu_{C=O}$ band at 1670 cm^{-1} (see below) in the above solution. The spectrum in CCl_4 recorded after 25 min boiling may thus be the spectrum of compound *6* solely: 3070 w, 2256 vs, 2220 s, 1705 s, 1629 s, 1596 s, 1490 m, 1446 m, 1314 w, 1270 m, 1201 m, 1123 w, 1074 w, 1027 w, 1002 w, 982 s, 695 and 690 d, vs, and 670 m. (b) Thiatriazole *4* (1.0 g) in CCl_4 (10 ml) was refluxed for 1 h. After cooling the solid material

Table 2. Bond lengths (Å) and bond angles (°), with estimated standard deviations.

Bond lengths		Bond angles	
N(1)−C(2)	1.273(7)	C(2)−N(1)−C(6)	115.1(5)
C(2)−N(3)	1.385(8)	N(1)−C(2)−N(3)	125.4(6)
N(3)−C(4)	1.364(8)	N(1)−C(2)−C(12)	118.0(6)
C(4)−C(5)	1.468(8)	N(3)−C(2)−C(12)	116.6(5)
C(5)−C(6)	1.377(9)	C(2)−N(3)−C(4)	122.7(5)
N(1)−C(6)	1.442(8)	N(3)−C(4)−C(5)	115.4(6)
C(6)−C(7)	1.396(8)	N(3)−C(4)−O(11)	120.9(5)
C(7)−C(8)	1.372(9)	C(5)−C(4)−O(11)	123.8(6)
C(8)−C(9)	1.405(10)	C(4)−C(5)−C(6)	117.6(6)
C(9)−C(10)	1.398(8)	C(4)−C(5)−C(10)	120.7(6)
C(5)−C(10)	1.402(9)	C(6)−C(5)−C(10)	121.7(5)
C(4)−O(11)	1.267(8)	N(1)−C(6)−C(5)	123.8(5)
C(2)−C(12)	1.497(9)	N(1)−C(6)−C(7)	117.4(6)
C(12)−C(13)	1.414(9)	C(5)−C(6)−C(7)	118.8(6)
C(13)−C(14)	1.398(9)	C(6)−C(7)−C(8)	120.8(6)
C(14)−C(15)	1.377(8)	C(7)−C(8)−C(9)	120.6(5)
C(15)−C(16)	1.374(10)	C(8)−C(9)−C(10)	119.2(6)
C(16)−C(17)	1.380(9)	C(5)−C(10)−C(9)	119.0(6)
C(12)−C(17)	1.384(7)	C(2)−C(12)−C(13)	118.5(5)
Hydrogen bond:		C(2)−C(12)−C(17)	121.8(6)
N(3)−O(11)($-x$,1$-y$,$-z$)	2.827(12)	C(13)−C(12)−C(17)	119.7(6)
		C(12)−C(13)−C(14)	118.5(5)
		C(13)−C(14)−C(15)	120.1(6)
		C(14)−C(15)−C(16)	121.4(6)
		C(15)−C(16)−C(17)	119.4(6)
		C(12)−C(17)−C(16)	120.9(6)

Table 3. Deviations from least-squares planes (Å × 10^3). The deviations for those atoms used to define the plane are given in italicized numbers.

Atom	Plane I	Plane II	Atom	Plane I	Plane II
N(1)	*18*	−178	C(10)	*−2*	333
C(2)	*0*	37	O(11)	*29*	833
N(3)	*−29*	345	C(12)	63	*4*
C(4)	*−4*	491	C(13)	398	*−3*
C(5)	*−17*	225	C(14)	490	*0*
C(6)	*8*	−80	C(15)	248	*1*
C(7)	*0*	−332	C(16)	−86	*0*
C(8)	*3*	−246	C(17)	−180	*−2*
C(9)	*−6*	80			

was isolated by filtration. Recrystallization from benzene (15 ml), followed by washing with cold benzene (2 × 3 ml) and hexane gave *7* (0.3 g, 36 %). M.p. 241 – 242 °C. Lit. 241 °C.[25]

Formation of 7 *from* N-*phenyl benzimidoyl-chloride and silver cyanate.* *N*-Phenylbenzimidoyl chloride (1 g) and silver cyanate (1 g) in dry acetonitrile (10 ml) were heated to reflux for 1 h. The solvent was removed *in vacuo* and the remaining material taken up in a mixture of methylene chloride and an aqueous solution of sodium thiosulfate. The phases were carefully mixed by shaking, the methylene chloride layer dried over $MgSO_4$ and the solvent removed by evaporation *in vacuo*. The impure material obtained was recrystallized from benzene to yield *7*, (0.13 g, 13 % based on *N*-phenyl-benzimidoyl chloride). M.p. 239 °C. Lit. 241 °C.[25] The infrared spectrum (KBr) which exhibits a strong band at 1670 cm^{-1} ($\nu_{C=O}$) and two characteristic strong bands at 768 and 693 cm^{-1} due to phenyl [13] is identical with the infrared spectrum of *7* obtained from *4* (see above).

Thermal decomposition of 4 *in ethanol. Formation of* 10. Thiatriazole *4* (1.0 g) in abs. ethanol (5 ml) and 2 drops of triethylamine was heated to reflux for 30 min. Evaporation of the solvent *in vacuo* gave a mixture of sulfur and an oil which was extracted with ether. Filtration and evaporation of the ether left a slowly crystallizing oil (0.98 g), which was purified by dissolving in ether (10 ml) and extraction of the ether phase with 4 N hydrochloric acid (10 ml). The aqueous phase was extracted several times with ether and finally neutralized with 2 N sodium hydroxide (20 ml) causing separation of *10* which was taken up in ether. The ether phase was washed with water, dried over $MgSO_4$ and evaporated to dryness. The resulting oil (*10*) solidified rapidly, was digested with hexane and recrystallized from hexane/benzene. M.p. 101 – 102 °C.

Hydrolysis of 10. Compound *10* (5 × 10^{-4} mol) was dissolved in N hydrochloric acid (5 ml) and refluxed for 1.5 h. A crystalline material (40 mg), m.p. 120 – 121 °C, was obtained on cooling in ice. This was identified as benzoic acid by comparison with an authentic sample. (IR spectra, mixture m.p.). Ether extraction of the hydrolysis mixture gave an additional 14 mg benzoic acid (m.p. 121 – 122 °C) after recrystallization from water thus giving a combined yield of benzoic acid of 90 % based on *10*.

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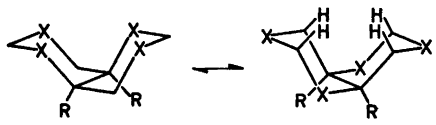
Reactions between Formaldehyde and Polyhydric Alcohols. IV.* Cyclic Acetals from *meso*- and D,L-2,3-Dimethylbutane-1,2,3,4-tetrol and Paraformaldehyde

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Dedicated to Professor K. A. Jensen on his 70th birthday

The acid catalyzed condensations of *meso*- and D,L-2,3-dimethylbutane-1,2,3,4-tetrol with paraformaldehyde have been studied. Possible reaction pathways are discussed and the results obtained by conformational analysis of two bicyclic acetals formed by these reactions are presented.



ΔG°_{300} (*1a*) = 0.0 kJmol^{-1}

ΔG°_{300} (*2a*) > +5.5 kJmol^{-1}

ΔG°_{300} (*2b*) > -11.2 kJmol^{-1}

Scheme 1. Chair-chair interconversions of *1a* (X = CH_2), *2a* (X = O) and *2b* (X = O). (*a*: R = H; *b*: R = CH_3).

Whereas only one chair-chair conformation is possible for *cis*-decalin (*1a*), the compound 2,4,7,9-tetraoxa-*cis*-decalin (*2a*) can adopt two different chair-chair conformations(*O*-inside and *H*-inside, Scheme 1), as pointed out by Mills.[2] On the basis of calculated and measured dipole moments and ^{1}H NMR measurements, Lemieux and Howard [3] have concluded that compound *2a* in benzene solution at 25 °C exists in the *O*-inside conformation to an extent of at least 90 %, corresponding to $\Delta G^\circ_{300} > 5.5$ kJ mol^{-1} for the conformational equilibrium.

The energetic and conformational consequences of the introduction of substituents in the 4- and 5-positions of 1,3-dioxane have been studied in detail by Eliel *et al.*[4,5] and from their results it can be calculated that the introduction of methyl groups at C-1 and C-6 in *cis*-tetraoxadecalin * should destabilize the *O*-inside conformation of *2b* by *ca.* 17 kJ mol^{-1} relative to the *O*-inside conformation of *2a* (Scheme 1).

In the course of studies of the reactions between formaldehyde and polyhydric alcohols we have therefore synthesized compound *2b* in order to test whether this destabilization of the *O*-inside conformation is sufficient to enforce the *H*-inside conformation of *2b*.

A second aim of our investigations has been to determine pathways for the reaction of formaldehyde with polyhydric alcohols so as to establish the mechanism whereby sugar alcohols undergo acetal formation. Rules for the prediction of the products, derived from a study of the structures of the glycitols,[2,6–8] have been known for decades but the mech-

* Part III, see Ref. 1.

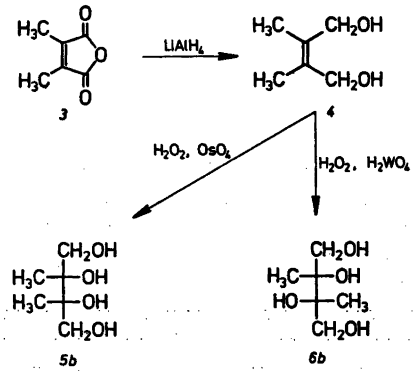
* The positional numerals are omitted for the sake of brevity.

anistic interpretation of these rules remains as a topic for future research.

The reactions between formaldehyde and the tetrols *meso*-erythritol (*5a*) [9,10] and L-threitol (*6a*) [8,11] have been studied previously.

RESULTS AND DISCUSSION

In order to synthesize *meso*- and D,L-2,3-dimethylbutanetetrol (*5b* and *6b*) with unambiguous stereochemistry the sequence shown in Scheme 2 was chosen.

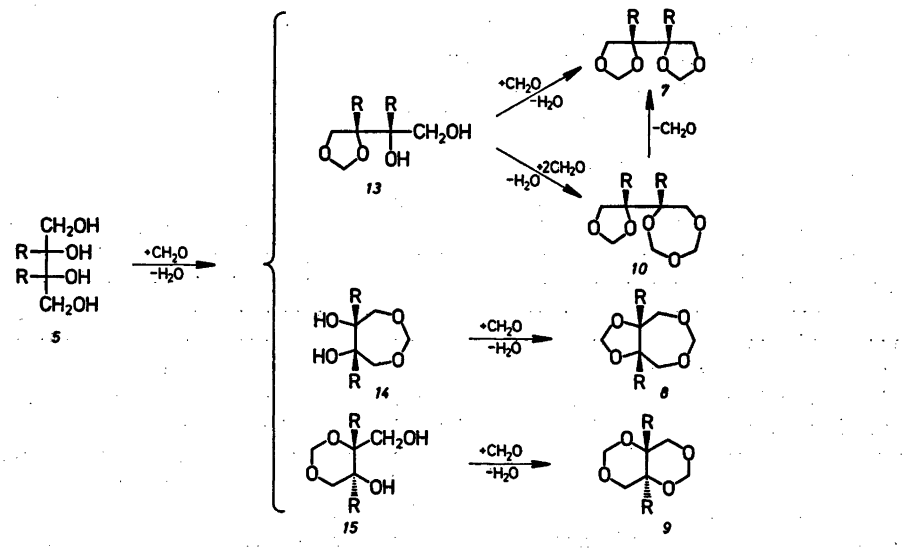


Scheme 2. Synthesis of *meso*- and D,L-2,3-dimethylbutanetetrol.

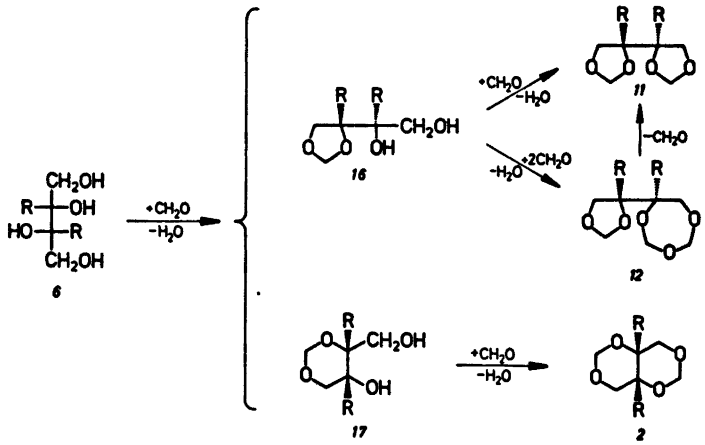
Reactions between 2,3-dimethylbutanetetrols and paraformaldehyde. The condensations of the tetrols (*5b* and *6b*) with paraformaldehyde were carried out in benzene. The workup procedure entailed filtration of the crude reaction mixture through alkaline alumnia and the isolation of monoacetals containing free hydroxy groups was therefore not possible.

meso-*2,3-Dimethylbutanetetrol* (5b). The condensation of *meso*-erythritol (*5a*) with two equivalents of methanal has previously been found to lead to the formation of the three possible diformals *7a*–*9a* (Scheme 3).[10] However, the reaction between *5b* and paraformaldehyde leads to only two isolable products, *7b* and *8b*. Gas chromatographic analysis of the latter product mixture revealed the existence of a third stable product (*9b*?) which could not be isolated; the latter compound constituted about 2 % of the yield as determined by GLC. When the reaction was carried out with a smaller amount of catalyst and stopped at an earlier stage, it was possible to isolate an unstable product with structure *10b*.

D,L-*2,3-Dimethylbutanetetrol* (6b). Lemieux and Howard [8] obtained the tetraoxadecalin, *2a*, from the reaction between L-threitol (*6a*) and dimethoxymethane, whereas the reaction with paraformaldehyde leads also to the bidioxolane,



Scheme 3. Reactions between *meso*-butanetetrols and paraformaldehyde (*a*: R=H; *b*: R=CH_3).



Scheme 4. Reactions between D,L-butanetetrols and paraformaldehyde (*a*: R=H; *b*: R=CH_3).

11a (Scheme 4).[11] The *trans* isomer of *8a* (Scheme 3) has not been obtained (*cf.* Ref. 9).

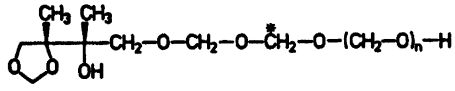
Compounds *2b* and *11b* are both formed by the reaction between *6b* and paraformaldehyde. It was possible to isolate a third product with structure *12b* when the reaction was performed using a smaller amount of catalyst.

Structure determination. The structures assigned to the six isolated compounds are consistent with the spectral data listed in the experimental section below.

Product distribution. The product distribution from the reaction between *5b* and paraformaldehyde was determined by GLC-analysis of the product mixture prior to separation. Workup after 70 h yielded a mixture containing 54 % of *7b*, 44 % of *8b* and 2 % of an unidentified compound. The ^{1}H NMR spectrum indicated a 56:44 ratio of *7b* and *8b*. Workup after *ca.* 45 h resulted in a mixture containing 40 % of *7b*, 43 % of *8b*, 15 % of *10b* and 2 % of the unidentified product. This clearly indicates that *10b* decomposes to give *7b*.

The reaction between *meso*-erythritol (*5a*) and paraformaldehyde under similar conditions has previously been shown to give a mixture of *7a*, *8a* and *9a* in the ratio 50:17:33.[10] It was therefore surprising that *9b* was not formed from *5b* and paraformaldehyde. It is not clear whether the *absence* of *9b* is a consequence of the conformation of the precursor *15b* (for which the more stable conformation is probably that with the two methyl groups equatorial [4,5]) or of kinetic control of the competitive formation of the precursors *14b* (acetal formation by two primary hydroxy groups) and *15b* (acetal formation by a primary and a tertiary hydroxy group).

The formation of *10b* is indicative of reaction between the precursor *13b* and formaldehyde oligomers. The preferred conformation of the $-C-O-C-O-C-$ grouping is all-*gauche* (all-g^+ *or* all-g^-),[12] and paraformaldehyde is thus helical. From inspection of molecular models it is evident that the least hindered position for attack of the free hydroxy group in *18* is at $-{}^*CH_2-$.



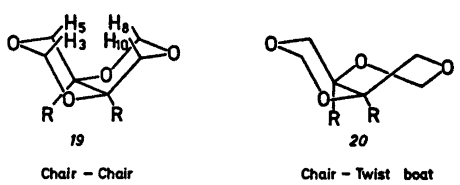
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The reaction mixture from D,L-*6b* and paraformaldehyde contained after 24 h compounds *2b*, *11b* and *12b* in the ratio 45:53:2. The use of a smaller amount of catalyst led to a mixture of the same compounds but in the ratio 44:44:12. The dioxolanyltrioxepane (*12b*) is thus formed at the expence of the bidioxolane (*11b*).

Conformational analysis. The main purpose of the present investigation was to ascertain whether the theoretically predicted destabilization of the *O*-inside conformation of *cis*-tetraoxadecalin (*2a*) by *ca.* 17 kJ mol^{-1} by the introduction of methyl groups at C-1 and C-6

would force compound *2b* to adopt predominantly the *H*-inside conformation (Scheme 1). The ^1H and ^{13}C NMR spectra recorded between 293 K and 183 K showed no temperature-dependent changes and the geminal coupling constant for the $O-CH_2-O$ protons of *2b* was found to be ± 6.6 Hz, consistent with a normal chair conformation.[13-15] The IR spectra of *2b* in tetrachloromethane solution and in potassium bromide were identical, indicating the same conformation in solution and in the solid state.

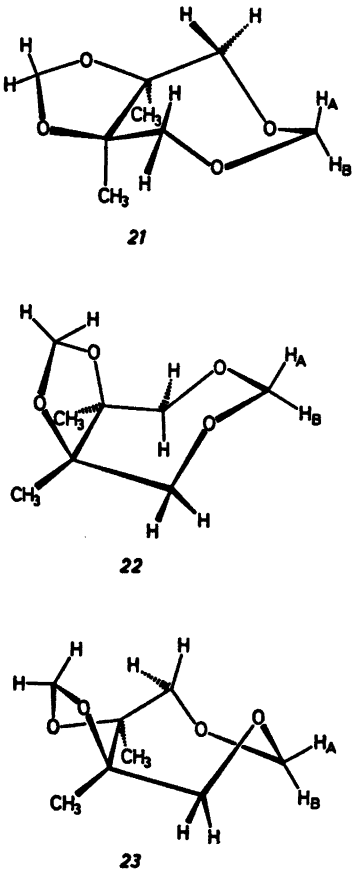
The dipole moments for *2b* in the *O*-inside and *H*-inside conformations were calculated by vector analysis [16] using bond lengths and valence angles from the known structure of 2-(*p*-chlorophenyl)-1,3-dioxane [17] and the reported dipole moment of 1,3-dioxane (2.13 D in benzene at 25 °C).[18] The calculated dipole moments were 4.25 D for the *O*-inside and 1.96 D for the *H*-inside conformation. The dipole moment of *2b* was measured at 25 °C and found to be 4.0 ± 0.1 in benzene and 3.9 ± 0.1 D in tetrachloromethane, *i.e.* in excellent agreement with the value calculated for the *O*-inside conformation. Destabilization of the *O*-inside conformation by *ca.* 17 kJ mol^{-1} is thus not sufficient to enforce the *H*-inside conformation. The H_3-H_{10} and H_5-H_{10} distances (*19*, Scheme 5) were calculated by the vector analysis to be 1.68 and 1.75 Å, respectively, and since these distances are less than twice the van der Waals' radius of hydrogen it is doubtful whether any destabilization of the *O*-inside conformation of *cis*-tetraoxadecalin will lead to the adoption of an undistorted *H*-inside conformation. Formation of a chair-twist boat conformer (*20*) might be expected to result from further destabilization of the *O*-inside conformation, although twist-boat 1,3-dioxane is estimated to be *ca.* 35 kJ mol^{-1} less stable than the chair conformer.[19]



Scheme 5. Chair-chair (*19*) and chair-twist boat (*20*) *H*-inside conformations of 1,6-dialkyl-*cis*-tetraoxadecalin.

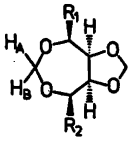
Burden and Stoddart have investigated 2,6-disubstituted *cis*-fused 3,5,8,10-tetraoxabicyclo-[5.3.0]decanes [9] and they claim that compounds of this type occur as an equilibrium mixture of two or more conformers. Three conformations are plausible for compound *8b* (Scheme 6) but an inspection of the Dreiding models of the three conformers (*21*–*23*) indicates conformation *23* to be the most favourable since it minimizes the methyl-methyl interactions.

The observed geminal coupling constant for the $O-CH_2-O$ protons in the dioxepane ring of *8b* was found to be ± 0.9 Hz. This value is numerically very small compared with the coupling constants reported for similar compounds (Scheme 7).[9,17,20] The geminal coupling constants, J_{AB}, will be about -6 Hz for con-



Scheme 6. Plausible conformations (*21*–*23*) of *Z*-1,7-dimethyl-3,5,8,10-tetraoxabicyclo[5.3.0]-decane (*8b*).

formations *21* and *22*, since the orientation of the $O-CH_2-O$ protons in the dioxepane rings of *21* and *22* is nearly the same as that of the $O-CH_2-O$ protons in the chair form of 1,3-dioxane, which displays $J_{gem} = -6.2$ Hz.[14] The orientation of the $O-CH_2-O$ group in conformation *23* corresponds to that in the twisted conformation of 1,3-dioxane, for which $J_{gem} = -2.8$ Hz.[20] The observed coupling constant thus indicates that compound *8b* adopts mainly conformation *23*.



R_1	R_2	J_{AB} Hz
$COOCH_3$	$COOCH_3$	-7.5
CH_2OCH_3	CH_2OCH_3	-7.5
H	$COOCH_3$	-6.7
H	CH_2OCH_3	-6.5
H	H	-6.1

Scheme 7. Reported geminal coupling constants (J_{AB}) of substituted *Z*-3,5,8,10-tetraoxabicyclo-[5.3.0]decanes.

EXPERIMENTAL

^1H NMR spectra were obtained using Varian T-60 A (60 MHz), Bruker HX-90 E (90 MHz) or Bruker HX-270 S (270 MHz) spectrometers (at *ca.* 30 °C unless otherwise stated). Proton-decoupled ^{13}C NMR spectra were obtained at 22.63 MHz using a Bruker WH-90 instrument. The Bruker WH-90 and HX-270 S systems were used in the Fourier transform mode. IR spectra were recorded on a Perkin-Elmer 337 Grating Infrared Spectrometer. Mass spectra were recorded on an AEI MS-902 mass spectrometer at 70 eV. The samples were introduced through a direct inlet system. Analytical GLC was performed using a Pye Unicam 104 gas chromatograph. (Columns: SE30 [10 % on Chromosorb W, 100/120 mesh] 4 mm × 1.64 m and Carbowax 1500 [10 % on Chromosorb W NAV, 60/80 mesh] 4 mm × 2.0 m. Flow: N_2, 30 ml min^{-1}). GLC separations were performed using a Perkin-Elmer F 21 "Preparative Gas Chromatograph". (Columns: Apiezon L [10 % on Chromosorb W NAV, 60/80 mesh] 6 mm × 1.8 m and SE30 [20 % on Chromosorb W NAV, 60/80 mesh] 6 mm × 1.8 m. Flow: N_2, 250 ml min^{-1}). Preparative layer chromatography (PLC) was carried out with 20 × 100 cm glass plates coated with 150 g of silica gel. (Eluent, ethyl acetate). The dipole moment of *2b* was determined from measurements of the dielectric constants (on a DM 01 instrument from Wissenschaftlich Technische Werkstätten, Weilheim, Germany) and densities of five solutions in benzene and two solutions in tetrachloromethane.

Z-2,3-Dimethyl-2-butene-1,4-diol (4) was prepared by the reduction of dimethylmaleic anhydride (*3*) with lithium aluminium hydride. To a stirred suspension of lithium aluminium hydride (30 g, 0.79 mol) in 1000 ml of ether was added a solution of *3* (76 g, 0.60 mol) in 1800 ml of ether at such a rate as to maintain reflux. The reaction mixture was then stirred and heated under reflux for 1 h. The excess of lithium aluminium hydride was destroyed by the addition of ethyl acetate (27 ml, 0.28 mol), and water (50 ml), sodium hydroxide (20 %, 23 ml) and water (105 ml) were then added successively. The mixture was filtered and the solid material extracted with ether for 24 h in a Soxhlet apparatus. The combined ether fractions were then dried with sodium sulfate and the ether removed by evaporation. The residue was distilled and the fraction boiling at 90 – 95 °C/7 Pa collected, yield of *4*: 52 g (76 %). On cooling the product in ice crystallization occurred. Recrystallization from anhydrous ether led to white hygroscopic needles, m.p. 37 – 38 °C. Anal. $C_6H_{12}O_2$: C, H. ^1H NMR (60 MHz, D_2O): δ 4.14 (4 H, s), 1.78 (6 H, s).

meso-*2,3-Dimethylbutanetetrol (5b)* was prepared by *cis*-hydroxylation[21] of *4*. To a stirred solution of *4* (29 g, 0.25 mol) and osmium tetroxide (0.25 g) in 100 ml of water was added a solution of hydrogen peroxide (0.25 mol) in 125 ml of water at such a rate as to maintain a reaction temperature of *ca.* 30 °C. The reaction mixture was then stirred for 2 h. After the addition of zinc dust (4 g) stirring was continued for a further 12 h. Filtration followed by evaporation under reduced pressure led to 29 g of crude product. The latter was dried by mixing with dry benzene followed by azeotropic evaporation. This procedure was performed twice after which the residue was cooled to −78 °C and 200 ml of pentane was added. After keeping the mixture in a refrigerator for 3 d small crystals could be observed, and when the mixture was allowed to warm up to room temperature the entire product crystallized. Recrystallization from ether/methanol (2:1) led to white hygroscopic crystals. Yield: 20.2 g (54 %), m.p. 84.5 – 85.5 °C. Anal. $C_6H_{14}O_4$: C, H. ^1H NMR (60 MHz, D_2O): δ 3.60 (4 H, s), 1.18 (6 H, s).

D,L-*2,3-Dimethylbutanetetrol (6b)* was prepared by *trans*-hydroxylation[22] of *4*. To a stirred solution of *4* (29 g, 0.25 mol) in 50 ml of water heated to 60 °C was added a solution of hydrogen peroxide (0.25 mol) and white, freshly prepared tungstic acid (0.3 g) in 50 ml of water at such a rate as to maintain a reaction temperature of 55 – 60 °C. The subsequent procedure was analogous to that used in the synthesis of *5b*. Yield: 14.6 g (39 %), m.p. 74 – 75.5 °C. Anal. $C_6H_{14}O_4$: C, H. ^1H NMR (60 MHz, D_2O): δ 3.62 *and* 3.60 (4 H, d), 1.18 (6 H, s).

Condensation of 2,3-dimethylbutanetetrols with paraformaldehyde. General method. A suspension of the tetrol (1.5 g, 0.01 mol) and paraformaldehyde (1.5 g, 0.05 mol of CH_2O) in 20 ml of benzene was heated under reflux in the presence of *p*-toluenesulfonic acid. The water formed was removed by means of a Dean-Stark trap. The reactions were monitored by GLC and on "completion" of the reaction sodium carbonate (0.5 g) was added so as to neutralize the catalyst. The reaction mixtures were filtered and the solvent removed by evaporation under reduced pressure.

meso-*2,3-Dimethylbutanetetrol and paraformaldehyde.* (Scheme 3, R = CH_3). The reaction was carried out using 0.1 g of *p*-toluenesulfonic acid as catalyst. The GLC-monitoring was carried out using a SE30 column at 150 °C. During the reaction formaldehyde sublimed into the reflux condenser and it was therefore necessary to add more paraformaldehyde (1.5 g after *ca.* 20 h and the same amount again after *ca.* 45 h). The crude reaction mixture was filtered through alkaline alumina using freshly distilled ethyl acetate (200 ml) as eluent, and subsequent evaporation of the solvent under reduced pressure yielded 1.54 g of product. GLC-analysis (Carbowax 1500, 150 °C) indicated the latter to be a mixture of 54 % of *7b*, 44 % of *8b* and 2 % of an unknown product. Cooling of the product mixture to *ca.* −20 °C led to the crystallization of compound *7b* and recrystallization of the latter from ether/ethanol (4:1) gave *ca.* 30 mg of meso-*4-(1-methyl-2,4-dioxolanyl-4-methyl-1,3-dioxolane* (7b), m.p. 54−55 °C. No attempts were made to isolate the remaining *ca.* 800 mg of *7b* in the product mixture. MS [*m/e* (% rel. int.)]: 173 (0.4, [M−H]), 87 (100), 86 (46), 57 (55), 44 (15), 43 (20). ^{1}H NMR (90 MHz, $CDCl_3$): δ 5.08 (2 H, s), 4.96 (2 H, s), 4.01 *and* 3.55 (4 H, AB-system, J ±8.5 Hz), 1.32 (6 H, s). ^{13}C NMR ($CDCl_3$): δ 95.28 (O−CH_2−O), 82.57 (*quat. C*), 72.53 (C−CH_2−O), 20.57 (CH_3−C). The identification of *7b* was based upon the ^{1}H NMR spectrum which displays J 0.0 Hz for the O−CH_2−O protons.[10,20,23]

Z-1,7-Dimethyl-3,5,8,10-tetraoxabicyclo[5.3.0]-decane (8b) was isolated from the product mixture by preparative GLC (Apiezon L, 130 °C). A small portion of the mixture was dissolved in ethanol and injected in 25 µl aliquots. *Ca.* 25 mg of *8b* was isolated, m.p. 41−43 °C.

MS: 174 (1, M), 173 (1), 144 (10), 131 (15), 114 (71), 112 (25), 101 (51), 100 (10), 99 (76), 87 (100), 86 (17), 85 (18), 84 (70), 71 (12), 69 (82), 58 (10), 57 (75), 45 (25), 44 (71), 43 (91), 41 (42).

^{1}H NMR (90 MHz, $CDCl_3$): δ 5.25 and 5.16 (2 H, AB-system, J ±0.9 Hz, O−C(4)H_2−O), 4.87 (2 H, s, O−C(9)H_2−O), 4.04 and 3.60 (4 H, AB-system, J ±12.9 Hz, C−CH_2−O) 1.26 (6 H, s, CH_3−C).

^{13}C NMR ($CDCl_3$): δ 95.96 (O−*C*(9)H_2−O), 92.45 (O−*C*(4)H_2−O), 83.15 (*C*1 and *C*7), 70.32 (C−CH_2−O), 19.27 (CH_3−C). The ^{1}H NMR and ^{13}C NMR spectra of compound *8b* indicate the presence of two different O−CH_2−O groups, one of which displays the zero geminal coupling constant indicative of the dioxolane ring.

meso-*6-(1-Methyl-2,4-dioxolanyl)-6-methyl-1,3,5-trioxepane* (10b) was obtained from the reaction between *5b* and paraformaldehyde using 0.05 g *p*-toluenesulfonic acid. After 20 h 1.5 g of paraformaldehyde was added. The reaction mixture was worked up after *ca.* 45 h, yielding *ca.* 1.5 g of crude product. GLC-analysis (SE30, 110−140 °C, 4° min^{-1}) indicated that the latter contained *ca.* 15 % of *10b*. A small portion of the product mixture was dissolved in ethanol and injected in 100 µl aliquots into the preparative gas chromatograph (SE30, 160 °C), yielding *ca.* 80 mg of *10b*, m.p. 32−34 °C.

MS: 203 (0.2, [M−H]), 117 (21), 87 (100), 86 (30), 57 (31), 44 (31), 43 (22).

^{1}H NMR (90 MHz, $CDCl_3$): δ 5.18 (1 H, s), 5.11 (2 H, s), 5.06 (1 H, s), 4.98 (2 H, s) [three different O−CH_2−O groups], 4.30 *and* 3.58 (2 H, AB-system, J ±9.1 Hz, *dioxolane* C−CH_2−O), 4.12 *and* 3.85 (2 H, AB-system, J ±13.4 Hz, *trioxepane* C−CH_2−O), 1.39 (3 H, s, CH_3−C), 1.31 (3 H, s, CH_3−C).

The structure of *10b* was assigned on the basis of the following observations: (a) The ^{1}H NMR spectrum indicates the presence of three different O−CH_2−O groups, two C−CH_2−O groups and two CH_3−C−O groups. Thus *10b* is formed by the reaction between *5b* and three equivalents of methanal. (b) The mass spectrum of *10b* displays abundant peaks at *m/e* 117 and 87, corresponding to rupture of the bond between the dioxolane and trioxepane rings.

D,L-*2,3-Dimethylbutanetetrol and paraformaldehyde.* (Scheme 4, R = CH_3). The condensation was carried out using 0.1 g of *p*-toluenesulfonic acid. The reaction time was *ca.* 20 h. After removal of solvent by evaporation 1.65 g of product mixture was obtained. GLC-analysis (SE30, 150 °C) indicated the composition: 45 % of *2b*, 53 % of *11b* and 2 % of *12b*. One g of the mixture was subjected to preparative layer chromatography, when two fractions were obtained (fraction 1: R_F = 0.2−0.4, fraction 2: R_F = 0.7). Fraction 1 was recrystallized from ether-ethanol (4:1), yielding *ca.* 330 mg of D,L-*1,6-dimethyl*-cis-*tetraoxadecalin* (2b), m.p. 101.0−101.5 °C. Anal. $C_8H_{14}O_4$: C, H.

MS: 174 (0.8, M), 144 (25), 114 (9), 87 (100), 84 (16), 69 (23), 57 (40), 44 (16), 43 (28), 41 (14).

^{1}H NMR (270 MHz, CD_2Cl_2, *ca.* 15 °C): δ 4.96 *and* 4.92 (4 H, AB-system, J ±6.6 Hz, O−CH_2−O), 3.67 *and* 3.61 (4 H, AB-system, J ±12.3 Hz, C−CH_2−O), 1.14 (6 H, s, CH_3−C). ^{13}C NMR ($CDCl_3$): δ 87.93 (O−CH_2−O), 72.01 (*C*1 and *C*6), 71.04 (C−CH_2−O), 16.40 (CH_3−C). The ^{1}H and ^{13}C NMR spectra are consistent with the presence of equivalent

pairs of $O-CH_2-O$, $C-CH_2-O$ and CH_3-C-O groupings. The geminal coupling constants for the two types of CH_2 protons are in good agreement with the reported values for compound *2a* (J ±6.25 and ±12.3 Hz, resp.).[3]

Fraction 2 from the PLC was purified by preparative GLC (SE30, 160 °C), yielding *ca.* 170 mg of D,L-*4-(1-methyl-2,4-dioxolanyl)-4-methyl-1,3-dioxolane* (11b), m.p. < 20 °C.

MS: 173 (0.4, [M−H]), 87 (100), 86 (45), 57 (49), 44 (17), 43 (19).

^{1}H NMR (90 MHz, $CDCl_3$): δ 5.02 (2 H, s), 4.98 (2 H, s), 4.15 *and* 3.58 (4 H, AB-system, J ±8.3 Hz), 1.30 (6 H, s). ^{13}C NMR ($CDCl_3$): δ 95.44 ($O-CH_2-O$), 83.09 (*quat. C*), 72.37 ($C-CH_2-O$), 21.45 (CH_3-C).

The structural assignment for compound *11b* is based upon the ^{1}H NMR spectrum which displays the zero geminal coupling constant for the $O-CH_2-O$ protons. Furthermore, the mass spectra of compounds *7b* and *11b* are identical.

D,L-*6-(1-Methyl-2,4-dioxolanyl)-6-methyl-1,3-5-trioxepane* (12b) was obtained from *6b* and paraformaldehyde using 20 mg of *p*-toluenesulfonic acid. GLC-analysis (SE30, 150 °C) indicated the following composition of the product mixture: 44 % of *2b*, 44 % of *11b* and 12 % of *12b*.

The PLC fraction with $R_F = 0.7$ was separated into *11b* and *12b* by preparative GLC (SE30, 170 °C), when *ca.* 20 mg of *12b* was obtained. MS: 203 (0.1, [M−H]), 117 (22), 87 (100), 86 (25), 57 (32), 44 (30), 43 (25).

^{1}H NMR (270 MHz, $CDCl_3$, *ca.* 15 °C): δ 5.01 (1 H, s), 4.98 (1 H, s), 4.97 (1 H, s), 4.93 (1 H, s), 4.93 *and* 4.78 (2 H, AB-system, J ±5.5 Hz) [three different $O-CH_2-O$ groups], 4.33 *and* 3.49 (2 H, AB-system, J 8.5 Hz, *dioxolane* $C-CH_2-O$), 4.05 *and* 3.82 (2 H, AB-system, J ±13.1 Hz, *trioxepane* $C-CH_2-O$), 1.29 (3 H, s, CH_3-C), 1.28 (3 H, s, CH_3-C). The spectral data for compound *12b* are very similar to those for compound *10b*.

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Arylation in the 5-Position of 1,4-Benzodiazepine 4-Oxides

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Dedicated to Professor K. A. Jensen on his 70th birthday

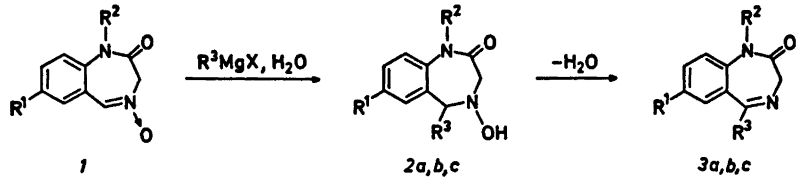
1,3-Addition of arylmagnesium halides to the 4,5-nitrone function in 1,4-benzodiazepine 4-oxides followed by dehydration or oxidation of the resulting substituted hydroxylamines opens up a new synthetic route to the pharmacologically active 1,4-benzodiazepines.

In previously [1] patented syntheses of 1,4-benzodiazepines the substituent in the 5-position was present in the 2-aminobenzophenones, which by various methods were attached to the $C^3-C^5-N^4$ unit before cyclization to the desired 1,4-benzodiazepines. With the present method,* reaction of arylmagnesium halides with 5-unsubstituted 1,4-benzodiazepine 4-oxides, it became possible to obtain 1,4-benzodiazepines with different substituents in the 5-position from the key compounds *1* or *4*.

Previously [2] 1,3-addition of Grignard reagents to nitrones has been described, but only with compounds not containing other "Grignard active" groups.

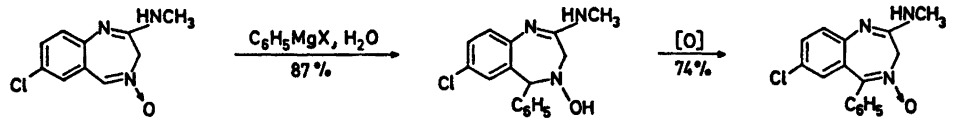
It was thus important to select the proper reaction conditions for the Grignard reaction as it was found that the Grignard-reactive groups R^1, $-N(R^2)-CO$ might have higher reactivity toward Grignard reagents in certain media than the nitrone function had. The stability of the arylating agent might also impose special reaction conditions. The different reaction conditions given here make this arylation method general.

* Patented during 1966—1975 by Grindstedværket A/S, Aarhus, Denmark.



1 *2a,b,c* *3a,b,c*

a, $R^1 = NO_2$; $R^2 = H$; $R^3 = C_6H_5$. *b*, $R^1 = Cl$; $R^2 = CH_3$; $R^3 = C_6H_5$.
c, $R^1 = Cl$; $R^2 = Et_2N(CH_2)_2$-; $R^3 = o$-FC_6H_4.



4 *5* *6*

Scheme 1.

When R^1 in *1* was NO_2 and R^2=H it was found necessary to use the unconventional Grignard reaction medium pyridine in order to get *2a*, which by dehydration gave the sedative *3a* (Nitrazepam). The use of pyridine as solvent in the Grignard reaction also proved to give reasonable yields in 5-phenylation of *1* with R^1=Cl and R^2=CH_3 giving *2b* shown to consist of two isomers, which could not be transformed into each other. The nature of the isomerism was not fully determined, but both isomers could be dehydrated to the well-known psychoactive drug *3b* (Diazepam). A similar case with two isomeric 4-hydroxy-1,4-benzodiazepines was described previously.[11]

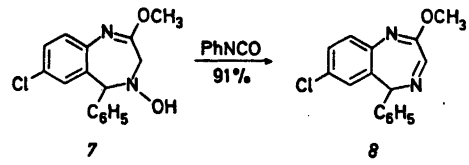
Oxidation of *3b* by *m*-chloroperoxybenzoic acid converted it to the 4-oxide,[7] which by reduction with sodium borohydride gave one of the isomers of *2b* obtained by the Grignard reaction.

The 5-phenylation of *4* gave 5, which was oxidized[12] with potassium hexacyanoferrate(III) to give a mixture of oxidation products, which were isomerized to *6* (Chlordiazepoxide).

When *6* was reduced[8] with lithium aluminium hydride another isomer of *5* was obtained. This isomer could again be oxidized[8] to *6*.

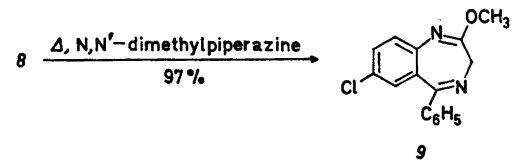
When the aryl group in the arylmagnesium halide contained an *o*-halogen substituent special reaction conditions had to be chosen in order to suppress the transformation of the arylmagnesium halide into a benzyne[9,10] before it reacted with the benzodiazepine 4-oxide. Thus we achieved high yields in the reaction of *1*, R^1=Cl, R^2=$CH_2CH_2N(Et)_2$ with *o*-fluorophenylmagnesium bromide when using the entrainer 1,2-dibromoethane in the *in situ* preparation of the Grignard reagent. The new hydroxylamine *2c* obtained was dehydrated by phenyl isocyanate to *3c* (Flurazepam).

The dehydration of the Grignard products – the cyclic hydroxylamines – was also investigated. Sometimes dehydration with phenyl isocyanate gave the best yields, but in most cases refluxing the hydroxylamine in *N,N'*-dimethylpiperazine gave the expected 3*H*-1,4-benzodiazepine. In the case of 2-alkoxy-4-hydroxy-5-phenyl-4,5-dihydro-3*H*-1,4-benzodiazepines the two dehydration procedures gave either the 5*H*- or the 3*H*-isomer. Thus *7* by dehydration with phenyl isocyanate gave the 5*H*-1,4-benzodiazepine *8*, whereas *7* on refluxing in *N,N'*-dimethylpiperazine gave the 3*H*-1,4-benzodiazepine *9*. Refluxing *8* in *N,N'*-dimethylpiperazine gave isomerization to *9*.



7 8

Scheme 2.



9

Scheme 3.

The structures of *8* and *9* were determined by ^{1}H NMR analysis, whereas other structures followed from syntheses and elemental analyses. The starting materials, *1* and *4*, were prepared by standard reactions from substituted benzaldehydes.[5]

EXPERIMENTAL

Preparation of Nitrazepam (*3a*)

1,3,4,5-Tetrahydro-4-hydroxy-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one (2a). (*1*: R^1=NO_2, R^2=H)[13] (4.42 g, 0.0200 mol) was suspended in dry pyridine (140 ml) and the suspension was stirred for 10 min at 35–40 °C and then cooled to 20 °C. A 2.6 M ethereal solution of phenylmagnesium bromide (18.4 ml, 0.0480 mol) was added dropwise at about 20 °C over a period of 7 min in a nitrogen atmosphere. The resulting brown suspension was cooled to 13 °C during the next 7 min. A mixture of ice (70 g) and conc. hydrochloric acid (20 ml) was added with stirring during 2 min. The tea-colored solution was cooled to 0 °C, seeded with *2a*, and stirred for 1 h at 0 °C. Water (225 ml) was added dropwise with stirring over a period of 40 min at 0 °C, whereafter the mixture was stirred overnight at 0 °C. The resulting crystals were isolated by filtration, washed with three 15 ml portions of water, and dried over calcium chloride. The dry crystals [5.26 g, m.p. 207–209 °C (decomp.)] were crystallized from acetone (90 ml) by cooling to –20 °C overnight to give 3.90 g (65 %) of pure *2a*, m.p. 219–220 °C (decomp.). The mother liquor and washings were evap-

orated to dryness from a water bath (40 °C) under reduced pressure, and the residue crystallized from acetone (15 ml) in the same way as above to give another 0.72 g (12 %) of *2a* of the same purity [*m.p.* 219–220 °C (decomp.)]. The total yield of *2a* was thus 4.62 g (77 %). Anal. $C_{15}H_{13}N_3O_4$: C, H, N.

Nitrazepam, 1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one (3a). *2a* (1.00 g, 0.00334 mol) was suspended in a mixture of anhydrous isobutyl acetate (10 ml) and 1,4-dimethylpiperazine (1 ml) at 20 °C and the suspension stirred for 10 min at 25 °C. Phenyl isocyanate (0.80 ml, 0.0075 mol) was added and the yellowish-white suspension heated to reflux over a period of 15 min. The evolution of carbon dioxide started at about 45 °C and stopped when reflux began. The slightly yellow suspension was cooled to 20 °C and methylene chloride (10 ml) was added with stirring. The white crystals were isolated by filtration, washed with methylene chloride (2+2 ml), and dried (80 °C) to give 0.64 g of 1,3-diphenylurea (90 %), m.p. 242–243 °C. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was crystallized from benzene (5 ml) to give 0.93 g of slightly impure *3a* (99 %), cream-colored crystals, m.p. 218–220 °C. Crystallization from acetonitrile (12 ml) with cooling to −20 °C overnight, filtration, washing with cold acetonitrile (1+1+1 ml), and drying (100 °C, 1 mmHg) gave 0.78 g of *2a* (83 %), almost white crystals, m.p. 226–227 °C. From the mother liquor and washings another crop of *3a*, 0.078 g (8 %), m.p. 225–226 °C was obtained. The total yield of *3a* was thus 0.858 g (91 %). The mixture melting point with an authentic sample was 226–227 °C. The product was further identified by the IR spectrum.

Preparation of Diazepam (*3b*)

7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide (1: R^1=Cl, R^2=CH_3). (*1*: R^1=Cl, R^2=H)[5] (1.11 g, 0.00525 mol) was suspended in methanol (30 ml) and the suspension heated to 60 °C with stirring. A solution of sodium methoxide [from 0.121 g of sodium (0.00525 mol) and methanol (40 ml)] was added in one portion. The clear solution was heated to boiling, and freshly distilled dimethyl sulfate (0.50 ml, 0.0055 mol) added in one portion. The mixture was heated under reflux for 1 h and then concentrated by distillation under reduced pressure to 3–4 ml. Ether (20 ml) and light petroleum (b.p. 40–60 °C) (30 ml) were added and the mixture cooled to −20 °C. A precipitate of a light reddish powder was isolated by filtration, stirred with water (10+5+5 ml) on the filter, and dried (50 °C, 0.1 mmHg, 1 h). The yield was 0.85 g, the main portion of which melted at 171–175 °C, while a small portion remained solid at 185 °C. Crystallization from ethanol–ether gave 0.65 g of (*1*: R^1=Cl, R^2=CH_3) (55 %), m.p. 175–176 °C. (Found: C 52.9; H 4.0; Cl 15.8 N 12.5. Calc. for $C_{10}H_9ClN_2O_2$: C 53.5; H 4.0; Cl 15.8; N 12.5).

7-Chloro-1,3,4,5-tetrahydro-4-hydroxy-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one, isomers A and B (2bA and 2bB). (*1*: R^1=Cl, R^2=CH_3) (7.88 g, 0.035 mol) was dissolved in dry pyridine (100 ml). A 1.37 M solution of phenylmagnesium bromide in ether (35 ml, 0.048 mol) was added dropwise with stirring at about 15 °C for 40 min. The resulting brick-red colored suspension was stirred overnight at room temperature. The next day the suspension was cooled to 0 °C and added with stirring to a mixture of crushed ice (200 g), and concentrated hydrochloric acid (100 ml), while the temperature was kept below −5 °C. Ether (200 ml) was added, and stirring continued for 20 min, while the temperature rose to 20 °C. The mixture was filtered, and the greyish-brown filter cake washed with water (40 ml), ether (40+40+40 ml), and dried (2 h, 80–100 °C). The resulting greyish-brown crystals (2.1 g) were crystallized from boiling benzene (45 ml), crystallization taking place by standing overnight at 5 °C. The crystalline precipitate was isolated by filtration, washed with benzene (2+2 ml), and dried (100 °C, 1 h) to give 1.05 g of grey crystals of *2bB*, m.p. 190 °C (Kofler hot stage). Evaporation of the mother liquor to 10 ml gave another 0.30 g of *2bB* with the same melting point. A sample of *2bB* (100 mg) was crystallized from benzene (3 ml) to give 72 mg of material with the same melting point. This material was analysed. Anal. $C_{16}H_{15}ClN_2O_2$: C, H, N.

The combined aqueous mother liquor and washings were separated from the ether and extracted once more with ether (120 ml). The combined ethereal extracts (about 400 ml) were washed with 6 % sodium hydrogen carbonate solution (35 ml), and dried over anhydrous magnesium sulfate. The ether was evaporated under reduced pressure from a water bath (60 °C), and the residue (9.8 g) stirred with light petroleum (b.p. 40–60 °C) (100 ml) at about 40 °C. The resulting suspension of crystals was filtered, the crystals washed with light petroleum (b.p. 40–60 °C) (20+20+20 ml), and dried at 80 °C to give 7.2 g of a brown powder. Crystallization from benzene (110 ml) then gave 2.68 g of *2bA*, m.p. 208 °C (Kofler hot stage). 100 mg of this product was recrystallized from a 2:3 mixture of benzene and light petroleum (b.p. 40–60 °C) (10 ml) to give 95 mg of *2bA*, white needles, m.p. 209 °C (Kofler hot stage), identical (IR spectra and mixture melting point) with the product obtained by sodium borohydride reduction of 4-oxide[7] of *3b*. Anal. $C_{16}H_{15}N_2O_2$: C, H, N.

Evaporation of the benzene mother liquor from the 2.68 g of *2bA* to 20 ml followed by cooling to 5 °C overnight gave another crop

of crystals (1.57 g) consisting of a mixture of *2bA* and *2bB*. Crystallization experiments showed this mixture to consist of equal amounts of the *A* and *B* isomers, bringing the total yield of *2bA* to 3.46 g (33 %) and of *2bB* to 2.13 g (20 %).

All attempts to transform *2bA* into *2bB*, or *vice versa*, by seeding during crystallization were unsuccessful.

*Diazepam, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (*3b*). 2bA* (1.00 g, 0.00330 mol) was dissolved in dry pyridine (10 ml) at 20 °C. Freshly distilled phenyl isocyanate (0.72 ml, 0.0066 mol) was added in one portion with stirring, The clear colorless solution was heated to 95 °C over a period of 25 min, and kept at this temperature until evolution of carbon dioxide stopped (about 1 h). The reaction mixture was worked up as described above for the dehydration of *2a* to give a voluminous residue, which was recrystallized from methanol (2 ml) at −20 °C to give 0.715 g of *3b* (76 %); white crystals, m.p. 128−130 °C, mixture melting point with an authentic sample 129−131 °C. The product was further identified by the IR spectrum.

When 100 mg of *2bB* was subjected to the above reaction conditions 40 mg of *3b* (43 %), white crystals, m.p. 126−128 °C, mixture melting point 129−131 °C was obtained.The product was further identified by the IR spectrum.

Preparation of Chlordiazepoxide (*6*)

*7-Chloro-2-methylamino-4-hydroxy-5-phenyl-4,5-dihydro-3*H*-1,4-benzodiazepine, isomers* A *and* B *(*5A *and* 5B*). 4*[5] (16.8 g, 0.0750 mol) was suspended in technical methylene chloride (150 ml) and the suspension cooled to 0 °C with stirring. A 2 M ethereal solution of phenylmagnesium bromide (82 ml, 0.164 mol) was added dropwise at about 5 °C over a period of 90 min. The resulting suspension was allowed to warm to room temperature with stirring over a period of 1 h. The suspension was then cooled to 0 °C and N hydrochloric acid (165 ml, 0.165 mol) added dropwise with stirring at about 5 °C over a period of 20 min. The mixture was stirred 20 min at 0−5 °C and the resulting white crystals filtered off, washed with methylene chloride (25+25 ml) and water (30+30+30 ml), and dried in the air at 60 °C for 2 h to give 16.9 g of *5A* (75 %), m.p. 180−184 °C. From the organic phase of the combined filtrate and washings another crop of *5* (4.4 g) was obtained, which contained about 1 g (5 %) of the starting material. Crystallization from methylene chloride (100 ml) gave 2.8 g of *5A*, m.p. 180 °C (Kofler hot stage), the total yield of *5A* thus being 19.7 g (87 %).

500 mg of the above 16.9 g *5A* was dissolved in boiling acetone (5 ml) the solution filtered, and the filtrate concentrated to 1.5 ml and left overnight at −20 °C. The resulting crystals were isolated by filtration, washed with cold acetone (1 ml), and dried at 60 °C to give 425 mg, m.p. 185−188 °C (decomp.). Anal. $C_{16}H_{16}ClN_3O$: C, H, Cl, N.

Reduction of purified, commercial chlordiazepoxide (1.0 g) with lithium aluminium hydride according to Sternbach [8] gave 0.38 g of *5B* (38 %), m.p. 196−198 °C (decomp.). The sample showed the same infrared absorption as our sample above. Anal. $C_{16}H_{16}ClN_3O$: C, H, Cl, N.

*Chlordiazepoxide, 7-chloro-3-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide (6).* Oxidation of *5A* or *5B* with potassium hexacyanoferrate (III) according to the directions given previously [12] gave a 74 % yield of *6*, m.p. 238−239 °C (lit.[8] 236 °C).

Preparation of Flurazepam (*3c*, 2HCl)

*7-Chloro-1-[2-(diethylamino)ethyl]-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide (*1: R^1=Cl, R^2=$(CH_2)_2NEt_2$). (*1*: R^1=Cl, R^2=H) (80.0 g, 0.380 mol) was added to a solution of sodium methoxide [from 9.66 g of sodium (0.420 mol) and methanol (200 ml), followed by evaporation of 150 ml of methanol] in methanol (50 ml) and *N,N*-dimethylformamide (800 ml). The clear solution, which became turbid after 30 s, was stirred for 30 min at room temperature. A solution of 2-chlorotriethylamine [prepared from the hydrochloride (72.3 g, 0.420 mol)] in dry toluene (440 ml) at 5 °C was added in one portion. The mixture was heated with stirring for 1 h on the water bath (100 °C). The resulting light orange suspension was evaporated to dryness under reduced pressure from a water bath (70 °C). Methylene chloride (300 ml) was added to the residue and the suspension poured on to a mixture of ice (1000 g), water (1000 ml), and 3 N hydrochloric acid (133 ml, 0.400 mol). The aqueous phase was removed, washed with methylene chloride (200+200 ml), and adjusted to pH 9−10 at 0 °C with 3 N sodium hydroxide (133 ml, 0.400 mol). The oil which separated was extracted with methylene chloride (200+200+200 ml). The combined methylene chloride extracts were dried with Drierite (Sikkon, Fluka). Removal of the methylene chloride by distillation from a water bath (40 °C), finally at 15 mmHg, gave 99.3 g (84 %) of a tea-colored oil.

The oil (99.3 g) was dissolved in warm ether (200 ml) and left for 48 h at −30 °C. The resulting cake of crystals was separated by decantation of the supernatant ether, washed with cold ether (15+15 ml), and dissolved in warm methanol (30 ml). Ether (300 ml) was added and the solution left overnight at −20 °C. The resulting crystalline precipitate was isolated by filtration, washed with cold ether

(25+25 ml), and dried (20 °C, 0.5 mmHg) to give 48.0 g of (*1*: R^1=Cl, R^2=$(CH_2)_2NEt_2$) (41 %) as white crystals, m.p. 87–89 °C. The mother liquor and washings were evaporated to dryness from a water bath (45 °C) under reduced pressure, and the residue crystallized from ether (100 ml) at −20 °C and isolated as above to give another 23.4 g of (*1*: R^1=Cl, R^2=$(CH_2)_2NEt_2$) (20 %) as slightly yellow crystals, m.p. 86–88 °C. The total yield of (*1*: R^1=Cl, R^2=$(CH_2)_2NEt_2$) was thus 71.4 g (61 %). A sample melting at 88–90 °C was analysed. Anal. $C_{15}H_{20}ClN_3O_2$: C, H, Cl, N.

Crude 7-chloro-1-[2-(diethylamino)ethyl]-5-(o-fluorophenyl)-1,3,4,5-tetrahydro-4-hydroxy-2H-1,4-benzodiazepin-2-one (2c). A stirred suspension of magnesium turnings (1.18 g, 0.0049 mol) in a mixture of 1,2-dibromoethane (1.5 g, 0.008 mol) and tetrahydrofuran (20 ml, free of water and peroxide), was heated under nitrogen in a dry flask to about 60 °C to initiate evolution of ethylene. A solution of (*1*: R^1=Cl, R^2=$(CH_2)_2NEt_2$) (1.49 g, 0.0048 mol) in a mixture of 1,2-dibromoethane (2.5 g, 0.013 mol), 1-bromo-2-fluorobenzene (1.22 g, 0.007 mol), and tetrahydrofuran (20 ml, free of water and peroxide) was then added dropwise with stirring at 40–42 °C over a period of 45 min. The resulting dark, red-brown solution was heated to 50 °C and stirred at this temperature for 30 min. The viscous mixture was added in one portion to ice-cold 0.14 N hydrochloric acid (150 ml, 0.021 mol). The resulting red-yellow, turbid solution was filtered to remove 0.4 g unreacted magnesium. The pH of the solution was adjusted to about 5 with 3 N NaOH (2 ml), and it was then extracted continuously with methylene chloride for 6 h.

The methylene chloride extract was washed with 20 % $KHCO_3$ (50 ml+50 ml) and dried over anhydrous calcium sulfate. The light brown filtrate was evaporated to about 150 ml. Methanol (50 ml) was added. Carbon black (0.2 g) was added, and the suspension was stirred under gentle reflux for 40 min. The filtrate was stirred with silica gel (3 g, Merck type G) for 10 min. The filtrate was evaporated to dryness (70 °C/10 mmHg), and the remaining oil was used in the next reaction without further purification.

Flurazepam, 7-chloro-1-[2-(diethylamino)ethyl]-5-(o-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one dihydrochloride (3c), 2HCl. The impure *2c* was dehydrated with phenyl isocyanate according to the directions given above, and the crude oil was treated with a solution of hydrogen chloride in 2-propanol to give a 46 % overall yield of *3c*, 2HCl, m.p. 199–203 °C. Previously [14] found 190–220 °C.

Dehydration experiments with 7-chloro-2-methoxy-4-hydroxy-5-phenyl-4,5-dihydro-3*H*-1,4-benzodiazepine (*7*).

7 was prepared by a Grignard reaction in tetrahydrofuran in a 70 % yield after directions given previously.[15]

7-Chloro-2-methoxy-5-phenyl-5H-1,4-benzodiazepine (8). *7* (6.2 g, 0.0205 mol) was dissolved in a mixture of isobutyl acetate (60 ml) and 1,4-dimethylpiperazine (6 ml) at 20 °C and the solution stirred for 5 min at 25 °C. Phenyl isocyanate (4.85 ml, 0.0456 mol) was added and the solution heated to reflux over a period of 15 min. The evolution of carbon dioxide started at about 40 °C and stopped when reflux began. The yellow suspension was cooled to 25 °C and methylene chloride (100 ml) was added with stirring. The white crystals were isolated by filtration, washed with methylene chloride (30+30 ml), and dried (60 °C) to give 4.0 g of 1,3-diphenylurea (92 %), m.p. 243 °C. The combined filtrate and washings were evaporated to dryness under reduced pressure from a water bath (70 °C). Boiling cyclohexane (100 ml) was added to the yellowish brown residue whereby most of the residue dissolved. The mixture was left overnight at 5 °C and the solution decanted from insoluble, oily material. The cyclohexane solution was evaporated to dryness under reduced pressure from a water bath (90 °C). The light yellow, very viscous residue (6.45 g) was distilled from an oil bath (178–182 °C) to give 5.29 g (91 %) of *8*, $b.p._{0.3}$ 162–165 °C. Anal. $C_{16}H_{13}ClN_2O$: C, H, Cl, N.

The ^1H NMR spectrum ($CDCl_3$) of *8* showed a singlet at δ 6.5 and a doublet at δ 4.8–4.9 representing, respectively, one proton at position 3 and one proton at position 5.

7-Chloro-2-methoxy-5-phenyl-3H-benzodiazepine (9). *8* (2.0 g, 0.0070 mol) and 1,4-dimethylpiperazine (3.5 ml) were heated under reflux over a period of 1 h. The tea-colored solution was evaporated to dryness under reduced pressure from a water bath (100 °C). The very viscous residue was distilled from an oil bath (175–185 °C) to give 1.93 g (97 %) of *9*, $b.p._{0.1}$ 141–148 °C. The product crystallized on standing at 60 °C for three days, whereafter it was powdered in a mortar and homogenized to give a slightly yellow powder, m.p. 85–87 °C (softening from 79 °C). (Found: C 67.4; H 4.6; Cl 12.4; N 10.1; OCH_3 10.0. Calc. for $C_{16}H_{13}ClN_2O$: C 67.5; H 4.6; Cl 12.5; N 9.9; one OCH_3 10.8).

100 mg of the above material was crystallized from light petroleum (1 ml) (b.p. 50 °C) to give 55 mg of *9*, m.p. 86–88 °C. (Found: C 67.6; H 4.8; Cl 12.3; N 9.7; OCH_3 10.5).

The ^1H NMR spectrum ($CDCl_3$) of both samples of *9* showed a singlet at δ 4.0 representing two protons at position 3.

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The Configurational Stability of Stilbene Anion Radicals

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Dedicated to Professor K. A. Jensen on his 70th birthday

The anion radicals of *cis* (C) and *trans* (T) stilbene were generated electrochemically and by pulse radiolysis. Cyclic voltammetric studies showed that C^- is formed at a potential about 50 mV more cathodic than T^- and has a lifetime greater than 1 s in dimethyl formamide. Electrochemical generation of C^- in the same solvent in the ESR cavity resulted in a resolved spectrum of C^- and once again showed that the lifetime was of the order of seconds. Under the conditions of pulse radiolysis, the lifetime of C^- and T^- in tetrahydrofuran was found to be much less, *i.e.* of the order of several μs. The isomeric anion radicals were found to be spectrally distinct. T^- has an absorption maximum at 500 nm while the corresponding absorption for C^- is at 516 nm. The molar extinction of T^- at the maximum is about two times that of C^- at the maximum. Thus, for the first time, it was possible to show the configurational stability of C^- by three independent techniques. The results reported here have very strong implications on previous work in which longer lifetimes of C^- were postulated but the spectral data reported correspond to T^-.

Electron spin resonance,[1–3] ultraviolet[3] and Raman[4] spectral studies have indicated that the same radical anion species is obtained when either *cis*- or *trans*-stilbene is reduced electrochemically or by alkali metals. The latter has been interpreted to indicate that isomerization (1), where C^- and T^- refer to the *cis* and *trans* anion radicals of the corresponding stilbene, is rapid.

$$C^- \rightleftharpoons T^- \qquad (1)$$

More recently, it has been proposed that in hexamethylphosphortriamide (HMPT), in which the ions exist in the free state, reaction (1) has a relaxation time of about 650 s.[5] We now report evidence from three independent techniques which confirms the lability of C^- and shows that the spectral characteristics of the isomeric monoanions of the stilbene are indeed different.

Cyclic voltammetric reduction of both C and T in dimethylformamide (DMF) containing Bu_4NBF_4 (0.2 M) in the presence of neutral alumina[6] results in the observation of two consecutive reversible or quasi-reversible one electron redox couples.[7] The potential at which the dianion is formed, 2.700 V *vs.* SCE (Fig. 1A), was found to be independent of which of the isomers, C or T, was reduced.[8] The potentials for formation of the monoanions, on the other hand, were dependent upon which isomer was being reduced. The potential difference between the first and second charge transfers was 465 mV for the reduction of C and 515 mV for reduction of T. That is, T is reduced about 50 mV more readily than C. When the direction of voltage sweep was changed about 300 mV negative of the reduction peak for C and cycled once again the reduction peak appeared at the same potential on the second cycle (Fig. 1B). After the second cycle, the potential was held negative of the reduction peak for 15 s before reversing the direction of sweep. Under the latter conditions, the peaks for oxidation of the anion radical and for reduction of the stilbene in the diffusion layer were shifted 50 mV

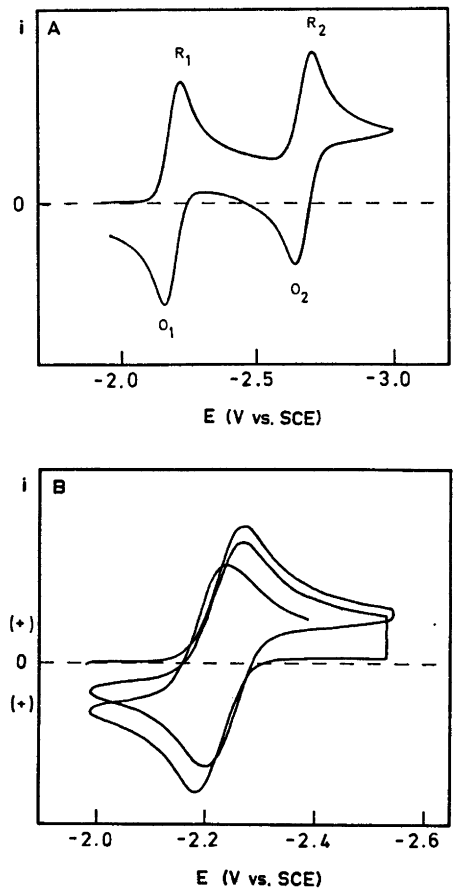


Fig. 1. Cyclic voltammetric reduction of stilbenes in DMF containing Bu_4NBF_4 at a platinum disc electrode. Voltage sweep rate equal to 100 mV s^{-1}. (A) *trans*-Stilbene, (B) *cis*-stilbene.

more positive which indicates that isomerization of C^- and T^- had occurred. Similar behaviour was observed when the cathodic scan for the reduction of C was carried out beyond the potential for reduction of C^-, showing that the stilbene dianion (S^{2-}) is oxidized to T^- under voltammetric conditions.

The ESR spectrum of C^- was obtained by reduction of C at a platinum gauze electrode in a flow cell placed in the cavity of the spectrometer. The residence time in the cell was about 1 s which indicates that under these conditions C^- has a lifetime greater than 1 s. The experimental spectrum (Fig. 2A) is nearly identical to the simulated spectrum (Fig. 2B)

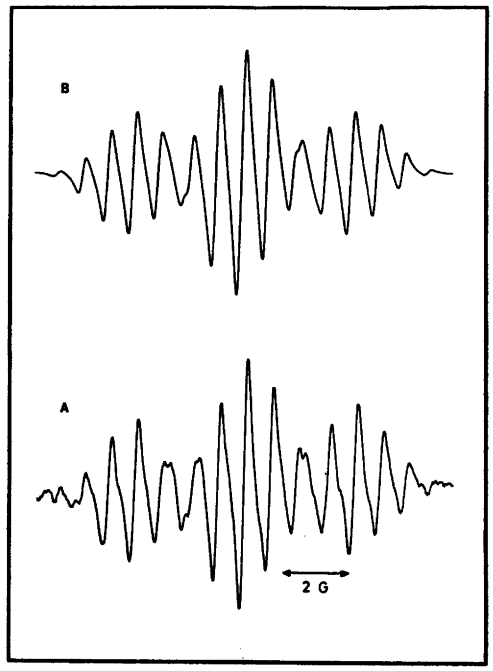


Fig. 2. ESR spectrum of *cis*-stilbene anion radical generated electrochemically in DMF (A), and the simulated spectrum (B).

with coupling constants, $a^H = 3.3$ G for the ethylenic protons and $a^H = 0.8$ G for six equivalent ring protons. An interpretation consistent with the spectra is that the *ortho* and *para* protons give identical coupling while coupling to the *meta* protons is too small to be observed with the resolution obtained. The most interesting feature of the spectrum (Fig. 1A) is the unusual width, much more narrow than expected for a π-radical. The sum of the coupling constants $[(2 \times 3.3) + (6 \times 0.8) = 11.4$ G] is less than half of the sum of that reported for the *trans* radical.[8] The small coupling constants of the ring protons indicate that the major portion of the spin density of C^- resides on the ethylenic carbons. Following the arguments of Bernal, Rieger and Fraenkel,[11] we estimate that the spin density resides 80 % on the ethylenic carbons and 20 % in the rings. The latter corresponds to a bond angle of about 135° between the phenyl rings and the ethylenic carbons.[17]

Pulse radiolysis of C in THF allowed us to obtain, for the first time, the visible absorp-

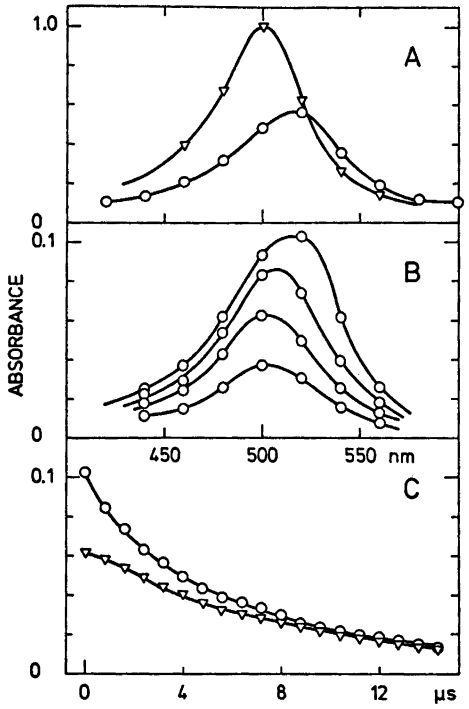


Fig. 3. Visible absorption spectra of *trans*-(upper) and *cis*-(lower) immediately after pulse radiolysis in tetrahydrofuran (A). Absorption spectra measured at various times after pulse radiolysis of *cis*-stilbene showing both decay and a blue shift of the absorption maximum (B). The decay of the visible absorption spectrum from *cis*-stilbene anion radical at various wavelengths (C).

tion spectrum of C^-. The spectra of the *cis* and *trans* radicals are shown in Fig. 3A. The absorption maximum for the *cis* anion radical appears at 516 nm as compared to 500 nm for T^-. The intensity of the absorption maximum for C^- is significantly lower than that for T^- The latter is consistent with the observations of Szwarc [5] who found an initial decrease in the absorption at 500 nm upon adding C to solutions of T^-. The latter study gave no indication of a shift in the absorption maximum which must mean that $C^-$was not formed in appreciable concentration. The radical anions generated from C by pulse radiolysis in THF at room temperature had half-lives of the order of only 6 μs. The decay of the visible absorption spectrum is illustrated in Figs. 3B and 3C. The decay of the absorption maximum is accompanied by a definite shift to lower wavelengths. The first spectrum shown, taken after 0.8 μs, is that of the nearly pure *cis* anion radical while the spectrum recorded after 12 μs revealed an absorption maximum at about 503 nm. The change in absorption maximum is most likely due to the isomerization reaction (1). If the latter is the case, we estimate that the half-life of reaction (1) under these conditions to be of the order of about 5 μs.[12]

The results presented above conclusively show that the *cis* and *trans* anion radicals of stilbenes are spectrally distinct species. The lifetime of C^- is short and dependent upon the environment in which it is generated. This work has definite bearing upon previous studies and forces one to conclude that reactions carried out with anion radicals generated from C actually had the *trans* configuration when the spectral data reported were characteristic of T^-.

EXPERIMENTAL

trans-Stilbene was reagent grade and used without further purification.

cis-Stilbene was prepared by photochemical isomerization of *trans*-stilbene and purified by column chromatography on a neutral alumina column.

DMF was distilled and passed through a column of active neutral alumina immediately before use. Cyclic voltammograms were recorded using standard equipment and neutral alumina in the cell as described previously.[6] The working electrode was made from 1 mm platinum wire sealed in soft glass and polished.

The flow cell for the ESR measurements was essentially identical to the flow cell described by Cauquis [14] with the modification that the counter electrode was placed very close to the working electrode in an arrangement similar to that published by Goldberg and Bard.[15] The solution was continuously purified by passing it through a layer of neutral alumina placed in the upper part of the cell during the experiment. This makes it possible to attain a high steady state concentration of a shortlined electrode product in the ESR cavity. The construction and performance of this cell will be published elsewhere.[16]

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17. Some doubt exists as to whether or not the spectrum shown in Fig. 2 is that of pure C^- since Professor M. Szwarc has recently informed us that a resolved spectrum of C^- much more similar to that of T^- than to Fig. 2 has been obtained in his laboratory.

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Structure and Dipole Moment of 1,6-Dioxa-6aλ^4-thiapentalene from Microwave Spectra

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Dedicated to Professor K. A. Jensen on his 70th birthday

The microwave spectra of 1,6-dioxa-6aλ^4-thiapentalene and its 3,4-D_2 derivative have been analysed. No evidence of valence tautomerism has been found. All observations so far indicate a planar molecule with C_{2v} symmetry. The electric dipole moment has been found to be 3.07 ± 0.04 D.

Compounds of the type illustrated in Fig. 1 where X, Y, Z are O, S, Se *etc.* have been quite intensively studied in recent years, with the aim of establishing the character of the so-called "electron rich three-center bond" X−Y−Z.[1] Numerous X-ray crystallographic studies in the X=Y=Z=S case (trithiapentalene) have revealed curious properties of this bond. Thus it has been found that even in the case of symmetrical substitution different S−S-bond lengths may result (*cf.* Ref. 2 and further references cited there). Such findings led to speculations as to whether the molecule exhibited valence tautomerism as depicted in Fig. 2, or, equivalently, whether the molecule has a double minimum potential in its electronic ground state. Molecular orbital calculations could not rule out such ideas.[1]

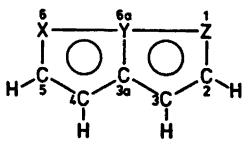


Fig. 1. Homologues of 1,6,6aλ^4-trithiapentalene considered as systems with a specific structure.

ESCA measurements in the vapour phase seemed to indicate a C_{2v}-symmetric structure,[3] and this was very strongly supported by a structure determination by electron diffraction (ED).[4] Large amplitudes of vibration of the SS-bonds were taken as evidence of the "bell-clapper" effect suggested in Ref. 1. This effect in fact means that the central (6a) sulfur atom is situated either in a flat single minimum, or in a double minimum with a rather low central hump. ED can hardly discriminate between these possibilities since an average over the Boltzmann distribution is observed. The controversy has therefore not been entirely settled.

Microwave spectroscopy should be particularly well suited to enlighten such matters for several reasons (see below). Attempts to study trithiapentalene failed however, because the vapour pressure of this substance is too low at room temperature. Corkill, Cox and Ewart made an attempt to study the molecule in a heatable spectrometer, but the substance decomposed.[5]

1,6-Dioxa-6aλ^4-thiapentalene (X=Z=O, Y=S in Fig. 1) does have sufficient vapour pressure and in fact turned out to have a very intense microwave spectrum. Assuming a rigid, planar model with C_{2v} symmetry, approximate

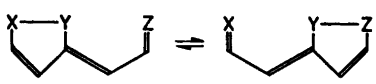


Fig. 2. Homologues of 1,6,6aλ^4-trithiapentalene considered as mixtures of valence tautomers.

moments of inertia and the rotational spectrum could be predicted with sufficient accuracy to allow an assignment of the spectrum as we shall see below.

EXPERIMENTAL

Microwave spectra were obtained on a Hewlett-Packard MRR-spectrometer.

The parent molecule and the deuterated sample were prepared according to the description by Reid and Webster.[6] The vapour pressure was estimated on a Pirani manometer to be *ca.* 0.1 mmHg at 25 °C.

Measurements of microwave frequencies in the ground state of both isotopic species were made at −10 °C at a pressure in the range 0.01 − 0.03 mmHg. The excited states at room temperature are in the same range of pressure. The accuracy of the frequencies is *ca.* 0.03 MHz. (*cf.* Tables 1 − 7).

ASSIGNMENTS AND INTERPRETATIONS

Due to their extreme sensitivity to the model we at first had some difficulty in assigning the

Table 1. Rotational transitions of dioxathiapentalene in the vibrational ground state.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	Frequency/MHz ν_{obs}	$\nu_{obs} - \nu_{calc}$ [a]
2	2	0	3	3	1	22588.530	0.006
2	2	1	3	3	0	22653.840	−0.005
3	2	2	4	3	1	25669.550	0.011
3	2	1	4	3	2	25339.710	−0.048
4	3	1	5	4	2	33966.330	0.017
6	0	6	7	1	7	18359.080	0.009
7	1	7	8	0	8	20258.530	−0.015
10	0	10	11	1	11	27665.910	0.021
11	0	11	12	1	12	30055.560	0.002
11	1	11	12	0	12	30030.550	−0.014
12	0	12	13	1	13	32451.670	0.023
12	1	12	13	0	13	32439.300	−0.010
12	1	11	13	2	12	35093.660	0.013
12	2	11	13	1	12	34677.660	−0.002
13	5	9	13	6	8	29793.260	−0.013
13	6	8	13	7	7	35590.510	0.002
13	0	13	14	1	14	34851.070	−0.007
13	1	13	14	0	14	34845.040	−0.013
14	5	9	14	6	8	28415.350	0.005
14	6	8	14	7	7	35275.330	0.001
15	5	11	15	6	10	29578.710	−0.018
15	6	10	15	7	9	35159.230	0.007
16	6	10	16	7	9	34399.030	0.002
17	3	15	17	4	14	30405.940	−0.005
17	4	14	17	5	13	28224.600	−0.001
17	5	13	17	6	12	29826.740	0.004
17	6	12	17	7	11	34672.280	0.002
18	2	16	18	3	15	31253.390	0.015
18	4	15	18	5	14	29718.870	−0.010
18	6	12	18	7	11	32712.560	0.010
18	6	13	18	7	12	34469.180	0.005
19	6	14	19	7	13	34346.070	−0.015
20	4	17	20	5	16	33424.440	0.001
20	6	14	20	7	13	29812.910	0.030
20	6	15	20	7	14	34352.340	0.001
24	5	19	24	6	18	24585.530	0.003
24	7	17	24	8	16	33372.530	−0.025
27	6	21	27	7	20	25645.750	0.021
29	6	23	29	7	22	31468.850	−0.015
29	7	22	29	8	21	26375.550	0.005

[a] Rms deviation: 0.0160.

Table 2. Rotational transitions of dioxathiapentalene in the $v_a = 1$ vibrational state.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	Frequency/MHz ν_{obs}	$\nu_{obs} - \nu_{calc}$ [a]
7	0	7	8	1	8	20624.620	−0.001
8	0	8	9	1	9	22944.225	−0.035
8	1	8	9	0	9	22755.535	0.016
8	2	7	9	1	8	23585.450	0.019
12	3	10	12	4	9	21858.705	−0.061
12	4	9	12	5	8	24617.095	0.040
18	5	13	18	6	12	22813.210	0.016
19	4	15	19	5	14	18807.555	0.024
19	5	14	19	6	13	21403.850	0.009
20	5	15	20	6	14	20452.730	−0.007
21	4	17	21	5	16	23692.590	0.011
21	5	16	21	6	15	20182.725	−0.016
24	6	18	24	7	17	23488.925	0.011
25	6	19	25	7	18	23249.810	−0.030

[a] Rms deviation: 0.0294.

Table 3. Rotational transitions of dioxathiapentalene in the $v_s = 1$ vibrational state.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	Frequency/MHz ν_{obs}	$\nu_{obs} - \nu_{calc}$ [a]
2	2	0	3	3	1	22576.600	−0.069
3	2	2	4	3	1	25654.565	0.031
8	0	8	9	1	9	22938.680	0.010
8	1	8	9	0	9	22746.655	−0.089
8	2	7	9	1	8	23559.030	0.045
9	0	9	10	1	10	25294.735	0.025
9	1	9	10	0	10	25195.065	0.014
13	3	11	13	4	10	23108.870	−0.026
19	4	15	19	5	14	18800.090	0.008
19	5	14	19	6	13	21533.170	0.008
20	5	15	20	6	14	20555.230	0.002
21	5	16	21	6	15	20248.940	0.017
22	5	17	22	6	16	20765.615	−0.001
24	6	18	24	7	17	23609.140	−0.002
25	6	19	25	7	18	23322.055	0.020

[a] Rms deviation: 0.0390.

Table 4. Rotational transitions of dioxathiapentalene in the $v_a' = 1$ vibrational state.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	Frequency/MHz ν_{obs}	$\nu_{obs} - \nu_{calc}$ [a]
2	2	1	3	3	0	22640.700	0.030
7	0	7	8	1	8	20518.810	0.005
7	1	7	8	0	8	20153.540	0.016
8	1	8	9	0	9	22627.280	−0.016
8	2	7	9	1	8	23421.605	−0.014
12	3	10	12	4	9	21940.375	−0.017
18	5	13	18	6	12	23090.920	−0.012
19	4	15	19	5	14	18785.850	−0.077
19	5	14	19	6	13	21652.355	0.024
21	4	17	21	5	16	23552.215	0.045
21	5	16	21	6	15	20307.280	−0.002
25	6	19	25	7	18	23386.020	0.006

[a] Rms deviation: 0.0345.

Table 5. Rotational transitions of dioxathiapentalene in the $v_a = 2$ vibrational state.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	Frequency/MHz ν_{obs}	$\nu_{obs} - \nu_{calc}$ [a]
8	1	7	9	2	8	26967.560	0.081
9	2	8	10	1	9	26644.410	0.007
10	0	10	11	1	11	27696.900	0.015
11	5	7	11	6	6	29889.150	−0.009
11	2	10	12	1	11	32140.690	0.004
12	0	12	13	1	13	32490.170	−0.018
13	5	9	13	6	8	29556.010	−0.028
14	0	14	15	1	15	37298.110	−0.040
14	1	14	15	0	15	37295.260	−0.012
15	5	11	15	6	10	29343.920	0.033
18	6	12	18	7	11	32444.500	−0.012
20	6	14	20	7	13	29561.770	0.004

[a] Rms deviation: 0.0353.

Table 6. Rotational transitions of dioxathiapentalene in the $v_s = 2$ vibrational state.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	Frequency/MHz ν_{obs}	$\nu_{obs} - \nu_{calc}$ [a]
11	2	10	12	1	11	32111.540	−0.003
12	0	12	13	1	13	32467.730	0.003
36	9	27	36	10	26	32413.230	−0.029
38	9	29	38	10	28	33209.650	0.002
38	10	28	38	11	27	38352.370	−0.012
39	10	29	39	11	28	36381.480	0.034
42	10	32	42	11	31	36302.550	0.003

[a] Rms deviation: 0.0232.

Table 7. Rotational transitions of 3,4-D_2-dioxathiapentalene in the vibrational ground state.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	Frequency/MHz ν_{obs}	$\nu_{obs} - \nu_{calc}$ [a]
7	0	7	8	1	8	19791.140	−0.032
7	1	7	8	0	8	19590.970	0.074
8	0	8	9	1	9	22053.825	0.009
8	1	8	9	0	9	21955.470	−0.011
9	0	9	10	1	10	24342.360	−0.018
9	1	9	10	0	10	24295.280	−0.014
18	5	13	18	6	12	18240.115	−0.044
20	4	16	20	5	15	23107.780	−0.066
20	5	15	20	6	14	18046.380	0.004
21	5	16	21	6	15	19288.340	−0.010
21	6	15	21	7	14	21692.090	−0.001
22	5	17	22	6	16	21438.950	0.018
23	5	18	23	6	17	24355.080	0.016
23	6	17	23	7	16	20429.460	0.040
24	6	18	24	7	17	21152.415	0.052
25	6	19	25	7	18	22876.465	−0.046
26	6	20	26	7	19	25516.710	0.031

[a] Rms deviation: 0.0392.

Table 8. Term values (cm^{-1}) calculated from Boltzmann factors for the $v_a = 1$, $v_s = 1$ and $v_a' = 1$ states.

Transition J''	K_{-1}''	K_{+1}''	J'	K_{-1}'	K_{+1}'	ν_a	ν_s	ν_a'
15	2	13	15	3	12	234	278	314
19	5	14	19	6	13	170	168	248
21	5	16	21	6	15	238	298	252
22	5	17	22	6	16	234	268	262
23	5	18	23	6	17	205	241	246
25	6	19	25	7	18	189	239	281
26	6	20	26	7	19	187	237	297
27	6	21	27	7	22	227	247	316
Mean values						211	247	277

spectra. However, once a suitable model was found, the turning point of the $J_{1,J-1} - J_{2,J-2}$ Q-branch was easily located. Low-J R lines were found by assuming zero inertial defect I.D. (defined in Table 9). Spectra of five excited vibrational states were also assigned for the parent molecule. The spinweights 10:6 were very helpful in the assignments. Two of the three most prominent excited states were found to have spinweights opposite to the ground state, while the third had the same spinweights. They are referred to as the $v_a = 1$, $v_a' = 1$ and $v_s = 1$ states respectively (see below), the remaining are the $v_a = 2$ and $v_s = 2$ states, both having the same spinweights as the ground state. We have tried in vain to assign the $v_a' = 2$ state, the number of lines with comparable intensity being very large. The index "a" means that the state concerned has an antisymmetric vibrational wave function, and "s" a symmetric with respect to the twofold axis of the C_{2v} point group. The term values of the $v = 1$ states have been determined from relative intensity measurements, *cf.* Table 8. These values have been corroborated by the far-IR and Raman spectra (see below).

The spectrum of the deuterated molecule was sufficiently well predicted by the same model to be easily assigned. Several attempts have been made to observe the spectrum of the ^{34}S-substituted molecule in natural abundance (4 %). But since this spectrum is of an intensity comparable to that of the $v_a' = 2$ state, we have not been successful so far.

Tables 9 and 10 contain the rotational and centrifugal distortion constants obtained by standard least squares fits based on the rigid rotor/centrifugal distortion model.[7] The centrifugal distortion constants for the ground state (*cf.* Table 10) have been kept fixed at their ground state values in all the remaining fits. This procedure is justified by the actual goodness of these fits (*cf.* the standard error cited at the bottom of the tables, which should

Table 9. Rotational constants (MHz) and inertial defects (uÅ²) (I.D. $= I_c - I_a - I_b$)[a]

Molecule/state	*A*	*B*	*C*	ID
Parent, G.S.[b]	4234.7556(13)	1677.60185(87)	1201.58046(81)	0.00359(16)
$v_a = 1$	4223.6115(15)	1676.84772(89)	1202.50119(84)	−0.76881(15)
$v_a = 2$	4212.7454(24)	1676.0595(13)	1203.41205(56)	−1.53129(33)
$v_s = 1$	4232.4713(19)	1675.8359(11)	1202.03472(92)	−0.53721(22)
$v_s = 2$	4230.1801(11)	1674.09777(69)	1202.44316(66)	−1.05780(12)
$v_a' = 1$	4233.7674(20)	1668.5049(10)	1195.4475(10)	0.49104(19)
3,4D$_2$,G.S.	3860.2278(22)	1653.90721(92)	1157.83237(89)	0.00100(18)

[a] $I_a = 505376/A$ *etc.* [b] G.S.: vibrational ground state.

Table 10. Centrifugal distortion constants [a] for the parent molecule (kHz).

Δ_J	Δ_{JK}	Δ_K	δ_J	δ_K
0.0618(25)	0.1225(41)	0.319(26)	0.01784(32)	0.1600(62)

[a] According to Watson.[9]

be comparable to the uncertainty of the frequencies, provided the model is perfect).

The stark effect of the $2_{20}-3_{31}$ transition has been analysed to give the electric dipole moment (*cf.* Table 11). The value determined agrees very well with calculated values.[10]

The crucial question regarding the observed excited states is whether they correspond to separate vibrational modes as we have tacitly assumed above, or whether they are vibrational levels of increasing quantum number of a bell-clapper vibration. We believe that the last possibility can be definitely ruled out for the following reasons: (*i*) A potential which would reproduce the observed energy level pattern is almost inconceivable. (*ii*) To two of the excited states we have been able to assign the corresponding doubly excited states, as was mentioned above. The fact that we have not been able to assign the $v_a' = 2$ state is not very disturbing in this connection, since we know already that it cannot belong to the v_a manifold, $v_a = 2$ having been assigned. (*iii*) The differences $A_o - A_k$ *etc.* and $(\text{I.D.})_o - (\text{I.D.})_k$ of Table 12 depend linearly to within a few per cent on the vibrational quantum numbers, which is a strong indication of harmonic behaviour (see below). (This conclusion has of course not been confirmed for the v_a' states.) (*iv*) The inertial defect is negative for two of the fundamental vibrational states. For excited states of an in-plane vibration one would normally expect a positive inertial defect.

Having ruled out this possibility we now want to determine which, if any, of the states belong to the bell-clapper vibration.

Being antisymmetric with respect to the C_s operation, *cf.* Fig. 3a. there are two possibilities: either v_a or v_a' are the quantum numbers to be associated with this vibration. Since the vibrations seem to be predominantly harmonic we are looking for two normal coordinates Q_a and Q_a' which would be expected to give rise to such low fundamental states (normal frequencies) as have been found. Model calculations based on rough estimates of the force constants suggest the possibilities of Fig. 3 for these and for Q_s. Clearly the "butterfly" vibration, Fig. 3c, and the Q_s motion, Fig. 3b, must be nearly degenerate, so if one is assumed

Table 11. Stark shifts $\Delta\nu$ (MHz) and derived dipole moments for the $2_{20}-3_{31}$ transition.

	Field strength V/cm			
	418.4 [a]		499.0	
M	$\Delta\nu$	μ	$\Delta\nu$	μ
0	−2.08	3.107	−3.015	3.136
1	−2.57	3.015	−3.725	3.043
2	−4.57	3.071	−6.445	3.058
μ_{av}	3.07 ± 0.04 D			

[a] Obtained by calibration on COS with $\mu = 0.71521$ D[9].

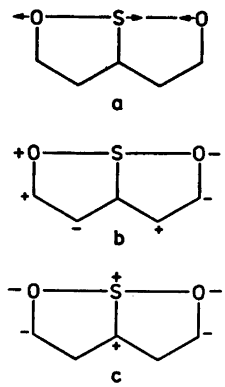


Fig. 3. Tentative normal coordinates for the three lowest modes: (a) The "bell-clapper" vibration, (b) the Q_s vibration, (c) the "butterfly" vibration, (see text).

Table 12. Differences between ground state values and excited state values divided by the vibrational quantum number.

v_k	$(A_0-A_k)/v_k$	$(B_0-B_k)/v_k$	$(C_0-C_k)/v_k$	$(ID_0-ID_k)/v_k$
$v_a=1$	11.1441	0.7541	−0.9207	−0.76881
$v_a=2$	11.0010	0.7712	−0.9158	−0.77044
$v_s=1$	2.2843	1.7659	−0.4543	−0.53721
$v_s=2$	2.2878	1.7520	−0.4313	−0.53070
$v_a'=1$	0.9882	9.0970	6.1330	0.48745

to be low, the other is too. Normally the butterfly vibration is one of the lowest in fused ring systems like naphthalene, where it is located at 176 cm^{-1}, significantly lower than in the present case.[11] In conclusion: we believe that the coordinates of Fig. 3 are the most likely candidates for the low frequency modes.

Assuming this interpretation Q_a and Q_a', must now be coupled by Coriolis interaction. This implies that the sign of the inertial defect cannot be trusted as an indicator of whether a vibration is in-plane or not. Indeed the force-field calculations mentioned above suggest that the lowest of the two should have negative inertial defect whether it is in-plane or not.

Therefore, we cannot decide unambiguously whether the bell-clapper motion or the butterfly motion is the lowest.

Provided the assignments made above are essentially correct Q_s is of A_2 symmetry in the C_{2v} point group. Therefore it is forbidden in infrared absorption. The far infrared spectrum in the solid state shows two pronounced absorption peaks at 204 and 268 cm^{-1} and only weak absorption in between. The Q_s vibration is allowed in Raman and the strongest peak is found at 238 cm^{-1} (from the Rayleigh line) thus corroborating the term values of Table 8 as well as the considerations above.

DISCUSSION

There are several ways in which double minimum potentials are known to manifest themselves in molecular spectra: (*i*) By direct transitions between near degenerate levels when the central potential hump is fairly high, and when such transitions are allowed, as in ammonia and similar systems (*cf.* Ref. 12 for a review). (*ii*) By irregular spacings between vibrational levels as in aniline, *p*-fluoro-aniline and similar molecules.[13] (*iii*) By a nonlinear (zig-zag) dependence of the rotational constants on the vibrational quantum numbers, as in ring-puckering molecules (*cf.* Ref. 14 for a review).

None of these effects have been observed in the present case: *Ad* (*i*) and (*ii*): Practically all lines of an intensity greater than 20 % of that of the corresponding lines in the ground state have been (or can be) assigned as described above, leaving no room for vibrational levels closer to the ground state than say 200 cm^{-1}. Considering the rather heavy mass of the sulfur atom this implies that if there is a hump at all then it must be very low, and most likely there is no such hump. We have already commented on (*iii*) above.

All evidence is in favour of the simplest possible assumption concerning the structure of 1,6-dioxa-6aλ^4-thiapentalene, namely that it is a single minimum structure. It remains to say that the very small, positive inertial defect leaves no serious doubt as to the planarity of the system.

We would like to conclude this paper with a few remarks on the intramolecular forces. Comparison with naphthalene (see above) makes it most likely that the fundamental at 211 cm^{-1} is to be associated with the butterfly vibration. Then 277 cm^{-1} is to be associated with the bell-clapper vibration, implying that the corresponding potential is in fact rather steep. Assuming that this vibration can be considered as an exclusive motion of the sulfur atom, in a harmonic potential with force constant f, we obtain $f=1.45$ mdyn/Å (145 N/m) corresponding to a classical amplitude in the ground state of *ca.* 1/20 of the S−O bond length.

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Short Communications

The Reactions of Ethyl 2-Furanacrylate with Grignard Reagents

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The action of Grignard reagents on diethyl furfurylidenemalonate has previously been studied in this laboratory.[1] Against this background, it seemed justified to allow Grignard reagents to react with the corresponding monocarboxylic ester, ethyl 2-furanacrylate. Our results considerably diverge from those reported by Maxim and Georgescu who have studied the reactions of this ester with some Grignard reagents and invariably found only double 1,2-addition products, *i.e.* unsaturated alcohols (*2* in Scheme 1).[2] It has, however, been shown that the purity of the magnesium used for the preparation of the Grignard reagent highly influences the course of the additions.[3] Because the commercial magnesium may be expected to be purer nowadays than 41 years ago, the discrepancies might be explained by this fact.

Our results show that the competing reactions in Scheme 1 occur. The observation that an α,β-unsaturated ester gives 1,4-, 1,2+1,4-, and/or double 1,2-addition in the same reaction is not new.[4,5]

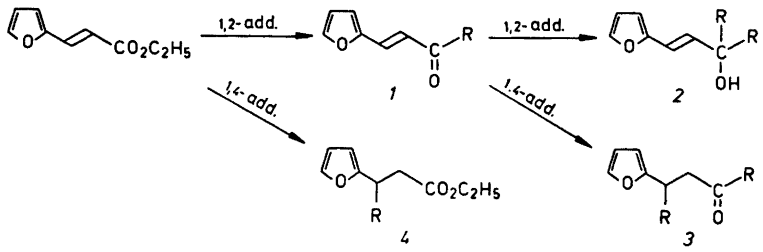
The primary 1,4-addition products are enolates that can scarcely react with another molecule of the Grignard reagent. The reaction sequence in the formation of the combined 1,2- and 1,4-adducts must therefore be that in the scheme. Munch-Petersen has previously come to the same result in connection with similar reactions.[6]

The principal course of the reactions depends on the alkyl group of the Grignard reagent. Methylmagnesium iodide gives *1* that reacts further to *2* and *3*, the ratio of which is 7:3. Any 1,4-addition is not observed. Isopropylmagnesium bromide gives *4* and *3* in the ratio 3:1. Isobutylmagnesium chloride reacts in the same way but gives more of *3* (the ratio of *4* and *3* is 1:4). The predominant product of *tert*-butylmagnesium chloride is *4* although small amounts of ethyl (5-*tert*-butyl-2-furan)propionate (1,8-addition product) and ethyl (3-*tert*-butyl-2,3-dihydro-2-furylidene)propionate (1,6-addition product) are observed. The 1,6-addition product easily rearranges to ethyl (3-*tert*-butyl-2-furan)propionate in acid ethereal solution. The three last-mentioned Grignard reagents do not give rise to any double 1,2-addition product (*2*).

Because the substrate is not varied and the Grignard reagents that have been studied are saturated and aliphatic, steric effects can explain the differences. In the first competition between 1,2- and 1,4-addition (to *2* and *4*), the 1,4-addition is more favoured the more branched the alkyl group is at the carbon atom that is bonded to the magnesium atom. Methylmagnesium iodide gives only 1,2-addition, the primary Grignard reagent (isobutyl) causes the formation of a small amount of 1,4-addition product, the secondary Grignard reagent (isopropyl) gives more 1,4-addition product than 1,2-addition, and if the alkyl group is tertiary the 1,2-addition is totally suppressed. In the second competition (from *1* to *2* and *3*), only the smallest alkyl group causes 1,2-addition in addition to 1,4-addition.

Another case (dialkyl isopropylidenemalonates) in which methylmagnesium iodide also gives double 1,2-addition in competition with 1,4-addition while other Grignard reagents react to give 1,4-addition and/or reduction of the carbon-to-carbon double bond has previously been reported.[7] The observation that methylmagnesium iodide gives double 1,2-addition to methyl cinnamate and that phenylmagnesium bromide predominantly reacts to give 1,4-addition might also be cited.[4]

If the present reactions are compared with those of diethyl furfurylidenemalonate [1] which has two ethoxycarbonyl groups in the α position instead of one in the present substrate, it is observed that ethyl 2-furanacrylate is not reduced by branched Grignard reagents as the malonate is under the same conditions. A reduction of the carbon-to-carbon double bond by branched Grignard reagents seems to require a stronger polarisation than that which one ethoxycarbonyl group induces. Another result of the weaker polarisation caused by only one ethoxycarbonyl group is the weakened tendency towards extension of the conjugated double bond system into the furan nucleus. In the present study, the 1,6- and 1,8-addition products of *tert*-butylmagnesium chloride were obtained in very small amounts while the proportions of these products are much greater when diethyl furfurylidenemalonate is allowed to react with the same Grignard reagent.



Scheme 1.

Experimental. Ethyl 2-furanacrylate was prepared in a good yield according to Galat.[8] The coupling constant (16 Hz) of the AB quartet in the ^{1}H NMR spectrum revealed that the *trans* isomer had formed.

The reactions between ethyl 2-furanacrylate and the Grignard reagents were performed as previously described for similar reactions with diethyl furfurylidenemalonate.[1] A fivefold excess of the Grignard reagents was used in all experiments. The reaction mixtures were analysed by GLC (stationary phase 3 % SE-30) and by combined GLC-MS. When it was possible, the components were isolated by preparative GLC and the ^{1}H NMR spectra taken on each separated substance.

Methylmagnesium iodide gave two compounds, 1,1-dimethyl-3-(2-furyl)-2-propenol (*2*, R = Me) and 4-(2-furyl)-2-pentanone (*3*; R = Me). The former compound was isolated. Both the NMR spectrum of this substance and the mass spectra contained the expected peaks. The ratio of the compounds (by GLC peak area) was about 70:30.

Isopropylmagnesium bromide gave two compounds, ethyl 3-(2-furyl)-4-methylpentanoate (*4*; R = Pr^i) and 2,6-dimethyl-5-(2-furyl)-3-heptanone (*3*; R = Pr^i). Both compounds were isolated. The NMR and mass spectra contained the expected peaks. The ratio of the compounds (by GLC peak areas) was 75:25.

Isobutylmagnesium chloride also gave two compounds, ethyl 3-(2-furyl)-5-methylhexanoate (4; R = Bu^i) and 2,8-dimethyl-3-(2-furyl)-4-nonanone (*3*; R = Bu^i). Both compounds, the ratio of which was 20:80, were isolated. The NMR and mass spectra of them contained the expected peaks.

tert-*Butylmagnesium chloride* gave one main product, ethyl 4,4-dimethyl-3(2-furyl)pentanoate (4; R = Bu^t), which was isolated. Because two by-products were identified by comparison of their mass spectra with those of the corresponding products of diethyl furfurylidenemalonate,[1] the mass spectrum of the isolated substance is given. MS [IP 70 eV; *m/e* (% rel. int.)]: 224 (5, M), 209 (1.5, M − Me), 179 (1.2, M − OEt), 167 (47, M − Bu^t), 137 (23, M − CH_2CO_2Et), 94 (100, M − Bu^t − CO_2Et), 81 (23, Furyl-CH_2), 57 (44, Bu^t), 19 (27, Et).

The gas chromatogram of the reaction products showed several smaller peaks. Two of the compounds due to these peaks were easily identified by MS. The compound that gave the peak next to the main peak was ethyl (5-*tert*-butyl-2-furan)propionate (1,8-addition product). MS [IP 70 eV; *m/e* (% rel. int.)]: 224 (23, M), 209 (100, M − Me), 179 (1.5, M − OEt), 150 (3, M − H − CO_2Et), 137 (41, M − CH_2CO_2Et), 135 (74, M − H − CO_2Et − Me), 121 (50), 43 (22, propen).

Ethyl (3-*tert*-butyl-2,3-dihydro-2-furylidene)-propionate (1,6-addition product) gave rise to peak No. 3. MS [IP 70 eV; *m/e* (% rel. int.)]: 224 (5, M), 222 (3, M − 2H), 209 (8, M − Me), 168 (43, M − C_4H_8), 139 (7), 137 (10, M − CH_2CO_2Et), 135 (10), 121 (32), 94 (100), 81 (19), 57 (64, Bu^t), 41 (19, propen), 29 (39, Et). In acid ether solution, the compound rearranged to ethyl (3-*tert*-butyl-2-furan)propionate. MS [IP 70 eV; *m/e* (% rel. int.)]: 224 (20, M), 209 (37, M − Me), 163 (34), 150 (26, M − H − CO_2Et), 137 (47, M − CH_2CO_2Et), 135 (28), 121 (100), 29 (20, Et.).

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Copper(II) Sulfate Dehydration of Alcohols

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Methods for the dehydration of alcohols to alkenes are legion, but the single step procedures mostly suffer from the disadvantage of double bond migration.[1,2] It is reported in the literature that anhydrous copper(II) sulfate may be used for the heterogeneous dehydration of secondary and tertiary alcohols in the liquid phase without this drawback.[3-7] In view of the limited data available and the fact that the method has not been much applied, we have undertaken a reinvestigation.

The literature procedure consists of the slow addition of the alcohol to the anhydrous salt at about 190 °C, such that the alkene is distilled off as formed.[3-6] The method as described requires that the temperatures chosen lie between the boiling points of the alkene and alcohol. Since copper(II) sulfate begins to decompose at temperatures not much in excess of 200 °C, the procedure has obvious limitations. The present work circumvented these difficulties by carrying out the reaction in a sealed tube.

Initial experiments were carried out to establish a standard procedure and these showed that satisfactory conversion required temperatures of not less than 180 °C and a reaction period of 24 h. Lower temperatures or shorter reaction times led to increased recoveries of unchanged alcohol. Equal weights of alcohol and anhydrous copper(II) sulfate were used throughout.

On completion of the reaction period the organic products were recovered by partition between water and ether. After drying and removal of ether, total recovery was confirmed and the crude product examined by GC-MS. When a main product was present, this was isolated by microdistillation or recrystallisation and characterised by IR, NMR and MS. The alcohols investigated together with the products of the reactions are given in Table 1.

The two tertiary alcohols (*1* – *2*) investigated provided the corresponding alkenes in excellent yields with only small amounts of unidentified products. Distillation gave pure alkenes whose identities were confirmed by IR, NMR and MS. [8,9]

The expected alkene was obtained in about 90 % yield from treatment of each linear secondary alcohol (*3* – *6*). The alkenes as obtained were contaminated by about 5 % of the corresponding dialkyl ether and were freed from this contamination by microdistillation under reduced pressure. The *trans*-alkene was isolated by way of the corresponding urea complex.[10] In each case the *cis*/*trans* ratio was about 1/3. GC-MS on a SCOT-column failed to reveal the presence of alkenes with shifted double bonds. Subsequent studies, involving chemical transformations, confirmed this finding.[11,12] The identity of 7-pentadecene was further confirmed by direct comparison with the authentic compound prepared by Wittig reaction.[11,13] Each *trans*-alkene gave satisfactory IR, NMR and MS data.[8,9]

The cyclic secondary alcohols (*7* – *8*) yielded complicated product mixtures where the cycloalkenes derived by 1,2-dehydration were the main products. However, the ether derived from the alcohol represented a significant by-product. The less sterically hindered alcohol (*8*) provided the larger quantity of ether,

Table 1. The products of the copper(II) sulfate dehydration of the alcohols (*1* – *12*).

Alcohol	Products (per cent)		
	Alkene	Ether	Unidentified
4-Propyl-4-heptanol (*1*)	88 [d]	–	12
5-Butyl-5-nonanol (*2*)	92 [d]	–	8
6-Undecanol (*3*)	86 [d]	8	6
7-Tridecanol (*4*)	88 [d]	10	2
8-Pentadecanol (*5*)	91 [d]	6	3
9-Heptadecanol (*6*)	89 [d]	6	5
2-Methylcyclohexanol (*7*)	45 [a]; 26 [b]	14	15
3-Methylcyclohexanol (*8*)	7 [b]; 48 [c]	38	7
1-Dodecanol (*9*)	5	90 [d]	5
1-Tridecanol (*10*)	5	94 [d]	1
1-Pentadecanol (*11*)	5	90 [d]	–
2-Phenylethanol (*12*)	–	94 [d]	6

[a] 1-Methylcyclohexene. [b] 3-Methylcyclohexene. [c] 4-Methylcyclohexene. [d] Isolated product gave satisfactory spectral data.

although the significance of this is unclear. The cyclohexenes were identified by comparison of their MS with the literature.[14]

The primary alcohols (*9 – 12*) all provided in excess of 90 % yields of the appropriate ether. About 5 % of alkene was present in most cases, together with a range of unidentified minor products. The aliphatic ethers were purified by recrystallisation from methanol, while diphenethyl ether was distilled. IR, NMR and MS data obtained for the ethers accorded with expectation.[15]

The above results show that the method now described is suitable for the preparation of alkenes from tertiary and secondary acyclic alcohols. A simple alternative synthesis of ethers derived from primary alcohols is also demonstrated. Cyclic secondary alcohols provide a mixture of products and in these cases the reaction is not immediately predictable.

Supplementary experiments carried out with reduced amounts of copper(II) sulfate indicate that 0.5 molar quantities of the salt are required for satisfactory alkene synthesis from tertiary and secondary alcohols. Only 0.05 molar quantities of salt are needed for ether synthesis from primary alcohols. Pilot experiments with other transition metal sulfates and copper salts indicate that these cannot replace copper-(II) sulfate in the alkene synthesis. The use of metal salts in liquid phase dehydration is largely unstudied,[1,2] and the mechanisms involved are unclear. Further work on the utility and mechanism of the reaction is in progress.

Experimental. The compounds *1 – 6* were prepared by standard Grignard procedures [16] and gave satisfactory physical data. Compounds *7 – 12* were commercial products (*purum* grade) from Fluka AG, Switzerland. Urea (*puriss.* grade) was obtained from the same firm. Copper(II) sulfate pentahydrate (*pro analysi* grade), Merck, Germany, was dried at 180 °C prior to use in order to remove all water of crystallisation.

Spectroscopic data were checked on a Unicam SP 200G infrared spectrometer, a JEOL C-60H NMR spectrometer, and a JEOL JMS D-100 mass spectrometer coupled to a JGC-20K gas chromatograph. Gas chromatography was accomplished using a commercially-available SE-30 SCOT-column obtained from SGE Pty. Ltd., Australia and guaranteed to have a NEFF-value in excess of 20 000.

Dehydration procedure. Anhydrous copper(II) sulfate (*ca.* 1 g) was sealed in a pyrex vial with an equal weight of the alcohol to be investigated. The vial was then maintained at 190 °C for 24 h. The vial was allowed to cool and then opened. The contents were shaken with a mixture of water (100 ml) and ether (50 ml). After separation the water layer was extracted with further portions of ether (2 × 50 ml), dried over anhydrous sodium sulfate, and the ether removed under reduced pressure. The total recovery was then found, quantitative in all cases, and the crude product examined by IR and GC-MS. Product distribution was determined on the basis of the total ion current trace from the GC-MS recorder.

trans-*Alkenes.*[10] The *cis/trans* alkene (*ca.* 1 g) was shaken for 24 h with a saturated solution of urea in methanol (20 ml). The resultant crystals were washed twice with small portions of saturated urea-methanol and then decomposed by the addition of water (20 ml). Ether extraction and evaporation under reduced pressure yielded the pure *trans*-alkene which was used to confirm identity spectroscopically.

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The Amino Acid Sequence of Soybean *(Glycine max)* Leghemoglobin *c*

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The amino acid sequences of leghemoglobins from soybean (*Glycine max*),[1,2] kidney bean (*Phaseolus vulgaris*),[3] broad bean (*Vicia faba*),[4] and yellow lupin (*Lupinus luteus*) [5] have been determined. All these proteins consist of single chains with molecular weight of about 15 000–16 000 daltons. The sequences of the leghemoglobins are closely similar, and they have features in common with those of animal origin. In this study the amino acid sequence of soybean leghemoglobin *c* ('fast' main component) is preliminarily reported. A detailed report will be given later.

Materials and methods. Leghemoglobin *c* and its apoprotein were prepared as previously described.[6,7] The apoprotein was digested with trypsin, chymotrypsin and thermolysin. The peptides obtained were separated by ion exchange chromatography, high voltage paper electrophoresis and paper chromatography.[8] Subtractive Edman and dansyl-Edman degradations, leucine aminopeptidase and carboxypeptidase A were used to elucidate the sequences of the peptides.[9] The *N*-terminal of the protein was determined by the Edman method and the *C*-terminal by hydrazinolysis.[9]

Results and discussion. The complete amino acid sequence of soybean leghemoglobin *c* is shown in Fig. 1 (p. 724). The *N*-terminal sequence of the protein is NH_2-Gly-Ala-Phe-Thr as determined by the Edman procedure. Hydrazinolysis of the protein gave equal amounts of lysine and phenylalanine.

Twenty-one tryptic peptides, one ditryptic peptide and free lysine were obtained from the tryptic hydrolysis of apoleghemoglobin *c*. The large number of tryptic peptides, as previously demonstrated by the fingerprint,[10] is a result of polymorphism at six positions (8, 20, 39, 79, 97, and 143) in the protein, giving six pairs of tryptic peptides in addition to eight single peptides. One Lys-Lys bond was found, at positions 140–141. The overlaps between the tryptic peptides were established with chymotryptic and thermolytic peptides. The polypeptide chain consists of 143 amino acids, one more than component *a*, giving an approximate molecular weight of 15 300 daltons for the apoprotein and 15 950 daltons for leghemoglobin *c*. The exact molecular weight cannot be calculated because of the polymorphism.

Leghemoglobin *c* contains two histidines at positions equivalent to those of other known leghemoglobins. In all leghemoglobins the distance between the histidines is 30 amino acids, two residues longer than in animal globins. Soybean leghemoglobin *c*, like component *a*,[1] kidney bean leghemoglobin *a* [3] and snake bean (*Vigna sinensis*) leghemoglobin,[11] has no sulfur-containing amino acids. This, however, is not an invariable rule for leghemoglobins, because broad bean leghemoglobin I contains two methionines [4] and lupine leghemoglobin I one methionine.[5]

The sequence of soybean leghemoglobin *c* differs completely from that of component *a* at only six positions (1, 31, 91, 114, 126 and 143). In addition, there is an insertion of lysine after position No. 140. At the positions of polymorphism one alternative is always identical to that of component *a*.

Leghemoglobin *c* has been reported to be resolved into two fractions on a DEAE-column,[12] and the amino acid composition of the fractions was found to differ slightly.[12] The difference, however, is not in congruence with the sequence elucidated here. The only difference we found between the fractions was in the *C*-terminal. The fraction first emerging from the DEAE-column has lysine and the later fraction phenylalanine as the *C*-terminal amino acid.

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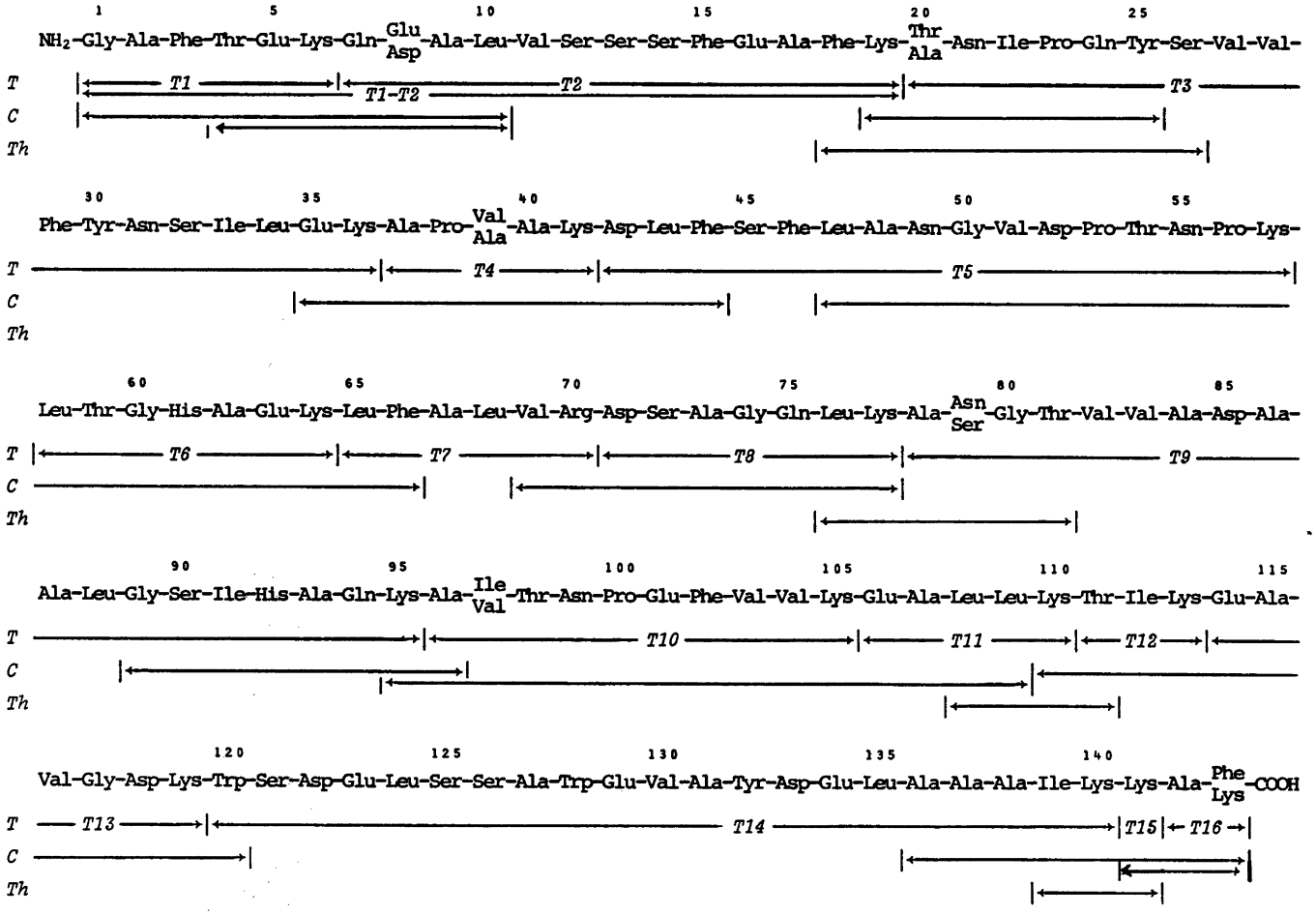


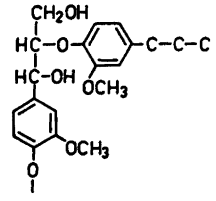
Fig. 1. The amino acid sequence of soybean leghemoglobin *c*. Tryptic peptides (*T*), chymotryptic peptides (*C*) and thermolytic peptides (*Th*) are indicated by horizontal lines.

On the Synthesis of Lignin Model Compounds of the Arylglycerol-β-aryl Ether Type

INGMAR BERNDTSSON and
KNUT LUNDQUIST

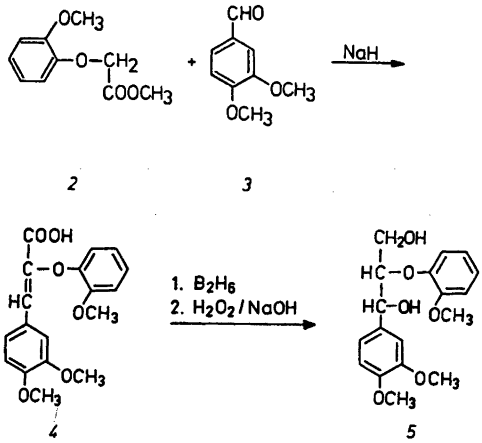
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In a previous paper [1] new synthetic routes for the preparation of lignin model compounds of the arylglycerol-β-aryl ether type (*1*) were described. Syntheses of such model compounds have also recently been reported by Nakatsubo *et al.*[2] One of the syntheses of model compound *5* described in Ref. 1 involves preparation of aryloxycinnamic acid *4* by a Perkin reaction [1] or by a Claisen reaction followed by hydroboration of acid *4* (Scheme 1). The present paper



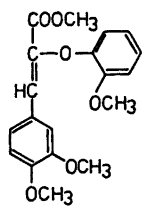
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describes the synthesis of acid *4* by the Claisen reaction and improvements of the yield in the conversion of this compound into compound *5* by hydroboration. In addition the separation of the two possible diastereomers of compound *5* by ion exchange chromatography is described.

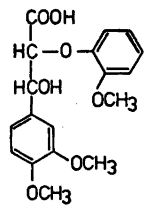


Scheme 1.

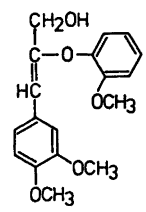
Synthesis of acid *4* by a Perkin reaction gave only poor yields.[1] Attempts have been made to increase the yield by replacing acetic anhydride with pivalic anhydride in the reaction mixture. However, the yield of acid *4* was about the same. Preparation of acid *4* by the Claisen reaction from the methyl ester of 2-methoxyphenoxyacetic acid (*2*) and veratraldehyde (*3*) using sodium hydride as a condensing agent (Scheme 1) gave a higher yield. We have made a series of experiments to find suitable conditions for this synthesis. The best results were obtained when an equimolar solution of the ester and the aldehyde was dropped into a suspension of sodium hydride in toluene at 60 °C (yield 41 %). Under these conditions the acid *4* is obtained and not its methyl ester (*6*), which was the expected product. Apparently the ester *6* formed is saponified in the reaction mixture (the condensation reaction involves an elimination of the elements of water) or, possibly, during an initial phase of the work-up procedure. Lower reaction temperatures result in the formation of considerable amounts of acid *7*. If the reaction temperature is increased, the disproportionation of the aldehyde (*cf.* Ref. 3) becomes important, which in our case



6



7



8

resulted in the presence of considerable amounts of 3,4-dimethoxybenzyl alcohol and veratric acid in the reaction mixtures. Similarly, dropping a solution of the ester alone into a solution of veratraldehyde containing sodium hydride favors the disproportionation reaction.

Earlier [1] hydroboration experiments with acid *4* gave model compound *5* in about 40 % yield. From hydroboration studies by Brown and co-workers [4] it was concluded that it should be possible to increase the yield considerably by modifying the reaction conditions. In fact, treatment with diborane at a lower temperature together with modifications of the subsequent

oxidation with hydrogen peroxide gave compound *5* in a considerably higher yield (68 %). Hydroboration of acid *4* gives the pure *erythro* form of compound *5*.[1] Preparation of this compound by other described methods for the synthesis of arylglycerol β-aryl ethers (Refs. 1 and 2 and literature cited therein) must involve a separation of diastereomers.

One way of obtaining the *erythro* as well as the *threo* form of compound *5* is the separation of these compounds from reaction products consisting of mixtures of the two diastereomers; several synthetic routes which provide such mixtures are known (Ref. 1 and literature cited therein). We wish to report that this separation can be done by chromatography on an anion exchanger, using borate solution as an eluent. The *threo* form is eluted more rapidly than the *erythro* form, which implies that the latter compound gives the strongest borate complex (*cf.* Ref. 5). This result is in accord with electrophoresis studies [6] of the two borate complexes.

Experimental. Conditions for TLC were those given in Ref. 1. The *methyl ester of 2-methoxyphenoxyacetic acid (2)* was prepared from 2-methoxyphenoxyacetic acid and methanol with H_2SO_4 as a catalyst (m.p. 33 °C, lit.[7] 33 °C). Alternatively compound *2* can be prepared from guaiacol and methyl chloroacetate.[7]

Preparation of α-(2-methoxyphenoxy)-3,4-dimethoxycinnamic acid (4) by the Claisen reaction. Compound *2* (15.0 g) and veratraldehyde (12.7 g) were dissolved in toluene (87 ml). The solution was dropped from a pressure-equalizing funnel into a stirred suspension of NaH (8.6 g of 55 – 60 % NaH in mineral oil) in toluene (20 ml) kept at 60 °C. Initially a few ml of the solution was added. As indicated by the evolution of hydrogen, the reaction started after 15 min. The rest of the solution was added during 1 h. Stirring at 60 °C was continued for 7 h. The reaction mixture was neutralized with conc. hydrochloric acid-water (1:1) and transferred to a separatory funnel by means of ether (375 ml) and 5 % $NaHCO_3$ solution (250 ml). After shaking, the layers were separated and the ether layer extracted with 2 × 250 ml of 5 % $NaHCO_3$ solution. The combined aqueous layers were acidified (pH 2) with conc. hydrochloric acid-water (1:1) under stirring. A crystalline precipitate was formed (18.9 g), which was treated with 75 ml of ether overnight. The residue (13.9 g) consisted according to TLC essentially of acid *4*. Recrystallization from acetone gave 10.4 g of acid *4* with m.p. 202 °C, lit.[1] m.p. 202 °C (yield 41 %).

Hydroboration of α-(2-methoxyphenoxy)-3,4-dimethoxycinnamic acid (4). Acid *4* (990 mg) was treated with B_2H_6 as described in Ref. 1, but the reaction mixture was kept at 0 °C. The reaction can be followed by direct examination of the reaction mixture by TLC, although the chromatograms only approximately reflect the composition of the final reaction product obtained after oxidation with H_2O_2. It was found suitable to interrupt the reaction, when an intermediate (or by-product) with R_F value 0.41 tends to disappear. This compound has been isolated by column chromatography according to the procedure described in Ref. 1 and has been identified by spectral data as compound *8*. [^{1}H NMR spectrum (δ units; solvent, chloroform-*d*): 2.2 (1 H, s; OH), 3.74 (3 H, s; OCH_3), 3.84 (3 H, s; OCH_3), 3.90 (3 H, s; OCH_3), 4.16 (2 H, s; $-CH_2-$), 6.08 (1 H, s; vinyl proton), ≈ 7.0 (7 H, m; aromatic protons)]. After 5 h the reaction mixture was oxidized with H_2O_2 and worked up as described in Ref. 1, with the exception that H_2O_2 was added to the reaction mixture prior to 3 M NaOH (which was added slowly during a 15 min period). Crystallization from ether gave 0.57 g of the *erythro* form of compound *5* (m.p. 98 °C, lit.[6] 97.5 – 98.5 °C). An additional amount (0.11 g) of the same compound was obtained from the mother liquor (column chromatography [1]). Yield: 68 %.

Separation of the two diastereomers of compound 5 by ion exchange chromatography. A column of an anion exchanger (QAE-Sephadex A-25, 20 g) was packed and several hundred ml of the eluent [0.06 M $K_2B_4O_7$ in water-acetone (4:1)] was allowed to pass the column. A mixture of the diastereomers of compound *5* (0.15 g) was dissolved in 9 ml of the eluent and applied to the column. The eluate was collected in 5 ml fractions which were pooled on the basis of an examination by TLC. The organic constituents were separated from the pooled fractions by chloroform extraction. From fractions 17 – 21, 89 mg of the *threo* form of compound *5* (^{1}H NMR) was obtained, and fractions 22 – 26 contained 40 mg of the *erythro* form of compound *5* (^{1}H NMR).

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Synthesis of Bullatenone and Bullatenone-related Substances

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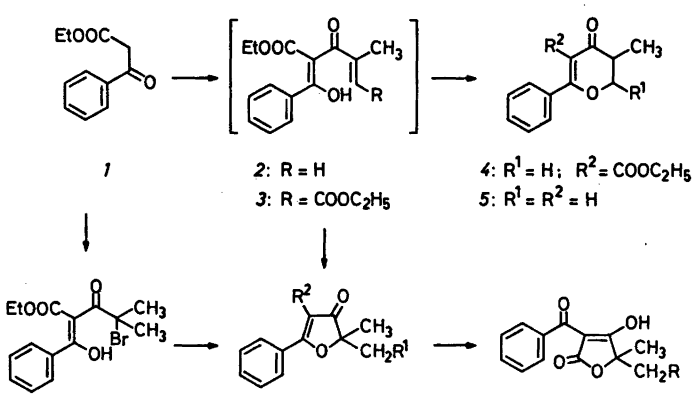
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In 1954 an optically inactive unsaturated ketone, bullatenone, was isolated from *Myrtus bullata* and a dihydro-γ-pyrone formulation *5* was proposed for the compound.[1] Subsequently bullatenone was shown to have the structure *10* and its structure was confirmed by synthesis from the not easily accessible 4-hydroxy-4-methyl-1-phenylpent-2-yl-1-one.[2] Since 3(2*H*)-furanones recently have been considered as potentially useful intermediates for the synthesis of tetronic acids,[3–8] it appeared that routes leading to these intermediates could in principle lead to a facile synthesis of bullatenone. This is, in fact, the case and we here report a facile synthesis of bullatenone and some bullatenone-related substances. The work was performed to make available reference compounds for biosynthetic studies.

One possible route involves cyclisation of (2-alkenoyl)-3-keto esters to give either dihydrofuranones [5,7] or dihydropyrones.[7,9] Reaction of the ethoxymagnesium salt of ethyl benzoylacetate (*1*) with 1 equiv. of methacryloyl chloride did not result in the isolation of *2*, but gave directly the dihydro-4-pyrone *4*, which could be hydrolyzed and decarboxylated smoothly to *5*, the compound originally thought to be bullatenone.[1] Alternatively, *1* was reacted with *trans*-3-ethoxycarbonylmethacryloyl chloride to give, not *3*, but the desired 3(2*H*)-furanone *7*. Hydrolysis and decarboxylation resulted in the formation of *8*. Attempts to transform *7* to bullatenone (*10*) were not successful.

The other route used for preparing 3(2*H*)-furanones involves cyclisation of α-haloacyl-3-keto esters.[3,4,6,8] *1* was reacted with 1 equiv. of 2-bromo-2-methylpropanoyl bromide to give 100 % of *6*, which upon cyclisation with triethylamine in toluene afforded 4-ethoxycarbonylbullatenone (*9*). Hydrolysis and decarboxylation of *9* resulted in the formation of bullatenone (*10*). Although no attempt has been made to optimise yields, the latter synthesis constitutes a highly convenient synthesis of bullatenone.

The occurrence of a 3(2*H*)-furanone system in a natural product has apparently not been encountered before and the biosynthesis of bullatenone is, therefore, of interest. Possibly, the biosynthesis can involve reaction of mevalonic acid with a benzoic acid derivative in a not easily explainable manner; but it also seems attractive to consider bullatenone as formed from citraconic or mesaconic acid and a shikimic acid derivative in a manner simulated in the synthesis of *8* from *3* *via* *7*. The drawbacks of such a biogenetic scheme is that a decarboxylation of *8* to bullatenone (*10*) could not be carried out *in vitro* and that benzoylacetate to our knowledge has not been found in nature. The reasons for taking such a biosynthetic hypothesis into consideration is that 3(2*H*)-furanones carrying a carboxyl group in the 4-position easily can be transformed into tetronic acids (compounds *7* and *9* are transformed with alkali to the tetronic acids *11* and *12*, respectively, in high yields and in almost pure form) and that α-fumaroyl-β-keto esters



via 3(2*H*)-furanones have been transformed directly to naturally occurring tetronic acid fungal metabolites.[5,7] These latter compounds apparently are biosynthesised from a polyketide and a C_4-compound being either oxaloacetate, fumarate, or malate.[10–12]

Experimental. All m.p.'s and b.p.'s are uncorrected. The ^{1}H NMR couplings are first order couplings.

Ethyl 5,6-dihydro-5-methyl-4-oxo-2-phenyl-(4H)-pyran-3-carboxylate (4). Methacryloyl chloride (26.1 g, 0.25 mol) was added dropwise, at a rate sufficient to maintain vigorous reflux, to a stirred ethereal solution of ethyl ethoxymagnesiobenzoylacetate (prepared [3] from 48.0 g (0.25 mol) of *1*). The mixture was then refluxed for 30 min, treated at 0 °C with ice and 60 ml of 2 M sulfuric acid. The ethereal phase and ether washings of the aqueous phase were dried and evaporated *in vacuo* giving the product as crystals, which upon washing with ether had m.p. 117–119 °C (52.6 g, 81 %). Anal. $C_{15}H_{16}O_4$: C, H. UV [ethanol (log ε)]: 217 (3.97), 242 (3.83), 288 (4.20) nm. ^{1}H NMR ($CDCl_3$): δ 1.05 (t, 3 H, *J* 7.0), 1.20 (d, 3 H, *J* 6.0), 2.5–3.1 (m, 1 H), 4.11 (q, 2 H, *J* 7.0), 4.0–5.0 (m, 2 H), 7.3–7.8 (aromatic, 5 H).

5,6-Dihydro-5-methyl-2-phenyl-4-pyrone (5). A solution of *4* (5 g, 0.019 mol) in 15 ml of 90 % sulfuric acid was heated slowly to 120 °C and kept at this temperature until evolution of CO_2 ceased. The solution was poured on ice, and the crystalline material was collected and washed with water to give 3.1 g (87 %) of material, m.p. 95–97 °C (lit.[2] m.p. 96–97 °C). UV in agreement with lit.[2] ^{1}H NMR ($CDCl_3$): δ 1.16 (d, 3 H, *J* 7.0), 2.3–3.0 (m, 1 H), 4.0–4.8 (m, 2 H), 5.97 (s, 1 H), 7.2–7.9 (aromatic, 5 H).

Ethyl (α-bromoisobutyryl)-benzoylacetate (6) was obtained from 2-bromo-2-methylpropanoyl bromide (87.4 g, 0.38 mol) and ethyl benzoylacetate (73.0 g, 0.38 mol) as described for *4*. The product was obtained as a yellow oil (129 g, 100 %) and used in crude form (Found: Br 23.0 %. Calc. for $C_{15}H_{17}BrO_4$: 23.4 %).

Ethyl 4,5-dihydro-5-ethoxycarbonylmethyl-5-methyl-4-oxo-2-phenylfuran-3-carboxylate (7). Ethyl benzoylacetate (19.2 g, 0.1 mol) and *trans*-β-ethoxycarbonylmethacryloyl chloride (15.8 g, 0.09 mol) were reacted as described for *4*. The combined ether extracts were shaken 5 min with concentrated hydrochloric acid (10 ml), washed twice with sodium hydrogencarbonate and once with water. After drying (Na_2SO_4) and evaporation of the solvent *in vacuo* 28.4 g of a reddish oil was left. On distillation the fraction with b.p. 180–190 °C/0.11 mmHg was collected. Redistillation gave 14.5 g (49 %) of yellow oil, b.p. 176–182 °C/0.12 mmHg, n_D^{23}: 1.5481, UV[ethanol (log ε)]: 213 (4.11), 246 (3.84), 293 (4.20) nm. ^{1}H NMR ($CDCl_3$): δ 1.27 (2 × t, 6 H, *J* 7.0), 1.56 (s,3 H), 2.96 (s, 2 H), 4.20 (2 × q, 4 H, *J* 7.0), 7.3–8.0 (aromatic, 5 H). Anal. $C_{18}H_{20}O_6$: C, H.

2-Carboxymethyl-2-methyl-5-phenyl-3(2H)-furanone (8). Compound *7* (1.0 g, 0.0031 mol) was treated with 90 % sulfuric acid as described for *5*, poured on ice and extracted with ether. The ether extracts were dried (Na_2SO_4) and the solvent removed *in vacuo* leaving 0.86 g of viscous oil, which soon solidified. Recrystallisation from ethyl acetate/light petroleum afforded 0.68 g (80 %) of colourless plates of m.p. 135–137 °C (lit.[13] m.p. 130–140 °C). UV[ethanol (log ε)]: 218 (3.91), 225 (3.88), 240 (3.87), 295 (4.19), 302 (4.20) nm, (lit.[13] UV[ethanol (log ε)]: 242 (4.02), 295 (4.32), 304 (4.33) nm). The NMR data were in accordance with those published.[13]

Ethyl 4,5-dihydro-5,5-dimethyl-4-oxo-2-phenylfuran-3-carboxylate (9). A mixture of *6* (85.2 g, 0.25 mol) and triethylamine [3] (50 ml) in dry toluene (700 ml) was stirred at room temperature for 30 min, heated gradually to the b.p. and allowed to cool for 1 h. The precipitate was filtered off and washed with toluene. The toluene phase was evaporated *in vacuo* to give after recrystallisation from tetrachloromethane 27.7. g (43 %) of prisms m.p. 60–62 °C. Anal. $C_{15}H_{16}O_4$: C, H. UV [ethanol (log ε)]: 213 (4.12), 247 (3.83), 292 (4.22) nm. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, 3 H, *J* 7.0), 1.53 (s, 6 H), 4.28 (q, 2H, *J* 7.0), 7.2–8.0 (aromatic, 5 H).

Bullatenone (10). Hydrolysis and decarboxylation of *9* (5.2 g, 0.02 mol) in 90 % sulfuric acid (15 ml) as described for *5* gave 2.9 g (77 %) of *10*, m.p. 64.5–67.0 °C. On recrystallisation from light petroleum the m.p. was 66–67 °C undepressed by admixture with authentic bullatenone. The IR spectra of *10* and authentic bullatenone were superimposable. ^{1}H NMR ($CDCl_3$): δ 1.50 (s, 6 H), 5.93 (s, 1 H), 7.3–7.8 (aromatic, 5 H).

3-Benzoyl-5-carboxymethyl-5-methyltetronic acid (11). Compound *7* (1.0 g, 0.0031 mol) was converted to *11* as described.[7] After work-up the product came out as 0.84 g of oil, which solidified. The crystals were thoroughly ground in ether leaving 0.55 g (65 %) of analytically pure *11*, m.p. 140–143 °C. Anal. $C_{10}H_{12}O_6$: C, H. UV [ethanol (log ε)]: 235 (4.32), 283 (4.00)nm. ^{1}HNMR (CD_3OD): δ 1.56 (s, 3 H), 3.03 (2 × s, 2 H), 7.5–8.3 (aromatic, 5 H).

3-Benzoyl-5,5-dimethyltetronic acid (12). Compound *9* (5.2 g, 0.02 mol) was converted to *12* as described [7] to give 3.6 g of solidified oil, which upon trituration with ether afforded 3.3 g (89 %) of *12*, m.p. 74–75 °C. Anal. $C_{13}H_{12}O_6$: C, H. UV [ethanol (log ε)]: 237 (4.33), 284 (4.00) nm. ^{1}H NMR (CD_3OD): δ 1.53 (s, 3 H), 1.57 (s, 3 H), 7.4–8.5 (aromatic, 5 H).

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The Reactions of Lignin during Neutral Sulfite Pulping. Part VIII.* Sulfonic Acids Isolated after Treatment of Spruce Wood Meal with Neutral Sulfite

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The behaviour of the main structural elements of lignin under the conditions of neutral sulfite pulping has been the subject of extensive model experiments.[2-6]

The present work is concerned with the isolation and identification of some of the main monomeric sulfonic acids formed during the treatment of finely divided wood with neutral sulfite.

Spruce wood meal (200 g), pre-extracted with acetone, was treated with a solution of sodium sulfite (120 g) in water (1600 ml) at 180 °C for 3 h in a stainless steel autoclave (initial pH ~ 10, final pH ~ 7). The resulting pulp (120 g; lignin content according to Klason 15.7 %) was washed with water and the total liquor concentrated to 2000 ml. Continuous extraction with methylene chloride removed 840 mg of lipophilic material. This was not investigated further. To the remaining aqueous solution, Hyamine 10-X ** (80 g) was added to precipitate polymeric lignosulfonic acids.[7] After centrifugation, tetrabutylammonium sulfate (80 g) was added to the remaining solution. The low-molecular weight lignosulfonic acids were continuously extracted as ion pairs [8] with methylene chloride. Evaporation of the solvent gave a mixture of sulfonates which were acetylated with acetic anhydride-pyridine (1:1) at room temperature. Excess acetic anhydride and pyridine were removed by evaporation under reduced pressure and water was added. The aqueous suspension was treated with active carbon to remove undissolved material (probably acetylated carbohydrates) and the acetylated sulfonic acids were then converted into methyl esters (23.6 g) as previously [9] described. Column chromatography on silicic acid (Bio-Sil A 100 – 200 mesh, Bio-Rad Lab., Richmond, California) using ethyl acetate as solvent removed 3.6 g of polymeric material. The remaining low-molecular weight acetylated sulfonic acid methyl esters were separated in a liquid chromatograph (Chromatromix Inc., Berkeley, California) using silicic acid (Bio-Sil A 200 – 325 mesh) as the stationary phase. The flow rate in a 25.4 × 1000 mm column was 1 ml/min. The individual sulfonic acid esters were identified by comparing the ^{1}H NMR and mass spectra with those of authentic samples (for references, see below).

The mixture of sulfonates was first separated using cyclohexane-ethyl acetate (1:1) as solvent system. Four fractions were obtained in yields of 1.6, 6.2, 2.7 and 4.9 g. On further column chromatography, only fractions 2 and 4 gave pure components. The composition of the other fractions was too complex to allow successful separation.

Fraction 2 (6.2 g) was subjected to chromatography but now using cyclohexane-ethyl acetate (3:2) as solvent system. Five subfractions were obtained in yields of 360, 160, 1060, 945 and 1285 mg.

Subfraction 3 (1.06 g) was further chromatographed using cyclohexane-ethyl acetate (7:3) as solvent system. Pure methyl methanesulfonate [6] (300 mg) was obtained. ^{1}H NMR: δ 2.96 (s, 3 H, Me), 3.84 (s, 3 H, OMe).

* Part VII, see Ref. 1.

** Benzyldimethyl {2-[2-(*p*-1,1,3,3-tetramethylbutylcresoxy)ethoxy]ethyl}ammonium chloride monohydrate (Rohm and Haas Co.).

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The Reactions of Lignin during Neutral Sulfite Pulping. Part VIII.* Sulfonic Acids Isolated after Treatment of Spruce Wood Meal with Neutral Sulfite

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Subfraction 4 (945 mg) was also separated using the same solvent system. Two fractions were obtained in yields of 200 and 410 mg. In the first fraction, α-methoxysulfonyl-β-acetoxymethyl-4-acetoxy-3-methoxystyrene (*1*) [2] was present as major component. The second fraction contained mainly dimethyl 1-(4-acetoxy-3-methoxyphenyl)-ethane-1,2-disulfonate (*2*).[2,4] In both fractions the main component was accompanied by small amounts of methyl 1,2-di-(4-acetoxy-3-methoxyphenyl)-ethane-1-sulfonate (*3*),[3] as indicated by the mass spectra.

Subfraction 5 (1285 mg) was separated using cyclohexane-ethyl acetate (3:2) as solvent system. Two fractions (315 and 110 mg) were obtained, both containing compound *2* as major component. Mass spectral fragmentation of the first and second fraction indicated the presence of small amounts of compound *3* and methyl 1-(4-acetoxy-3-methoxyphenyl)-3-acetoxypropane-1-sulfonate (*4*),[1] respectively.

Fraction 4 (4.9 g) was separated using cyclohexane-ethyl acetate (2:3) as solvent system. One component could be obtained in pure form and identified as dimethyl 1-(4-acetoxy-3-methoxyphenyl)-propane-1,3-disulfonate (*5*) [10] (2200 mg).

The sulfonic acids hitherto isolated and identified as their acetylated methyl esters (*1* – *5*) are the dominating monomeric components of the resulting complex reaction mixture (TLC). They are all products expected to be formed by neutral sulfonation of known structural elements [11] of the "uncondensed" [12] type. Thus, compounds *1* and *2* should arise from terminal (=phenolic) or inner (=non-phenolic) arylglycerol-β-aryl ether structures.[2] Compound *3* should originate from 1,2-diaryl-propane-1,3-diol structures,[3] and compounds *4* and *5* from end groups of the coniferaldehyde [1] and coniferyl alcohol [10] types, respectively. The formation of compounds *1* – *5* presupposes the existence or the sulfitolytic liberation of the respective structure as a free phenol.

The isolation of these compounds supports the validity of the sulfonation schemes proposed for the aforementioned structural elements on the basis of the results from model studies.[1–4] Additional acids suggested in these schemes are likely to be present, albeit in minor amounts, in the fractions not successfully separated so far.

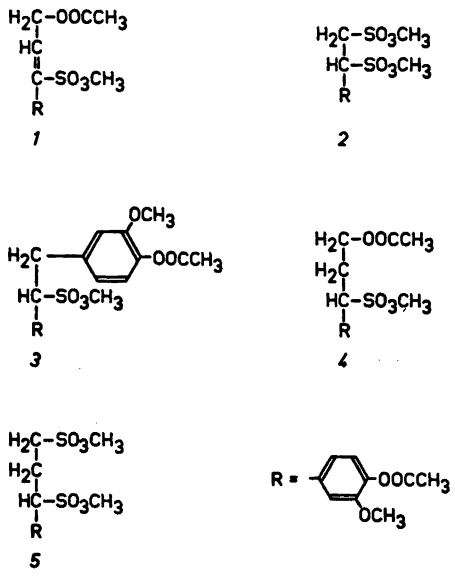


Fig. 1. Acetylated sulfonic acid methyl esters isolated after treatment of spruce wood meal with neutral sulfite, acetylation, methylation and column chromatography.

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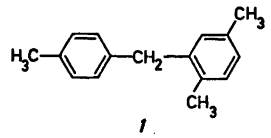
Metal Ion Oxidation. V.[1] The Mechanism of the Dehydrodimerization of *p*-Xylene by Perchloric Acid and Iron(II) Chloride in Acetic Acid

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One of us has earlier reported on the reaction between iron(III) chloride and methylbenzenes and its mechanism.[2-4] The reaction shows similarities with the anodic oxidation of methylbenzenes yielding biaryls and/or diphenylmethanes.[2] In a recent report it was shown that selective formation of diphenylmethanes can be achieved by the oxidation of methylbenzenes using various iron(III) salts (chloride, sulfate, nitrate, acetate and perchlorate) in the presence of perchloric acid using acetic acid as a solvent at elevated temperatures.[5] In the absence of perchloric acid the yields were low. In many cases the reaction was catalytic in iron(III) and the authors proposed that reoxidation of iron(II) was caused by atmospheric oxygen. We have been studying a similar reaction for some time and now report results showing that the proposed mechanism [5] is not correct and that the reoxidation of iron(II) is caused by perchloric acid.

When iron(II) chloride is heated with *p*-xylene in acetic acid and in the presence of perchloric acid *1* is formed (Table 1, run No. 1).



1

The yield of *1* is better than 100 % if one assumes that iron(III) is the oxidant responsible for converting *p*-xylene into *1*. The reaction would then require 2 mol of iron(III) for each mole of *1* formed. A prolonged reaction time (run No. 2) increased the yield of *1* slightly. Runs Nos. 3 and 4 show that no reaction takes place in the absence of either iron(II) or perchloric acid. Reactions 1 – 4 were carried out in an air atmosphere. Run No. 5 was carried out under pure nitrogen (with careful exclusion of oxygen) which resulted in a slightly lower yield of *1* but the reaction was still catalytic. Thus, the results of these experiments (runs Nos. 1 – 5) do not indicate that oxygen plays a major role in the oxidation of iron(II) to iron(III). Finally, we carried out a reaction where perchloric acid was replaced by trifluoromethanesulfonic acid, a strong non-oxidizing acid (run No. 6); a low yield of *1* was obtained.

We believe that an explanation of our results is consistent with the assumption that the major reaction path involves perchloric acid oxidation of iron(II) to iron(III). The latter species acts as the oxidant towards *p*-xylene. Oxygen plays only a minor role in the reoxidation of iron(II) as indicated by the result of run No. 6. It is well known that perchloric acid is a weak oxidant at room temperature but a strong one at temperatures above 100 °C.[6] The results from run No. 4 shows that perchloric acid is not the oxidant responsible for the formation of *1*.

We have also studied the reaction between iron(III) chloride and *p*-xylene in acetic acid and verified the low yield of *1* previously reported.[5] This is, however, not surprising. When iron(III) chloride is heated in acetic acid a red iron(III) compound is formed. At room temperature it precipitates from acetic acid.[6,7] We found that the red iron(III) compound is inactive as an oxidant. Since some oxidation took place between iron(III) chloride and *p*-xylene in acetic acid, there are probably other iron(III) compounds formed acting as oxidants. A recent report on the analysis of

Table 1. Yields of *1* in the oxidation of *p*-xylene (80 mmol) in acetic acid (50 ml) at 114 °C for 5 h.

Run No.	$FeCl_2$/mmol	$HClO_4$[a]/mmol	*1*[b]/mmol	mol of *1*/mol of $FeCl_2$
1	5	40	2.91	0.58
2	5	40	3.17[c]	0.63[c]
3	5	0	0	0
4	0	40	0	0
5	5	40	2.72[d]	0.54[d]
6	5	0	0.31[e]	0.06[e]

[a] 70 % $HClO_4$. [b] Determined by GLC. [c] 17 h. [d] In a pure nitrogen atmosphere. [e] In the presence of CF_3SO_3H (40 mmol).

the products in the reaction between iron(III) chloride and acetic acid shows that four iron(III) compounds are formed depending on the reaction conditions.[7] One of these is the red precipitate mentioned above. We have found that treating solid iron(III) chloride with a solution of dichloromethane containing slightly more than one equivalent of acetic acid produces a soluble iron(III) species. This compound reacts with methylbenzenes producing diphenylmethanes selectively. A full report of this reaction will be published later.

Experimental. Commercial anhydrous iron (III) chloride and acetic acid was used without further purification. The reaction conditions are given in Table 1. The reaction mixtures were worked up adding dichloromethane (100 ml), washing the solution 3 times with water. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated by evaporation *in vacuo*. The resulting solution was analyzed by GLC using an HP 6830 A instrument equipped with an electronic integrator on a 2 m × 0.3 cm 3 % OV-17 on Chromosorb W column. *1* was identified by comparison with an authentic sample (GLC, MC). The yields were determined by calculation of the GLC peak areas after calibration of *1* against a standard.

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Protonation and Deprotonation of Enamines. III. On the Use of Ion Exchange Resin for Selective Protonation of Enamines

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In a previous paper[1] we reported that the isomeric enamines obtained from isopropyl methyl ketone and morpholine, *1a* and *2a* (Fig. 1), could be easily separated by treating the tautomeric mixture with gaseous HCl or trifluoroacetic acid (which can afford a selective protonation of *1a*). We have studied whether this technique could be improved to achieve the separation either by column chromatography or by batch procedure using acid ion exchange resin. Different types of resins were examined for enamine protonating abilities. "Amberlyst 15", a macroreticular resin with aromatic sulfonic acid groups and only *ca.* 2 % of moisture content, was found to be suitable. Other strongly acidic resins of the polystyrene – divinylbenzene type have a moisture content of *ca.* 50 % causing hydrolysis of the enamines. Drying of these resins evidently altered their properties since the dried resins were almost devoid of enamine protonating ability when used in pentane. The enamines studied were the tautomeric equilibrium mixtures obtained from morpholine and isopropyl methyl ketone, (*1a*, *2a*), cyclopentyl methyl ketone, (*1b*, *2b*) and cyclohexyl methyl ketone, (*1c*, *2c*). The isomer distribution (pentane solution, room temperature) was *1a*:*2a* = 29:71, *1b*:*2b* = 9:91 and *1c*:*2c* = 65:35.

Results. Preliminary experiments have shown that adding Amberlyst 15 to a pentane solution of *1a* and *2a* (equilibrium mixture) changed the isomeric distribution with a relative increase of *2a*, *i.e.* a selective protonation of *1a*. A study of the effects of amount of resin (1 – 3 equivalents/*1a*), concentration of enamine solution (0.5 – 2.0 M) and temperature (−78 to 25 °C) showed that neither the amount of resin nor the enamine concentration affected selectivity. The temperature is the only important factor, and that low temperature does not give selectivity, is probably due to a rapid non-



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selective *N*-protonation and a too slow selective rearrangement to the alternative *C*-protonated immonium salt. At 0 °C and even at room temperature the reaction is selective and gives an isomer ratio in solution *2a*:*1a* = 95:5 (^{1}H NMR), with yields 90 – 95 % (internal standard, ^{1}H NMR) after 30 min. No change was found by increasing this time to 2 h. A preparative scale experiment (40 mmol) gave 86 % as isolated yield with the isomer ratio *2a*:*1a* = 92:8. Evaporation of the solvent inevitably gave some isomerisation since ^{1}H NMR analysis prior to removal of solvent showed 95 % *2a*. Selectivity was also found in benzene solution; after 30 min at room temperature an isomer distribution *2a*:*1a* = 92:8 was obtained. In chloroform no selectivity was found.

Unfortunately, the scope of the method is limited since no selectivity in protonation by ion exchange resin was observed to occur with the enamines obtained from cyclopentyl methyl and cyclohexyl methyl ketone. However, using trifluoroactic acid as described in Ref. 1, *2b* was obtained in 98 % isolated yield with a purity better than 99 %, and *2c* was obtained in quantitative yield with a purity of 96 %.

Attempts at liberating the enamine from the resin bound immonium salt by treatment with base have not been successful. The immonium salt seems to be rather inert and tightly bound to the resin, such that not even a concentrated solution of amine base at reflux temperature was effective in liberating the enamine.

Experimental. The enamines were prepared by the titanium tetrachloride method.[2] Ion exchange resin Amberlyst 15 was purchased from FLUKA and washed with diethyl ether, finally with pentane boiling from a piece of sodium in a Soxhlet apparatus and dried *in vacuo* over solid paraffin. The capacity was determined to 4.5 mmol H^+/g of resin.

Selective protonation of 1a *using Amberlyst 15.* To 6.21 g of a mixture of *1a*:*2a* = 30:70 (40 mmol) in 40 ml of pentane was added 3.00 g of Amberlyst 15. After stirring at room temperature for 30 min the resin was removed by filtration and washed three times with 5 ml of pentane. A few crystals of 1,8-bis(dimethylamino)naphthalene were added to the filtrate to suppress acid catalyzed isomerisation and the solvent was removed by evaporation under reduced pressure giving 3.72 g of enamine *2a*:*1a* 92:8, (86 % calculated from the amount of *2a* present in the starting material). When this isomeric mixture was dissolved in 25 ml of pentane and treated with 0.60 g of Amberlyst 15 the amount of *1a* dropped to less than 5 % within 30 min.

Acknowledgements. A grant from CNRS (France) to R.C. and financial support from the Swedish Natural Science Research Council are gratefully acknowledged.

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Organic Electrosyntheses. X.[1] Preparation of *N*-Benzylidene-*tert*-butylamine-*N*-oxide

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The title compound *3* has been used as a radical scavenger[2] and is normally obtained by isomerization of the corresponding oxaziridine[3] or by direct condensation of the components.[4] We here describe a simple procedure for its preparation using as a key step the cathodic reduction of 2-methyl-2-nitropropane *1*:

$$(CH_3)_3CNO_2 \xrightarrow[HCl/EtOH]{4e^- + 4H^+} (CH_3)_3CNHOH,HCl$$

1 85 – 90 % *2*

$$\xrightarrow[NaOAc/EtOH]{C_6H_5CHO} (CH_3)_3C-N(\downarrow O)=CHC_6H_5$$

80 – 85 % *3*

The electrochemical reduction of *tert*-nitroalkanes to the corresponding *N*-alkylhydroxylamines is a general reaction proceeding with high yields[5] and in the present case has the advantage that the crude, wet *2* from the permanganate oxidation of *tert*-butylamine[2] can be used directly in the electrolytic step.[6] Furthermore, the product *N*-*tert*-butylhydroxylamine *2* from simple evaporation of the catholyte (giving the hydrochloride) is also used without purification for the condensation with benzaldehyde in the presence of sodium acetate to form the desired nitrone *3* in about 50 % overall yield from *tert*-butylamine on a 0.5 mol scale. Thus the handling of the free base of *2* (which is easily oxidized)[2] is avoided and manual work kept at a minimum by using essentially the same approach as for 2-methyl-2-nitrosopropane.[6] Also unsophisticated electrolytic

selective *N*-protonation and a too slow selective rearrangement to the alternative *C*-protonated immonium salt. At 0 °C and even at room temperature the reaction is selective and gives an isomer ratio in solution *2a*:*1a* = 95:5 (^{1}H NMR), with yields 90 – 95 % (internal standard, ^{1}H NMR) after 30 min. No change was found by increasing this time to 2 h. A preparative scale experiment (40 mmol) gave 86 % as isolated yield with the isomer ratio *2a*:*1a* = 92:8. Evaporation of the solvent inevitably gave some isomerisation since ^{1}H NMR analysis prior to removal of solvent showed 95 % *2a*. Selectivity was also found in benzene solution; after 30 min at room temperature an isomer distribution *2a*:*1a* = 92:8 was obtained. In chloroform no selectivity was found.

Unfortunately, the scope of the method is limited since no selectivity in protonation by ion exchange resin was observed to occur with the enamines obtained from cyclopentyl methyl and cyclohexyl methyl ketone. However, using trifluoroactic acid as described in Ref. 1, *2b* was obtained in 98 % isolated yield with a purity better than 99 %, and *2c* was obtained in quantitative yield with a purity of 96 %.

Attempts at liberating the enamine from the resin bound immonium salt by treatment with base have not been successful. The immonium salt seems to be rather inert and tightly bound to the resin, such that not even a concentrated solution of amine base at reflux temperature was effective in liberating the enamine.

Experimental. The enamines were prepared by the titanium tetrachloride method.[2] Ion exchange resin Amberlyst 15 was purchased from FLUKA and washed with diethyl ether, finally with pentane boiling from a piece of sodium in a Soxhlet apparatus and dried *in vacuo* over solid paraffin. The capacity was determined to 4.5 mmol H^+/g of resin.

Selective protonation of 1a *using Amberlyst 15.* To 6.21 g of a mixture of *1a*:*2a* = 30:70 (40 mmol) in 40 ml of pentane was added 3.00 g of Amberlyst 15. After stirring at room temperature for 30 min the resin was removed by filtration and washed three times with 5 ml of pentane. A few crystals of 1,8-bis(dimethylamino)naphthalene were added to the filtrate to suppress acid catalyzed isomerisation and the solvent was removed by evaporation under reduced pressure giving 3.72 g of enamine *2a*:*1a* 92:8, (86 % calculated from the amount of *2a* present in the starting material). When this isomeric mixture was dissolved in 25 ml of pentane and treated with 0.60 g of Amberlyst 15 the amount of *1a* dropped to less than 5 % within 30 min.

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Organic Electrosyntheses. X.[1] Preparation of *N*-Benzylidene-*tert*-butylamine-*N*-oxide

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The title compound *3* has been used as a radical scavenger[2] and is normally obtained by isomerization of the corresponding oxaziridine[3] or by direct condensation of the components.[4] We here describe a simple procedure for its preparation using as a key step the cathodic reduction of 2-methyl-2-nitropropane *1*:

$$(CH_3)_3CNO_2 \xrightarrow[HCl/EtOH]{4e^- + 4H^+} (CH_3)_3CNHOH,HCl$$

1 85 – 90 % *2*

$$\xrightarrow[NaOAc/EtOH]{C_6H_5CHO} (CH_3)_3C-N(\downarrow O)=CHC_6H_5$$

80 – 85 % *3*

The electrochemical reduction of *tert*-nitroalkanes to the corresponding *N*-alkylhydroxylamines is a general reaction proceeding with high yields[5] and in the present case has the advantage that the crude, wet *2* from the permanganate oxidation of *tert*-butylamine[2] can be used directly in the electrolytic step.[6] Furthermore, the product *N*-*tert*-butylhydroxylamine *2* from simple evaporation of the catholyte (giving the hydrochloride) is also used without purification for the condensation with benzaldehyde in the presence of sodium acetate to form the desired nitrone *3* in about 50 % overall yield from *tert*-butylamine on a 0.5 mol scale. Thus the handling of the free base of *2* (which is easily oxidized)[2] is avoided and manual work kept at a minimum by using essentially the same approach as for 2-methyl-2-nitrosopropane.[6] Also unsophisticated electrolytic

equipment can be applied. Only a divided cell is indispensable; the electrolysis can be run in unattended constant current mode with a simple rectifier, as there is no risk of over-reduction in strongly acidic medium (hydrogen evolution).

Experimental. N-tert-*Butylhydroxylamine hydrochloride, 2.* Crude *1*[2] (0.6–0.7 mol) was reduced at a mercury cathode in 1:1 conc. hydrochloric acid/ethanol at 30–40 °C in a conventional H-type cell[4] of about 400 ml catholyte volume surrounded by a water bath. A current of 2–3 A from an ordinary selenium rectifier (some voltage ripple is easily tolerated) was found satisfactory (very little hydrogen evolution from the cathode) for about 90 % of the reaction. Towards the end the current was reduced stepwise to minimize hydrogen evolution. Alternatively potentiostatic control at −0.9 V *vs.* Ag | AgCl | 4 NHCl has been used.[6] Work-up by simple evaporation of the catholyte gave the crude hydrochloride of *2*.[6] To obtain a dry product the solid residue was evaporated once more with 75–100 ml of absolute ethanol.

The procedure has been tested on a larger scale (up to 2.5 mol) in a similar cell of 1000 ml capacity with automatic temperature control by means of a contact thermometer in the cathode compartment regulating a magnetic valve and a heat exchanger. The yield of recrystallized *2* hydrochloride (1:5 absolute ethanol/ether) was usually 75–85 %, m.p. 182–183 °C (uncorr.). The cathodic reduction of pure *1* to *2* has been found to give 90 % yield (or more) by anodic polarography.[5]

N-*Benzylidene*-tert-*butylamine*-N-*oxide, 3.* Crude, dry *2* hydrochloride (0.5–0.6 mol) was refluxed for 45–48 h with the equivalent amount of anhydrous sodium acetate in absolute ethanol (150–200 ml) and 5 % less of benzaldehyde (the amount based on a 90 % conversion in the cathodic reduction step). After cooling to room temperature dichloromethane (200 ml) and water (200–300 ml) was added, and after separation the aqueous phase was extracted further with 2 x 25 ml of dichloromethane. The combined organic extracts were washed with aqueous sodium bicarbonate and water, dried over anhydrous magnesium sulfate, and the solvent evaporated to give about 85 % of crude *3*, m.p. 65–71 °C, with a slight smell of benzaldehyde. Recrystallization from 1:3 dichloromethane/light petroleum gave 70–75 % of pure *3* (white needles), m.p. 73–74 °C (uncorr., lit[4] 75–76 °C) with a further small crop of 5–6 %, m.p. 69–71 °C by working up the mother liquor.

This step, tested with pure *2* hydrochloride, gave a total yield of 84–85 % of recrystallized *3*. The use of an equivalent amount of benzaldehyde gave greater losses, as several recrystallizations were necessary to remove traces of unreacted benzaldehyde. It was found advantageous to use an equimolar amount of sodium acetate for neutralization, as 25 % less or excess both resulted in a 10–15 % lower yield of the pure nitrone. The procedure has not been tested with other carbonyl compounds, but ought to be generally applicable.

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The Reactions of Lignin during Neutral Sulfite Pulping. Part VII.* The Behavior of Structural Elements Containing Carbonyl Groups

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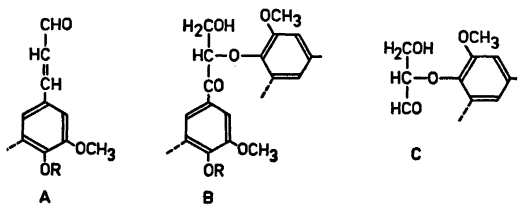
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The reactions of carbonyl-containing lignin units of the coniferaldehyde (A), phenacyl aryl ether (B) and glyceraldehyde-α-aryl ether (C) types with neutral sulfite under technical conditions have been investigated.

All three types undergo extensive sulfonation and suffer cleavage of aryl alkyl ether bonds. These reactions result in the removal of the aldehydic groups in end units (types A and C), in the separation of adjacent units (type B) and in the formation of potential chromophoric groups of the catechol type (types A, B and C).

The significance of these reactions for the behaviour of lignin in neutral sulfite pulping is briefly discussed.

It is known that carbonyl-containing units in lignins participate in the sulfonation process in acidic media.[2] In particular, structural elements containing conjugated carbonyls, such as coniferaldehyde (A) [3,4] and the 1,3-ketol (B) [5] type of structure, have been shown to undergo sulfonation. Aldehydic groups in "detached" side chains (C),[6,7] which constitute another type of 1,3-ketol structure, have been suggested [8] to react in a way analogous to type B.



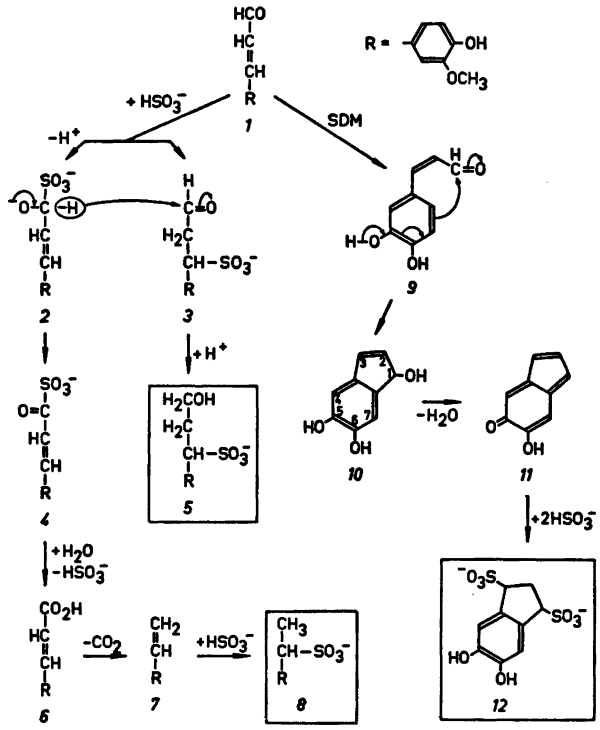
In continuation of our work on the reactions of lignin during neutral sulfite pulping, the behaviour of carbonyl-containing model compounds representing the structural types A – C under the conditions of this pulping process has now been studied.

RESULTS AND DISCUSSION

(a) *Neutral sulfonation of coniferaldehyde and of coniferaldehyde methyl ether.* After treatment of coniferaldehyde (*1*) with neutral sulfite, two monosulfonic acids (*5* and *8*) and one disulfonic acid (*12*) were isolated as the acetylated methyl esters (see Experimental). Plausible pathways of formation for these main products are outlined in Scheme 1.

It is suggested that compounds *5* and *8* are formed by a hydride transfer, the doubly charged bisulfite addition product *2* acting as hydride donor and the aldehydic α-sulfonic acid *3* as acceptor. The dehydrogenated product (*4*), being an α-keto sulfonic acid, readily undergoes hydrolytic cleavage affording ferulic acid (*6*). The latter, probably in the tautomeric quinone methide form which is a vinylogous β-keto acid, looses carbon dioxide and the resulting 4-vinylguaiacol (*7*) adds bisulfite to give sulfonic acid *8*. The facile conversion of ferulic acid (*6*) into *8* was shown in a separate experiment using similar sulfonation conditions. The hydrogenated product (*5*) was isolated. Disproportionation of compound *1* prior to sulfonation can be excluded since the reduced

* Part VI, see Ref. 1.



Scheme 1. Neutral sulfonation of coniferaldehyde (*1*). In this and the following schemes the sulfonic acids are depicted as anions. Isolated products are framed. SDM = sulfitolytic demethylation.

product, coniferyl alcohol, under the conditions used, would yield the corresponding α,γ-disulfonic acid [9] (*cf.* also Ref. 10).

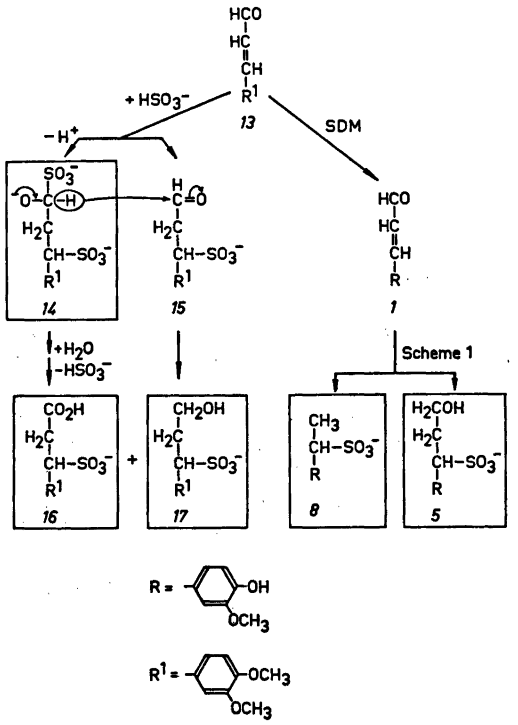
The formation of the disulfonic acid *12* is suggested to be initiated by the sulfitolytic demethylation (SDM) of *1* to give *9*, followed by ring closure to yield 1,5,6-trihydroxyindene (*10*). The latter may be regarded as a cyclic dihydroxydehydrocinnamyl alcohol which is able to form the extended cyclic quinone methide *11*. A 1,8- (or 1,6-) and a subsequent 1,2- (or 1,6-) addition of bisulfite gives the symmetrical dihydroxydisulfonic acid *12*. The behaviour of the hypothetical trihydroxyindene (*10*) towards neutral sulfite is thus analogous to that of (acyclic) *p*-hydroxycinnamyl alcohols, like coniferyl alcohol,[9] and of β-aryl-substituted *p*-hydroxycinnamyl alcohols (α-hydroxymethyl-substituted stilbenes).[1]

Similar demethylation and subsequent cyclization reactions of the coniferaldehyde type of units should also take place upon treatment with other strong nucleophiles (*e.g.* hydrogen sulfide ions). However, the reactions can only be demonstrated if the trihydroxyindene intermediate is immediately trapped to give a stable compound (*e.g.*, by sulfonation) and thus prevented from polymerization.

Treatment of *1* with acidic sulfite afforded the aldehydic sulfonic acid *3* which decomposed during the attempted separation and purification (as the acetylated methyl ester). The formation of coniferaldehyde hydrosulfonic acid groups (*3*) during acidic sulfonation has been previously supported by numerous model experiments and compound *3* has been detected as the sodium salt in the spent liquor from the treatment of red alder with acidic sulfite.[11]

Coniferaldehyde methyl ether (*13*), similarly treated with neutral sulfite, afforded the sulfonic acids, *5*, *8*, *14*, *16* and *17* as major products (Scheme 2). The phenolic α-sulfonic acids *5* and *8* are apparently formed from *1* by the route shown in Scheme 1. Compound *1* arises from the

starting compound (*13*) by partial sulfitolytic demethylation. Further sulfitolytic demethylation of *1* should ultimately result in the formation of the dihydroxyindanedisulfonic acid *12* as outlined in Scheme 1. However, this expected sulfonation product was obviously formed from *13* in such a small amount that it was not found in the reaction mixture. The formation of com-



Scheme 2. Neutral sulfonation of coniferaldehyde methyl ether (*13*).

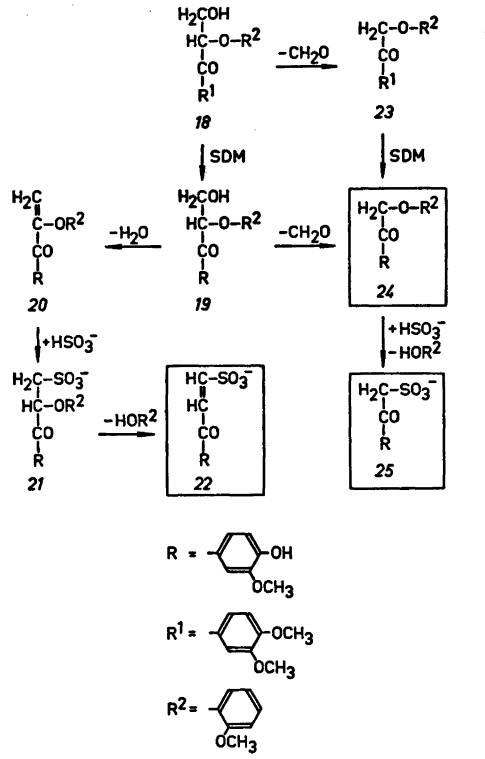
pounds *16* and *17* may again be explained by a hydrogen transfer. The (isolated) disulfonic acid (*14*), formed by addition of 2 mol of bisulfite to *13*, serves as hydride donor and the aldehydic α-sulfonic acid *15*, formed by addition of 1 mol of bisulfite to *13*, as acceptor.

The mechanism of formation of *16* and *17* is thus analogous to that proposed for the formation of ferulic acid (*6*) and of compound *5*, respectively (Scheme 1). However, unlike ferulic acid, the non-phenolic carboxylic acid *16*, formed by hydrolysis of the corresponding α-keto sulfonic acid intermediate, cannot undergo decarboxylation.

Compound *13* is a vinylog of veratraldehyde which has been reported [12] to be in part oxidized by bisulfite to give veratric acid. Attempts to find veratryl alcohol, which would be the reduced product in the case of a disproportionation, have been unsuccessful.

In addition to the sulfonic acids depicted in Scheme 2, the reaction mixture from compound *13* also contained small amounts of 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxypropane-1,3-disulfonic acid, obviously formed by sulfitolytic demethylation of *14*, and of its dehydration product, 1-(4-hydroxy-3-methoxyphenyl)-2-propene-1,3-disulfonic acid.

(b) *Neutral sulfonation of the 1,3-ketol* 18. Treatment of compound *18* (Scheme 3) with neutral sulfite resulted in extensive sulfitolytic cleavage of the alkyl aryl ether bonds. This was revealed by the high yield of methanesulfonic acid and by the phenolic nature of the majority of the other reaction products. Besides methane-



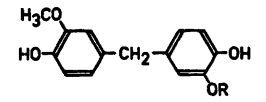
Scheme 3. Neutral sulfonation of 1-(3,4-dimethoxyphenyl)-2-guaiacoxy-3-hydroxypropane-1-one (*18*).

sulfonic acid, the keto sulfonic acids *22* and *25* were the main components of the water-soluble fraction (see Experimental). The formation of these keto sulfonic acids is tentatively outlined in Scheme 3. Demethylation is suggested to be the initial step of the formation of *22*. The resulting phenolic 1,3-ketol intermediate *19* loses one molecule of water to give the unsaturated ketone 20.[13] The latter adds bisulfite affording the keto sulfonic acid *21* which, by a β-elimination, loses the aroxy substituent as guaiacol to yield *22*. The other keto sulfonic acid (*25*) obviously arises by elimination of formaldehyde from either the starting compound *18* or its demethylation product *19*, followed by sulfitolytic cleavage of the phenacyl aryl ether linkage in *24*. The latter reaction step was demonstrated by treating authentic *24* with neutral sulfite and isolating *25* in high yield.[14]

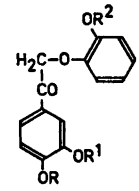
It has previously [5] been reported that compound *18*, when treated with a sulfite solution of pH 6 at 135 °C for 5 h, gives a fair yield of the corresponding keto sulfonic acid (*21*, with R^1 instead of R). This non-phenolic sulfonic acid was not obtained in the neutral sulfonation of the present work, obviously due to the different conditions used (pH 7, 180 °C, 3 h). Under these conditions, sulfitolytic demethylation of *18* and elimination of guaiacol from the suggested intermediate *21* appear to dominate. Treatment of *18* with acidic sulfite (*cf.* also Ref. 5) afforded the keto sulfonic acid (*21*, with R^1 instead of R) in a yield of about 25 %.

The chloroform-ether soluble fraction from the neutral sulfite treatment of *18* contained small amounts of guaiacol, bis(4-hydroxy-3-methoxyphenyl)methane (*26*) and acetoveratrone (*32*) as well as the demethylation products catechol, ω-guaiacoxyacetoguaiacone (*24*), compounds *27*–*29*, acetoguaiacone (*30*) and its isomer (*31*).

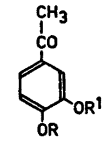
The formation of compound *24* has already been discussed. Compounds *26* and *27* arise by condensation of the eliminated formaldehyde with guaiacol (*27*) or with guaiacol and catechol (*26*). The formation of acetoveratrone (*32*) must involve the reductive cleavage of the phenacyl aryl ether bond in *23*. Acetoguaiacone (*30*) and its isomer (*31*) are formed by partial sulfitolytic demethylation of *32*.



26 R = CH_3
27 R = H



28 R = R^1 = H, R^2 = CH_3
29 R = R^1 = CH_3, R^2 = H

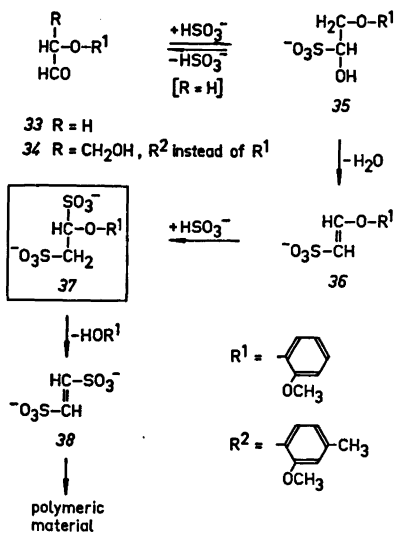


30 R = H, R^1 = CH_3
31 R = CH_3, R^1 = H
32 R = R^1 = CH_3

Phenolic structures of type B should behave like compound *19*, an intermediate in the formation of the unsaturated keto sulfonic acid *22*.

Neutral sulfonation of (2-methoxyphenoxy)-acetaldehyde (33). Like the carbonyl compounds *1*, *13* and *18*, compound *33* undergoes extensive cleavage of the alkyl aryl ether linkages when treated with neutral sulfite. Considerable amounts of guaiacol and catechol were found in the chloroform-ether extract. The water-soluble fraction contained several low-molecular constituents as well as polymeric material. Methanesulfonic acid and 1-(2-methoxyphenoxy)ethane-1,2-disulfonic acid (*37*, Scheme 4) were isolated from this fraction. The formation of the latter product is suggested to proceed *via* bisulfite-addition to the aldehydic group, elimination of water from the resulting α-hydroxy sulfonic acid (*35*) and a further bisulfite addition across the double bond in *36*. Attempts to isolate the unsaturated sulfonic acid *38*, expected to be formed by elimination of guaiacol from *37*, were unsuccessful.

Compound *34*, a better model for the detached side-chain structures of type C, behaves similarly when treated with neutral sulfite. Large amounts of creosol and 4-methyl catechol were obtained, indicating an almost quantitative cleavage of the 2-aryl ether bond and a partial cleavage of the methyl aryl ether bond in *34*. The water-soluble fraction contained methanesulfonic acid and a high-molecular weight material, probably formed by polymerization of unsaturated sulfonic acids arising by a course of reaction analogous to that shown in Scheme 4.



Scheme 4. Neutral sulfonation of guaiacoxyacetaldehyde (*33*).

CONCLUSIONS

All carbonyl-containing structural elements investigated here undergo sulfonation when subjected to the conditions of neutral sulfite pulping. In the case of structures A (coniferaldehyde end groups), the reaction sequences involved lead to the formation of carbonyl-free sulfonic acids and thus remove conjugated (chromophoric) systems (*cf.* also Ref. 1). Structures B (phenacyl aryl ether structures) afford phenacylsulfonic acids and other keto sulfonic acids with a concomitant cleavage of the phenacyl aryl ether linkage by β-elimination, sulfitolysis or reduction. This cleavage implies separation of two adjacent lignin units (fragmentation). Structures C ("detached" glyceraldehyde end groups) give a similar cleavage of the aryl alkyl ether bond accompanied by extensive polymerization of the liberated sulfonated aliphatic moiety.

All three types of carbonyl structures (A, B and C) undergo extensive sulfitolytic demethylation with formation of methanesulfonic acid. This reaction may give rise to catechol groups which are potential chromophores. The elimination of chromophoric groups by sulfite-addition to carbonyl-containing lignin structures (see above) may thus be counteracted by the generation of potential chromophores of the catechol type. An analogous behaviour has recently [1] been observed with intermediates of the stilbene type.

The fragmentation due to the cleavage of phenacyl aryl ether bonds in structural elements of type B may be partly compensated by the condensation of the liberated formaldehyde with phenolic structures. The expected formation of benzyl sulfonic acids, arising by condensation of hydroxymethanesulfonic acid with phenolic structures or by sulfonation of benzyl alcoholic structures (*cf.* Ref. 15), was not observed in these experiments, presumably due to the somewhat lower pH used.

EXPERIMENTAL

Melting points are corrected. Evaporations were carried out under reduced pressure.

Model compounds. The model compounds used were prepared as previously described: *1*,[16] *6*,[17] *13*,[16] *18*,[5] *33*,[18] *34*.[19]*

Sulfite treatment, work-up procedure, chromatographic, ^1H NMR and *MS methods* have been previously described.[20,15]

^1H NMR spectra. The spectra of the chloroform-ether soluble products and the acetylated sulfonic acid methyl esters showed all the expected signals exhibited by aromatic and olefinic protons (range: δ 6.6–7.9), methoxyl protons (ester and ether) (range: δ 3.5–3.9), aliphatic protons (range: δ 2.6–5.9) and acetoxyl protons (aromatically bound: δ 2.25–2.32, aliphatically bound: δ 2.0–2.1).

The chemical shifts and the integrals of the signals were all in agreement with the proposed structures.

Mass spectra. The methyl esters of the (acetylated) sulfonic acids gave peaks of varying intensity for the molecular ions. The main fragmentations are due, in the order given, to the losses of keten (–42) from aromatic acetyl groups, of $\cdot SO_2OCH_3$ (–95) or HSO_2OCH_3 (–96) from aliphatic sulfonic acid methyl ester groups and of acetic acid (–60) from aliphatic acetyl groups.

Treatment of model compounds with sulfite solutions

The following (acetylated) sulfonic acid methyl esters were isolated (Table 1) and

* Part of the compound *34* used in this work was prepared by Drs. Tapio Hase, Leila Lahtinen and Aarno Klemola, Helsinki, Finland, following an improved synthetic procedure (see *Acta Chem. Scand. B 31* (1977) 501), and kindly put at our disposal.

Table 1. Treatment of model compounds containing carbonyl groups with sulfite solutions.[a]

Compound (mg)	Conditions	Products (mg)	
		Chloroform-ether extract	Sulfonic acids, isolated as (acetylated) methyl esters
1 (500)	NS	(60); SM, P	(525); *5* (155), *8* (290) (m.p. 74.5–75.0°) *12* (80), MSA+some P
1 (1000)	AS	(60); P	(920)[b]
13 (500)	NS	(60); V, VE	(1100); *5*+product of demethylation and dehydration from *14* (270),[b] *8* (60), *14*+product of demethylation from *14* (290),[b] *16* (45) (m.p. 94.5–96.5°), *17* (60), MSA
18 (1000)	NS	(450); G (250), C (25), *24* (35), *26*+*30* (65),[b] *27*+*28*+*29* (40),[b] *31*+*32* (25)[b]	(1500); *22* (220) (m.p. 107.5–108.0°), *25* (350), MSA
18 (500)	AS	(375); SM	Methylated *21* (150)
33 (2000)	NS	(1040); G, C	(1720); *37* (260), MSA (290), P
34 (1000)	NS	(665); Cr, MC	MSA, P

[a] *Abbreviations.* Conditions: NS, treatment with neutral sulfite (pH 7) at 180 °C for 3 h. AS, treatment with acidic sulfite (pH 1.5) at 135 °C for 7 h. Products: C, catechol; Cr, creosol; G, guaiacol; MC, 4-methylcatechol; MSA, methanesulfonic acid; P, polymeric material; SM, starting material; V, vanillin; VE, veratraldehyde. [b] The components were indentified by MS without complete separation from each other.

identified by the ^{1}H NMR (60 MHz, $CDCl_3$) and mass spectral data given.

(1) *After neutral sulfonation (pH 7, 180 °C, 3 h) From compund* 1:*

1-(4-Acetoxy-3-methoxyphenyl)-3-acetoxypropane-1-sulfonic acid methyl ester (methyl ester of *5*-diacetate). ^{1}H NMR: δ 1.98 (s, 3 H, Ac), 2.29 (s, 3 H, Ac), 2.6 (broad s, 2 H, CH_2), 3.67 (s, 3 H, OMe), 3.84 (s, 3 H, OMe), 4.2 (broad s, 3 H, CH+CH_2), 7.00 (3 H, ar). MS *m/e* (rel. int.): 360 (M, 6), 318 (M−42, 23), 223 (M−42−95, 10), 222 (M−42−96, 3), 163 (M−42−95−60, 100).

1-(4-Acetoxy-3-methoxyphenyl)ethane-1-sulfonic acid methyl ester (methyl ester of *8*-acetate). m.p. 74.5–75.0 °C. ^{1}H NMR: δ 1.78 (d, 3 H, al. Me), 2.27 (s, 3 H, Ac), 3.67 (s, 3 H, OMe), 3.80 (s, 3 H, OMe), 4.33 (q, 1 H, CH), 7.00 (3 H, ar). MS *m/e* (rel. int.): 288 (M, 5), 246 (M−42, 25), 151 (M−42−95, 100), 150 (M−42−96, 27).

5,6-Diacetoxyindane-1,3-disulfonic acid diethyl ester (dimethyl ester of *12*-diacetate). ^{1}H NMR: δ 2.27 (s, 6 H, Ac), 3.09 (2 H, CH_2), 3.82 (s, 6 H 2×OMe), 4.92 (2 H, 2×CH), 7.51 (2 H, ar). MS *m/e* (rel. int.): 422 (M 3), 380 (M−42, 28), 338 (M−2×42, 41), 285 (M−42−95, 83), 243 (M−2×42−95, 100), 242 (M−2×42−96, 8), 190 (M−42−2×95, 4), 189 (M−42−95−96, 34), 148 (M−2×42−2×95, 10), 147 (M−2×42−95−96, 48).

From compound 13:

1-(4-Acetoxy-3-methoxyphenyl)-3-acetoxypropane-1-sulfonic acid methyl ester. For identification, see above.

1-(4-Acetoxy-3-methoxyphenyl)ethane-1-sulfonic acid methyl ester. For identification, see above.

1-(3,4-Dimethoxyphenyl)-3-acetoxypropane-1,3-disulfonic acid dimethyl ester (dimethyl ester of *14*-acetate). ^{1}H NMR: δ 2.13 (s, 3 H, Ac), 2.96 (broad s, 2 H, CH_2), 3.67 (s, 3 H, OMe), 3.87 (9 H, 3×OMe), 4.2 (broad s, 1 H, CH), 5.6 (broad s, 1 H, CH), 6.89 (3 H, ar). MS *m/e* (rel. int.): 426 (M, 9), 331 (M−95, 13), 271 (M−95−60, 26), 175 (M−95−60−96, 100).

1-(4-Acetoxy-3-methoxyphenyl)-3-acetoxypropane-1,3-disulfonic acid dimethyl ester (dimethyl ester of demethylated *14*-diacetate). MS *m/e* (rel. int.): 454 (M, 1), 412 (M−42, 9), 352 (M−42−60, 9), 317 (M−42−95, 9), 257 (M−42−95−60, 43), 161 (M−42−95−60−96, 100).

1-(4-Acetoxy-3-methoxyphenyl)-2-propene-1,3-disulfonic acid dimethyl ester (dimethyl ester of demethylated and dehydrated *14*-acetate).

* All compounds investigated here gave methanesulfonic acid methyl ester in addition to the sulfonic acid esters mentioned.

MS *m/e* (rel.int.): 394 (M, 2), 352 (M−42, 7), 257 (M−42−95, 22), 161 (M−42−95−96, 100).

3-(3,4-Dimethoxyphenyl)-3-methoxysulfonyl-propionic acid methyl ester (dimethyl ester of *16*). m.p. 94.5−96.5°. ^{1}H NMR: δ 3.29 (d, 2 H, CH_2), 3.62 (s, 3 H, OMe), 3.69 (s, 3 H, OMe), 3.87 (6 H, 2×OMe), 4.73 (1 H, CH), 6.91 (3 H, ar). IR (KBr): 1725 (s, C=O) cm^{-1}. MS *m/e* (rel. int.): 318 (M, 13), 223 (M−95, 100), 222 (M−96, 19), 191 (M−96−31, 10), 181 (M−137, 80), 164 (M−95−59, 10).

1-(3,4-Dimethoxyphenyl)-3-acetoxypropane-1-sulfonic acid methyl ester (methyl ester of *17*-acetate). ^{1}H NMR: δ 1.98 (s, 3 H, Ac), 2.6 (broad s, 2 H, CH_2), 3.67 (s. 3 H, OMe), 3.84 (6 H, 2×OMe), 4.2 (broad s, 3 H, CH+CH_2), 6.89 (3 H, ar). MS *m/e* (rel. int.): 332 (M, 9), 237 (M−95, 2), 177 (M−95−60, 100).

From compound 18:

1-(4-Acetoxy-3-methoxybenzoyl)ethene-2-sulfonic acid methyl ester (methyl ester of *22*-acetate). ^{1}H NMR: δ 2.32 (s, 3 H, Ac), 3.89 (6 H, 2×OMe), 7.19 (d, 1 H, ol, *J* 15.3 Hz), 7.24 (1 H, ar), 7.67 (2 H, ar), 7.84 (d, 1 H, ol, *J* = 15.3 Hz). IR (KBr): 1660 (s. C=C−C=O) cm^{-1}. MS *m/e* (rel. int.): 314 (M, 9), 272 (M−42, 100), 177 (M−42−95, 24), 176 (M−42−96, 22), 151 (M−42−95−26, 53).

4-Acetoxy-3-methoxyacetophenone-ω-sulfonic acid methyl ester (methyl ester of *25*-acetate). ^{1}H NMR: δ 2.30 (s, 3 H, Ac), 3.89 (s, 3 H, OMe), 3.96 (s, 3 H, OMe), 4.67 (s, 2 H, CH_2), 7.00 (1 H, ar), 7.56 (2 H, ar). MS *m/e* (rel. int.): 302 (M, 6), 260 (M−42, 89), 151 (M−42−109, 100).

From compound 33:

1-(2-Methoxyphenoxy)ethane-1,2-disulfonic acid dimethyl ester (dimethyl ester of *37*). ^{1}H NMR: δ 3.50 (s, 3 H, OMe), 3.69 (s, 3 H, OMe), 3.76 (s, 3 H, OMe), 4.1−4.8 (broad s, 3 H, CH+CH_2), 6.6−7.1 (broad s, 4 H, ar). MS *m/e* (rel. int.): 340 (M, 33), 245 (M−95, 100), 244 (M−96, 28), 150 (M−2×95, 80), 149 (M−95−96, 22), 148 (M−2×96, 23), 124 (32).

(2) *After acidic sulfonation (pH 1.5, 135°C, 7 h)*

From compound 1:

3-(4-Acetoxy-3-methoxyphenyl)propionic aldehyde-3-sulfonic acid methyl ester (methyl ester of *3*-acetate). MS *m/e* (rel.int.): 221 (M−95, 2), 220 (M−96, 7), 179 (M−42−95, 13), 178 (M−42−96, 100).

From compound 18:

1-(3,4-Dimethoxyphenylbenzoyl)-1-(2-methoxyphenoxy)ethane-2-sulfonic acid methyl ester (methyl ester of methylated *21*). ^{1}H NMR: δ 3.73 (s, 3 H, OMe), 3.84 (s, 3 H, OMe), 3.87 (s, 3 H, OMe), 3.89 (s, 3 H, OMe), 4.22 (d, 2 H, CH_2), 5.96 (tr, 1 H, CH), 6.89 (5 H, ar), 7.60 (2 H, ar). MS *m/e* (rel. int.): 410 (M, 7), 315 (M−95, 5), 314 (M−96, 20), 192 (M−95−123, 8), 165 (M−245, 100), 150 (M−245−15), 124 (22).

From the chloroform-ether soluble fractions after the neutral sulfonations, the following compounds were isolated by TLC or LC (phenolic compounds as acetates) and identified by ^{1}H NMR and/or GC-MS (comparison with authentic samples, if available): guaiacol, creosol, catechol, 4-methylcatechol, vanillin and veratraldehyde as well as compounds *24* and *26−32*.

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Cathodic Behaviour of Vinylic Sulfones in Weakly Acidic Media

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Four vinylic sulfones, namely, (*E*)-2-phenylvinyl phenyl sulfone, (*E*)-1-methyl-2-phenylvinyl phenyl sulfone, (*Z*)-1,2-diphenylvinyl phenyl sulfone, and 2,2-diphenylvinyl phenyl sulfone, and the cyclic unsaturated sulfone benzo[*b*]thiophene-1,1-dioxide have been reduced at a mercury cathode in *N*,*N*-dimethylformamide solution. The four open-chain compounds yield the corresponding olefins and benzenesulfinate ion (in one case some saturation of the double bond was also observed). The cyclic sulfone is hydrogenated, forms a hydrodimer, or polymerizes, depending on the acidity of the medium.

Polarography and cyclic voltammetry show that with one exception, the reduction potential is shifted in the anodic direction as the acidity of the solvent increases, phenol being used as the proton donor. From the electroanalytical data and the preparative results, a detailed mechanism for the cathodic cleavage is presented.

The electrochemical reduction of sulfones is characterized by cleavage of a carbon-sulfur bond rather than reduction of the sulfonyl group. For example, alkyl aryl sulfones are split at a mercury cathode to yield alkanes and arenesulfinate ions.[1,2] In their extensive study of sulfones, Horner and Neumann [1] included allyl phenyl sulfone, which was found to yield propene and benzenesulfinate ion. Hydrogenation of the allylic double bond was not observed. On the other hand, if a carbon-carbon double bond is conjugated with a sulfonyl group, cathodic reduction of the double bond seems to take place. Phenyl vinyl sulfone is thus converted to ethyl phenyl sulfone,[3] and methyl vinyl sulfone similarly is supposed to yield ethyl methyl sulfone.[4] However, other workers report that methyl vinyl sulfone may be cathodically cleaved to ethene and methanesulfinate ion,[5] the evidence being, however, indirect.

A non-electrochemical cleavage of some phenyl substituted vinyl aryl sulfones with aluminium amalgam in water-containing tetrahydrofuran has been reported by Pascali and Umani-Ronchi.[6] Under their conditions, olefins were exclusively produced.

Since *a priori* different reaction modes are possible, we considered it worth-while to make an electrochemical study of substituted vinyl phenyl sulfones. They might be cathodically cleaved to yield olefins, or the double bond might become saturated. A third possibility is hydrodimerization, an electrochemical reac-

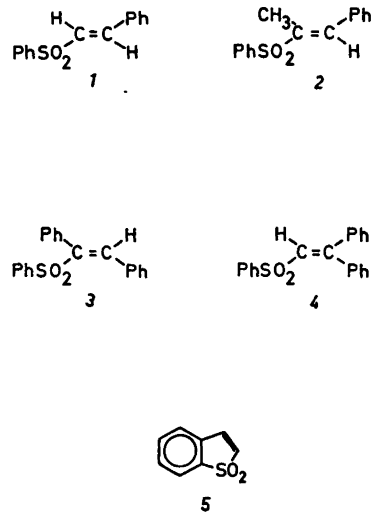


Fig. 1. Unsaturated sulfones investigated in this work.

Table 1. Polarographic and coulometric data for sulfones studied in this work.

Compound	Conc. $\times 10^3$/M	$E_{1/2}$/V [a] [i_d/μA]			$E_{1/2}$/V [b] [i_d/μA]		Coulometry F mol^{-1} (at potential V) [a]	[b]
$PhSO_2CH=CHPh$ (*1*)	1.00	−1.15	−1.63	−1.91	−1.05	−1.87	2.1	2.1
		[2.7]	[2.0]	[3.8]	[4.5]	[4.0]	(−1.4)	(−1.4)
$PhSO_2CMe=CHPh$ (*2*)	1.55	−1.48	−1.70	−2.25	−1.41	−2.17	1.9	2.1
		[5.0]	[3.0]	[2.0]	[9.0]	[4.0]	(−1.6)	(−1.5)
$PhSO_2CPh=CHPh$ (*3*)	1.30	−1.20	−1.60		−1.20	−1.60	2.7	2.3
		[6.0]	[4.0]		[12]	[10]	(−1.4)	(−1.4)
$PhSO_2CH=CPh_2$ (*4*)	1.30	−1.19	−1.85		−0.98	−1.91	2.3	2.1
		[7.0]	[5.0]		[7.0]	[5.5]	(−1.45)	(−1.25)
Benzo[*b*]thiophene-1,1-dioxide (*5*)	2.40	−1.07	−1.59	−2.1	−1.02	−1.97	1.0	2.2
		[7.0]	[5.5]	[7.5]	[16]	[10]	(−1.3)	(−1.3)

[a] In 0.1 M tetraethylammonium perchlorate (TEAP) in DMF. Potential *vs.* Ag,AgI/0.1 M TBAI in DMF. [b] In 0.1 M TEAP in DMF. Phenol present in a concentration 4–5 times that of the substrate. Potential *vs.* Ag,AgI/0.1 M TBAI in DMF.

Table 2. Cyclic voltammetry cathodic peak potentials (E_{pc}) for the vinylic sulfones, saturated analogs, and unsaturated hydrocarbons studied in this work. Sweep rate 80 mV s^{-1}.

Compound	E_{pc}/V [a] Unsat. sulfone				E_{pc}/V [a] Sat. sulfone [b]	E_{pc}/V [a] Unsat. hydrocarbon [c]	
$PhSO_2CH=CHPh$ (*1*)	−1.23	−1.77	−2.06		−1.76	−2.12	
$PhSO_2CMe=CHPh$ (*2*)	−1.42	−1.83	−2.21		−1.75	−2.20	
$PhSO_2CPh=CHPh$ (*3*)	−1.25	−1.59	−1.71	−2.09	−1.67	−1.72	−2.08
$PhSO_2CH=CPh_2$ (*4*)	−1.23	−1.49	−1.99		−1.75	−1.97	
Benzo[*b*]thiophene-1,1-dioxide (*5*)	−1.15	−1.80	−2.03		−1.90	–	

[a] Potential *vs.* Ag,AgI/0.1 M TBAI in DMF. [b] Obtained through cat. hydrogenation of the vinylic double bond of the corresponding unsat. sulfone. [c] Derived from *1*–*4* through replacement of $PhSO_2$ by H. For their origin, see the text.

tion which has in fact been observed for methyl vinyl sulfone in an attempted mixed coupling reaction.[5]

The present paper deals with the cathodic behaviour of some α,β-unsaturated sulfones. They are shown in Fig. 1 and will be referred to as *1*–*5*. These compounds have been studied with conventional polarography, cyclic voltammetry at a hanging mercury drop electrode, microcoulometry, and gram-scale electrolysis followed by product identification. All experiments have been made in *N*,*N*-dimethylformamide (DMF), and the cathode material has been mercury.

RESULTS

Polarography. The results are summarized in Table 1. In the absence of a proton donor, sulfones *1*, *2*, and *5* give three waves, and *3* and *4* give two. It is obvious that the first step (reversible) is much less cathodic than the reduction wave for simple aromatic sulfones under similar experimental conditions. For example, methyl phenyl sulfone exhibits a wave (irreversible) at −1.73 V.[7] In the presence of phenol as proton donor, the polarograms are simpler; only two waves are visible. The height of the first one is about twice that of the first wave in aprotic medium (except for *4*) and its half-wave potential has moved to a less cathodic potential (except for *3*).

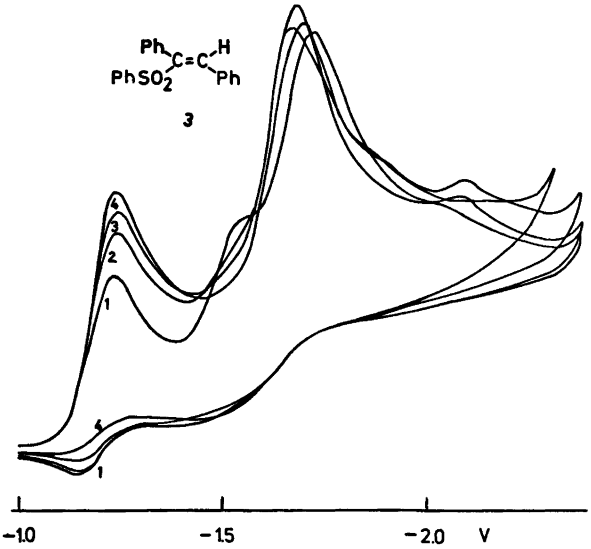


Fig. 2. Cyclic voltammograms of (*Z*)-1,2-diphenylvinyl phenyl sulfone in DMF. Sweep rate 600 mV s^{-1}, substrate conc. 10^{-3} M. Phenol/substrate ratio 0, 1, 2, and 4 for traces 1, 2, 3, and 4, respectively. Potential *vs.* Ag,AgI/0.1 M TBAI in DMF.

Cyclic voltammetry. The peak potentials are given in Table 2. The behaviour of two representative substrates, *3* and *4*, upon addition of increasing amounts of phenol, is depicted in Figs. 2 and 3. In the absence of phenol (trace 1), three peaks are obtained on the forward sweep. Addition of phenol causes the first peak to increase at the expense of the second one. When the concentration of phenol is four times that of the substrate, two peaks of similar height are obtained (trace 4). The first step, which is totally reversible in the absence of phenol, is then irreversible. The reversibility is separately demonstrated in Fig. 4 for substrate *3*. The influence of phenol on the peak potentials of the first step is shown in Fig. 5. It is important to note the invariance of $E_{1/2}$ for sulfone *3*.

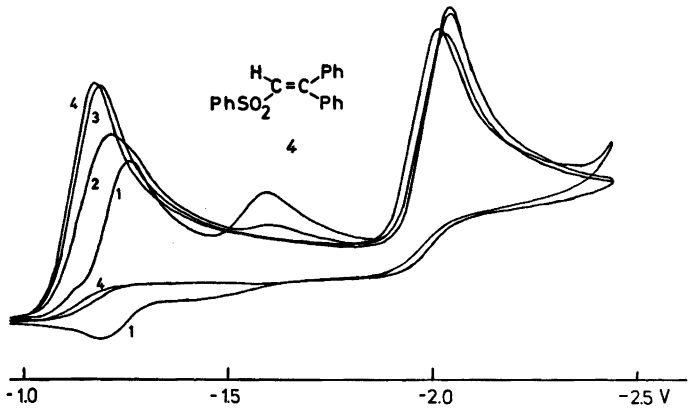


Fig. 3. Cyclic voltammograms of 2,2-diphenylvinyl phenyl sulfone in DMF. Sweep rate 600 mV s^{-1}, substrate conc. 10^{-3} M. Phenol/substrate ratio 0, 0.5, 1.5, and 4 for traces 1, 2, 3, and 4, respectively. Potential *vs.* Ag,AgI/0.1 M TBAI in DMF.

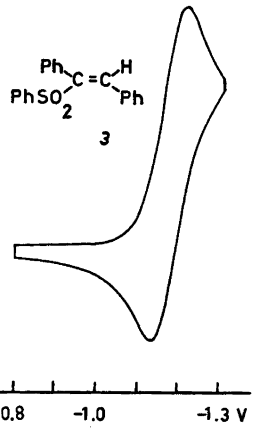


Fig. 4. Cyclic voltammogram of (*Z*)-1,2-diphenylvinyl phenyl sulfone in aprotic DMF. Sweep rate 250 mV s^{-1}, substrate conc. 10^{-3} M. Potential *vs.* Ag,AgI/0.1 M TBAI in DMF.

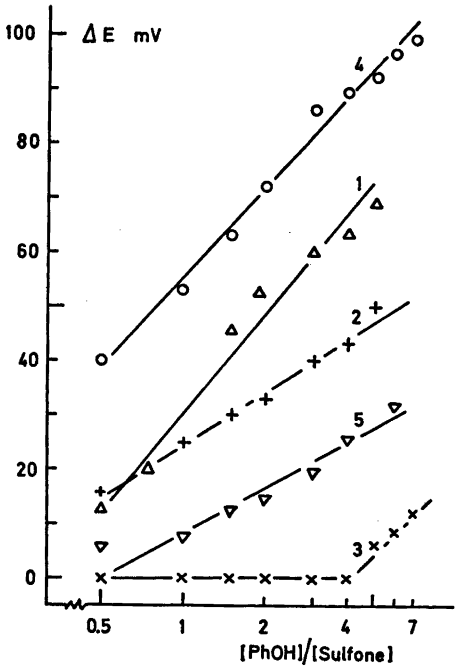


Fig. 5. Shift in potential of the first peak (relative to the aprotic value) in cyclic voltammetry as a function of phenol concentration

The unsaturated hydrocarbons corresponding to *1*–*4*, in which the benzenesulfonyl group is replaced by a hydrogen atom, were separately investigated under the same conditions as the sulfones. The results are given in Table 2. The peak potentials are seen to correspond to the third peak in the voltammograms of the unsaturated sulfones, suggesting that the latter yield the unsaturated hydrocarbons at the cathode.

Coulometry. Runs at the potential of the first polarographic plateau show that 2 electrons per molecule are consumed in protic media (Table 1). In aprotic medium, this number is 1 for sulfone *5* but about 2 for all others.

Preparative electrolyses. Table 3 shows the conditions of electrolysis and the compounds isolated from each experiment. It should be noted that the yields of products represent lower limits because of workup losses. Remarkably, two runs gave almost quantitative yield.

A positive identification of benzenesulfinate ion was made in one case (compound *3*, run with phenol) through conversion to methyl phenyl sulfone.[1,7] In the other runs with *1*–*4*, benzenesulfinate ion was left unidentified.

DISCUSSION

Obviously, the electrochemical reduction of *1*–*4* leads to unsaturated hydrocarbon in a two-electron process both in aprotic and phenol-containing DMF. A comparable amount of saturated hydrocarbon is also obtained with *3*. When the rate of protonation of the radical anion is low (voltammetry in carefully dried DMF), two one-electron steps are observed before hydrocarbon reduction. The dianion formed in the second step can undergo β-elimination followed by protonation to give sulfinate ion and unsaturated hydrocarbon, or it can be protonated (*3*) to give the mono-anion corresponding to the saturated sulfone.

In phenol-containing DMF, the change in height and position of the first peak in the voltammograms is interpreted in terms of an ECE mechanism. The C reaction is protonation of the radical anion of the substrate. Increasing the phenol concentration causes the peak potential to shift in the anodic direction, except for *3*. This behaviour is well-known for many other activated olefins, *e.g.*, aromatic ethylenic ketones.[8]

The unsaturated hydrocarbons resulting from cleavage of *1*–*4* are further reduced between

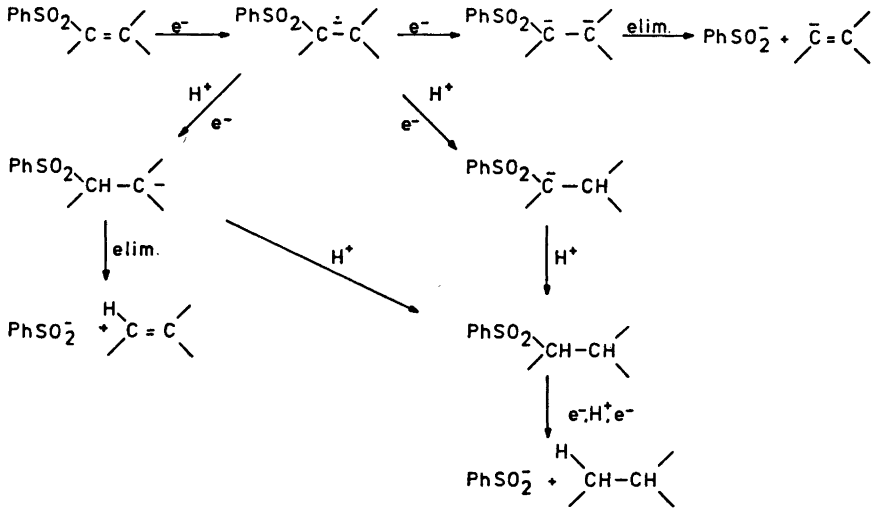
Table 3. Preparative electrolyses of unsaturated sulfones.

Substrate	Cathode potential [a] V	Phenol/substrate initial molar ratio	Coulometry F mol^{-1}	Products (% yield)
1	−1.1	3.2	1.87	Polystyrene (43)
2	−1.4	4.1	1.95	*trans*-*β*-Methylstyrene (54)
3	−1.5	5.1	3.6 [b]	*trans*-Stilbene (15) Bibenzyl (24)
3	−1.5	0	1.63	*trans*-Stilbene (25) *cis*-Stilbene (5) Bibenzyl (20)
4	−1.4	6.8	2.3 [b]	1,1-Diphenylethene (98)
5	−1.0	5 [c]	2.67	Dihydro-*5* (98)
5	−1.3	3.0	1.2	Hydrodimer of *5* (12)
5	−1.3	0	0.43	Unidentified polymer

[a] *vs.* Ag,AgI/0.1 M TBAI, DMF. [b] Run was interrupted after passage of this charge. [c] Acetic acid instead of phenol.

−1.7 and −2.2 V in a two-electron process. The radical anions of the hydrocarbons are protonated fast even in the absence of phenol, except that of *3*, which forms stilbene. The radical anion of stilbene is long-lived in dry DMF.

The mechanism found in this work for the cathodic cleavage of the *α*,*β*-unsaturated sulfones is different from that of the cleavage of alkyl phenyl sulfones or allyl phenyl sulfone, even though the net outcome is the same. The detailed path is shown in Scheme 1. In the absence of a proton donor, the radical anion is stable and may be further reduced to a dianion at more negative potential, as in a cyclic voltammetry experiment. With increasing proton availability, protonation of the radical anion followed by a second electron transfer occurs to produce an organic anion. Depending on the nature and positions of the substituents at the double bond, protonation will take place at the *α* or *β* carbon with respect to the sulfonyl group. In the former case, a *β*-benzenesulfonyl radical is formed, which after reduction to carbanion undergoes *β*-elimination (left route in Scheme 1) to yield unsaturated hydrocarbon.



Scheme 1. Mechanism of cleavage of substituted vinyl sulfones in DMF.

On the other hand, if protonation gives an α-benzenesulfonyl radical, this will after reduction to carbanion become protonated to the saturated sulfone (right route in Scheme 1). The latter mode is observed for *3*. The possibility that the saturated sulfone undergoes a base-catalyzed β-elimination at the cathode to give unsaturated hydrocarbon will be discussed below.

The cyclic sulfone *5* does not undergo elimination of sulfinate ion in a ring-opening reaction. The reason for this is that the double bond activating the sulfonyl group is also an *ortho* substituent to the same group. After electron transfer to the double bond, the sulfinate ion would be a poor leaving group because of its excess of negative charge.

With sulfone *3*, some formation of dihydro-*3* has also been observed.[10] In fact, *3* behaves rather differently compared to *1*, *2*, and *4*. The proton donor concentration has a negligible influence on the peak potential of the first step. This suggests that delocalisation of the anionic charge by the substituents at the double bond is excellent. The stability of the anion radical may allow some homogeneous electron exchange with saturated sulfone,[11] at least in weakly acidic media. In this way, one can explain the formation of bibenzyl from dihydro-*3* rather than *via* hydrogenation of stilbenes, which would be prohibitively slow at the potential actually employed.

Concerning the stereochemistry, only *trans*-stilbene is formed from *3* in the presence of phenol. The starting material is *trans* with respect to the two phenyl groups,[12] and the elimination step is fast enough to preserve the stereochemistry. In aprotic medium, some *cis*-stilbene is also formed. We may assume that the intermediate anion has a longer life-time so that its geometry partially changes. The resulting anion can give both *cis* and *trans* stilbene through elimination of benzenesulfinate ion.

In conclusion, the present investigation has shown that some substituted vinylic sulfones undergo cleavage at a mercury cathode to yield unsaturated hydrocarbons. Nevertheless, the electrochemical characteristics of the reaction (potential influenced by the acidity of the medium) are typical of hydrogenation reactions of activated olefins.

EXPERIMENTAL

Syntheses. (*E*)-2-Phenylvinyl phenyl sulfone (*1*, Fig. 1) was prepared from benzaldehyde and methyl phenyl sulfone according to Field.[13] Yield 33 %, m.p. 72–73 °C, lit.[13] 74–75 °C. The ^{1}H NMR spectrum at 60 MHz showed two peaks belonging to an AB pattern at δ 6.65 and 6.92 ($CDCl_3$), together 1 H. A complex pattern at δ 7.1–8.0, 11 H, contained the other half of the AB pattern and the aromatic proton signals. The coupling constant, 16.2 Hz, established the *E* configuration.

(*E*)-1-Methyl-2-phenylvinyl phenyl sulfone (*2*) was prepared in analogy with (*1*) from benzaldehyde and ethyl phenyl sulfone. Yield 13 %, m.p. 92–93 °C, lit.[14] 90 °C.

(*Z*)-1,2-Diphenylvinyl phenyl sulfone (*3*) was similarly prepared from benzaldehyde and benzyl phenyl sulfone. Yield 44 %, m.p. 187–188 °C, lit.[12] 183 °C.

2,2-Diphenylvinyl phenyl sulfone (*4*) was prepared from benzophenone and methyl phenyl sulfone following the general procedure.[13] Yield 69 %, m.p. 113–114 °C, lit.[15] 114–115 °C.

Benzo[*b*]thiophene-1,1-dioxide (*5*) was available from previous work.[7]

The ^{1}H NMR spectra of *2*–*5* were in agreement with those reported in the literature.[13–15]

For comparison purposes, compounds *1*–*5* were converted to their saturated analogs by catalytic hydrogenation at atmospheric or slightly elevated pressure, using 10 % palladium on charcoal as catalyst and ethanol containing a trace of conc. hydrochloric acid as solvent.

The unsaturated hydrocarbons derived from *1*–*4* through replacement of $PhSO_2$ by H were available in the laboratory (styrene, and *trans*-stilbene), prepared independently through electrolysis of cinnamyl alcohol,[16] (β-methyl styrene), or obtained from a preparative electrolytic reduction of *4* (1,1-diphenyl ethene).

Polarography and cyclic voltammetry. The DMF used for the electroanalytical work was purified as described earlier.[7] Tetrabutylammonium iodide (TBAI) was from Fluka AG, polarography grade. A Tacussel Tipol three-electrode polarograph was used with a sweep rate of 2.5 mV s^{-1}. The supporting salt concentration was 0.15 M, and that of the substrate, 5×10^{-3} M. The reference electrode was Ag,AgI/0.1 M TBAI in DMF. Cyclic voltammograms on a hanging mercury drop were recorded using the same solvent and substrate concentration. A standard instrumentation from Tacussel was used. The voltammograms were either registered on a storage oscilloscope and photographed, or on a Houston XY recorder.

Coulometry. A 0.1 M solution of tetraethylammonium perchlorate in DMF was used as supporting electrolyte. The mercury cathode, about 7 cm^2, was agitated by a magnetic stirring bar, and the platinum anode was separated *via* a glass frit. The catholyte was purged with

nitrogen. The potentiostat and coulometer were a Tacussel type ASA 4SHT and IG4 10A, respectively. Runs were made on 20–30 mg of substrate. To the runs with a proton donor, a 3–5-fold molar excess of phenol was added. The initial current was about 30 mA. The run was stopped when the decay of current was more than 95 %.

Macroelectrolyses. Potentiostatic conditions were used for all macroelectrolyses. An H-type cell with the cathode and anode compartments separated by two glass frits was employed. The supporting electrolyte was 0.15 M TBAI in DMF. A Tacussel potentiostat with current integrator was used. The catholyte was purged with nitrogen. In each run, about 1 g of substrate was taken into work. After electrolysis, the catholyte was diluted with a ten- to twentyfold amount of water and extracted with ether. This was washed with water, dried over anhydrous sodium sulfate, filtered and evaporated. The residue was analyzed with GLC and ^{1}H NMR spectroscopy.

In one experiment, the catholyte was extracted with hexane and the extract was analyzed for electrolysis products. An excess of methyl iodide was then added to the DMF phase, which was heated to 50 °C for 3 h in order to convert the benzenesulfinate ion to methyl phenyl sulfone. The DMF solution was diluted with water and extracted with ether as above.

The compounds isolated from the different runs (Table 3) were easily identified through comparison, with authentic samples and/or literature data.

The electrolysis of *5* in aprotic DMF gave a dark green catholyte, which on workup yielded an unidentifiable, polymeric product. In the presence of phenol, a run with 1.5 g of *5* gave 180 mg of white crystals. These were sparingly soluble in most solvents and had a melting point above 300 °C. A mass spectral analysis (AEI 902 instrument) gave the molecular weight 334, indicating that a hydrodimer of *5* had been formed. We are not sure of its structure, but *a priori*, *β,β*-coupling is most likely.[5]

The low electricity consumption in the run of *3* in aprotic DMF probably depends on the substrate being destroyed by base, generated at the cathode. It could be demonstrated in a test experiment that sodium hydroxide in DMF decomposes *3* on heating.

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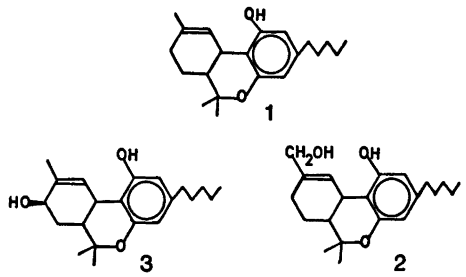
The Crystal and Molecular Structure at —165 °C of the 1:1 Molecular Complex Formed by 8β-Hydroxy-Δ^9-tetrahydrocannabinol and *N,N*-Dimethylformamide

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The structure of the title compound $C_{21}H_{30}O_3.C_3H_7NO$ has been determined by X-ray methods using 2431 reflections collected by counter methods at −165 °C. The complex crystallizes in the space group $P2_1$ with two formula units per unit cell. Cell dimensions at −165 °C are: $a = 8.815(4)$ Å; $b = 10.256(5)$ Å; $c = 12.877(3)$ Å; $\beta = 102.27(3)°$. The structure model was refined to a conventional R of 0.047 and a weighted R_w of 0.045. The side chain shows a fully extended all-*anti* conformation and the cyclohexene and pyrane rings are in the half-chair conformation. The variations of the bond angles and deviations from planarity in the benzene ring, indicate strains in the aromatic system. The phenolic group is bent 0.126 Å out of the plane of the benzene ring. The angle between this plane and a plane through the cyclohexene ring is 40.4°. Along the twofold axes run channels where the *N,N*-dimethylformamide molecules fit. The carbonylic group of the dimethylformamide molecules acts as acceptor for hydrogen bond [2.733(3) Å] from the 8β-hydroxylic group. The phenolic group acts as donor for another hydrogen bond [2.701(3) Å] along the *a*-axes to the 8β-hydroxylic group in a molecule one cell edge away.

The metabolism of Δ^9-tetrahydrocannabinol (Δ^9-THC) (*1*), the predominant active principle of marihuana, has been extensively investigated (for a review, see Mechoulam *et al.*[1]) The major pathway so far identified, is through hydroxylation of the C11 position to give 11-hydroxy-Δ^9-THC (*2*). Another important metabolite, 8β-hydroxy-Δ^9-THC (*3*) has been isolated from incubation of Δ^9-THC with rabbit liver homogenate.[2]



Both metabolites are biologically active in *Rhesus* monkeys and it has been suggested that these compounds are indeed the active forms of Δ^9-THC on the molecular level.[3]

We have earlier studied the structure of the Δ^9-THC skeleton in form of the psychotomimetically inactive Δ^9-THC Acid B (THCAB).[4] However, a fuller understanding of the activity of the cannabinoids requires more information than at the present is available. The effect of different environments on the conformation, and the mode of interaction between Δ^9-THC and other molecules, are important topics to be investigated. Of particular interest would be a complex between a cannabinoid and a model molecule for the receptor. As 8β-hydroxy-Δ^9-THC is an "active" molecule and also forms a stable 1:1 complex with *N,N*-dimethylformamide (DMF), we found this system well suited for a structural study.

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Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations. The anisotropic temperature factor is given by: $\exp\{-2\pi^2[u_{11}(a^*h)^2+\ldots+2u_{23}(b^*c^*kl)]\}$.

Atom	x	y	z	$U11$	$U22$	$U33$	$U12$	$U13$	$U23$
O1	.5618(2)	.3397(0)	.7100(1)	.0144(10)	.0209(11)	.0177(10)	.0037(9)	.0022(8)	−.0016(9)
C2	.4275(3)	.4083(4)	.7103(2)	.0150(14)	.0186(15)	.0155(14)	.0020(13)	.0041(11)	.0029(13)
C3	.3021(2)	.3733(4)	.6298(2)	.0371(17)	.0289(17)	.0314(15)	−.0012(13)	.0099(13)	−.0013(13)
C4	.1597(3)	.4346(3)	.6223(2)	.0174(14)	.0201(16)	.0162(14)	−.0000(13)	.0035(11)	.0033(13)
C5	.1434(3)	.5270(4)	.6983(2)	.0139(14)	.0212(16)	.0171(14)	−.0021(12)	.0042(12)	.0019(12)
C6	.2691(3)	.5603(4)	.7788(2)	.0170(14)	.0189(16)	.0140(14)	−.0013(13)	.0067(11)	−.0010(12)
C7	.4176(3)	.5070(4)	.7836(2)	.0135(13)	.0186(16)	.0137(13)	−.0016(12)	.0046(11)	.0014(12)
C8	.5610(3)	.5466(4)	.8657(2)	.0119(13)	.0192(15)	.0142(13)	.0011(12)	.0026(11)	−.0004(12)
C9	.5826(3)	.6917(4)	.8851(2)	.0154(14)	.0235(16)	.0146(14)	.0037(13)	.0040(11)	−.0019(13)
C10	.7207(3)	.7491(4)	.9150(2)	.0183(15)	.0228(16)	.0190(15)	.0012(13)	.0030(12)	−.0042(13)
C11	.8716(3)	.6751(4)	.9285(2)	.0121(13)	.0279(19)	.0223(15)	−.0021(13)	.0023(12)	−.0061(14)
C12	.8496(3)	.5274(4)	,9192(2)	.0143(14)	.0271(18)	.0192(15)	.0017(13)	−.0004(12)	−.0002(14)
C13	.7069(3)	.4921(4)	.8334(2)	.0114(13)	.0210(16)	.0136(13)	.0039(12)	.0034(11)	−.0005(12)
C14	.6865(3)	.3484(4)	.8049(2)	.0172(14)	.0175(15)	.0169(14)	.0050(12)	.0026(11)	.0024(13)
O15	.2540(2)	.6455(3)	.8574(2)	.0154(10)	.0257(12)	.0202(10)	.0011(9)	.0050(6)	−.0087(9)
C16	.7356(4)	.8932(4)	.9386(3)	.0172(16)	.0275(19)	.0550(24)	−.0029(15)	.0073(16)	−.0157(18)
O17	.9603(2)	.7234(3)	.8540(2)	.0147(9)	.0295(13)	.0283(11)	−.0002(10)	.0086(9)	−.0039(10)
C18	.6449(4)	.2636(4)	.8923(3)	.0340(18)	.0234(18)	.0245(17)	.0006(15)	.0053(15)	.0066(14)
C19	.8259(4)	.2914(4)	.7683(3)	.0214(16)	.0235(18)	.0283(17)	.0076(14)	.0060(14)	.0019(14)
C20	.0266(3)	.4039(4)	.5304(2)	.0168(14)	.0214(16)	.0204(16)	−.0044(13)	.0024(12)	−.0010(14)
C21	.0231(3)	.4942(4)	.4345(2)	.0184(14)	.0223(16)	.0176(14)	−.0008(13)	.0021(12)	−.0001(13)
C22	−.1174(3)	.4664(4)	.3455(2)	.0143(14)	.0280(18)	.0176(15)	−.0012(13)	.0010(12)	.0020(13)
C23	−.1340(3)	.5585(4)	.2511(2)	.0219(15)	.0301(18)	.0202(15)	−.0009(15)	.0010(12)	.0017(15)
C24	−.2925(4)	.5423(5)	.1757(3)	.0259(18)	.0608(27)	.0205(16)	.0024(19)	−.0003(14)	.0053(18)
O25	.7877(2)	.7266(3)	.6508(2)	.0249(12)	.0547(18)	.0314(13)	.0008(13)	.0067(10)	.0163(13)
C26	.6485(4)	.7543(4)	.6343(3)	.0344(19)	.0285(19)	.0253(18)	−.0019(16)	.0093(15)	.0105(16)
N27	.5383(3)	.6918(3)	.5655(2)	.0241(14)	.0226(14)	.0236(13)	.0019(12)	.0100(11)	.0050(12)
C28	.5746(5)	.5807(4)	.5058(3)	.0578(24)	.0238(19)	.0383(20)	−.0010(18)	.0214(19)	−.0033(17)
C29	.3761(4)	.7302(4)	.5508(3)	.0259(17)	.0341(21)	.0373(19)	.0010(16)	.0075(15)	.0074(17)

Atom	x	y	z	B	Atom	x	y	z	B
H31	.312(6)	.289(6)	.567(4)	4.4(14)	H51	.034(5)	.563(5)	.700(4)	2.7(12)
H81	.562(5)	.501(5)	.941(3)	1.2(9)	H91	.488(6)	.743(6)	.878(4)	3.6(13)
H111	.939(5)	.700(5)	1.003(4)	2.4(12)	H121	.953(5)	.490(5)	.901(4)	2.2(11)
H122	.843(6)	.485(6)	.992(4)	4.0(14)	H131	.721(5)	.540(5)	.764(4)	2.2(11)
H151	.144(7)	.661(7)	.847(5)	6.7(16)	H161	.631(6)	.954(6)	.918(4)	3.4(14)
H162	.807(5)	.926(5)	.893(3)	1.9(11)	H163	.785(7)	.928(7)	1.019(5)	6.6(16)
H171	.898(7)	.758(7)	.793(5)	9.0(18)	H181	.536(8)	.280(7)	.915(5)	7.1(17)
H182	.643(5)	.165(5)	.871(3)	1.4(10)	H183	.737(6)	.271(5)	.954(4)	3.7(13)
H191	.802(5)	.193(6)	.730(4)	3.0(12)	H192	.922(7)	.281(6)	.824(5)	4.3(15)
H193	.857(5)	.348(6)	.715(4)	1.7(11)	H201	−.083(5)	.407(5)	.554(3)	1.1(10)
H202	.036(6)	.301(5)	.515(4)	2.8(12)	H211	.020(6)	.596(5)	.462(4)	2.6(13)
H212	.125(5)	.477(5)	.404(4)	2.8(11)	H221	−.205(5)	.478(5)	.382(4)	2.0(11)
H222	−.113(6)	.372(6)	.323(4)	4.5(14)	H231	−.111(5)	.657(5)	.282(4)	2.8(12)
H232	−.044(5)	.536(5)	.209(4)	3.2(12)	H241	−.302(7)	.446(7)	.146(5)	6.4(16)
H242	−.308(7)	.605(7)	.117(5)	6.4(15)	H243	−.381(6)	.562(6)	.217(5)	5.5(15)
H261	.601(6)	.832(6)	.674(4)	4.2(13)	H281	.725(7)	.570(7)	.521(5)	5.8(17)
H282	.521(6)	.593(6)	.424(4)	3.5(12)	H283	.521(7)	.510(8)	.520(5)	7.1(18)
H291	.336(7)	.757(7)	.463(5)	6.9(17)	H292	.311(7)	.661(7)	.556(5)	7.5(16)
H293	.375(6)	.814(6)	.599(4)	2.7(13)					

EXPERIMENTAL AND STRUCTURE DETERMINATION

Synthetic [5] 8β-hydroxy-Δ⁹-THC was kindly supplied by Professor R. Mechoulam, Hebrew University, Jerusalem. Colourless prismatic crystals of the complex were formed by slow evaporation of a saturated solution of the compound in *N*,*N*-dimethylformamide.

Oscillation and Weissenberg diagrams indicated monoclinic symmetry. Systematic extinctions of the $0k0$ reflections for k odd uniquely determined the space group to $P2_1$ for the optically active compound. A computer-controlled Syntex $P\bar{1}$ four-circle diffractometer with graphite-monochromatized Mo$K\alpha$ radiation and equipped with a modified Enraf-Nonius liquid nitrogen cooling device was utilized for preliminary experiments and for the measurement of diffraction intensities. The work was carried out using a crystal of dimensions $0.3 \times 0.3 \times 0.1$ mm.

Unit cell parameters were determined by a least-squares treatment of the angular coordinates of 15 symmetry independent reflections with 2θ-values from 22 to 45°. The temperature at the crystal site was −165 °C.

Three-dimensional intensity data were recorded using the $\omega - 2\theta$-scanning mode, with scan speed variable from $2-12°$ min^{-1}, depending on the peak intensity of the reflections. Background counting time was equal to 0.35 × scan time on each side of the scan area. Reflections with 2θ-values larger than 45° which had integrated counts of less than 5 cps, determined in a 2 s scan over the reflections, were not measured.

The variations in the intensities of three standard reflections which were remeasured after every sixty reflections were random. Accordingly no corrections were applied to the intensity data for these variations.

The estimated standard deviations were taken as the square root of the total counts with a 2 % addition for experimental uncertainties. Of the 2793 symmetry-independent reflections measured ($2\theta_{max} = 60°$) 2431 had intensities larger than twice their standard deviations. These were regarded as "observed" reflections, and the remaining were excluded from further calculations. The intensities were corrected for Lorentz and polarization effects. The computer programs utilized in all calculations are part of a local assembly of programs for CYBER-74 described in Ref. 6.

Atomic form factors used were those of Doyle and Turner [7] for oxygen, carbon and nitrogen and those of Stewart *et al.*[8] for hydrogen.

The phase problem was solved by the MULTAN [9] program assembly. The structure model was refined with isotropic thermal parameters to a conventional R of 0.11. Introduction of anisotropic thermal parameters and least-squares refinement yielded an R of

0.08. At this point a difference Fourier map was calculated and all the 37 hydrogen atoms were found in the map. These were included in the calculations. Full-matrix least-squares refinement of all positional and anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms, resulted in a conventional R of 0.047 and a weighted R_w of 0.045.

Atomic parameters are listed in Table 1. 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.

CRYSTAL DATA

1:1 8β-Hydroxy-Δ^9-tetrahydrocannabinol N,N-dimethylformamide $C_{24}H_{37}NO_4$, $M = 403.5$ amu, space group: $P2_1$, cell dimensions at -165 °C: $a = 8.815(4)$ Å, $b = 10.256(5)$ Å, $c = 12.877(3)$ Å, $\beta = 102.27(3)°$, $V = 1137.5(8)$ Å^3, $Z = 2$, $D_{calc} = 1.178$ gcm^{-3}, $F(000) = 440$.

DISCUSSION

The observed molecular geometries and intramolecular distances and angles are shown in Fig. 1, together with the atomic labels used in this study. Selected dihedral angles are presented in Table 2. Compared to the structure determination of THCAB,[4] the standard deviations of the present study are improved by a factor of approximately three.

With the exception of the side chain, the bond angles, distances and conformation are close to the corresponding values in THCAB, and may all be regarded as normal values.

The influence on the conformation of the cyclohexene ring from the hydroxyl-substituent seems to be small, but significant. The dihedral angle C9–C10–C11–C12 has changed from -11.3 (15)° in THCAB to -7.6 (4)° in this structure, which gives a more distorted half-chair conformation, with C12 0.14 Å below and C13 0.61 Å above plane C (Table 3). The corresponding values in THCAB are 0.21 and 0.59 Å. The conformation of the pyran ring is also half-chair, with C13 0.41 Å above and C14 0.38 Å below a least-squares plane through O1, C2, C7, C8 (plane B, Table 3). The main

Table 2. Selected dihedral angles (°) with estimated standard deviations. The angles are positive in a righthand screw.

Angle	(°)
C7–C2–O1–C14	15.4(4)
C2–C7–C8–C9	138.2(3)
C2–C7–C8–C13	15.1(4)
C7–C8–C9–C10	−150.3(3)
C13–C8–C9–C10	−26.7(4)
C7–C8–C13–C12	−177.5(2)
C7–C8–C13–C14	−48.6(3)
C9–C8–C13–C12	55.3(3)
C9–C10–C11–C12	−7.6(4)
C10–C11–C12–C13	37.7(4)
C11–C12–C13–C8	−63.0(3)
C11–C12–C13–C14	171.0(2)
C8–C13–C14–O1	65.0(3)
C12–C13–C14–O1	−170.0(2)
C2–O1–C14–C13	−47.8(3)
C8–C7–C6–O15	−4.5°(4)
C3–C4–C20–C21	−90.0(3)
C4–C20–C21–C22	−176.7(2)
C20–C21–C22–C23	176.0(3)
C21–C22–C23–C24	−168.9(3)
C16–C10–C11–O17	−63.1(3)
O25–C26–N27–C28	1.9(5)
O25–C26–N27–C29	179.7(3)

Table 3. Deviations from least-squares planes (Å × 10^3). The deviations for those atoms used to define the plane are marked with an asterisk.*

Atom	Plane A	Plane B	Plane C
O1	69	4*	−1667
C2	22*	−9*	
C3	9*	−75	
C4	−25*		
C5	5*		
C6	28*	103	
C7	−40*	9*	−697
C8	−109	−4*	4*
C9	−1071	−898	−10*
C10	−1697		10*
C11	−1547		−4*
C12	−478		143
C13	−474	−415	−613
C14	397	381	−718
O15	126		
C16			64
O17			−1202
C18	1896		
C19	34	−30	
C20	−138		

* Angle between: A and C, 40.4°; A and B, 3.3°; B and C, 37.1°.

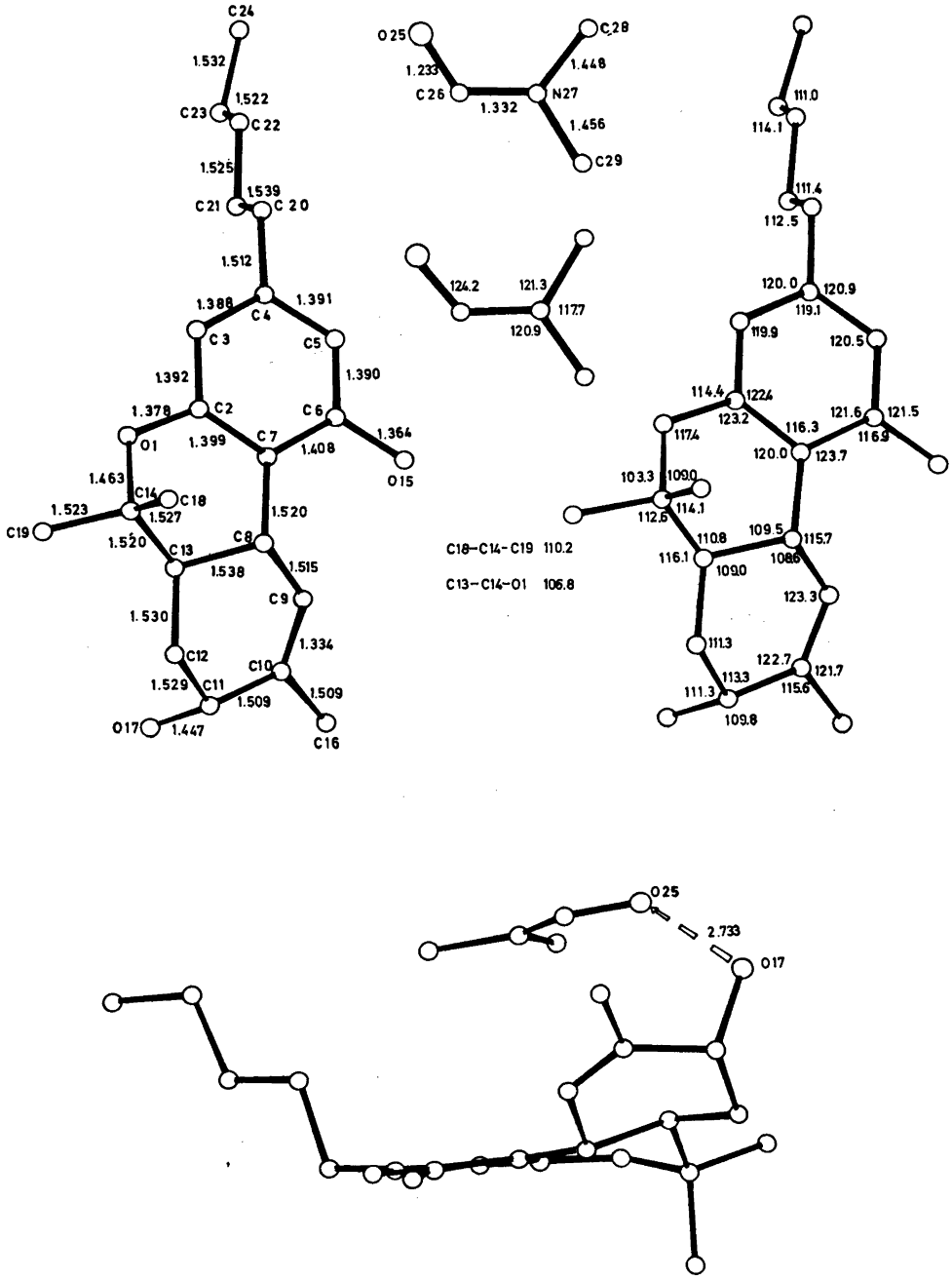


Fig. 1. Bond lengths (Å), bond angles (°) and a view along the plane of the benzene ring showing the conformation and relative orientation of the molecules. The estimated standard deviations are 0.003 – 0.004 Å for the bond lengths and 0.2 – 0.3° for the bond angles.

differences in geometry between the pyran rings in the two structures, are the opening of the C7–C2–O1 angle from 118.7 (7)° in THCAB to 123.2 (3)° in this structure and the closing of the C2–O1–C14 angle from 122.3 (7)° in THCAB to 117.4 (3)° in this structure. The dihedral angle C8–C13–C14–O1, which was calculated (Westheimer calculations) by Archer *et al.*[10] to be 56° in Δ^9-THC, is here 65.0(3) and in THCAB 60.8 (10)°. The differences may be explained by the effects of the intramolecular hydrogen bond to the pyran oxygen in THCAB. As in THCAB, the variations of the bond angles and the deviation from planarity in the benzene ring (Plane A), indicate strains in the aromatic system. The phenolic group (O15) is bent 0.126 Å out of the plane of the benzene ring and away from the C9 proton, which is situated only 2.25 Å away from O15. The dihedral angle C8–C7–C6–O15 is −4.5°, and the C9–H91···O15 angle is 121°.

In this low temperature determination the atomic positions in the pentyl side chain are fixed with low thermal vibrations and normal C(sp^3)–C(sp^3) single bond distances and angles. From the crystal structures of the cannabinoids hitherto studied: THCAB,[4] cannabicyclol,[11] cannabinol,[12] cannabidiol,[13] we do not find any preferred side chain conformation. In this structure there is a fully extended all-*anti* conformation. Compared to THCAB there is a rotation of 180° around the C4–C20 bond, and the side chain is on the same side of plane A as the cyclohexene ring (see Fig. 1). Structure activity relationship studies have shown that changes in the side chain are of importance for the psychotomimetic activity. For example, cannabinoids with a dimethylheptyl side chain are considerably more active than those with a pentyl side chain.[14] There is therefore good evidence for the assumption that the side chain is involved in a stereospecific interaction with a hydrophobic part of the receptor. Exactly in what way the 8β-OH-Δ^9-THC molecule will interact with a receptor is not possible to predict, but if we imagine the DMF-molecule as a part of the binding site, we suggest that this complex may represent the mutual orientation of the molecules also in a cell membrane.

As seen from Fig. 1 and the packing diagram

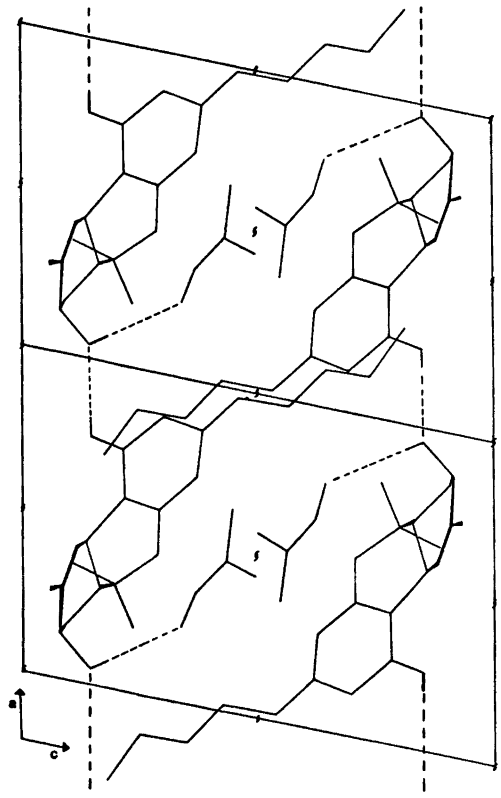


Fig. 2. The crystal structure of the complex as viewed along the *b*-axis.

in Fig. 2, the 8β-OH-Δ^9-THC and the DMF molecules are kept together by hydrogen bonds from the β-hydroxylic group to the carbonylic oxygen in DMF. The O17···O25 distance is 2.733(3) Å and the O17–H171···O25 angle is 146.9°.

The 8β-OH-Δ^9-THC molecules are also linked together by hydrogen bonds along the *a*-axis with the phenolic group in one molecule as hydrogen donor and the 8β-hydroxylic oxygen in another as acceptor. The O15···O17 distance is 2.701(3) Å and the angle O15–H151···O17 is 163.7°.

Between the columns of 8β-OH-Δ^9-THC molecules are channels running along the two-fold axis, into which the DMF molecules fit. These are very nearly planar with no atoms more than 0.01 Å out of the least-squares plane through the heavy atoms. Bond angles and distances agree well with the values observed

in a nitramin.DMF complex studied by Cobbledick and Small.[15]

There are only van der Waals contacts between the DMF molecules.

Acknowledgement. We wish to express our sincere thanks to Professor Raphael Mechoulam for supplying the raw material.

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^{13}C Nuclear Magnetic Resonance Spectra of Some 3-Acyltetronic Acids

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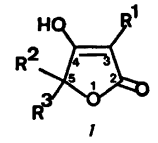
3-Acyltetronic acids (*1c*–*1h*) have been shown to exist in two monoenolic forms. The two forms give rise to two separate sets of signals in deuteriochloroform; in deuteriomethanol only one set of the lines is observed due to a fast interconversion between the two forms. This is opposite to the situation in tetronic acid (*1a*) and 5-methyltetronic acid (*1b*) in which only one form has been observed. The ^{13}C NMR spectra of the anions in alkaline solution show a high degree of delocalization of the excess charge.

Tetronic acid and substituted tetronic acids are normally formulated as having an enolic group at C-4 (*1*).[1] Several other enolic structures are possible, especially in the case of the 3-acetyltetronic acids. Recently Keukeleire *et al.*[2] have reported that a 300 MHz ^1H NMR spectrum of a 3-acyltetronic acid gave rise to two doublets at δ 4.61 and 4.48, respectively, each accounting for approximately half a proton. The spectrum of this product is explained on the basis of a 1:1 mixture of two enol forms.

As part of our interest in the structure of the naturally occurring tetronic acids we report the ^{13}C NMR spectra of several 3-acyl-5-methyltetronic acids together with a few other related tetronic acids (*cf.* Table 1). The assignments of the lines are based upon gated decoupled spectra and chosen so as to obtain internal consistency.

DISCUSSION

The ^{13}C NMR spectra of tetronic acid (*1a*) and 5-methyltetronic acid (*1b*) in deuteriomethanol show that these molecules exist in only one form. Five possibilities exist for the structure of this form, a keto form, three monoenolic forms, and a doubly-enolic form. From the gated decoupled spectrum of (*1b*) it can be established that one hydrogen is attached to C-3 as well as to C-5. This excludes in accordance with the recorded ^1H NMR spectra [2] the keto form, the doubly-enolic form, and one of the mono-enolic forms. Normal α,β-unsaturated ketonic carbon atoms give lines in the area downfield to δ 195.[4,5] Since neither *1a* nor *1b* give rise to any lines of this area, the second mono-enolic form with a keto group at C-4 can also be disregarded, implying that the structures of *1a* and *1b* indeed are represented by *1*. By accepting this structure the chemical shifts of the respective carbon atoms in *1a* and *1b*, given in Table 1, are in



1

	R^1	R^2	R^3	Ref.
1a	H	H	H	9
1b	H	CH_3	H	9
1c	$COCH_3$	CH_3	H	10
1d	$COCH_2CH_3$	CH_3	H	10
1e	$CO(CH_2)_2CH_3$	CH_3	H	10
1f	$COCH(CH_3)_2$	CH_3	H	10
1g	$COCH_2C_6H_5$	CH_3	H	10
1h	COC_6H_5	CH_3	CH_3	
1i	$OCOC_2H_5$	CH_3	H	12
1j	$CO(CH_2)_2CH_3$	CH_2COOH	H	

Table 1. ^{13}C chemical shifts, δ,[a] of substituted tetronic acids.[b]

Compound	C-2	C-3	C-4	C-5	C-1′	C-2′	C-3′	C-4′	[α]/[β]
1a [c]	178.35	88.95	182.57	69.85					
1b [c]	177.25	88.30	185.57	77.51					
1b [d]	177.96	73.68	193.00	74.46					
1c (α)	175.75	97.26	195.24	81.87	188.43	19.56			0.71
1c (β)	167.70	100.19	200.24	76.34	194.46	22.35			
1d (α)	176.14	96.16	194.98	81.74	192.97	26.31	9.55		0.57
1d (β)	167.63	99.67	199.14	75.89	199.27	29.56	8.32		
1e (α)	175.88	96.35	194.72	81.48	191.93	34.18	19.23	13.38	0.71
1e (β)	167.31	99.41	199.99	75.89	197.65	36.90	18.19	13.38	
1e [c]	172.44	99.93	197.70	78.75	196.48	38.33	19.62	13.97	
1e [d]	172.37	91.03	194.92	72.45	195.37	36.97	13.77	8.75	
1f (α)	176.66	95.18	194.72	81.67	196.67	31.32	18.58		0.63
1f (β)	167.44	98.69	200.44	75.87	202.58	33.72	18.26		
1g (α)	176.07	96.61	195.05	81.93	188.43	38.40			0.45
1g (β)	167.63	99.73	199.86	75.95	195.05	41.78			
1h (α)	177.96	97.46	195.24	87.97	183.55				1.0
1h (β)	166.33	94.40	206.10	82.65	188.68				
1i (β)	166.33	94.60	192.19	73.75	166.14				
1j [c,e]	172.90	100.45	194.20	79.33	197.58	36.90	20.14	13.97	
1j [d,f]	177.57	96.61	198.23	78.94	200.44	41.97	18.78	13.32	
2	182.57	88.30	193.75	84.40	161.85				
3 [g]	161.20	99.93	181.21	101.49	205.25	20.73			
4 [h]	164.45	103.50	195.18	39.57	201.28	20.60			

[a] Uncertainty ±0.07 ppm. [b] Dissolved in deuteriochloroform unless otherwise stated. The methyl group at C-5 gave δ 17.0±0.5 in all compounds exept in *1h* in which $\delta(CH_3)$ 23.78. [c] Dissolved in deuteriomethanol. [d] 100 mg of the compound dissolved in 1.3 ml D_2O+0.05 ml 40 % NaOD. [e] $\delta(CH_2)$ 36.06 and δ(COOH) 172.18. [f] $\delta(CH_2)$ 39.89 and δ(COOH) 178.55. [g] δ(C-6) 99.93. [h] δ(C-6) 70.30.

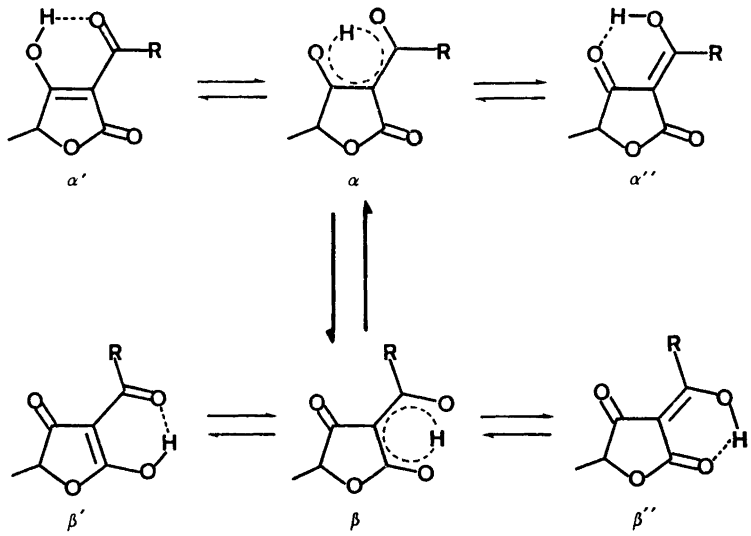
agreement with values found for carbon atoms in other compounds.[4,5] The possibility of the existence of a rapid equilibration between two enolic forms of *1a* and *1b*, as in the case of *1e* in deuteriomethanol, seems unjustified in view of the chemical shifts of C-4 and C-2.

The ^{13}C NMR spectra of 3-acyl-5-methyltetronic acids *1c*–*1h* in deuteriochloroform reveal the existence of two forms occurring in different proportion. The gated decoupled spectra show that C-5 in these compounds is attached to one hydrogen, whereas C-3 carries no hydrogen. This implies that these compounds have to be considered as being of the mono-enolic type. Several types of tautomeric equilibria are conceivable in such a system (Scheme 1).

Each of the equilibria $\alpha' \rightleftharpoons \alpha \rightleftharpoons \alpha''$ and $\beta' \rightleftharpoons \beta \rightleftharpoons \beta''$ will give rise to only one set of NMR signals, since the interconversion between the three forms is fast compared to the time scale in NMR spectroscopy. Shapet'ko *et al.*[6] have suggested the nonexistence of form α' and α'' as well as of β' and β'' in acyclic β-diketones. The enolic form of the latter compounds is completely described by a structure of either the α-type or the β-type. This can be rationalized in the sense of the potential function of the enolic hydrogen having only one minimum. This is contrary to the situation in six-membered cyclic β-ketoaldehydes in which the ^{13}C NMR spectrum has been shown to be a superposition of two forms equivalent to either α' and α'' or β' and β''.[7] The potential function of the enolic proton will in that case contain two minima separated by a small potential barrier. The chemical shifts, δ, of the carbon atoms are then given by [7]

$$\delta = p_a\delta_a + p_b\delta_b \quad [a = \alpha' \text{ (or } \beta'\text{)}, \; b = \alpha'' \text{ (or } \beta''\text{)}]$$

in which δ_a and δ_b are the chemical shifts in the tautomeric forms a and b, whereas p_a and p_b are the temperature dependent probabilities of these forms. A change of the value for the



Scheme 1.

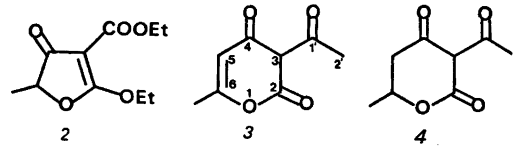
chemical shift, δ, between different temperatures will therefore be observable if two potential minima are present.

The ^{13}NMR spectrum of *1e* in deuteriochloroform has been investigated in the temperature range from −40 to 54 °C. The values of the chemical shifts of all the carbon atoms turned out to be completely independent of the temperature. This indicates that the forms α', α'', β', and β'' are not present in case of *1e*. It seems not unreasonable to extend this conclusion to all the compounds *1c*−*1h*. The two forms present in these compounds are therefore most likely represented by α and β. However, these structures do not imply that the distance from the enolic proton to the two oxygen atoms are equal, but implies the existence of only one potential minimum for the proton.

The assignments of the lines to the carbon atoms of the two forms given in Table 1 are based upon the difference in intensity of the lines. The α-form has been considered to be the form giving δ (C-2) ≈ 175 in order to achieve consistency with *1b*. As can be seen from the table this means that the β-form in most cases is the dominating form in deuteriochloroform ($[\beta] > [\alpha]$).

The effect of different substituents R^1 in *1c*−*1g* is clearly seen to influence the ratio between the two forms ($[\alpha]/[\beta]$) and the value of the chemical shift of C-1′. The chemical shifts of C-4 and C-2 are rather insensitive to changes in R^1. It can, furthermore, be calculated that the difference in the values of C-1′ between the two forms is independent of the nature of the substituent. This suggests that the structures of the two forms are the same for all the compounds and, thus, independent of R^1. The values of δ(C-2), δ(C-4), and δ(C-1′) are most easily rationalized by considering the two O−H distances as being almost identical in the α-form, whereas the β-form is best represented by the structure β' since δ(C-1′) in this form is almost in the area expected for normal α,β-unsaturated ketonic carbon atoms.

3-Ethoxycarbonyl-5-methyltetronic acid (*1i*) exists only in one form in deuteriochloroform. By comparing the value of δ(C-4) for *1i* with the corresponding value for 2-ethoxy-3-ethoxycarbonyl-5-methyl-4,5-dihydrofuran-4-one (*2*) it seems justified to regard *1i* as being of the β-



2 *3* *4*

type. This is opposite to the situation found for the unsubstituted tetronic acid.

The unusual stability as well as the IR and UV spectra of 3-acyltetronic acids [1] are not in agreement with the behaviour of the corresponding six-membered ring compounds, *e.g.* the ^{13}C spectrum of 3-acetyl-6-methyl-2,3-dihydropyran-2,4-dione (*3*) and 3-acetyl-6-methyl-2,3,5,6-tetrahydropyran-2,4-dione (*4*). The latter two exist only in one form in deuteriochloroform (Table 1). The difference in behaviour of these two types of compounds is therefore likely to be connected to the special hydrogen bonding system present in the case of the 3-acyltetronic acids.

The ^{13}C NMR spectrum of *1e* in deuteriomethanol gives rise to only one set of signals (Table 1). This can be explained in terms of a fast interconversion between the α- and β-form due to the exchange of the enolic hydrogen with the solvent. For carlosic acid (*1j*), being almost insoluble in deuteriochloroform, nothing can be said about the possible existence of two forms. Only one form is present in deuteriomethanol (Table 1).

3-Acyltetronic acids are only slightly soluble in water. In alkaline solution the anion is formed. The ^{13}C chemical shift values for the anion of *1b*, *1e* and *1j* are given in Table 1. The values of δ(C-4) and δ(C-1′) are in the area normally found for α,β-unsaturated ketonic carbon atoms, suggesting a high degree of delocalization of the excess charge. This is in agreement with the results obtained for the open form of carolic acid.[8]

We have recently shown [8] that carolic acid has to be considered as a 4:5 mixture of (*E*)- and (*Z*)-methyl-(2′-tetrahydrofurylidene)-tetrahydrofuran-2,4-dione (*5*), respectively. This ratio can be considered quite strange, since steric factors in the proposed hydrated precursor, 3-(ω-hydroxybutyryl)-5-methyltetronic acid, do not give reason for suggesting any deviation from a 1:1 ratio of the two enolic ether forms of carolic acid. We believe that the reason for deviation can be explained from our observation that the 3-acyltetronic acids exist in two forms, α and β, the β-form being the major form present. Therefore, it is suggested that the proportion, in which *5α* and *5β* exist in the solvent, is the main factor controlling the formation of carolic acid (*5*) with an *E*/*Z* ratio of 4:5.

EXPERIMENTAL

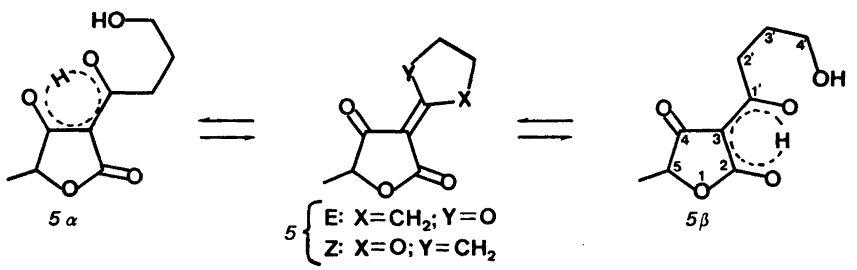
The ^{13}C NMR spectra were recorded on a Jeol FX60 spectrometer. 8K data points were used with a pulse length of 6 μs corresponding to a 60° flip angle. The spectral width was 4000 Hz. The magnetic field was stabilized by internal deuterium lock on the signal from the solvents. The probe temperature was 30 °C. All chemical shifts were measured as δ (ppm downfield to TMS). In case of variable temperature measurement the sample temperature was controlled with a standard variable temperature controller and determined with an accuracy of ±1 °C.

3-Benzoyl-5,5-dimethyltetronic acid (1h). Prepared from 2-chloro-2-methylpropanoyl chloride and ethyl benzoylacetate,[11] m.p. 74–75 °C (Anal. $C_{13}H_{12}O_4$: C, H, O).

Carlosic acid (1j). Isolated from single flask cultures of *Penicillium charlesii* NRRL 1887. Details will be discussed in another paper.[13]

Compound *2* was prepared according to Ref. 11.

3-Acetyl-6-methyl-2,3,5,6-tetrahydropyran-2,4-dione (4). Dehydracetic acid (7.05 g, 0.042 mol) was hydrogenated at room temperature in glacial acetic acid (50 ml) with 0.5 g of 5 % Pd/C as catalyst in a low pressure hydrogenation apparatus. The uptake of 0.042 mol of hydrogen took 4 h. The reaction mixture was poured into water (100 ml) and the pH value



Scheme 2.

was adjusted to 7. Extraction with ether and removal of acetic acid from the etheral phase by extraction with saturated sodium hydrogen carbonate, drying and subsequent evaporation of the solvent under reduced pressure gave 5.3 g (70 %) of crude *4* with m.p. 88 – 92 °C. Recrystallization from ethanol raised the m.p. to 95 – 97 °C (lit.[14] 100 °C).

Acknowledgement. The authors are indebted to Dr. C. W. Hesseltine, U. S. Department of Agriculture, Agricultural Research Service, Peoria, Illinois, for supplying the *Penicillium charlesii* NRRL 1887.

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Chlorinated Polycyclic Compounds. II. Reactions of 9,10-Dichloroanthracene with 1,1-Dichloroethylene and Related Reactions

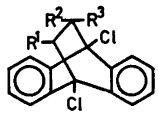
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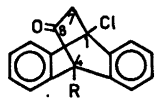
The reaction of 9,10-dichloroanthracene with 1,1-dichloroethylene gave low yields of the Diels-Alder adduct and 1,5-dichlorodibenzobicyclo-[3,2,1]octadien-4-one. Convenient syntheses for the latter and several related compounds from the Diels-Alder adduct of 9,10-dichloroanthracene and *trans*-1,2-dichloroethylene are described.

In the first part of this series [1] it was shown that the Diels-Alder reaction between 9,10-dichloroanthracene (DCA) and *cis*- or *trans*-1,2-dichloroethylene gives chloro ketones as side products. As these synthetically interesting ketones can be obtained from the Diels-Alder products, the synthesis of analogous compounds from DCA and chloro olefins was attempted.

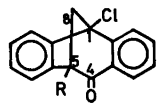
The reaction between DCA and 1,1-dichloroethylene failed to give satisfactory yields of the Diels-Alder adduct. At lower temperatures (150 – 200 °C) most of the DCA was recovered and at higher temperatures (200 – 250 °C) extensive polycondensation of dichloroethylene occurred. A small amount of the Diels-Alder adduct *1b* was isolated from the reaction mixture. The structure of *1b* was confirmed by its spectra and a further structure proof was furnished by the elimination of hydrogen chloride to give the chloro olefin *7*. This synthetically versatile compound could be obtained more conveniently by similar elimination from the Diels-Alder adduct of DCA and *trans*-1,2-dichloroethylene.[1] In addition to *1b*, a ketone was isolated, to which the structure *3b* was assigned in analogy with the results reported previously.[1] An alternative structure *2b* was supported by the fact that the ^{1}H NMR spectrum of the ketone contains only a sharp 2 H singlet besides the aromatic region and that it could also be obtained by acid hydrolysis of *7*.



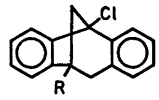
	R^1	R^2	R^3
1a	H	H	H
b	H	Cl	Cl
c	Cl	H	Cl



2a R = H
b R = Cl



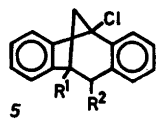
3a R = H
b R = Cl



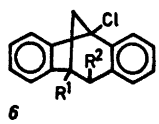
4a R = H
b R = Cl

To decide between *3b* and *2b* a dithioketal was prepared and desulfurized with Raney Ni. Two compounds were obtained, whose ^{1}H NMR spectra showed that neither could be *1a* and consequently structures *4a* and *4b* were assigned to the reduction products. Reduction of *3b* with sodium borohydride gave only one alcohol and the synthesis of both epimers *via* the alcohol-chloride-acetate-alcohol route [1] showed that it was the *endo* epimer *5d*. Dehalogenation with zinc and ethanol caused the removal of one chlorine atom from *3b* leading

to the ketone *3a* and reduction of the latter gave the *endo* alcohol *5a* as the sole reaction product. If a 1:1 mixture of phosphorus pentachloride and phosphorus oxychloride was used to effect the replacement of hydroxyl by chloride in the alcohols *5a* and *5d*, the product was 90 – 95 % *exo*, while thionyl chloride yielded comparable amounts of both epimers. In the reaction of *5a* with thionyl chloride, unlike *5d* and other similar cases,[1] also the [2,2,2] isomer *8c* was formed to a variable extent. The yield of the latter was found to be dependent on the acidity of the reaction mixture and with the use of a large excess of thionyl chloride with added gaseous hydrogen chloride the isomerization was complete. Acetolysis of the chlorides *5c*, *6c*, *5f* and *6f* using reaction times of 40

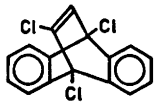


5

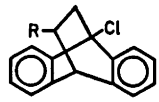


6

	R^1	R^2
5a, 6a	H	OH
b	H	OAc
c	H	Cl
d	Cl	OH
e	Cl	OAc
f	Cl	Cl



7



8a	R = OH
b	R = OAc
c	R = Cl

min – 24 h gave 80 – 95 % of the *exo* acetates, but with addition of 10 % of sulfuric acid, only the *endo* acetates were obtained. Treatment of these acetates *5b* and *5e* with 30 % sulfuric acid in acetic acid gave the dibenzobicyclo[2,2,2]octadiene derivatives *8b* and *2a*, respectively. These equilibration results are in good agreement with other observations on the relative thermodynamic stability of the *endo* and *exo* substituted dibenzobicyclo[3,2,1]-octadiene derivatives and the corresponding [2,2,2] isomers.[2]

In most of the dibenzobicyclo[3,2,1]octadiene derivatives discussed here the *syn*- and *anti*-8-protons have different chemical shifts in ^{1}H NMR and it is possible to correlate the signals with the appropriate protons. In compounds unsubstituted at C-5, the assignment is based on the coupling constants between 5-H and *anti*-8-H.[3] In the 5-chloro derivatives having an *endo*-4-substituent, no couplings, except the geminal coupling between the 8-protons, are visible, while all the *exo* epimers exhibit a long-range splitting of about 1.5 Hz between the *endo*-4-proton and one of the 8-protons. The proton involved is assumed to be the *anti*-8-proton in accordance with the concept of W-geometry required for this coupling.[4] When no information based on the coupling constants was available, the chemical shifts were tentatively assigned to the 8-protons making use of the observation that in the compounds discussed above and in other analogous cases,[3] the *syn*-8-proton absorbs at a higher field than the epimeric *anti*-8-proton, when the C-4 substituent has *endo* configuration. In *exo*-4-substituted compounds the order is reversed. The stereostructure at C-4 is evident from the coupling constants between the 4- and 5-protons,[3] when the latter is present, while in the 5-chloro derivatives it is based on the rule that in the derivatives of dibenzobicyclo[3,2,1]-octadiene, both unsubstituted [3] and those bearing chloro substituents at the 1- and 5-positions,[1] the *endo*-4-proton absorbs at a higher field than the epimeric *exo*-4-proton.

EXPERIMENTAL

For general experimental conditions see Ref. 1.

Reaction of DCA with 1,1-dichloroethylene. A mixture of 24.7 g (0.1 mol) of DCA [5] and 291 g (3.0 mol) of 1,1-dichloroethylene was heated at 210 °C for 24 h in a 1 l stainless steel pressure vessel. After cooling a pressure of 20 atm due to HCl remained. The reaction mixture consisted mainly of hard black porous material that was ground in a mortar and the powder refluxed with 500 ml of acetone for 4 h. The solution was filtered and boiled for 20 min with 10 g of activated charcoal. The solution was filtered and evaporated. The residue was distilled with steam to remove all volatile material and the two major components were separated on a silica gel column (100 g, elution with an 1:1 mixture of benzene and light petroleum) and purified by crystallization from EtOH. The first fraction gave 2.1 g (6.1 %) of 1,4,7,7-tetrachlorodibenzobicyclo[2,2,2]octadi-

ene (*1b*), m.p. 194 °C, δ 3.33 (2 H, s) +8 Ar-H, *m/e* 342(0.2), 246(100) and the second fraction 0.4 g (1.4 %) of 1,5-dichlorodibenzobicyclo-[3,2,1]octadien-4-one (*3b*), m.p. 155 °C, ν_{max} 1705 cm^{-1}, δ 3.57 (2 H, s) +8 Ar-H, *m/e* 288(17), 218(100).

Elimination of hydrogen chloride from 1c. The chloride *1c*[1] (10 g) was added to a solution of 10 g of KOH in 100 ml of EtOH and the mixture refluxed for 40 min. The cooled mixture was acidified with HCl and extracted twice with ether. The ethereal solution was dried and evaporated. The residue was dissolved in 200 ml of light petroleum and decolorized by passing it through a silica gel column (20 g). Concentration of the solution gave 6.7 g (75 %) of 1,4,7-trichlorodibenzobicyclo[2,2,2]octatriene (*7*), m.p. 101 °C, δ 6.84 (1 H, s) +8 Ar-H.

Hydrolysis of the unsaturated chloride 7. A mixture of 5.0 g of *7*, 60 g of H_2SO_4 and 40 g of HOAc was refluxed for 80 min. The hot reaction mixture was poured into ice water and the product isolated by ether extraction. TLC and ^{1}H NMR examination showed the presence of only one compound. The analytical sample was prepared by crystallization from EtOH and it was identical with the ketone *3b* obtained from the Diels-Alder reaction.

Preparation of the dithioketal of 3b *and desulfurization with Raney nickel.* The ketone *3b* (1.0 g) was dissolved in a mixture of 5 ml of 1,2-ethanedithiol and 5 ml of BF_3 etherate. After standing for 20 h at room temperature the mixture was poured into 100 ml of 10 % NaOH solution. The aqueous solution was extracted with ether and the ethereal layer dried and evaporated. The residue crystallized on standing to yield 0.88 g (72 %) of the dithioketal, m.p. 184 °C, δ 3.47 (2 H, AB q, J=11.6 Hz), 3.5–3.9 (4 H, m) +8 Ar-H.

A mixture of 0.8 g of the dithioketal and 20 g of Raney nickel in 200 ml of EtOH was refluxed until the reduction was complete (as shown by TLC). This took about 10 h. The solution was filtered and evaporated and the residue fractionated by TLC (elution with light petroleum). The two fractions were crystallized from EtOH to give 0.17 g (31 %) of 1-chlorodibenzobicyclo[3,2,1]octadiene (*4a*), m.p. 102 °C, δ 2.63 (*syn*-8-H), 2.87 (*anti*-8-H), 2.77 (*endo*-4-H), 3.22 (*exo*-4-H), 3.45 (5-H) +8 Ar-H, $J_{4,5}$=4.5 Hz, $J_{5,8}$=5.0 Hz, $J_{4,4}$=17.2 Hz, $J_{8,8}$= 9.2 Hz, *m/e* 240(49), 205(100) and 0.22 g (35 %) of 1,5-dichlorodibenzobicyclo[3,2,1]octadiene (*4b*), m.p. 72 °C, δ 2.98 (*syn*-8-H), 3.15 (*anti*-8-H), 3.19 (*endo*-4-H), 3.54 (*exo*-4-H) +8 Ar-H, $J_{4,4}$=17.0 Hz, $J_{8,8}$=9.2 Hz, *m/e* 274(37), 239(100).

Dechlorination of the ketone 3b. A mixture of 2.0 g of *3b*, 4.0 g of Zn-powder and 100 ml of EtOH was refluxed for 8 h. The ethanol was removed, the residue dissolved in acetone, the solution filtered and evaporated. Purification by TLC and crystallization from 80 % aqueous EtOH furnished 1.3 g (74 %) of 1-chlorodibenzobicyclo[3,2,1]octadien-4-one (*3a*), m.p. 95 °C, ν_{max} 1692 cm^{-1}, δ 3.14 (2 H, d), 3.99 (1 H, tr) +8 Ar-H, $J_{5,8}$=2.4 Hz, *m/e* 254(40), 219(100).

Reduction of the ketones 3a *and* 3b *with sodium borohydride.* A solution of 10 mmol of the ketone (2.55 g of *3a* or 2.89 g of *3b*) and 0.38 g (10 mmol) of $NaBH_4$ in 100 ml of EtOH was stirred for 40 min at room temperature. The solution was poured into water, HCl added and the aqueous solution extracted twice with ether. The ethereal solution was dried and evaporated. By TLC and ^{1}H NMR examination only one alcohol could be detected in each case; *3a* gave 1-chlorodibenzobicyclo[3,2,1]-octadien-*endo*-4-ol (*5a*), m.p. 143 °C, ν_{max} 3230 cm^{-1}, δ 2.72 (*syn*-8-H), 3.00 (anti-8-H), 4.83 (*exo*-4-H), 3.58 (5-H), 1.30 (OH) +8 Ar-H, $J_{4,5}$=5.3 Hz, $J_{5,8}$=5.3 Hz, $J_{8,8}$=11.0 Hz and *3b* gave 1,5-dichlorodibenzobicyclo[3,2,1]octadien-*endo*-4-ol (*5d*), m.p. 116 °C, ν_{max} 3520 cm^{-1}, δ 3.05 (*syn*-8-H), 3.21 (*anti*-8-H), 4.95 (*exo*-4-H), 2.58 (OH) +8 Ar-H, $J_{8,8}$=10.0 Hz. The analytical samples were crystallized from 80 % aqueous EtOH.

Reactions of the alcohols 5a *and* 5d *with thionyl chloride and phosphorus pentachloride.* The alcohol (1.0 g) was refluxed for 10 min with a mixture of 2.0 g of PCl_5 and 2.0 g of $POCl_3$. The hot mixture was carefully decomposed with water, the aqueous solution extracted twice with ether, the ethereal solution dried and evaporated. Approximate yields estimated from the ^{1}H NMR spectra are given. The analytical samples of the chlorides were prepared by TLC (elution with light petroleum) and crystallization from EtOH. *5a* gave 10 % of 1-*endo*-4-dichlorodibenzobicyclo[3,2,1] octadiene (*5c*), m.p. 112 °C, δ 2.73 (*syn*-8-H), 3.00 (*anti*-8-H), 5.50 (*exo*-4-H), 3.74 (5-H) +8 Ar-H, $J_{4,5}$=4.7 Hz, $J_{5,8}$=5.7 Hz, $J_{8,8}$=11.0 Hz and 90 % of the *exo* epimer *6c*, m.p. 89 °C, δ 3.18 (*syn*-8-H), 2.87 (*anti*-8-H), 4.97 (*endo*-4-H), 3.70 (5-H) +8 Ar-H, $J_{4,5}$=2.0 Hz, $J_{5,8}$=4.8 Hz, $J_{8,8}$=10.0 Hz. *5d* gave 5 % of 1-*endo*-4,5-trichlorodibenzobicyclo[3,2,1]octadiene (*5f*), m.p. 118 °C, δ 3.13 (*syn*-8-H), 3.38 (*anti*-8-H), 5.54 (*exo*-4-H), +8 Ar-H, $J_{8,8}$=11.0 Hz and 95 % of the *exo* epimer *6f*, m.p. 134 °C, δ 3.60 (*syn*-8-H), 3.15 (*anti*-8-H), 5.10 (*endo*-4-H) +8 Ar-H, $J_{8,8}$=10.4 Hz, $J_{4,8}$=1.5 Hz. *5d* (1.0 g) refluxed for 20 h with 10 ml of $SOCl_2$ gave 55 % of *5f* and 45 % of *6f*. Similarly *5a* gave 25 % of *5c*, 55 % of *6c* and 20 % of 1,8-dichlorodibenzobicyclo[2,2,2]octadiene (*8c*), m.p. 107 °C, δ 2.20 (*cis*-7-H), 2.68 (*trans*-7-H), 4.14 (8-H), 4.31 (4-H) +8 Ar-H, *cis*$J_{7,8}$=8.6 Hz, *trans*$J_{7,8}$=3.8 Hz, $J_{7,7}$=12.8 Hz, $J_{4,8}$= 2.6 Hz. Only *8c* was obtained, when 1.0 g of *5a* was refluxed with 50 ml of $SOCl_2$ for 8 h and a slow stream of HCl gas was bubbled into the reaction mixture.

Acetolysis of the chlorides 5c, 6c, 5f *and* 6f. Epimeric mixtures resulting from the reactions

of the corresponding alcohols with thionyl chloride were used as starting material. A mixture of 1.0 mmol of the chloride (0.28 g of dichloride or 0.31 g of trichloride), 0.20 g (1.2 mmol) of AgOAc and 10 g of HOAc was refluxed for 20 h. The acetic acid was removed under reduced pressure, the residue dissolved in acetone, the solution filtered and evaporated. Approximative yields are based on ^{1}H NMR. The analytical samples of the acetates were prepared by TLC (elution with a 1:1 mixture of benzene and light petroleum) and crystallization from EtOH. A mixture of *5c* and *6c* gave 5 % of 1-chlorodibenzobicyclo[3,2,1]octadien-*endo*-4-yl acetate (*5b*), m.p. 119 °C, ν_{max} 1730 cm^{-1}, δ 2.76 (*syn*-8-H), 2.89 (*anti*-8-H), 6.09 (*exo*-4-H), 3.80 (5-H), 2.00 (OAc) +8 Ar-H, $J_{4,5}=5.5$ Hz, $J_{5,8}=5.2$ Hz, $J_{8,8}=11.0$ Hz and 95 % of the *exo* epimer *6b*, m.p. 99 °C, ν_{max} 1733 cm^{-1}, δ 3.02 (*syn*-8-H), 2.83 (*anti*-8-H), 5.71 (*endo*-4-H), 3.56 (5-H), 2.10 (OAc) +8 Ar-H, $J_{4,5}=2.0$ Hz, $J_{5,8}=5.4$ Hz, $J_{8,8}=$ 10.8 Hz. A mixture of *5f* and *6f* gave 15 % of 1,5-dichlorodibenzobicyclo[3,2,1]octadien-*endo*-4-yl acetate (*5e*), m.p. 166 °C, ν_{max} 1740 cm^{-1}, δ 3.23 (*syn*- and *anti*-8-H), 6.50 (*exo*-4-H), 2.10 (OAc) +8 Ar-H and 85 % of the *exo* epimer *6e*, m.p. 135 °C, ν_{max} 1730 cm^{-1}, δ 3.47 (syn-8-H), 3.13 (anti-8-H), 6.05 (endo-4-H), 2.13 (OAc) +8 Ar-H, $J_{8,8}=10.0$ Hz, $J_{4,8}=$ 1.5 Hz. When 10 % of H_2SO_4 was added to the reaction mixtures, only the *endo* acetates were obtained. When 0.2 g of *5b* was refluxed for 80 min with a mixture of 3 g of H_2SO_4 and 7 g of HOAc, the only product was 1-chlorodibenzobicyclo[2,2,2]octadien-8-yl acetate (*8b*), m.p. 98 °C, ν_{max} 1732 cm^{-1}, δ 1.92 (*cis*-7-H), 2.61 (*trans*-7-H), 4.98 (8-H), 4.46 (4-H), 1.82 (OAc) +8 Ar-H, *cis*$J_{7,8}=8.6$ Hz, *trans*$J_{7,8}=2.7$ Hz, $J_{7,7}=12.8$ Hz, $J_{4,8}=2.6$ Hz. Similarly, *5e* gave 1-chlorodibenzobicyclo[2,2,2]octadien-8-one (*2a*), m.p. 135 °C, ν_{max} 1730 cm^{-1}, δ 2.63 (7-H), 4.73 (4-H).

Hydrolysis of the acetates 5b, 6b, 5e, 6e *and* 8b. The acetate (0.5 – 1.0 g) was stirred with 20 ml of 10 % ethanolic KOH for 80 min at room temperature. Water and HCl was added and the alcohol was obtained by ether extraction and purified by crystallization from 80 % aqueous EtOH. *6b* gave 1-chlorodibenzobicyclo-[3,2,1]octadien-*exo*-4-ol, (*6a*), m.p. 138 °C, ν_{max} 3320 cm^{-1}, δ 2.94 (*syn*-8-H), 2.74 (*anti*-8-H), 4.45 (*endo*-4-H), 3.40 (5-H), 2.27 (OH) +8 Ar-H, $J_{4,5}=2.0$ Hz, $J_{5,8}=4.8$ Hz, $J_{8,8}=10.5$ Hz, *6e* gave 1,5-dichlorodibenzobicyclo[3,2,1]octadien-*exo*-4-ol (*6d*), m.p. 171 °C, ν_{max} 3260 cm^{-1}, δ 3.45 (*syn*-8-H), 3.10 (*anti*-8-H), 4.54 (endo-4-H), 2.83 (OH) +8 Ar-H, $J_{8,8}=10.0$ Hz, $J_{4,8}=1.5$ Hz and *8b* gave 1-chlorodibenzobicyclo[2,2,2]octadien-8-ol (*8a*), m.p. 104 °C, ν_{max} 3270 cm^{-1}, δ 1.61 (*cis*-7-H), 2.40 (*trans*-7-H), 3.76 (8-H), 4.02 (4-H), 2.38 (OH) +8 Ar-H, *cis*$J_{7,8}=8.6$ Hz, *trans*$J_{7,8}=2.4$ Hz, $J_{7,7}=$ 12.8 Hz, $J_{4,8}=2.6$ Hz. *5b* and *5e* gave the alcohols *5a* and *5d*, respectively, which were identical with those obtained from the reduction of the ketones *3a* and *3b*.

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Heteroaromatic Boron Compounds. XVI. Some New Heteroaromatic Borohydrides

SALO GRONOWITZ and ALF MALTESSON

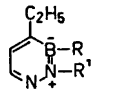
Division of Organic Chemistry 1, Chemical Center, University of Lund, P.O. Box 740, S-22007 Lund 7, Sweden

Dedicated to Professor K. A. Jensen on his 70th birthday

5-Methyl-4,5-borazarothieno[2,3-*c*]pyridine (*2c*) and 6-methyl-7,6-borazarothieno[3,2-*c*]pyridine (*3c*) have been synthesized. They showed no reducing or hydroborating properties.

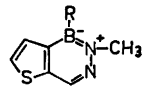
It has previously been demonstrated that 3,2-borazaropyridines, such as *1* and their thieno-fused analogues (*2a*, *3a*), from which they are prepared by Raney-nickel desulfurization, show pronounced aromatic properties.[1] Their dipole moments, UV and NMR spectra gave strong evidence that these compounds are isoelectronic with the corresponding pyridines. Aromatic substitution reactions could be carried out with these compounds [2–6] (for a recent review on boron-containing heterocycles *cf.* Ref. 7). We were therefore interested to find out if the aromatic nature of these ring systems was reflected in the reactivity and other properties of the corresponding boron hydrides.

Aromatic boron hydrides have been prepared in 1958 by Dewar by reduction of the corresponding B—Cl compound with lithium aluminium hydride in ether, as in the syntheses of 10,9-borazarophenantrene [8] and 2,1-borazaronaphthalene.[9] Alternatively, the B—OCH_3 derivative or some other ether was reacted with lithium aluminium hydride and aluminium chloride, as in the syntheses of 5,6-borazarobenz[*a*]antracene,[10] 5,4-borazaropyrene [11] and 3,4-methyl-4,3-borazaroisoquinoline.[12] Dewar and coworkers, however, did not study the reactions of these borohydrides. We used the latter approach for the synthesis of 5-methyl-4,5-borazarothieno[2,3-*c*]pyridine (*2c*) and 6-methyl-7,6-borazarothieno[3,2-*c*]pyridine (*3c*), which were obtained from *2a* and *3a* *via* the butoxy derivatives, *2b* and *3b*, in high yield. The latter compound was crystalline, m.p. 30 °C. The compounds showed strong absorption in the IR spectrum at 2550 and 2540 cm^{-1}, characteristic of B—H stretching.[13] These compounds are most probably monomers, since absorption at 1600 cm^{-1}, considered characteristic for dimers with B—H—B bridges,[14]



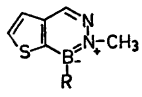
1

R = OH or CH_3
R = H or CH_3



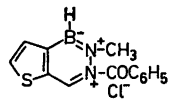
2

a R=OH
b R=OC_4H_9
c R=H

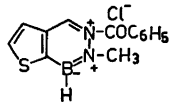


3

a R=OH
b R=OC_4H_9
c R=H



4



5

was not observed. Also in the mass spectrometer these compounds were monomeric.

The NMR spectra were in accordance with the suggested structures, although the B–H resonance could not be observed, most probably due to the high quadrupole moment of the boron.[12] However, indirect evidence for the presence of the BH group was obtained in the form of long-range coupling to one of the thiophene hydrogens, which persisted when the resonance from all other protons were decoupled, and therefore must be due to the B–H proton. The thiophene hydrogen to which it couples is most probably the 3-hydrogen. The assignment is based on chemical shift comparison with the shifts of 2- and 3-bromo-7,6-borazaro[3,2-*c*]pyridines.[15] Also the theory of the shortest zig-zag path for this long-range coupling [16] supports this assignment. Similar long-range couplings have been observed in 4,5-borazarothieno[2,3-*c*]pyridines.[17] Both *2c* and *3c* are very stable compounds and could be stored at room temperature under nitrogen for several years without discoloration or decomposition. They reacted very slowly with water or alcohols under evolution of hydrogen to give the corresponding B–OH or B-alkoxy derivatives. The reaction of *2c* with eight equivalents of butanol at 30 °C was followed by NMR. We found that after 4 h 75 % had reacted to give *2b*. *2c* and *3c* did not react with benzaldehyde, acetone, ethyl acetate or benzyl cyanide in anhydrous ether. Only starting material was recovered after 150 h at 25 °C. In the reaction of *2c* and *3c* with benzoyl chloride, a white precipitate was immediately formed, which most probably is the benzoylated derivative (*4* and *5*, respectively), as treatment with water caused hydrogen evolution and the formation of *2a* and *3a*, respectively, besides benzoic acid. It is well-known that 1-hexyne reacts easily with boranes such as disiamylborane.[17] However, *2c* and *3c* did not react with 1-hexyne. Only starting material was recovered after 150 h at 25 °C.

It is thus obvious that the boron hydrides *2c* and *3c* show no reducing power nor are they active as hydroborating agents. This behaviour thus provides additional evidence for the aromatic nature of the 3,2-borazaropyridines.

EXPERIMENTAL

Microanalyses were carried out at Dornis und Kolbe, Mikroanalytisches Laboratorium, Mülheim, a.d. Ruhr. IR spectra were recorded with a Perkin-Elmer 257 grating infrared spectrophotometer. ^{1}H NMR spectra were recorded with a Varian A-60 spectrometer. The MS were recorded on an LKB A 9000 spectrometer. GLC analyses were carried out on a Perkin-Elmer 900 gas-chromatograph using a flame ionization detector.

6-Methyl-7,6-borazarothieno[3,2-c]pyridine (3c). A solution of 10.4 g (0.0625 mol) of 7-hydroxy-6-methyl-7,6-borazarothieno[3,2-*c*]-pyridine [17] in 150 ml of butanol and 100 ml of benzene in a flask connected with a water separator was refluxed for 6 h. The solvents were distilled off, finally at 0.13 Pa. The residue, which still contained 3.4 g of butanol, was dissolved in 500 ml of anhydrous ether cooled to −20 °C, and 1.25 g (0.0325 mol) of lithium aluminium hydride suspended in 300 ml of anhydrous ether was added dropwise under nitrogen during 15 min with vigorous stirring. Then 0.625 g of aluminium chloride in 100 ml of anhydrous ether was added. The temperature was allowed to rise to room temperature and the mixture was refluxed for 2 h. The precipitate was allowed to sediment and the supernatant was transferred with nitrogen pressure through a glass filter to a flask and the ether was evaporated over nitrogen, giving the title compound as colorless needles. The product was sublimed at 75 °C/0.25 Pa and the sublimation apparatus opened in a glove box, yielding 7.2 g (77 %) of the title compound, m.p. 30 °C; IR (KBr) B–H 2540 cm^{-1}; NMR ($CDCl_3$): δ 7.73 (1, d, H-2, J_{23} = 5.0 Hz), 7.34 (1, dd, H-3, J_{23} = 5.0 Hz, J_{37} = 0.6 Hz), 8.43 (1, s, H-4), 3.88 (3, s, NCH_3); *m/e* (%): 150 (100), 149 (42), 122 (47), 121 (14). (Found: C 48.54; H 4.89; B 7.01; N 18.89; S 21.5. Calc. for $C_6H_7BN_2S$: C 48.04; H 4.71; B 7.20; N 18.67; S 21.37).

5-Methyl-4,5-borazarothieno[2,3-c]pyridine (2c). From 10.4 g (0.0625 mol) of 4-hydroxy-5-methyl-4,5-borazarothieno[2,3-*c*]pyridine,[17] 0.65 g (0.0172 mol) of lithium aluminium hydride and 0.625 g of aluminium chloride, 8.35 g (89 %) of the title compound, b.p. 84.0–85.5 °C/0.65 Pa was obtained in the same way as described above; IR (KBr): B–H 2550 cm^{-1}; NMR ($CDCl_3$): δ 7.40 (2, s, thiophene protons), 8.45 (1, s, 7-H), 3.85 (3, s, NCH_3); *m/e* (%): 150 (100), 149 (40), 122 (51), 121 (15). (Found: C 48.21; H 4.80; B 7.11; N 18.70; S 21.48. Calc. for $C_6H_7BN_2S$: C 48.04; H 4.71; B 7.20; S 21.37).

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Preparation of Some 2-Bromo-2-deoxy-D-hexopyranoses

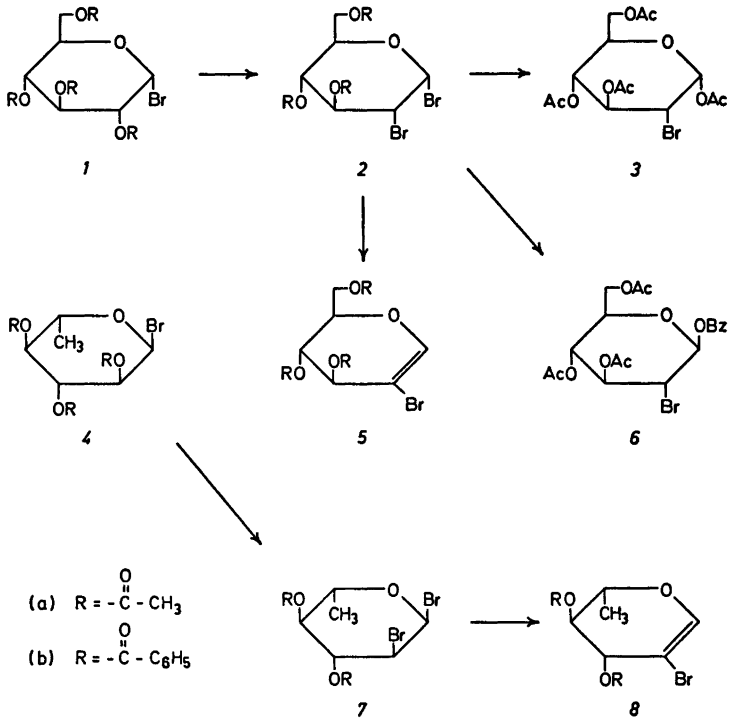
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Dedicated to Professor K. A. Jensen on his 70th birthday

The zinc bromide catalyzed reaction of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (*1a*) with acetyl bromide gives tri-*O*-acetyl-2-bromo-2-deoxy-α-D-glucopyranosyl bromide (*2a*). A similar treatment of tri-*O*-acetyl-6-deoxy-α-L-glucopyranosyl bromide (*4a*) yields di-*O*-acetyl-2-bromo-2,6-dideoxy-α-L-glucopyranosyl bromide (*7a*). An analogous reaction takes place with the corresponding benzoylated pyranosyl bromides. The 2-bromo-2-deoxy bromides form 2-bromo-1,2-dideoxy-hex-1-enopyranoses on treatment with triethylamine.

In previous work it was shown that reaction of tri-*O*-acetyl-D-xylopyranosyl bromide with acetyl bromide and zinc bromide led to the formation of di-*O*-acetyl-2-bromo-2-deoxy-D-xylopyranosyl bromide in moderate yield.[1] A better yield was obtained when the corresponding benzoylated bromide was treated with benzoyl bromide. Other acetylated or benzoylated pentopyranosyl bromides did not give bromo-deoxy-pentoses by this treatment.



The reaction of some acetylated and benzoylated hexopyranosyl bromides with acetyl bromide and zinc bromide has now been studied.

When tetra-*O*-acetyl-α-D-glucopyranosyl bromide (*1a*) was treated with anhydrous zinc bromide and acetyl bromide it was converted into tri-*O*-acetyl-2-bromo-2-deoxy-α-D-glucopyranosyl bromide (*2a*). After *ca.* 20 h at room temperature the reaction mixture no longer contained *1a*. When the mixture was worked up *2a* could be crystallized in *ca.* 30 % yield. An NMR spectrum showed that the material in the mother liquor contained rather large amounts of *2a* and the α-1-acetate (*3*);[3] besides, some of the unsaturated compound (*5a*) was present. Treatment with hydrogen bromide converted the 1-acetate (*3*) into the bromide (*2a*) and after this treatment additional amounts of *2a* could be obtained. Treatment of the mother liquor from this product with silver benzoate yielded *ca.* 10 % of the known[3] β-1-benzoate (*6*). The 1-acetate (*3*) is probably formed by zinc bromide catalyzed reaction of *2a* with acetic anhydride;[3] the latter arising from the acetic acid cleaved off from *1a*.

It was found necessary to use a rather large amount of zinc bromide to catalyse the reaction of *1a* with acetyl bromide. With smaller amounts of zinc bromide the reaction was slower and coloured by-products were formed. No reaction took place when *1a* was treated with acetyl bromide in the presence of boron trifluoride or aluminium tribromide.

The dibromo compound (*2a*) has been previously obtained, together with other isomers, by addition of bromine to tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranose.[2,4,5] The reaction described above gave *2a* as the only detectable bromo-deoxy bromide.

A similar treatment of tri-*O*-acetyl-6-deoxy-α-L-glucopyranosyl bromide (*4a*) gave the 2-bromo-2-deoxy bromide (*7a*) in *ca.* 50 % yield. The benzoylated bromides, *1b* and *4b*, also reacted with acetyl bromide and zinc bromide to give the corresponding 2-bromo-compounds *2b* and *8b*, respectively, in yields comparable to those obtained with the acetates.

Attempts to prepare bromo-deoxy compounds from acetylated galacto- or mannopyranosyl bromides were unsuccessful. In the pentose series it was also found that only xylopyranosyl bromide gives a bromo-deoxy bromide.[1] Thus, only pyranosyl bromides with the substituents at C2, C3, and C4 *trans*-oriented react with acetyl bromide and zinc bromide.

The mechanism for the formation of *2* from *1*, or *7* from *4*, is probably the same as that proposed for the reaction of tri-*O*-acetyl-D-xylopyranosyl bromide with acetyl bromide-zinc bromide,[1] and for the reaction of pentosyl bromides with dibromomethyl methyl ether.[6] The latter reagent is not, however, suited for the preparation of bromo-deoxy-hexoses.

The bromides *2a* and *2b*, as well as *7a* and *7b*, eliminated hydrogen bromide readily when treated with triethylamine to give the 1,2-unsaturated products *5a* and *5b* and *8a* and *8b*, respectively. The latter compounds rearrange to 2,3-unsaturated bromides with acetyl bromide. This will be described in a forthcoming paper.

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 HA-100 or on Bruker HX-90E instruments in deuteriochloroform solution using tetramethylsilane as internal reference. Optical rotations were measured in chloroform solution on a Perkin Elmer 141 instrument.

Tri-O-acetyl-2-bromo-2-deoxy-α-D-glucopyranosyl bromide (2a). A mixture of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (*1a*) (10.0 g), anhydrous zinc bromide (20 g), and acetyl bromide (25 ml) was stirred at room temperature for 20 h. A ^1H NMR spectrum of a sample then showed that the major component present was *2a*; besides, signals corresponding to smaller amounts of the α-1-acetate (*3*)[3] and of the unsaturated compound (*5a*) were seen. The starting material (*1a*) was no longer present. The mixture was diluted with dichloromethane (100 ml) and poured on ice. The organic phase was washed three times with 4 N hydrochloric acid and once with aqueous $NaHCO_3$, dried, filtered through carbon, and evaporated. The syrupy residue was crystallized from ether-pentane to give 3.0 g (28.5 %) of *2a*, m.p. *ca.* 85 °C.

To the material in the mother liquor was added 15 ml of a 30 % solution of hydrogen bromide in glacial acetic acid and the mixture was kept for 1 h at room temperature. Dichloromethane was then added and the solution was washed with water and aqueous $NaHCO_3$, dried and evaporated. The residue was crystallized from ether-pentane to give an additional 2.8 g of *2a*, bringing the total yield to 55 %. The product was sufficiently pure for most purposes, but could be recrystallized from ether-pentane, or from cyclohexane,

m.p. 92–93 °C, $[\alpha]_D^{20}$ 259° (*c* 5) (reported [4] m.p. 92–93 °C, $[\alpha]_D$ +260 °). A ^{1}H NMR spectrum confirmed the structure.

The material in the mother liquor contained some *2a* as seen from an NMR spectrum. It was dissolved in acetonitrile and stirred with silver benzoate for 3 h. Filtration through carbon and evaporation gave a yellow syrup which was dissolved in dichloromethane and washed with aqueous $NaHCO_3$. The solvent was evaporated and the residue was crystallized from ether-pentane to give 800 mg (12 %) of tri-*O*-acetyl-1-*O*-benzoyl-2-bromo-2-deoxy-β-D-glucopyranose (*6*), m.p. 150–153 °C. Recrystallization from ether-pentane gave the pure product, m.p. 157–158 °C, $[\alpha]_D^{20}$ 10.5° (*c* 2.8) (reported [3] m.p. 161–162 °C, $[\alpha]_D^{28}$ 15.3°). ^{1}H NMR: δ 6.08 (H1), 4.09 (H2), 5.44 (H3), 5.09 (H4), 3.99 (H5), 4.37 (H6), 4.10 (H6′); J_{12} = 9.0 Hz, J_{23} = 10.4, J_{34} = 9.1, J_{45} = 9.8, J_{56} = 4.5 $J_{56'}$ = 2.6, $J_{66'}$ = 12.5.

Di-O-acetyl-2-bromo-2,6-dideoxy-α-L-glucopyranosyl bromide (7a). A mixture of tri-*O*-acetyl-6-deoxy-α-L-glucopyranosyl bromide [7] (2.0 g), acetyl bromide (20 ml) and anhydrous zinc bromide (4 g) was stirred at room temperature for 3 h. Work up as described above gave 1.9 g of a product which was crystallized from ether-pentane to give 1.1 g (51 %) of *7a*, m.p. 126–128 °C. Recrystallization from ether-pentane gave the pure product, m.p. 130–132 °C, $[\alpha]_D^{20}$ −355° (*c* 1.4). Anal. $C_{10}H_{14}Br_2O_5$: C, H, Br. ^{1}H NMR: δ 6.44 (H1), 4.13 (H2), 5.56 (H3), 4.89 (H4), 4.33 (H5), 1.26 (H6). J_{12} = 3.7 Hz, J_{23} = 10.8, J_{34} = 9.2, J_{45} = 9.9, J_{56} = 6.1.

Tri-O-benzoyl-2-bromo-2-deoxy-α-D-glucopyranosyl bromide (2b). A mixture of tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (*1b*) (5.0 g), acetyl bromide (35 ml), and zinc bromide (10 g) was stirred at room temperature for 15–20 h. Work up as described above and crystallization from ether-pentane gave 3.12 g (66 %) of *2b*, m.p. 151–155 °C. Recrystallization from the same solvent gave 2.35 g (50 %) of pure product, m.p. 165–167 °C, $[\alpha]_D^{20}$ 141° (*c* 3.8). Anal. $C_{27}H_{22}Br_2O_7$: C, H. ^{1}H NMR: δ 6.62 (H1), 4.45 (H2), 6.18 (H3), 5.76 (H4), 4.82 (H5), 4.71 (H6), 4.49 (H6′). J_{12} = 3.5 Hz, J_{23} = 10.5, J_{34} = 9.3, J_{45} = 10.0, J_{56} = 2.5, $J_{56'}$ = 5, $J_{66'}$ = 12.5.

Di-O-benzoyl-2-bromo-2,6-dideoxy-α-L-glucopyranosyl bromide (7b). Tri-*O*-benzoyl-6-deoxy-α-L-glucopyranosyl bromide [7] (*4b*) (1.0 g), acetyl bromide (6 ml) and zinc bromide (2 g) were stirred at 5 °C for 18 h. Work up as described above gave 1 g of product which was crystallized from ether-pentane to give 483 mg (52 %) of *7b*, m.p. 90–94 °C. Recrystallization from ether-pentane gave the pure product, m.p. 91–92 °C, $[\alpha]_D^{20}$ −125° (*c* 4.1). Anal. $C_{20}H_{18}Br_2O_5$: C, H. ^{1}H NMR: δ 6.50 (H1), 4.38 (H2), 6.07 (H3), 5.33 (H4), 4.58 (H5), 1.40 (H6); J_{12} = 3.6 Hz, J_{23} = 10.9, J_{34} = 9.5, J_{45} = 9.5, J_{56} = 6.1.

Tri-O-acetyl-2-bromo-1,2-dideoxy-D-arabino-hex-1-enopyranose (5a). A solution of *2a* (1.0 g) and diethylamine (2.0 ml) in dichloromethane (4 ml) was kept at 0 °C for 30 min. It was then diluted with dichloromethane, washed with 4 N hydrochloric acid and with aqueous $NaHCO_3$, dried and evaporated. The residue was a syrup (802 mg, 99 %) which was pure as seen from TLC and ^{1}H NMR. A sample was purified by preparative TLC (ether-pentane (1:1)), $[\alpha]_D^{20}$ 19.1° (*c* 2.2). Anal. $C_{12}H_{15}BrO_7$: C, H. ^{1}H NMR: δ 6.83 (H1), 5.57 (H3), 5.27 (H4), 4.1–4.6 (H5–H6); $J_{13} \simeq 1$ Hz, J_{34} = 4.8, $J_{45} \simeq 4.8$. Crude *5a* has been prepared in the same way by Hurd and Jenkins.[9]

Tri-O-benzoyl-2-bromo-1,2-dideoxy-D-arabino-hex-1-enopyranose (5b) was prepared as described above from 500 mg of *2b*. The crude product (425 mg) was purified by preparative TLC, using 3 elutions with ether-pentane (1:2), to give 376 mg (87 %) of pure *5b* as a syrup, $[\alpha]_D^{20}$ −92.7° (*c* 1.1). Anal. $C_{27}H_{21}BrO_7$: C, H. ^{1}H NMR: δ 6.98 (H1), 6.02 (H3), 5.85 (H4), 5.0–4.5 (H5–H6); J_{34} = 4.0 Hz, J_{45} = 4.0 Hz.

Di-O-acetyl-2-bromo-1,2,6-trideoxy-L-arabino-hex-1-enopyranose (8a) was prepared analogously from 706 mg of *7a*. The crude product (457 mg) was purified by preparative TLC (ether-pentane 1:1) to give 350 mg (63 %) of *8a* as a syrup, $[\alpha]_D^{20}$ 1.8° (*c* 3.8). Anal. $C_{10}H_{13}BrO_5$: C, H. ^{1}H NMR: δ 6.77 (H1), 5.50 (H3), 5.02 (H4), 4.27 (H5), 1.33 (H6); $J_{13} \simeq 0.8$ Hz, J_{34} = 4.8, J_{45} = 6.3, J_{56} = 6.8.

Di-O-benzoyl-2-bromo-1,2,6-trideoxy-L-arabino-hex-1-enopyranose (8b) was also prepared in the same way from 1.0 g of *7b*. The crude product crystallized from ether to give 369 mg (47 %) of *8b*, m.p. 106–108 °C. Recrystallization from ether gave the pure product, m.p. 109–110 °C, $[\alpha]_D^{20}$ 19.9° (*c* 3.4). Anal. $C_{20}H_{17}BrO_5$: C, H. ^{1}H NMR: δ 6.89 (H1), 6.00 (H3), 5.51 (H4), 4.53 (H5), 1.51 (H6); J_{13} = 1 Hz, $J_{15} \simeq 0.8$, J_{35} = 1, J_{45} = 5.4, J_{56} = 6.8.

Microanalyses were performed by Novo Microanalytical Laboratory.

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Some Substitution Reactions of 2-(2′-Thienyl)pyrimidine and 2-(3′-Thienyl)pyrimidine

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Dedicated to Professor K. A. Jensen on his 70th birthday

2-(2′-Thienyl)pyrimidine (*1*) and 2-(3′-thienyl) pyrimidine (*2*) have been prepared from the corresponding thiopheneamidine hydrochlorides through reaction with 3-(dimethylamino)propenal. Isomer distributions in nitration of *1* and *2* under different conditions and in bromination with bromine in acetic acid have been determined. The isomeric nitro and bromo derivatives were separated and their structures determined by NMR spectroscopy.

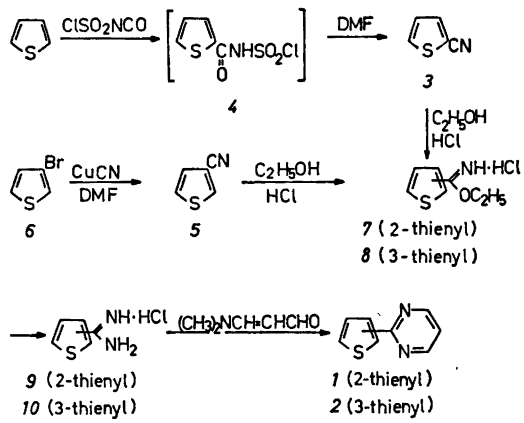
The directing properties of the 2-pyrimidyl group as a substituent on thiophene in electrophilic substitution are discussed.

In connection with a study of the chemistry of biaryls of heterocyclic type [1] we have synthesized 2-(2′-thienyl)pyrimidine *1* and 2-(3′-thienyl) pyrimidine *2* and have investigated some nitration and bromination reactions with the purpose of evaluating the directing effects of a 2-pyrimidyl group on electrophilic substitution in the thiophene series. The synthesis, nitration with mixed acids, and lithiation of *1* and *2* have been the subject of a short communication published when the major part of this work was finished.[2]

RESULTS

Synthesis and spectral properties. Our synthesis of *1* and *2* followed the usual pathway for the preparation of 2-arylpyrimidines,[3] as outlined in Scheme 1.

2-Cyanothiophene (*3*) was obtained in a convenient one-pot procedure through reaction of thiophene with chlorosulfonyl isocyanate,[4] giving 2-thiophenecarboxamido-*N*-sulfochloride (*4*) which was then treated with dimethylformamide [5] — a simpler method than the usual three step procedure.[6] 3-Cyanothiophene (*5*) was prepared by refluxing 3-bromothiophene (*6*) and copper(I) cyanide in dimethylformamide.[7] By reaction with ethanol and hydrogen chloride in ether, *3* and *5* were converted to the thiophenecarboimidic acid ethyl ester hydrochlorides (*7* and *8*), which after treatment with ammonia in ethanol gave the thiopheneamidine hydrochlorides (*9*) and (*10*). In the final step, *9* and *10* were reacted with 3-(dimethylamino)-propenal [8] to give *1* and *2*, respectively.

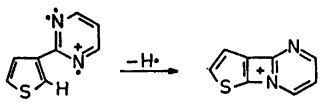


Scheme 1.

The ^{1}H NMR spectra of *1* and *2* (see experimental part) are well resolved, and from the

known coupling constants of thiophene [9] and pyrimidine,[10] each band can be assigned to a definite hydrogen, thus facilitating the structure determination of the substitution products. In the mass spectrometer, *1* and *2* show a small tendency to fragmentation (see experimental part) with the molecular ion giving the base peak and, in addition to M − 1 in the case of *2*, the only peak with an intensity higher than 12 % is $m/e = 109$. This is probably the thiophenenitrile ion, in analogy with the dominance of benzonitrile ion in spectra of 2-phenylpyrimidines.[11]

The intensity of M − 1 is 8 % in spectrum of *1*, but 19 % in the spectrum from *2*. The greater tendency to hydrogen abstraction in the 3-substituted thiophene may, as in the case of 2- and 3-(2′-pyridyl)thiophene,[12] be due to the presence of a reactive α-thienyl centre near one of the nitrogen atoms (*cf.* Scheme 2).



Scheme 2.

Reactions of 1 *and* 2. 2- and 3-Nitrothiophene and other deactivated thiophenes undergo nitration with nitric acid in trifluoroacetic acid at 25 °C.[13] Under the same conditions, *1* reacted rather slowly. After reaction for 48 h at 25 °C between equimolar amounts of substrate and reagent, NMR analysis showed the reaction mixture to contain about 90 % of starting material. The reaction was considered not to have preparative value, but was used for a comparison of the reactivity of *1* and *2* with 2-nitrothiophene on a semiquantitative basis.

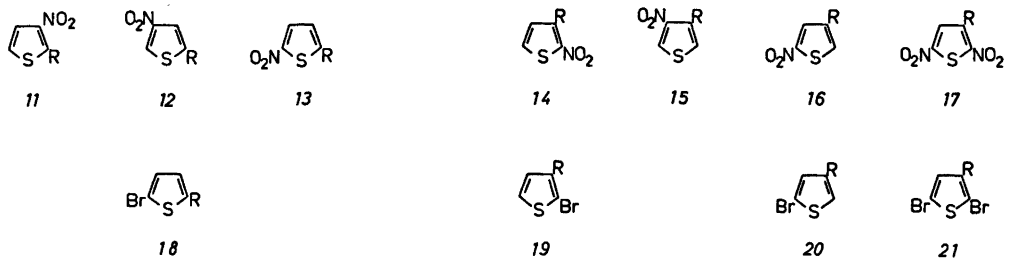
However, *1* and *2* were shown to be stable towards 98 % sulfuric acid, as neither decomposition nor sulfonation could be detected by NMR analysis after reaction for 24 h at 25 °C. Thus, 100 % nitric acid in 98 % sulfuric acid was chosen as the reagent for nitration of *1* and *2*. In order to study the nitration under essentially non-acidic conditions, *1* and *2* were also treated with an excess of acetyl nitrate in acetic anhydride. Brominations of *1* and *2* were performed with molecular bromine in acetic acid containing sodium acetate to neutralize the hydrogen bromide formed in order to prevent the precipitation of the hydrobromides of the pyrimidines involved.

The results of these reactions are summarized in Table 1. In all cases but one, mixtures of products were obtained. Efforts to separate these products by GLC were without success. (The isomers of 4-(nitrophenyl)pyrimidine separate on SE-30.[14]) However, mainly by PTLC all compounds but one (*11*) could be isolated in pure form. The structures of the compounds were determined by MS and ^{1}H NMR (see experimental part). The quantitative analysis of the mixtures obtained after work-up was performed by integration of the ^{1}H NMR spectra of the crude products.

Nitration with mixed acids (entries 1 and 3) gave mononitro isomers in high yield. The 2-isomer *1* gave a mixture of 2-(4′-nitro-2′-thienyl) pyrimidine (*12*) and 2-(5′-nitro-2′-thienyl)pyrimidine (*13*) in about equal amounts. The assignments of *12* and *13* were based on the presence in the ^{1}H NMR spectra of thiophene doublets with coupling constants of 1.5 and 4.4 Hz, respectively. Nitration of *2* was much more selective, and gave predominantly 2-(5′-nitro-3′-thienyl)pyrimidine (*16*), $J_{2'4'} = 1.7$ Hz. Small amounts of 2-(4′-nitro-3′-thienyl)pyrimidine (*15*), $J_{2'5'} = 3.8$ Hz and 2-(2′-nitro-3′-thienyl)pyrimidine (*14*), $J_{4'5'} = 5.5$ Hz, were also isolated.

Acetyl nitrate (entries 2, 4, and 5) gave a less selective reaction and a lower yield of nitrated products. Thus *1* now yielded *13* as the main product and the amount of *12* formed is even less than that of 2-(3′-nitro-2′-thienyl)pyrimidine (*11*). The nitration of *2* under these conditions was even more complex; *14* now became the main isomer, and the amount of *15* was approximately the same as in the nitration with mixed acids, while much less of *16* was formed. This is partly due to further nitration of *16* to a dinitro derivative (*cf.* entries 4 and 5). ^{1}H NMR and MS show that this compound contains two nitro groups bonded to the thiophene nucleus and the ^{13}C spectrum demonstrates that the dinitro derivative is 2-(2′,5′-dinitro-3′-thienyl)pyrimidine (*17*). In ^{13}C NMR the unsubstituted thiophene carbon shows a direct C − H coupling constant of 184 Hz. In a series of monosubstituted thiophenes, J(C5H5) was found to vary between 184 and 199 Hz and J(C4H4) between 164 and 177 Hz (the highest

Table 1. Some substitution reactions and their products. R = 2-pyrimidyl.



11 *12* *13* *14* *15* *16* *17*

18 *19* *20* *21*

Entry	Reaction [a]	Composition of isolated material/ mol %					Yield
		11	*12*	*13*			
1	*1* + HNO_3,H_2SO_4, 0 °C, 1 h	–	47	53			93
2	*1* + $AcONO_2$, 25 °C, 3 h	11	5	84			70
		14	*15*	*16*	*17*	*2*	
3	*2* + HNO_3,H_2SO_4, 0 °C, 1 h	4	4	92	–	–	93
4	*2* + $AcONO_2$, 25 °C, 48 h	39	5	19	19	18	59
5	*2* + $AcONO_2$, 25 °C, 96 h	45	5	7	43	–	62
				18			
6	*1* + Br_2,AcOH,NaOAc, 25 °C, 0.5 h			100			93
		19		*20*	*21*	*2*	
7	*2* + Br_2,AcOH,NaOAc, 25 °C, 18 h	24		36	12	28	59
8	*2* + Br_2,AcOH,NaOAc, 25 °C, 18 h	39		32	29	–	73

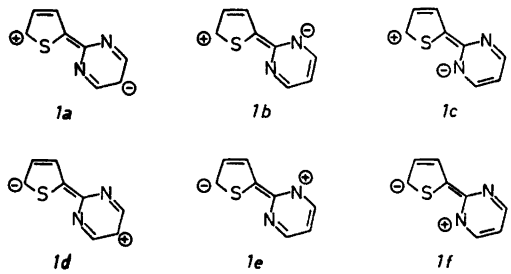
[a] Acetyl nitrate was used in 12-fold excess, nitric acid and bromine in equimolar amounts, except in entry 8 where 1.7 equiv. of bromine were used.

values are observed when the substituent is a nitro group).[15] The coupling constant obtained from *17* is at first sight not typical for a C_4–H_4 coupling, but that assignment seems to be reasonable considering the electronegative character of the substituents (*cf.* also ^{13}C NMR of compound *21*, see below). Further proof of the structure is obtained from the fact that *2*, which at 0 °C gives *16* mainly, upon nitration with mixed acids, first at room temperature and then at 100 °C, produces *17*, exclusively. The nitro group in *16* is also expected to deactivate position 4 (*ortho*) more than position 2 of the thiophene nucleus. Our results also indicate that *14* is more deactivated towards further nitration than *16*, and that *1* is more reactive than *2* towards nitration with acetyl nitrate.

Also in the bromination *1* was more reactive than *2*, and gave 2-(5′-bromo-2′-thienyl)pyrimidine (*18*) selectively and in high yield. As in the case of nitration with acetyl nitrate, *2* also gave a more complex product mixture than *1* upon bromination, and the reaction was further complicated by a bromine-consuming side reaction giving some unstable by-products. 2-(2′-Bromo-3′-thienyl)pyrimidine (*19*), $J_{4'5'} = 5.7$ Hz, 2-(5′-bromo-3′-thienyl)pyrimidine (*20*), $J_{2'4'} = 1.2$ Hz, and 2-(2′,5′-dibromo-3′-thienyl)pyrimidine (*21*), were the products isolated. The structures of the two monobromo derivatives are obvious from the characteristic coupling between the two remaining thiophenic protons. The structure of *21* seems reasonable from those of the monobromo derivatives, and was confirmed by its ^{13}C NMR spectra, which showed a direct coupling constant from the unsubstituted thiophene carbon of 178 Hz, assigned as J(C4H4). The value is somewhat larger than usually observed but the assignment is justified by a comparison with ^{13}C-parameters[15b] of 2,3-

dibromo-4-formylthiophene, J(C5H5) 194 Hz, and 2,5-dibromo-3-formylthiophene, J(C4H4) 178 Hz.

Our results (*cf.* entries 1 and 3), differ somewhat from those reported in Ref. 2, according to which *1* reacts with mixed acids (conditions not specified) to give *12* and *13* in the ratio 65:35 (*cf.* entry 1). From the same reaction with *2* as substrate, only 2-(5′-nitro-3′-thienyl)pyrimidine *16* was observed as product by the French workers (*cf.* entry 3).



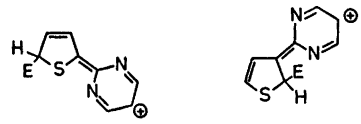
Scheme 3.

DISCUSSION

According to classic electronic theory the 2-pyrimidyl group with its amidine-like structure can be expected to be a −I-substituent. For the resonance hybrid of *1* in the ground state, structures Ia−If (Scheme 3) may contribute. However, structures Ie and If, with a positive charge on the nitrogen, are of less importance, indicating that the −M-effect of the 2-pyrimidyl group is of greater importance than its +M-effect.

A comparison of the ^{1}H NMR shifts ($CDCl_3$) of H5 (δ 7.00) and H5′ (7.45) in *1* with the corresponding protons in unsubstituted pyrimidine (7.36)[10] and thiophene (7.30),[16] respectively, indicates the operation of a −M-effect of the pyrimidine ring in the ground state. The same conclusion can be drawn in the case of *2*, where the ^{1}H NMR ($CDCl_3$) shows the signal from H5 at 7.08.

On the other hand, it seems reasonable to consider the possibility that the 2-pyrimidyl group of *1*, through a +M-effect, could stabilize transition states following attacks of electrophiles in the 5- (or 3-) position of the thiophene nucleus. In *2* the same +M-effect should favour electrophilic attack in the 2-position (*cf.* Scheme 4).



Scheme 4.

In protonated *1* and *2* such stabilization is much less likely as the pyrimidinic moiety will be doubly charged.

The results in entries 1 and 3 indicate that the pyrimidine ring acts as a *meta*-directing substituent[17] in the nitration in mixed acids. However, the results from entries 2 and 4 imply that the pyrimidine ring in nitration with acetyl nitrate in neutral medium acts more as an *ortho-para*-directing group. These differences may be due to differences in the nitration process. Thus, it could be possible that acetyl nitrate reacts via an addition-elimination reaction. (Furan and furan derivatives carrying electronegative substituents undergo this type of nitration[18] and in the case of 4-phenylpyrimidine acetyl nitrate adds to the pyrimidine ring to give 2,4-diacetoxy-6-phenyl-1,3,5-trinitro-1,2,3,4-tetrahydropyrimidine in high yield.[14]) However, to our knowledge, no examples of such addition-elimination nitration are known from the thiophene series and no acetoxy compounds were observed in the reaction mixtures from entries 2, 4 and 5. Further, acetyl nitrate in acetic anhydride or *N*-nitropicolinium salts in acetonitrile give almost identical results in nitration of 2-acetylthiophene,[19] indicating that acetyl nitrate gives a normal electrophilic nitration with this deactivated thiophene.

A more plausible explanation for the difference in directing power is that the substrate in concentrated sulfuric acid reacts in a protonated form, *i.e.* the orientational pattern in entries 1 and 3 is caused by a protonated pyrimidine ring, while that in entries 2 and 4 is caused by an unprotonated ring.

Protonation on a pyrimidine nitrogen must increase the −I-effect of the 2-pyrimidyl group, but can also, as mentioned above, be expected to inhibit an eventual +M-effect of the substituent, both phenomena favouring substitution in the 4-position of *1* and the 5-position of *2*.

Selective bromination in the 5-position, as in entry 6, is observed for thiophenes substituted in position 2 by for example halogens (−I, +M) and carbonyl-containing groups (−I, −M). Strong +M-substituents also give reaction in the 3-position, whereas strong −M-substituents, such as complexed carbonyl groups, lead to 4- and 5-substitution.[17]

Our results then exclude strong +M or strong −M as a characterization of the 2-pyrimidyl group, and this together with the *ortho-para* pattern obtained in entry 2, justifies the above assumption of a +M-character of the 2-pyrimidyl group.

The orientation in the acetyl nitrate nitration (entries 4 and 5) and bromination (entries 7 and 8) of *2* differs from most 3-substituted thiophenes in giving approximately equal amounts of 2- and 5-substitution, but seems to be reasonable for strong −I, weak +M-substituent.

The facile dibromination and especially dinitration of *2* is quite interesting but somewhat difficult to explain at present.

An isomer distribution similar to that in entries 7 and 8 is obtained by the bromination of 3-phenylthiophene with bromine in acetic acid. This reaction was shown to be reversible due to the presence of the hydrogen bromide formed, and the isomer distribution was explained as the result of thermodynamic product control.[20] However, in our brominations, the hydrogen bromide was neutralized by sodium acetate, and hence thermodynamic control is less likely.

The relatively large amount of *ortho*-substitution (*cf.* entries 2, 4, and 5) could also, tentatively, be explained by the assumption that the 2-pyrimidine group has a −M character but also coordinates the electrophile to one of its nitrogens before an intramolecular substitution process occurs. In such a case, the orientational consequences of an eventual −M-effect of the substituent could be masked. However, it is known that thiophenes substituted in the 2-position by −I, −M groups react more slowly than the corresponding 3-isomers.[13] A comparison of entries 2 and 4 or 6 and 7 makes it clear that in the cases of *1* and *2* the 2-substituted isomer reacts faster than the 3-substituted, indicating that the 2-pyrimidyl group can hardly be a −M-substituent. The discussion above leads to the conclusion that the 2-pyrimidyl group in *1* and *2* acts as −I, −M-substituent in strongly acidic medium, but as a −I, +M-substituent under neutral conditions.

Comparison with literature data confirms these assumptions. In mixed acids, *1* and 2-nitrothiophene give a similar substitution pattern,[21] whereas the latter does not react with acetyl nitrate in acetic anhydride.[22]

To obtain more detailed data on the reactivity of 2-nitrothiophene towards bromine,[23] we tested the conditions used in entry 6, but after reacting for 3 h only starting material could be found by GLC.

A semiquantitative comparison of the nitration with nitric acid of 2-nitrothiophene, *1* and *2* in strongly acidic medium (trifluoroacetic acid [13]) was made by following the disappearance of starting material by ^{1}H NMR and the following order of reactivity was obtained: 2-nitrothiophene > *2* > *1*. Here *2* is more reactive than *1* (*cf.* above) confirming the −I, −M-character in strongly acidic medium.

In comparison with the corresponding 4-pyrimidyl derivatives, [1b] *1* and *2* appear to be more reactive under non-acidic conditions. The 4-pyrimidyl derivatives do not react with acetyl nitrate, and 4-(3′-thienyl)pyrimidine gives no bromination products with bromine in acetic acid at room temperature, but reacts sluggishly at 50 °C.

EXPERIMENTAL

Melting points are uncorrected. The ^{1}H NMR spectra were recorded with Varian A60, JEOL PMX-60, or JEOL MH-100 spectrometers; data given below refer to recordings at 60 MHz in $CDCl_3$ solution if not otherwise stated. ^{13}C NMR spectra were obtained in $(CD_3)_2CO$ solution on a JEOL-FX60 NMR spectrometer. The shifts were determined from proton decoupled spectra with TMS as internal standard. The MS were recorded on an LKB A 9000 spectrometer. GLC analyses were carried out on a Varian aerograph 1400 gas chromatograph equipped with a flame ionization detector.

Separations by preparative thin layer chromatography (PTLC) were performed on silica gel plates, 20 × 20 cm, layer thickness 2 mm (Merck Fertigplatten, Kiselgel 60 F_{254}). The fractions were isolated by methanol extraction of the silica gel. 3-Bromothiophene,[24] 3-(dimethylamino)propen-2-al [8] and 100 % nitric acid [25] were prepared according to methods described in the literature.

2-Cyanothiophene 3. Chlorosulfonyl isocyanate (85.0 g, 0.60 mol, 52.1 ml) was rapidly added

to a stirred solution of thiophene (168.2 g, 2.00 mol) in dry ether (100 ml) and dry benzene (150 ml). The reaction mixture was kept at 35 °C until crystals began to precipitate. More chlorosulfonyl isocyanate (198.2 g, 1.40 mol, 122.0 ml) in dry benzene (250 ml) was then added over a period of 1 h, during which time the temperature rose but was kept at 50 °C with a water bath. After complete addition, the mixture was stirred at 50 °C for another h and was then left at room temperature over night.

The flask, now containing a precipitate of 2-thiophenecarboxamido-*N*-sulfochloride *4*, was placed in a cooling bath (ice/salt). Dimethylformamide (308 g, 4.20 mol) was added at such a rate that the temperature could be kept at 10 – 12 °C (about 20 min was required). The mixture was stirred at 30 °C for 30 min and was then poured onto 400 g of ice. After separating the organic layer, the water phase was extracted twice with benzene (100 ml). The combined organic layers were washed with water, $NaHCO_3$ and dried over $MgSO_4$. Evaporation and distillation yielded 148.6 g (72 %) of the title compound, b.p. 77 – 78 °C/1.6 kPa; n_D^{20} 1.5619. Lit.[26] 72 °C/1.3 kPa, n_D^{20} 1.5612.

3-Cyanothiophene 5. A stirred mixture of 3-bromothiophene (162.6 g, 1.00 mol), copper (I) cyanide (106.0 g, 1.20 mol), and dimethylformamide (150 ml) was refluxed for 3 h. The hot dark reaction mixture was poured into a warm solution of sodium cyanide (200 g) in water (600 ml). The mixture was vigorously shaken and then extracted twice with benzene (200 + 100 ml). The extract was washed with 10 % aqueous sodium cyanide (100 ml) and water. After drying ($MgSO_4$) and removal of solvent, distillation yielded 69.6 g (64 %) of the title compound, b.p. 82 – 83 °C/1.6 kPa, n_D^{24} 1.5603. Lit.[26] 77 °C/1.3 kPa; n_D^{20} 1.5632.

2-Thiophenecarboximidic acid ethyl ester hydrochloride 7. A stirred solution of 2-cyanothiophene (54.6 g, 0.50 mol) and absolute ethanol (34.5 g, 0.75 mol) in dry ether (300 ml) was cooled to 0 °C (solid CO_2/ethanol) and saturated with dry hydrogen chloride passed into the solution at such a rate that the temperature could be kept between 0 – 5 °C (about 1 h). The cooling bath was removed and the mixture was stirred for 24 h. The crystalline precipitate formed was then filtered off, rapidly washed with dry ether, and dried *in vacuo* over silica, yielding 86.2 g (91 %) of the title compound, m.p. 126 – 128 °C (decomposition to amide, m.p. 177 – 178 °C). Lit.[27] 123 – 126 °C; 176 °C.

The ^1H NMR spectrum of the free imido ester was recorded on a carbon tetrachloride solution (dried over $CaCl_2$) obtained by shaking CCl_4, 7 and cold 1.0 M NaOH (1.1 equiv.) followed by separation of the aqueous layer: δ 7.43 (s, broad, disappears after addition of D_2O, N=H), 7.39 (3-H, dd, 1 H), 7.30 (5-H, dd, 1 H), 6.95 (4-H, dd, 1 H), 4.28 (CH_2, q, 2 H), 1.37 (CH_3, t, 3 H). J (H3,H4) 3.6 Hz, J (H3,H5) 1.3 Hz, J (H4,H5) 4.9 Hz, J (CH_2-CH_3) 7.0 Hz.

3-Thiophenecarboximidic acid ethyl ester 8 was prepared in 91 % yield from *5* by the same procedure as for 7. The title compound forms white crystals, m.p. 126 – 129 °C (decomposition to amide, m.p. 180 – 181 °C). Lit.[28] m.p. for amide 179 – 180 °C. ^1H NMR (CCl_4) of the free imido ester: δ 7.33 (s, broad, disappears after addition of D_2O, N=H), 7.65 (H-2, dd, 1 H), 7.27 (4-H, dd, 1 H), 7.15 (5-H, dd, 1 H), 4.27 (CH_2, q, 2 H), 1.35 (CH_3, t, 3 H). J(H2,H4) 1.6 Hz, J(H2,H5) 2.7 Hz, J (H4,H5) 5.0 Hz, J (CH_2-CH_3) 7.0 Hz.

2-Thiophenecarboxamidine hydrochloride 9. 2-Thiophenecarboximidic acid ester hydrochloride (82.2 g, 0.43 mol) was added in portions to a stirred solution of ammonia (16.3 g, 0.96 mol) in absolute ethanol (200 ml) and the mixture was then stirred at room temperature. Samples of the mixture were made alkaline with dilute NaOH and extracted with ether. The ether phase was analyzed on TLC (Al_2O_3/acetone; R_F amidine 0.05 R_F imido ester 0.90). After 48 h reaction all imido ester was consumed. Eventual ammonium chloride was then filtered off, and after evaporation of the solvent, 67.8 g (97 %) of the title compound was obtained, m.p. 177 – 178 °C. Lit.[27] 176 °C.

To get a sample for ^1H NMR, *9* was treated with cold 2.5 M NaOH (1.5 equiv.). An oil separated which crystallized in the refrigerator. Filtration and drying *in vacuo* yielded 2-thiophenecarboxamidine, m.p. 109 – 110 °C. ^1H NMR (DMSO-d_6): δ 7.63 – 7.43 (H-3 and H-5, m, 2 H), 7.03 (4-H, dd, 1 H), 6.43 (s, broad, disappeared after addition of D_2O, $C(NH)NH_2$, 3 H). J (H3,H4) 3.5 Hz, J (H4,H5) 5.0 Hz.

3-Thiophenecarboxamidine hydrochloride 10 was prepared from *8* in 98 % yield by the same procedure as for *9*. The title compound forms white crystals, m.p. 177 – 178 °C. ^1H NMR of the free amidine (m.p. 107 – 108 °C) in DMSO-d_6 gave an AA'X spectrum: δ 7.98 (H-2, t, 1 H), 7.52 (H-4 and H-5, d deformed, 2 H), 6.38 [$C(NH)NH_2$, s, 3 H disappears after addition of D_2O].

2-(2-Thienyl)pyrimidine 1. To a stirred solution made from sodium (2.30 g, 0.100 mol) and methanol (75 ml), 2-thiophenecarboxamidine hydrochloride (16.3 g, 0.100 mol) was added in portions followed by 3-(dimethylamino)propen-2-al (9.91 g, 0.100 mol), after which the mixture was refluxed for 6 h. The sodium chloride formed was filtered off from the hot reaction mixture and washed twice with hot methanol (10 ml). The combined methanol phases were kept at – 15 °C overnight. Filtration yielded 9.99 g of the title compound as almost white crystals, m.p. 90 – 92 °C. The mother liquor was evaporated and the residue recrystallized from aqueous methanol yielding an additional 2.01 g of the title compound, m.p. 89 – 90 °C, total yield 12.0 g (74 %). To prepare an analytically pure material, the crude product was dissolved in 2 M HCl and the solution was treated with charcoal. After filtration, 5 M NaOH was added

to pH ≈ 3, yielding white crystals. m.p. 92–93 °C. (Found: C 59.1; H 3.69; N 17.2; S 19.8. Calc. for $C_8H_6N_2S$ (162.22): C 59.27; H 3.73; N 17.27; S 19.73). ^{1}H NMR: δ 8.63 (H-4 and H-6, d, 2 H), 8.00 (H-3′, dd, 1 H), 7.45 (H-5′, dd, 1 H), 7.12 (H-4′, dd, 1 H), 7.00 (H-5, t, 1 H). J(H4,H5) 4.9 Hz, J(H3′,H4′) 3.6 Hz, J(H3′, H5′) 1.3 Hz, J(H4′,H5′) 4.9 Hz. *m/e* (%): 162 (100, M$^+$), 161 (8, M$^+$ –H), 135 (4, M$^+$ –HCN), 109 (52, M$^+$ – C_3H_3N), 82 (9), 45 (12), 39 (11).

2-(3-Thienyl)pyrimidine 2 was prepared from *10* in 76 % yield by the same procedure as for *1*. The title compound forms white prisms, m.p. 101–102 °C. (Found: C 59.3; H 3.80; N 17.0; S 19.6. Calc. for $C_8H_6N_2S$ (162.22): C 59.27; H 3.73; N 17.27; S 19.27). ^{1}H NMR: δ 8.73 (H-4 and H-6, d, 2 H), 8.28 (H-2′, dd, 1 H), 7.90 (H-4′, dd, 1 H), 7.33 (H-5′, dd, 1 H), 7.08 (H-5, t, 1H). J(H4,H5) 4.9 Hz, J(H2′,H4′) 1.2 Hz, J(H2′,H5′) 3.1 Hz, J(H4,H5) 5.2 Hz. *m/e* (%): 162 (100, M$^+$), 161 (19, M$^+$–H), 135 (6, M$^+$–HCN), 109 (50, M$^+$–C_3H_3N), 81 (8), 45 (2), 39 (8).

Nitration of 2-(2′-thienyl)pyrimidine (1) with nitric and sulfuric acids. 1 (324 mg, 2.00 mmol) was dissolved in 98 % sulfuric acid (3.5 ml). To the yellow solution 100 % nitric acid (83 µl, 2.0 mmol) was added at 0 °C. The mixture was allowed to stand at 0 °C for 1 h and was then poured onto ice (*ca.* 20 g). After collecting the resulting precipitate on a filter, it was stirred with a saturated solution of $NaHCO_3$ at 80 °C for 15 min. After cooling, filtration yielded 374 mg (90 %) of yellow crystalline powder, m.p. 160–185 °C.

TLC indicated two products and the mixture was chromatographed by PTLC (30 mg/plate, elution 4 times with dicloromethane) which gave two bands. From the band at $R_F = 0.40$ (356 nm: weak greyish-violet), 2-(4′-nitro-2′-thienyl)pyrimidine (*12*) was obtained as almost white crystals, m.p. 157–158 °C. (Found: C 46.8; H 2.50; N 19.6. Calc. for $C_8H_5N_3O_2S$ (207.2): C 46.4; H 2.43; N 20.3). ^{1}H NMR: δ 8.77 (H-4 and H-6, d, 2 H), 8.48 (H-3′ or H-5′, d, 1 H), 8.38 (H-3′ or H-5′, d, 1 H), 7.25 (H-5, t, 1 H); J(H4,H5) 4.8 Hz, J(H3′,H5′) 1.5 Hz. *m/e* (%): 207 (100, M$^+$), 191 (2, M$^+$–O), 177 (7, M$^+$–NO), 162 (7, M–CHS), 161 (26, M$^+$–NO_2), 149 (43, M$^+$–NO–CO). ν_{max} (KBr): 1515, 1335 cm^{-1}

From the band at $R_F = 0.51$ (356 nm: strong dark violet), 2-(5′-nitro-2′-thienyl)pyrimidine (*13*) was isolated as pale yellow crystals, m.p. 201–202 °C. (Found: C 46.6; H 2.48; N 19.5. Calc. for $C_8H_5N_3O_2S$ (207.2): C 46.4; H 2.43; N 19.6). ^{1}H NMR: δ 8.78 (H-4 and H-6, d, 2 H), 7.93 (H-3′ and H-4′, s, 2 H, separated in HMPT: δ 8.32, d, and 8.00, d, J(H3′,H4′) = 4.4 Hz), 7.28 (H-5, t, 1 H). J(H4,H5) 4.8 Hz. *m/e* (%): 207 (100), 191 (2), 177 (20), 161 (20), 149 (28), assignments see for compound *12*. ν_{max} (KBr) 1490, 1330 cm^{-1}. Through integration of the ^{1}H NMR spectrum of the crude product the ratio of *12* to *13* was determined to be 46:54.

The first filtrate above was neutralized to pH = 7 (NaOH, $NaHCO_3$) and was then extracted with dichloromethane (3 × 10 ml). The combined organic phases were washed (H_2O) and dried ($MgSO_4$) Evaporation of solvent yielded 12 mg of product. TLC together with ^{1}H NMR showed it to consist of *1* and *12* in ratio 1:3.

Nitration of 2-(2′-thienyl)pyrimidine (1) with acetyl nitrate. 100 % nitric acid (1.0 ml) was mixed with acetic anhydride (3.0 ml), with cooling. The mixture was kept at room temperature for 15 min, then cooled to 0 °C and added to a cold (0 °C) solution of *1* (324 mg, 2.00 mmol) in acetic anhydride (3.0 ml). The reaction mixture was then put in a water bath (25 °C). White needles (for identification, see below) began to precipitate after some minutes, but were redissolved after about half an hour when a slightly exothermic reaction started. After 3 h, all starting material was consumed (GLC). The yellow precipitate formed was collected on a filter and washed with cold acetic anhydride, which afforded 104 mg (25 %) of 2-(5′-nitro-2′-thienyl)pyrimidine (*13*) (NMR, IR), m.p. 201–202 °C, contaminated with traces of other isomers (TLC). The filtrate was poured onto ice, neutralized (5 M NaOH) and the mixture was extracted with dichloromethane (3 × 15 ml). The extract was washed (H_2O), dried ($MgSO_4$), and evaporated to give 186 mg (45 %) of yellow crystalline material, m.p. 140–170 °C. This consisted of a mixture of 2-(3′,2′-thienyl)pyrimidine (*11*), 2-(4′-nitro-2′-thienyl)pyrimidine (*12*), and 2-(5′-nitro-2′-thienyl)pyrimidine (*13*) in the proportions 2:1:9, which could be shown as follows. The major product (*13*) was identified from the ^{1}H NMR spectrum of the mixture. This spectrum did not allow an unambiguous identification of the other products. Therefore 50 mg of the mixture was chromatographed by PTLC (50 mg/plate, elution 4 times with dichloromethane), which gave two bands. One band (R_F 0.54; 356 nm: strong dark violet) contained *13*, and from the other band (R_F 0.43; 356 nm: weak bright violet) 11 mg of crystalline material, m.p. 70–105 °C was isolated. The spectroscopical data showed it to be a 2:1 mixture of *11* and *12*: ^{1}H NMR (100 MHz, acetone-d_6) 2-(3′-nitro-2′-thienyl)pyrimidine: δ 8.85 (H-4 and H-6, d, 2 H), 7.82 (H-4′ or H-5′, d, 1 H), 7.54 (H-4′ or H-5′, d, 1 H), 7.47 (H-5, t, 1 H). J(H4,H5) 5.0 Hz, J(H4′,H5′) 5.5 Hz. 2-(4′-Nitro-2′-thienyl)pyrimidine: δ 8.86 (H-4 and H-6, d, 2 H), 8.73 (H-3′ or H-5′, d, 1 H), 8.37 (H-3′ or H-5′, d, 1 H), 8.45 (H-5, t, 1 H). J (H4,H5) 5.0 Hz, J(H3′,H5′) 1.7 Hz. Integration gave the ratio between *11* and *12* = 2:1.

The MS of the mixture showed no other peaks than those expected from 2-(mononitrothienyl)-pyrimidines. ν_{max} (KBr) 1518, 1505, 1334 cm^{-1}.

When the NMR parameters of all three isomers in $CDCl_3$ were known, the spectrum of the first isolated mixture could be interpreted, integration giving the product distribution given above.

In one run of the experiment, the precipitate formed in the beginning of the reaction was collected on a filter, giving 210 mg of white needles, m.p. 128 °C (decomp. evolution of nitrogen oxides). The product was identical (IR, mixed melting point) with an authentic sample of 2-(2′-thienyl)pyrimidine mononitrate prepared by adding nitric acid to a saturated solution of *1* in acetonitrile. (Found: C 42.4; H 3.14; N 18.6. Calc. for $C_8H_7N_3O_3S$ (225.2) requires C 42.66; H 3.13; N 18.66). ^{1}H NMR (CH_3CN): The same as (*1*). ν(KBr) 2500 cm^{-1} (broad, $=\overset{+}{N}H$).

Nitration of 2-(3′-thienyl)pyrimidine (2) with nitric and sulfuric acids. The same nitration procedure as above was used. Starting from 2.00 mmol of *2*, filtration of the acidic water phase yielded after neutralization of the precipitate 351 mg (85 %) 2-(5′-nitro-3′-thienyl) pyrimidine *16* as a slightly beige powder, m.p. 189 – 190°. Sublimation (13 Pa, 150 °C) gave pale yellow prisms, m.p. 191.0 – 191.5°. (Found: C 46.5; H 2.25; N 19.8. Calc. for $C_8H_5N_3O_2S$ (207.2): C 46.4; H 2.43; N 20.3). ^{1}H NMR: δ 8.80 (H-4 and H-6, d, 2 H), 8.72 (H-2′ or H-4′, d, 1 H), 8.47 (H-2′ or H-4′, d, 1 H), 7.24 (H-5, t, 1 H). J(H4,H5) 4.9 Hz, J(H2′,H4′) 1.7 Hz. *m/e* (%): 207 (100), 191 (2), 177 (12), 161 (11), 149 (29). ν_{max} (KBr) 1498, 1330 cm^{-1}.

After neutralization, the acidic water phase was extracted with dichloromethane, yielding 32 mg of semicrystalline oil. This was chromatographed by PTLC (32 mg/plate; elution 3 times with ether) which gave four bands. Two of these, at R_F 0.63 and 0.56, were of minor magnitude and contained (IR) traces of starting material (*2*) and (*16*), respectively. From the third band (R_F 0.47, 356 nm: pale violet), 2-(4′-nitro-3′-thienyl)pyrimidine *15* was isolated as white prisms, m.p. 93 – 94 °C. ^{1}H NMR: δ 8.78 (H-4 and H-6, d, 2 H), 8.13 (H-2′ or H-5′, d, 1 H), 7.88 (H-2′ or H-5′, d, 1 H), 7.27 (H-5, t, 1 H). J(H4,H5) 4.8 Hz, J(H2′,H5′) 3.8 Hz. *m/e* (%): 207 (100), 191 (2), 177 (45), 162 (7), 161 (8), 149 (33). ν_{max} (KBr) 1505, 1348 cm^{-1}.

From the fourth band (R_F 0.38, 356 nm: strong dark violet) 2-(2′-nitro-3′-thienyl)pyrimidine *14* was isolated as yellow needles m.p. 117 – 118 °C. ^{1}H NMR: δ 8.85 (H-4 and H-6, d, 2 H), 7.52 (H-4′ or H-5′, d, 1 H), 7.32 (H-4′ or H-5′, d, 1 H), 7.35 (H-5, t, 1 H). J(H4,H5) 4.8 Hz, J(H4′,H5′) 5.5 Hz. *m/e* (%): 207 (100), 191 (2), 177 (40), 161 (16), 149 (79). ν_{max} (KBr) 1495, 1328 cm^{-1}.

Nitration of 2-(3′-thienyl)pyrimidine (2) with acetyl nitrate. The amounts of substrate, nitric acid and acetic anhydride and the way of mixing them were the same as above. After 48 h, the reaction mixture was poured onto ice. The mixture was neutralized to pH = 7 with 5 M NaOH and then extracted with dichloromethane (3 × 10 ml). The extract was washed (H_2O), dried ($MgSO_4$) whereupon evaporation yielded 265 mg of yellow semicrystalline product, m.p. 75 – 110 °C.

Through a combination of PTLC (100 mg/plate, elution 4 times with dichloromethane) and ^{1}H NMR analysis the product was shown to consist of 12 % starting material *2* together with *14*, *15*, *16* and 2-(2′,5′-dinitro-3′-thienyl)-pyrimidine *17* in the molar proportions of 48:6:23:23. The dinitro compound *17* was not isolated, but was shown (IR, NMR, TLC) to be identical with authentic *17* (see below).

Repetition of this experiment with prolonged reaction time (96 h) led to 294 mg product which by NMR analysis ($CDCl_3$) was shown to contain no starting material but the same nitrated products as above, now in the molar proportions of 45:5:7:43.

2-(2′,5′-Dinitro-3′-thienyl)pyrimidine (17). 100 % nitric acid (0.5 ml) was added to a solution of *2* (162 mg, 1.00 mmol) in 98 % sulfuric acid (2 ml). The mixture was heated to 100 °C for one hour, and after cooling the solution was poured onto ice. The precipitate formed was collected on a filter, washed with water, $NaHCO_3$ solution, and water, yielding 113 mg (45 %) of the title compound as a yellow powder, m.p. 125 – 126 °C. Recrystallization from methanol afforded pale yellow prisms, m.p. 126 – 127 °C. (Found: C 38.9; H 1.59; N 22.2. Calc. for $C_8H_4N_4O_4S$ (252.2): C 38.10; H 1.60; N 22.22). ^{1}H NMR: δ 8.85 (H-4 and H-6, d, 2 H), 8.17 (H-4′, s, 1 H), 7.38 (H-5, t, 1 H). J(H4,H5) 5.0 Hz. ^{13}C NMR: δ 129.8 (C4′). J(C4′,H4′) 183.7 Hz. *m/e* (%): 252 (76, M$^+$), 236 (1, M$^+$ – O), 222 (1, M$^+$ – NO), 206 (4, M$^+$ – NO_2), 190 (3, M$^+$ – NO_2 – O), 176 (27, M$^+$ – NO_2 – NO), 164 (32, M$^+$ – NO_2 – CNO), 160 (18, M – 2NO_2), 148 (100, M$^+$ – NO_2 – NO – CO). ν_{max} (KBr) 1518, 1322 cm^{-1}.

2 in sulfuric acid as above was also treated with 2 equiv. of nitric acid for 1 h at 40 °C. Under these conditions, however, only mononitrated product was obtained.

Bromination of 2-(2′-thienyl)pyrimidine (1). Bromine (320 mg, 102 µl, 2.0 mmol) was added to a solution of *1* (324 mg, 2.00 mmol) and sodium acetate (330 mg, 4.0 mmol) in acetic acid (10 ml), whereupon the flask was stoppered. After 30 min all starting material was consumed (GLC) and the reaction mixture poured onto ice. The resulting suspension was neutralized with solid sodium carbonate and then extracted with ether (3 × 20 ml). The extract was washed (H_2O), dried ($MgSO_4$), and evaporated to give 447 mg 93 % of 2-(5′,bromo-2′-thienyl)pyrimidine *18* as a yellow-white solid, m.p. 99 – 101 °C. TLC (silica gel, dichloroethane or ether) showed, in addition to the application spot (polar impurity), only one spot. Sublimation (140 °C, 13 Pa) afforded white crystals, m.p. 100 – 101 °C. (Found: C 40.0; H 1.99; Br 34.1. Calc. for $C_8H_5N_2BrS$ (241.2): C 39.85; H 2.10; Br 33.14). ^{1}H NMR: δ 8.60 (H-4 and H-6, d, 2H), 7.70 (H-3′, d, 1 H), 7.07 (H-4′, d, 1 H), 7.03 (H-5, t, 1 H). J (H4,H5) 4.8 Hz, J(H3′,H4′)

3.9 Hz. *m/e* (%): 240, 242 (79, 78, M^+), 187, 189 (25, 25, $M^+ - C_3H_3N$), 161 (100, $M^+ - Br$), 108 (7, $M^+ - Br - C_3H_3N$).

Bromination of 2-(3'-thienyl)pyrimidine (2). The same procedure as in the above bromination was used. The reaction was followed by GLC (OV1, 1.9 m, 160°). After 18 h, starting material still remained, but the ratio of products to starting material did not increase further. Work-up as above afforded 324 mg of yellow oil. GLC of this oil showed three peaks which according to MS were caused by starting material, mono- and dibrominated products, respectively. The oil also contained (TLC) some unstable polar, yellow product which slowly decomposed to tars. To remove these impurities, an ether solution of the crude product was filtered through silica gel, which was then washed with ether. After removal of solvent from the combined filtrates a colourless oil (304 mg) was obtained. (GLC of this material was identical with that of the crude product.) When the NMR spectra of the single pure components had been recorded (see below) this mixture could be shown by NMR analysis to consist of starting material *2*, 2-(2'-bromo-3'-thienyl)pyrimidine *19*, 2-(5'-bromo-3'-thienyl) pyrimidine *20*, and 2-(2',5'-dibromo-3'-thienyl)-pyrimidine *21* in the molar proportions 28:24: 36:12.

To simplify separation work, the experiment was repeated with an excess of bromine in order to eliminate starting material from the product mixture. When 3.4 mmol of bromine (174 µl) was added in small portions over a period of 18 h, practically all starting material was consumed. Work-up as above yielded a semicrystalline oil (392 mg) which according to NMR analysis consisted of *19*, *20*, and *21* in the molar proportions 39:32:29. PTLC of this product (75 mg/plate, elution 5 times with dichloroethane) gave 3 bands visible in light of 254 nm. From the first band (R_F 0.73) white crystals, m.p. 85–86 °C, were obtained which according to spectral data were identified as 2-(2',5'-dibromo-3'-thienyl)pyrimidine (*21*). (Found: C 30.8; H 1.85; Br 50.31. Calc. for $C_8H_4N_2Br_2S$ (320.8): C 30.02; H 1.26; Br 49.94). ^1H NMR: δ 8.78 (H-4 and H-6, d, 2 H), 7.65 (H-4', s, 1 H), 7.18 (H-5, t, 1 H). J(H4,H5) 4.8 Hz. ^{13}C NMR: δ 133.4 (C4'). J(C4'H4') 178.2 Hz. *m/e* (%): 322, 320, 318 (50, 100, 48, M^+), 269, 267, 265 (13, 25, 13, $M^+ - C_3H_3N$), 251, 249 (63, 63, $M^+ - Br$), 160 (29, $M^+ - 2Br$), 107 (33, $M^+ - 2Br - C_3H_3N$).

The second band (R_F 0.54) yielded white crystals, m.p. 103–104 °C, identified as 2-(5'-bromo-3'-thienyl)pyrimidine (*21*). (Found: C 40.1; H 2.20; Br 32.7. Calc. for $C_8H_5N_2BrS$ (241.2): C 39.9; H 2.10; Br 33.1). ^1H NMR: δ 8.67 (H-4 and H-6, d, 2 H), 8.13 (H-2', d, 1 H), 7.83 (H-4', 1 H), 7.08 (H-5, t, 1 H). J(H4,H5) 4.8 Hz, J(H2',H4') 1.2 Hz. *m/e* (%): 242, 240 (100, 97, M^+), 239 (11, $M^+ - H$), 189, 187 (32, 32, $M^+ - C_3H_3N$), 161 (38, $M^+ - Br$), 108 (5, $M^+ - Br - C_3H_3N$).

From the third band (R_F 0.43), white crystals, m.p. 83–85 °C, identified as 2-(2'-bromo-3'-thienyl)pyrimidine (*19*), were isolated. (Found: C 40.2; H 2.20; Br 32.67. Calc. for $C_8H_5N_2BrS$ (241.2): C 39.9; H 2.10; Br 33.1). ^1H NMR: δ 8.77 (H-4 and H-6, d, 2 H), 7.61 (H-4', d, 1 H), 7.25 (H-5', d, 1 H), 7.15 (H-5, t, 1 H). J(H4,H5) 4.8 Hz, J(H4',H5') 5.7 Hz. *m/e* (%): 242, 240 (79, 79, M^+), 239 (3, $M^+ - H$), 189, 187 (25, 25, $M^+ - C_3H_3N$), 161 (100, $M^+ - Br$), 108 ($M^+ - Br - C_3H_3N$).

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The Crystal and Molecular Structure of Cannabinol

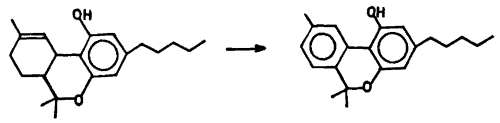
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The structure of cannabinol, $C_{21}H_{26}O_2$, has been determined by X-ray methods using 1401 reflections collected by counter methods. The compound crystallizes in the space group $P2_1/c$ with two molecules in the asymmetric unit. Cell dimensions are: $a = 20.658(8)$ Å; $b = 18.166(4)$ Å; $c = 13.517(5)$ Å; $\beta = 133.56°$ (2). The structure model was refined to a conventional R of 0.135 and a weighted R of 0.091. The main difference between the two conformers in the asymmetric unit is found in the pentyl side chain. Both phenyl rings in each molecule are planar and the mean angle between the two planes is 20.2°. The mean bond distance between the phenyl rings is 1.46(2) Å. The phenol group is bent out of the benzene plane in both molecules. There is one *inter*-molecular hydrogen bond [2.73(2) Å] from a phenol group to a pyrane oxygen, and one between the phenol groups in the two molecules [2.92(2) Å]. The cannabinol molecule is considerably more planar than the Δ^9-tetrahydrocannabinol molecule. While the methyl substituent on the cyclohexene ring in Δ^9-tetrahydrocannabinol is 2.6 Å above a plane through the phenol ring, the corresponding distance in cannabinol is only 1.2 Å.

Cannabinol (CBN, *2*) was the first cannabinoid to be structurally defined and synthesized.[1] CBN is rarely found in freshly prepared marihuana,[2] since Δ^9-tetrahydrocannabinol (Δ^9-THC, *1*) is the precursor of CBN. In the presence of oxygen Δ^9-THC is slowly converted into CBN. The aromatisation of the cyclohexene ring leads to a loss of the psychotomimetic activity,[3] however, in experiments with rabbits a synergistic effect occurs [4] when CBN is given together with Δ^9-THC.

Current interest in structural activity relationship of cannabinoids and how interaction between them alters biological activity, has created a need for detailed structural information on the conformation and hydrogen-bonding pattern. We found that a comparison of the three-dimensional structure of the "active" Δ^9-THC molecule and the "*in*active" CBN would be of interest.



1 2

EXPERIMENTAL AND STRUCTURE DETERMINATION

A square plate-formed crystal of dimensions $0.05 \times 0.16 \times 0.16$ mm was used for the crystallographic work. A computer-controlled Syntex $P\bar{1}$ four-circle diffractometer with graphite-monochromatized Mo$K\alpha$ radiation was utilized in the preliminary experiments and the collection of intensity data. The axial solutions (computer program written by R. Sparks which is part of the diffractometer program library) from the angular coordinates of eleven reflections indicated a monoclinic system. The systematic absences found in a fast low-angle data collection implied the space group $P2_1/c$ with eight molecules in the unit cell, *i.e.* two molecules per asymmetric unit. Cell dimensions and their standard deviations were determined by a least-squares treatment of the angular coordinates of eleven reflections with 2θ-values between 4.5 and 11°.

Three-dimensional intensity data were recorded using the ω-2θ scanning mode with scan speed variable from 3° to 12° min^{-1} de-

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Table 1. Fractional atomic coordinates and thermal parameters for nonhydrogen atoms. The anisotropic temperature factor is given by $\exp\{-2\pi^2[U_{11}(a^*h)^2+U_{22}(b^*k)^2+U_{33}(c^*l)^2+2U_{12}(a^*b^*hk)+2U_{13}(a^*c^*hl)+2U_{23}(b^*c^*kl)]\}$. Estimated standard deviations in parentheses.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C16A	.2838(14)	.0863(12)	−.0584(22)	.066(17)	.079(19)	.115(20)	.022(15)	.062(17)	.007(16)
C17A	.6032(14)	.0856(11)	−.0522(21)	.101(19)	.067(17)	.098(18)	.009(15)	.086(17)	.009(15)
C18A	.4835(15)	.1459(11)	−.2804(17)	.125(21)	.082(18)	.024(13)	−.006(16)	.051(15)	.001(12)
C19A	.8016(14)	.3449(12)	.3463(18)	.078(19)	.085(21)	.036(14)	−.049(16)	.034(15)	−.043(14)
C20A	.7916(14)	.4150(12)	.3879(20)	.089(20)	.070(19)	.061(16)	−.016(16)	.057(16)	−.030(14)
C21A	.8681(14)	.4693(13)	.4555(21)	.051(17)	.050(17)	.063(17)	−.026(15)	.017(14)	.002(14)
C22A	.8636(15)	.5405(14)	.5029(23)	.068(20)	.068(20)	.095(22)	−.007(17)	.040(19)	.031(18)
C23A	.9342(16)	.5936(13)	.5523(23)	.091(20)	.077(20)	.109(22)	−.039(17)	.061(19)	−.016(17)
C16B	.4565(14)	.0847(12)	.3702(21)	.081(18)	.103(20)	.103(19)	−.012(15)	.073(17)	.046(15)
C17B	.2041(14)	.2120(11)	.4583(21)	.115(20)	.066(19)	.083(17)	.031(16)	.069(17)	.003(14)
C18B	.1065(15)	.2431(12)	.2105(22)	.077(20)	.099(21)	.081(19)	.019(16)	.048(17)	.016(16)
C19B	.0880(18)	−.0919(15)	.4655(29)	.167(29)	.109(25)	.174(31)	−.012(21)	.155(29)	−.010(22)
C20B	.1148(21)	−.0870(17)	.5958(37)	.169(32)	.167(34)	.225(39)	−.043(25)	.171(33)	.006(30)
C21B	.0744(31)	−.1423(30)	.6229(40)	.233(53)	.422(74)	.155(41)	−.036(47)	.168(44)	.000(44)
C22B	.1177(25)	−.1653(23)	.7444(39)	.147(37)	.219(46)	.167(37)	−.065(31)	.107(36)	−.022(37)
C23B	.0783(23)	−.2244(19)	.7681(32)	.200(39)	.190(38)	.144(31)	−.019(29)	.131(32)	−.005(27)

Atom	x	y	z	B	Atom	x	y	z	B
O1A	.5649(7)	.2163(6)	−.0789(12)	3.5(3)	O1B	.1090(9)	.1233(7)	.2790(12)	4.7(3)
C2A	.6052(12)	.2303(10)	.0550(18)	3.6(5)	C2B	.1508(13)	.0595(10)	.3564(17)	3.7(5)
C3A	.6773(13)	.2770(11)	.1312(19)	3.9(5)	C3B	.1022(12)	.0171(11)	.3716(19)	4.3(5)
C4A	.7211(13)	.2951(10)	.2667(20)	4.2(5)	C4B	.1366(15)	−.0475(12)	.4404(21)	4.7(5)
C5A	.6878(12)	.2614(10)	.3170(18)	4.1(5)	C5B	.2226(14)	−.0679(10)	.5049(19)	4.7(5)
C6A	.6169(12)	.2156(10)	.2406(18)	3.3(4)	C6B	.2700(12)	−.0249(10)	.4888(16)	2.7(4)
C7A	.5681(10)	.1972(8)	.1020(16)	1.7(4)	C7B	.2346(12)	.0420(10)	.4092(17)	2.9(4)
C8A	.4858(12)	.1542(10)	.0087(18)	3.0(4)	C8B	.2759(11)	.0884(10)	.3775(17)	2.9(4)
C9A	.4251(13)	.1395(10)	.0214(18)	3.6(5)	C9B	.3450(12)	.0645(9)	.3896(16)	3.1(4)
C10A	.3461(13)	.1021(11)	−.0803(20)	4.0(5)	C10B	.3811(13)	.1121(11)	.3551(19)	4.4(5)
C11A	.3273(14)	.0748(11)	−.1929(21)	5.8(6)	C11B	.3464(12)	.1810(12)	.3130(21)	6.1(6)
C12A	.3885(14)	.0912(11)	−.2034(20)	4.9(5)	C12B	.2780(14)	.2055(11)	.2979(20)	5.2(5)
C13A	.4646(13)	.1293(10)	−.1110(19)	3.9(5)	C13B	.2416(12)	.1605(10)	.3312(18)	3.2(4)
C14A	.5260(13)	.1404(11)	−.1321(18)	3.6(5)	C14B	.1673(15)	.1835(12)	.3189(23)	5.4(5)
O15A	.5859(8)	.1819(6)	.2961(11)	4.0(3)	O15B	.3551(8)	−.0436(7)	.5530(12)	4.7(3)

pending on the intensity of the reflection, and a scan range from $2\theta(\alpha_1) - 0.8°$ to $2\theta(\alpha_2) + 0.8°$. Background counting time was equal to 0.35 × (scan time) on each side of the scan range. The temperature was kept constant within 2° at 24 °C. The variations in the intensities of three standard reflections which were remeasured after every hundred reflections, were random. Accordingly no corrections were applied to the intensities for these variations.

The estimated standard deviations were taken as the square root of the total counts with a 2 % addition of the net intensity for experimental uncertainties. Of the 4835 symmetry-independent reflections measured ($2\theta_{max} = 45°$), 1401 had intensities larger than twice their standard deviations. These were regarded as observed reflections, and the remaining were excluded from further calculations. The intensities were corrected for Lorentz and polarization effects.[5]

The phase problem was solved by the MULTAN [6] program package. The structure model was refined to a conventional R of 0.17. The computer programs used as well as programs subsequently employed, are part of a local (Oslo) assembly of computer programs which is described in Ref. 7. Atomic scattering factors used were those of Doyle and Turner [8] for carbon and oxygen, and of Stewart *et al.* [9] for hydrogen. Because of the relatively small number of observed reflections anisotropic thermal parameters were only introduced for the atoms in the sidechains (eight atoms in each molecule, see Table 1) where the thermal motion is largest. This gives an overdetermination ratio of 5.3. Least-squares refinement lowered R to 0.16.

At this point the hydrogen atoms were placed in calculated positions and included in the structure factor calculations with estimated isotropic thermal parameters. The distances indicated two *inter*-molecular hydrogen bonds and the two hydrogens bonded to oxygens (A15 and B15) were placed accordingly. Full-matrix least-squares refinement of all non-hydrogen atomic parameters converged to a weighted R_w of 0.091 and an R of 0.135. The weights used in the refinement were $1/\sigma^2(F_{obs})$.

Atomic parameters for nonhydrogen atoms are given in Table 1, and those used for hydrogen atoms are listed in Table 2. A list of observed and calculated structure factors is available upon request from: Department of Chemistry, University of Oslo, Oslo 3, Norway.

CRYSTAL DATA

Cannabinol, $C_{21}H_{26}O_2$, $M = 310.43$ amu, space group $P2_1/c$, $a = 20.658(8)$ Å, $b = 18.166(4)$ Å,

Table 2. Calculated fractional coordinates and estimated thermal parameters for hydrogen atoms.

Atom	x	y	z	B	Atom	x	y	z	B
A3	.699	.299	.090	4.5	B3	.042	.034	.332	5.0
A5	.718	.272	.414	4.5	B5	.250	−.114	.563	5.0
A9	.440	.156	.106	4.5	B9	.370	.014	.424	5.0
A11	.273	.044	−.262	5.5	B11	.371	.215	.288	5.5
A12	.374	.073	−.287	5.0	B12	.256	.257	.267	5.5
A15	.579	.217	.340	4.5	B15	.374	−.089	.602	5.2
A16,1	.230	.060	−.141	6.0	B16,1	.474	.125	.341	6.0
A16,2	.265	.134	−.048	6.0	B16,2	.436	.041	.311	6.0
A16,3	.316	.056	.024	6.0	B16,3	.508	.072	.468	6.0
A17,1	.579	.035	−.086	6.0	B17,1	.245	.254	.489	6.0
A17,2	.637	.089	.047	6.0	B17,2	.237	.171	.526	6.0
A17,3	.644	.698	−.066	6.0	B17,3	.153	.228	.446	6.0
A18,1	.532	.153	−.280	6.0	B18,1	.059	.256	.210	6.0
A18,2	.442	.189	−.326	6.0	B18,2	.079	.224	.120	6.0
A18,3	.450	.100	−.330	6.0	B18,3	.143	.288	.235	6.0
A19,1	.853	.318	.430	6.0	B19,1	.024	−.076	.396	6.5
A19,2	.813	.356	.287	6.0	B19,2	.094	−.144	.452	6.5
A20,1	.786	.404	.454	6.5	B20,1	.101	−.036	.605	6.5
A20,2	.736	.439	.305	6.5	B20,2	.181	−.096	.667	6.5
A21,1	.872	.481	.388	6.5	B21,1	.052	−.182	.560	7.0
A21,2	.924	.444	.537	6.5	B21,2	.021	−.113	.597	7.0
A22,1	.867	.531	.579	7.0	B22,1	.136	−.124	.806	7.5
A22,2	.805	.564	.425	7.0	B22,2	.174	−.190	.774	7.5
A23,1	.925	.640	.583	7.5	B23,1	.122	−.238	.866	7.5
A23,2	.933	.605	.480	7.5	B23,2	.061	−.267	.710	7.5
A23,3	.994	.572	.633	7.5	B23,3	.024	−.202	.743	7.5

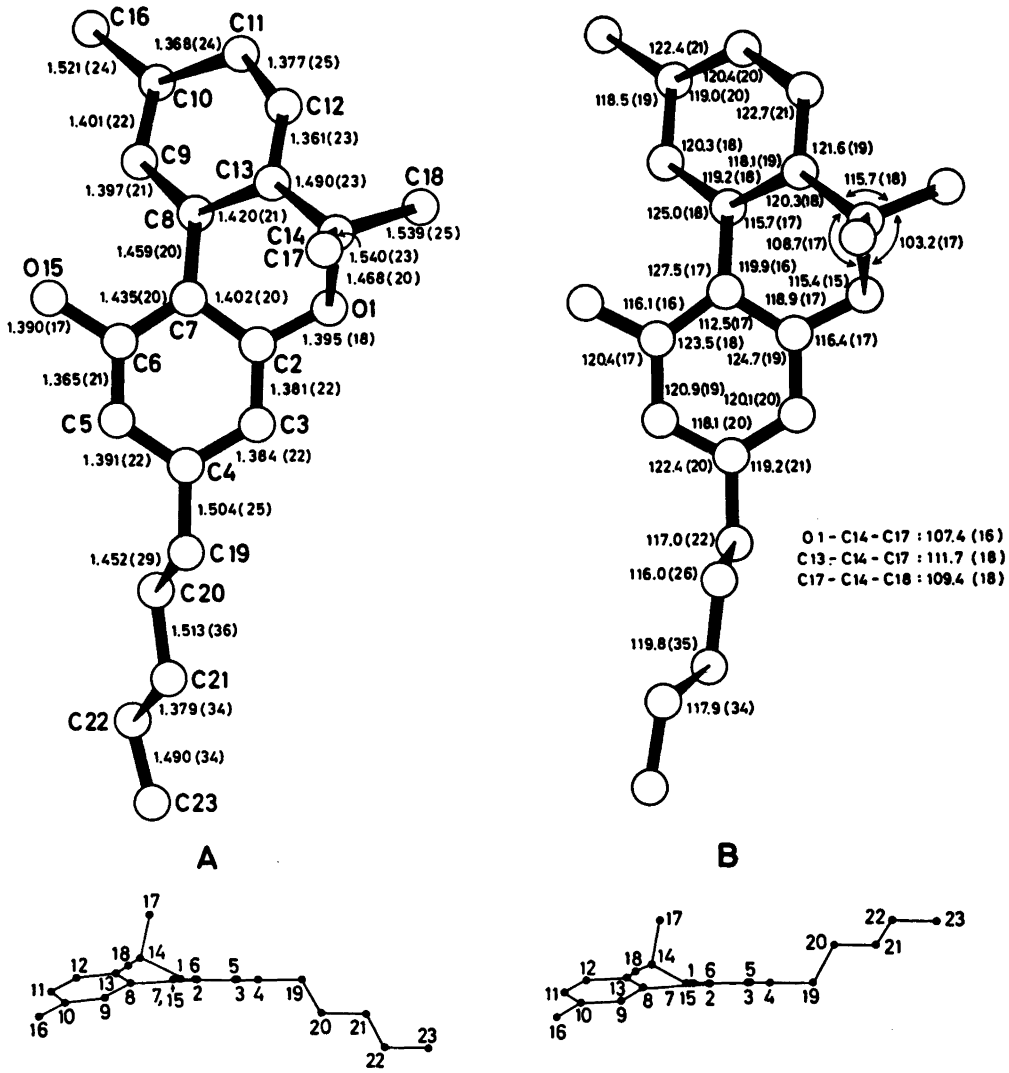


Fig. 1. Mean bond lengths (Å) and bond angles (°) and a view along the plane of the benzene ring, showing the conformation of the two molecules A and B, in the asymmetric unit.

$c = 13.517(5)$ Å, $\beta = 135.56°$ (2), $V = 3675.8(18)$ Å^3, $Z = 8$, $D_{calc} = 1.222$ g cm^{-3}, F (000) = 1344.

DISCUSSION

The molecular structure and atomic numbering is shown in Fig. 1, together with a view along the plane of the phenol ring, showing the conformation. The asymmetric unit in the crystal consists of two CBN molecules, denoted A and B, respectively. Bond lengths and angles are given in Table 3 and mean molecular parameters are shown in Fig. 1. The standard deviations are relatively high, so a detailed discussion of bond distances and angles is not meaningful. The mean values of the molecular parameters are all normal within their estimated standard deviations, except for the shortening of the C19 – C20 and C21 – C22 bonds, probably caused by the large thermal vibrations in the side chain.

Table 3. Molecular parameters (Å and °) with estimated standard deviations. See Fig. 1 for mean bond lengths and bond angles.

Bond length	A	B	Angle	A	B
O1–C2	1.40(2)	1.39(2)	C2–O1–C14	115(1)	115(2)
O1–C14	1.51(2)	1.43(2)	O1–C2–C3	117(2)	116(2)
C2–C3	1.37(2)	1.39(2)	O1–C2–C7	119(2)	119(2)
C2–C7	1.42(2)	1.38(2)	C3–C2–C7	124(2)	125(2)
C3–C4	1.41(2)	1.36(2)	C2–C3–C4	121(2)	119(2)
C4–C5	1.40(2)	1.38(2)	C3–C4–C5	116(2)	120(2)
C5–C6	1.35(2)	1.38(2)	C3–C4–C19	118(2)	120(2)
C6–C7	1.43(2)	1.44(2)	C5–C4–C19	126(2)	119(2)
C7–C8	1.46(2)	1.46(2)	C4–C5–C6	122(2)	119(2)
C8–C9	1.40(2)	1.39(2)	C5–C6–C7	124(2)	123(2)
C8–C13	1.43(2)	1.41(2)	C5–C6–O15	121(2)	120(2)
C9–C10	1.39(2)	1.42(2)	C7–C6–O15	115(2)	117(2)
C10–C11	1.38(2)	1.36(2)	C2–C7–C6	112(2)	113(2)
C11–C12	1.40(2)	1.35(3)	C2–C7–C8	120(2)	120(2)
C12–C13	1.34(2)	1.38(2)	C6–C7–C8	128(2)	127(2)
C13–C14	1.49(2)	1.49(2)	C7–C8–C9	127(2)	123(2)
C6–O15	1.42(2)	1.36(2)	C7–C8–C13	115(2)	117(2)
C10–C16	1.53(2)	1.51(2)	C9–C8–C13	118(2)	120(2)
C14–C17	1.52(2)	1.56(2)	C8–C9–C10	121(2)	120(2)
C14–C18	1.54(2)	1.54(2)	C9–C10–C11	121(2)	117(2)
C4–C19	1.51(2)	1.50(3)	C9–C10–C16	118(2)	119(2)
C19–C20	1.46(2)	1.44(3)	C11–C10–C16	121(2)	124(2)
C20–C21	1.52(3)	1.50(5)	C10–C11–C12	117(2)	124(2)
C21–C22	1.47(3)	1.28(4)	C11–C12–C13	125(2)	120(2)
C22–C23	1.47(3)	1.51(4)	C8–C13–C12	118(2)	118(2)
			C8–C13–C14	122(2)	118(2)
			C12–C13–C14	120(2)	123(2)
			O1–C14–C13	106(2)	111(2)
			O1–C14–C17	108(1)	107(2)
			O1–C14–C18	102(1)	105(2)
			C13–C14–C17	113(2)	111(2)
			C13–C14–C18	117(2)	114(2)
			C17–C14–C18	110(2)	109(2)
			C4–C19–C20	115(2)	119(3)
			C19–C20–C21	116(2)	117(3)
			C20–C21–C22	119(2)	121(5)
			C21–C22–C23	116(2)	120(4)

Dihedral angles (°) The angles are positive in a right-hand screw.

	A	B		A	B
C14–O1–C2–C7	−34.5(22)	−33.5(25)	O1–C2–C7–C8	−4.5(25)	−3.0(26)
C2–C7–C8–C13	19.3(24)	19.5(25)	C7–C8–C13–C14	5.4(26)	0.2(25)
C8–C13–C14–O1	−39.5(23)	−34.0(26)	C8–C13–C14–C17	78.3(24)	84.9(23)
C8–C13–C14–C18	−152.0(18)	−152.4(18)	C13–C14–O1–C2	53.6(19)	50.8(23)
C17–C14–O1–C2	−67.5(18)	−70.2(21)	C18–C14–O1–C2	176.7(15)	174.6(15)
C3–C4–C19–C20	117.5(20)	−98.9(32)	C4–C19–C20–C21	−174.0(18)	−169.6(32)
C19–C20–C21–C22	−178.7(19)	149.0(47)	C20–C21–C22–C23	−173.1(19)	−176.1(35)

Compared to the structure of the Δ^9-THC skeleton, studied in Δ^9-THC Acid B [10] and 8β-hydroxy-Δ^9-THC,[11] the aromatisation of the cyclohexane ring has led to considerable changes in the over-all geometry, as well as a loss of biological activity. CBN is a much more planar molecule than Δ^9-THC. The methyl group C16 is approximately 1.2 Å above the plane through the phenol ring in CBN, while the corresponding distance in Δ^9-

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.[a]

Atom	Plane A1	Plane B1	Atom	Plane A2	Plane B2
O1	7	2	O1	846	757
C2	*10*	*9*	C2	531	423
C3	*5*	*19*	C7	52	12
C4	*−12*	*−34*	C8	*−9*	*−2*
C5	*5*	*18*	C9	*−10*	*0*
C6	*10*	*9*	C10	*24*	*7*
C7	*−17*	*−23*	C11	*−19*	*−12*
C8	−147	−130	C12	*−1*	*10*
C13	190	225	C13	*14*	*−3*
C14	750	680	C14	−24	5
O15	44	80	C16	−10	0
C19	26	60			

[a] Angle between: A1 and A2, 21.0°; B1 and B2, 19.4°.

THC is about 2.6 Å. It is obvious that this difference will be of great importance for the matching of the receptor and the drug molecule.

The two crystallographic independent molecules differ mainly in the conformation of the side chain. The side chain in molecule A is rotated 180° around the C4 – C19 bond compared to molecule B. Except for the C20 – C21 bond which is more eclipsed in B than in A [dihedral angle C19 – C20 – C21 – C22 is 149.0° (4.7) in B and −178.7° (1.9) in A], the chain in both molecules is fully extended and all-*anti*.

Both aromatic rings are nearly planar, but while all atoms directly attached to ring 2 (Plane A2 and B2, Table 4) are less than 0.05 Å out of the plane, both the phenol oxygen O15 and C8 are bent significantly out of the plane through ring 1 (Plane A1 and B1). The mean deviation from the plane is +0.062 Å for O15 and −0.138 Å for C8. The short distance between the hydrogen atom on C9 and the phenol group (2.25 Å) indicates considerable steric interaction which probably is the cause of this effect. The same result is also observed in the Δ^9-THC structures studied.[10,11] In all the four independent determinations of this parameter we observe a C7 – C6 – O15 bond angle less than 120° (mean value 117.1°), while Archer *et al.*[12] have calculated by Westheimer calculations the bond angle to open slightly (122 – 124°).

The bond angles in the phenol ring vary considerably in both molecules (112 – 125°) and in the same way as in the other cannabinoids studied.[10,11,13] This has been interpreted by strains in the aromatic system.[10] In the other aromatic ring in CBN the angles are not significantly different from benzene values.

The mean bond distance between the two phenyl rings is 1.46(2) Å, which is not significantly different from the corresponding bond in biphenyl in the crystalline phase [14] (1.494(3) Å).

The planes through the phenyl rings make an angle of 19.4° in molecule B and 21.1° in molecule A. The dihedral angle C2-C7-C8-C13 is 19.3° in A and 19.5° in B. In the two Δ^9-THC structures,[10,11] this angle was observed to 15.1° and 16.6°, while Westheimer calculations [12] gave 13° for CBN.

The pyrane oxygen in molecule A (O1A) acts as acceptor for a hydrogen bond [2.73(2) Å] from the phenol group (O15A) in another A molecule in position: $x, \frac{1}{2}-y, \frac{1}{2}+z$. There are no short contacts from the pyrane oxygen in molecule B to a hydrogen donor. The C14 – O1 bond distance is probably significantly longer in molecule A, [1.51(2) Å] where O1 is hydrogen-bond acceptor, than in B [1.43(2) Å]. There is another hydrogen bond [2.92(2) Å] from the phenol group in molecule B to the phenol group in A in position: $1-x, -y, 1-z$. There are no other contacts shorter than 3.5 Å between the heavy atoms in A and B.

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NMR Studies of Lignins. 1. Signals Due to Protons in Formyl Groups

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The occurrence of structural elements with formyl groups in spruce lignin (units of the coniferaldehyde, vanillin, α-aryloxyacrolein, and glyceraldehyde-2-aryl ether type) has been studied by ^{1}H NMR spectroscopy. Signals from formyl protons were found to be essentially due to coniferaldehyde and vanillin units. By integration the amount of coniferaldehyde units was determined as 4 %.

Proton NMR spectra of lignins have been published by several authors.[1–3] In most cases acetate derivatives of the lignins have been examined. A comprehensive ^{1}H NMR study of lignins and lignin model compounds was published as early as 1964.[4] In the present work a 270 MHz instrument has been used. The lignin spectra recorded with this instrument permit a more detailed interpretation than is possible with spectra taken with the lower frequency instruments used in earlier work. The present paper deals with a study of protons in formyl groups. Signals due to such protons have not been previously detected in NMR spectra of lignins.

A ^{1}H NMR spectrum of acetylated milled wood lignin (MWL) from spruce (Fig. 1) ex-

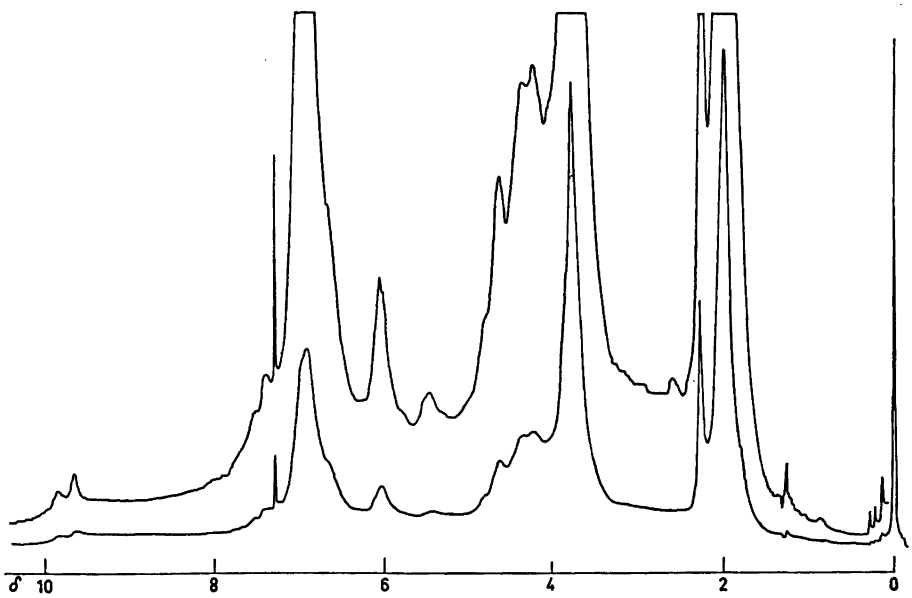


Fig. 1. ^{1}H NMR spectrum of acetylated MWL from spruce (solvent, $CDCl_3$).

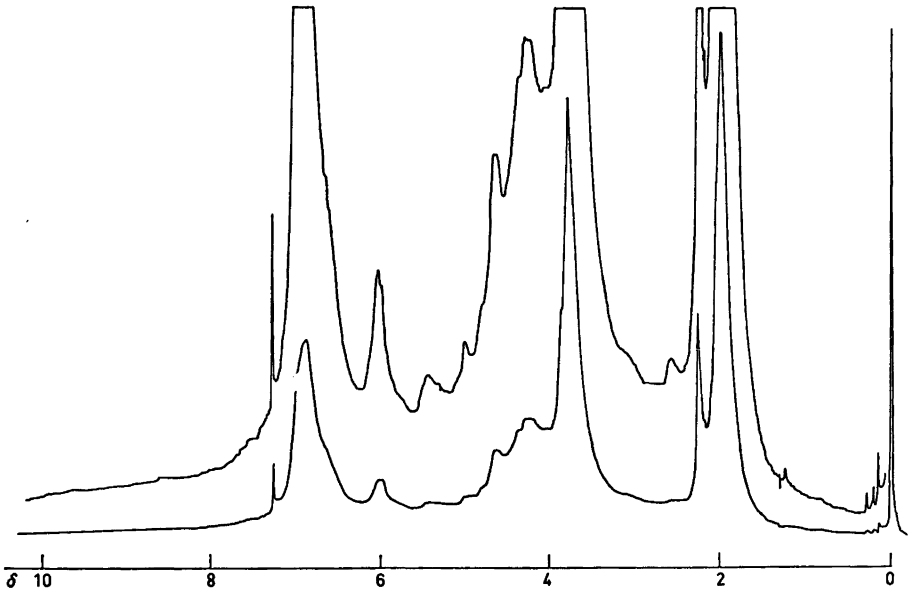
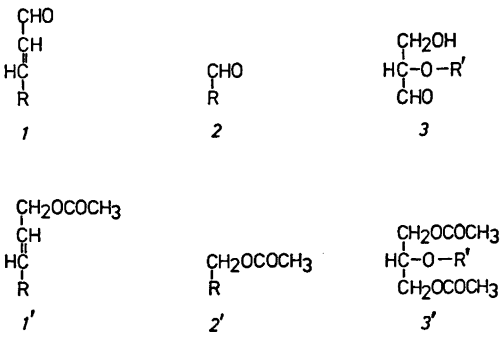
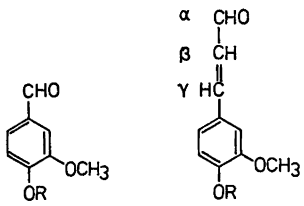


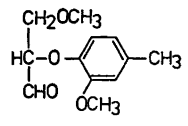
Fig. 2. ^{1}H NMR spectrum of borohydride reduced and acetylated MWL from spruce (solvent, $CDCl_3$).

hibits signals in the range $\delta = 9-10$ with δ values 9.64, 9.84, and 9.94 (shoulder) which can be attributed to protons in formyl groups. Spectra of borohydride reduced and, subsequently, acetylated MWL show no signals in this region (Fig. 2). Spectral studies of model compounds (Table 1) suggest that the peak at $\delta = 9.64$ is due to formyl groups in cinnamaldehyde units (*1*). The signals around $\delta = 9.9$ can be attributed to formyl groups in benzaldehyde units (*2*) or glyceraldehyde-2-aryl ether units (*3*) (Table 1). Spectra of nonderivatized MWL in dioxane-d_8 – D_2O (5:1) showed peaks at $\delta = 9.56$ and $\delta = 9.74$. It appears from Table 1 that these data support the interpretation of the results obtained with acetylated MWL. Spectra of a model compound for units of type *3*, compound *8*, in dioxane – water medium



R= aromatic nucleus of the guaiacyl type
R'= guaiacylpropane unit





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Table 1. NMR data for formyl protons in model compounds *4*–*8* (for further model compound data, see Refs. 1, 5, 6, and 7).

Compound	δ Value		J/Hz
	$CDCl_3$	Dioxane-d_8–D_2O	
4 [a]	9.86	9.77	–
5	9.94		–
6 [b]	9.67	9.58	7.8
7	9.70		7.5
8	9.88	9.70	1.5

[a] δ Values for aromatic protons *ortho* to the formyl group were 7.42 (J=2 Hz) and 7.47 (J=2 and 8 Hz).

[b] δ_{H_γ}=7.42 (J=16 Hz), δ_{H_β}=6.62 (J=16 and 7.8 Hz).

showed a comparatively weak formyl proton peak due to partial hydration of the carbonyl group. Acetylation of compound *8* and examination of the product by NMR (solvent, deuteriochloroform) showed that the formyl group had to a large extent disappeared. The experiments with compound *8* and data presented below suggest that the peak in Fig. 1 at δ=9.84 (as well as the peak at δ=9.74 in the spectrum of nonderivatized lignin) is essentially due to benzaldehyde units of type *2* linked to the lignin by ether bonds; the shoulder at δ=9.94 may reflect the presence of minor amounts of benzaldehyde units with a *p*-acetoxy group (Table 1). The spectrum of acetylated compound *8* showed a small peak at δ=9.43 which can be explained by partial conversion into α-aryloxyacrolein.[5] A very small peak at the same position was found in spectra of acetylated MWL. This may be due to formation of α-aryloxyacrolein structures from units of type *3* in the original MWL. However, since structural elements with α-aryloxyacrolein groups have been suggested as being present in untreated lignin,[6] there is an alternative explanation for the occurrence of this peak.

From integrations of the aldehyde peaks in spectra of acetylated MWL the proportion of units carrying formyl groups was determined to be 7 %. The number of cinnamaldehyde units could be estimated as 4 %, which is in good agreement with results obtained by other methods.[8] Similar results were obtained from integrations of spectra of nonderivatized MWL.

To verify the assignments of the formyl proton signals in Fig. 1, a difference spectrum (Fig. 3) was made by subtracting the spectrum of acetylated MWL in Fig. 1 from the spectrum of borohydride reduced and acetylated MWL in Fig. 2. This was made by the computer connected to the NMR spectrometer. In the difference spectrum negative peaks appear at δ=9.84 and 9.64 corresponding to signals due to formyl protons. The negative peak at δ=7.40

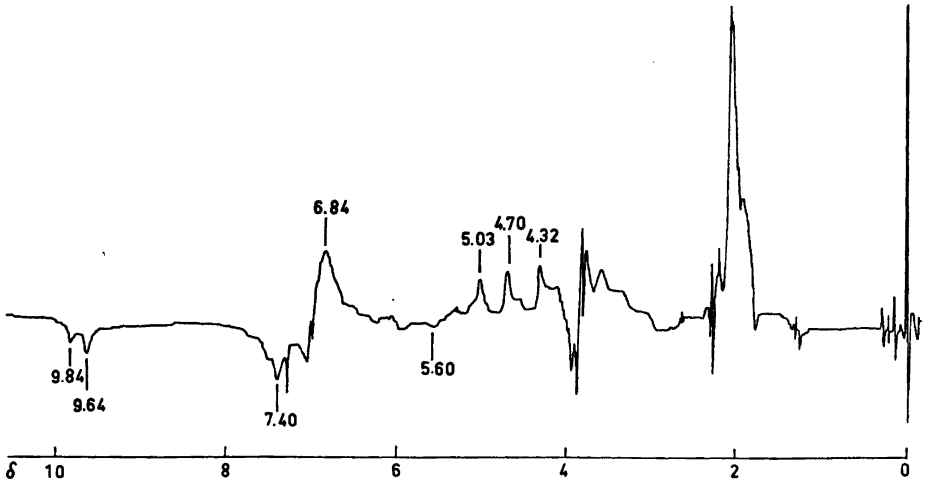
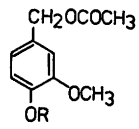
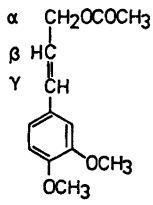


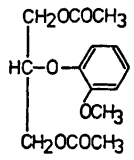
Fig. 3. Difference spectrum obtained by subtraction of the spectrum in Fig. 1 from the spectrum in Fig. 2.



9 R=CH_3
10 R=$COCH_3$



11



12

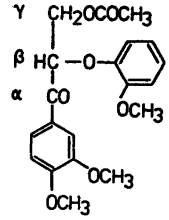
and the positive peak at $\delta = 6.84$ are expected from shifts in signal positions caused by exchange of units of types *1* and *2* for units of types *1'* and *2'*. Some of the protons in units of types *1* and *2* give rise to signals around $\delta = 7.40$ (footnotes in Table 1). The signals from the corresponding protons in units of types *1'* and *2'* appear at other positions (footnote in Table 2; according to experiments with model compounds signals due to aromatic protons in units of types *1'* and *2'* are located close to $\delta = 6.84$). The positive peaks at $\delta = 4.70$ and $\delta = 5.03$ agree well with signals of methylene protons in units of types *1'* and *2'* (Table 2). The difference spectrum also exhibits a positive peak at $\delta = 4.32$. This peak can be attributed to presence of units of type *3'* in borohydride reduced and acetylated MWL (Table 2). Since on borohydride reduction – acetylation units of type *3* are converted into units of type *3'* this provides support for the occurrence of units of type *3* in the original MWL. The size of the 4.32 peak in the difference spectrum seems to be of the order expected from studies [5] of the number of glyceraldehyde-2-aryl ether units in spruce ignin.

Table 2. NMR data for methylene protons in model compounds *9* – *12* (solvent, $CDCl_3$).

Compound	δ Value	J/Hz
9	5.04	–
10	5.07	–
11 [a]	4.71	6.5
12	4.33	5.0

[a] $\delta_{H\gamma} = 6.60$ ($J = 16$ Hz), $\delta_{H\beta} = 6.16$ ($J = 16$ and 6.5 Hz).

Data obtained in the present studies, supplemented with results from estimates [5] of the number of glyceraldehyde-2-aryl ether units in lignin, suggest that the number of formyl protons in MWL from spruce is about 0.09/OCH_3. The total amount of carbonyl groups in MWL from spruce has been determined as 0.20/OCH_3.[8] Thus the number of ketone groups would be about 0.11/OCH_3. It is plausible that ketone units of the α-aryloxypropiophenone type are present in lignin. A model compound for



13

such structures, compound *13*, has been examined. The δ value for Hβ was 5.62 ($J = 7.1$ and 3.7 Hz) and signals from aromatic protons *ortho* to the carbonyl group were observed at $\delta = 7.68$ and $\delta = 7.85$. However, no pronounced negative peaks appeared at these δ values in the difference spectrum (Fig. 3). Consequently, the present studies suggest that the number of α-aryloxypropiophenone structures in lignin is small. To explain the rest of the carbonyl groups, we think that units of quinone and quinonoid types should be considered.[9]

EXPERIMENTAL

^1H NMR spectra were taken with a 270 MHz instrument working in the pulse Fourier mode (Bruker WH 270). Solvents were chloroform-*d* (internal reference TMS) and dioxane-d_8 – D_2O (5:1) (internal reference was the sodium salt of 3-(trimethylsilyl)propanesulfonic acid). Temperatures were in the range 300 – 308 K (lignin spectra were all run at 308 K). In the case of lignin preparations the concentration was about 0.1 g in 0.5 ml solvent.

Acetylations were performed by treatment with acetic anhydride – pyridine (1:1) for 24 h. Lignin acetates were precipitated by dropping the reaction mixture into ether. The products were purified by precipitation in ether from a chloroform solution.

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Tobacco Chemistry. 42. Structure Elucidation and Synthesis of 3,3-Dimethyl-7-hydroxy-2-octanone, a New *Seco* Nor-carotenoid Constituent of Greek Tobacco

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A new C_{10} *seco* nor-carotenoid was isolated from Greek *Nicotiana tabacum* L. and shown by spectroscopic methods and synthesis to be (+)-3,3-dimethyl-7-hydroxy-2-octanone.

Previous studies have revealed the presence in tobacco of a large number of compounds, which in all probability arise by initial oxidative cleavage of the polyene chain of cyclic carotenoids and subsequent chemical alterations.[1] Until now, however, only a few *seco* nor-carotenoids have been encountered. The present communication describes the structure elucidation and synthesis of a new C_{10} *seco* compound.

RESULTS

The title compound (*1*) was isolated in a minute amount by repeated preparative liquid and gas chromatography of a polar, volatile fraction, B 8,[2] obtained from an extract of sun-cured Greek tobacco. Accurate mass measurements, which had to be performed on the $[M-33]^+$ ion of mass 139 due to the low abundance of the molecular ion (*m/e* 172), established that the new compound (*1*) had the composition $C_{10}H_{20}O_2$. Its ^{13}C NMR spectrum confirmed the presence of ten carbon atoms and revealed that these comprised four methyl, three sp^3 methylene, one oxygen-carrying sp^3 methine, one non-protonated sp^3 carbon and one carbonyl carbon. The latter was part of a methyl ketone group, a conclusion based upon the fact that the ^1H NMR spectrum contained a methyl singlet at δ 2.12 and the IR spectrum an absorption band at 1705 cm^{-1}.

The second oxygen atom was accomodated by a hydroxyl substituent attached to the sp^3 methine carbon as shown by IR absorption at 3600 and 3450 cm^{-1} and by the presence of a one-proton sextet at δ 3.81 in the ^1H NMR spectrum. Irradiation at this frequency made a methyl doublet at δ 1.18 collapse to a singlet. Since, conversely, irradiation at the frequency of the methyl doublet converted the sextet to a broadened triplet, it followed that the hydroxyl-carrying methine carbon was attached to a methyl and a methylene group. The remaining two methyl groups, giving rise to singlets at δ 1.12 in the ^1H NMR spectrum, must be linked to the non-protonated sp^3 carbon atom.

On the basis of these results three possible structures, A, B and C, which all incorporate the remaining two sp^3 methylene groups, could be formulated for the new tobacco constituent (*1*). An examination of the mass spectrum, which displayed diagnostically important peaks at *m/e* 172 (M), 139, 129, 111, 86, 69, 45 and 43, strongly favoured structure A, *i.e.* 3,3-dimethyl-7-hydroxy-2-octanone for *1* (*cf.* Fig. 1). Thus compound A is expected to undergo a McLafferty rearrangement giving a $C_5H_{10}O$ ion of mass 86, compound B would yield an ion of mass 58, whereas no such rearrangement is possible in the fragmentation of compound C.

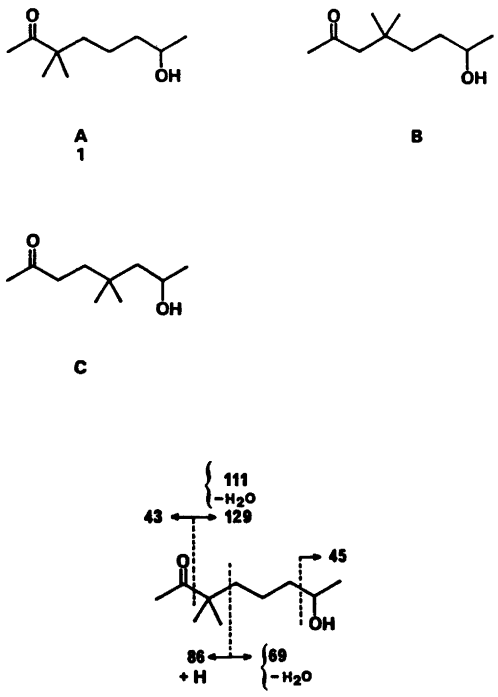
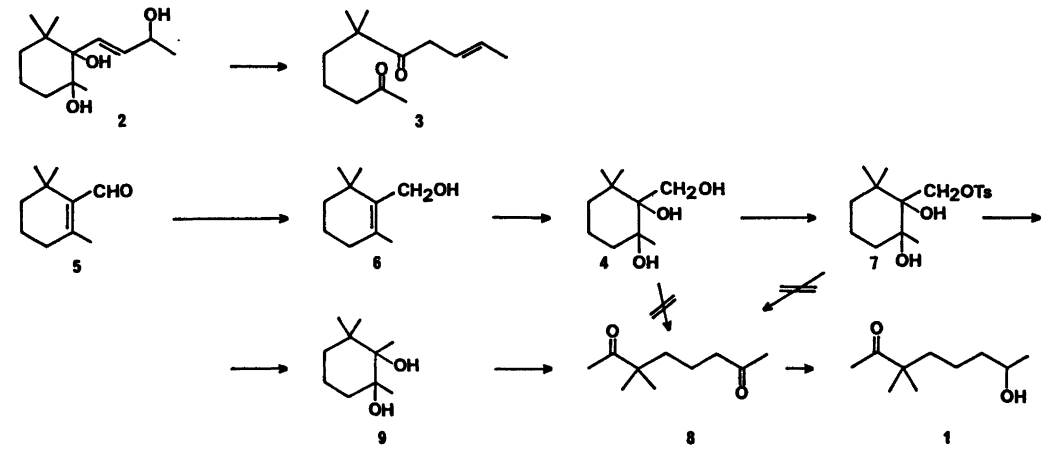


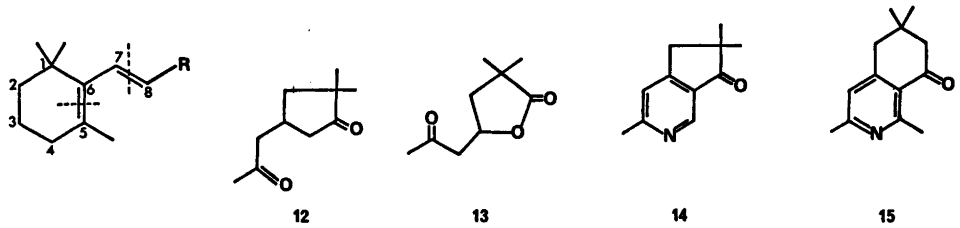
Fig. 1. MS fragmentation of compound *1*.

The structure proposed for *1* was confirmed by synthesis using a route, which involved a possibility to test a biogenetically plausible reaction (*vide infra*) analogous to the facile acid–catalysed rearrangement of the C_{13} nor-carotenoid triol (*2*) to the *seco* diketone (*3*).[3] Thus, attempts were made to convert the triol *4*, which was synthesized from β-cyclocitral (*5*) *via* hydroxylation of β-cyclogeraniol (*6*) using osmium tetroxide, as well as the monotosylate (*7*) to the desired diketone (*8*) under acidic conditions. However, these proved to be abortive and the required ring cleavage was instead achieved by chromic acid oxidation of 1,2,3,3-tetramethyl-1,2-cyclohexanediol (*9*), which was prepared from the tosylate (*7*) by LAH reduction. The diketone (*8*), which was isolated in 71 % yield, was subsequently reacted with a bulky reducing agent, lithium tri-*t*-butoxyaluminium hydride, at a low temperature (0 °C) in order to achieve selective reduction of the carbonyl group at C-7. The desired (±)-3,3-dimethyl-7-hydroxy-2-octanone (*1*), identical to the new tobacco constituent, was obtained in 35 % yield along with a similar amount of 3,3-dimethyl-2,7-octanediol (*10*) and a minor quantity of 3,3-dimethyl-2-hydroxy-7-octanone (*11*).

The new compound, (+)-*1*, whose absolute configuration could not be settled due to shortage of material, may be formed in tobacco from a cyclic carotenoid precursor by cleavage of the 5,6 and 7,8 bonds as indicated in Fig. 2. It thus constitutes an additional representative of the relatively few *seco* nor-carotenoids encountered in tobacco, which include the C_{10} diketone *12*,[4] the C_9 lactone *13* [5] and the alkaloids *14* and *15*.[4]



Scheme 1.



12 13 14 15

Fig. 2. Possible formation of *1*. Compounds *1* and *12 – 15* represent the known *seco* nor-carotenoids in tobacco.

EXPERIMENTAL

Optical rotations were measured on a Perkin-Elmer 141 polarimeter and IR spectra on Digilab FTS-14 and Perkin-Elmer 257 instruments. Mass spectra were recorded on an LKB 2091 instrument and accurate mass measurements were carried out on a Varian MAT 311 instrument at the Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm. Fourier transform ^{1}H NMR (100 MHz) and ^{13}C NMR (25.16 MHz) spectra were recorded in $CDCl_3$ solutions using TMS as internal standard on a Varian XL-100-12 spectrometer equipped with S-124 FT and disk accessories and controlled by a Varian 620/L computer. Analytical and preparative gas chromatography was performed on a Varian 1700 instrument using glass capillary columns (50 m × 0.37 mm) coated with HB 5100 and glass columns (2.8 m × 7 mm) packed with Carbowax 20 M (4 %) on Chromosorb G, respectively. High performance liquid chromatography was carried out using a Waters 6000 A solvent delivery system, a U6K injector and an R-401 differential refractometer.

Isolation of 3,3-dimethyl-7-hydroxy-2-octanone (1) from tobacco. A volatile, neutral fraction (B 8) [2] of an extract obtained from 295 kg of sun-cured Greek *Nicotiana tabacum* L. was chromatographed over silica gel using a light petrol/ether gradient. One of the sub-fractions obtained was separated further by liquid chromatography on columns packed with Bondapak C_{18}/Porasil (Waters) using water-acetone (60:40) as eluent and by preparative gas chromatography to give 1.8 mg of 3,3-dimethyl-7-hydroxy-2-octanone (*1*) as a colourless oil (Found: [M − 33]+ 139.1113. Calc. for $C_9H_{15}O$ [M − 33]+: 139.1123), $[\alpha]_{578}^{22}$ + 5.8° (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) bands at 3600 (m), 3450 (m) and 1705 cm^{-1}; ^{1}H NMR peaks at δ 1.12 (6 H, s), 1.18 (3 H, d, J = 6 Hz), 2.12 (3 H, s) and 3.81 (1 H, sextet, J = 6 Hz); ^{13}C NMR peaks at δ 21.06 (t), 23.51 (q), 24.28 (q), 24.43 (q), 25.03 (q), 39.74 (t), 39.92 (t), 47.81 (s), 67.41 (d) and 214.18 (s) (s = singlet, d = doublet, t = triplet and q = quartet refer to the single frequency off resonance decoupled spectrum); MS peaks at *m/e* (composition, %): 172 (M, 1), 139 ($C_9H_{15}O$, 4) 129 ($C_8H_{17}O$, 7), 111 (C_8H_{15}, 22), 86 ($C_5H_{10}O$, 35), 69 (C_5H_9, 100), 55 (C_4H_7, 30), 45 (C_2H_5O, 14) and 43 (C_2H_3O, 42).

Preparation of β-cyclogeraniol (6). A solution of 1.80 g of β-cyclocitral (*5*) in ether was treated with lithium aluminium hydride at room temperature for 2 h. The reaction mixture was diluted with aqueous H_2SO_4, extracted with ether, dried and evaporated. The residue was chromatographed over silica gel using ethyl acetate-hexane (20:80) as eluent, which furnished 0.80 g of β-cyclogeraniol (*6*) m.p. 40 – 42 °C (lit. 43 – 44°);[6] IR (KBr) bands at 3400 (m) and 1660 (w) cm^{-1}; ^{1}H NMR peaks at δ 1.06 (6 H, s), 1.76 (3 H, broad s) and 4.16 (2 H, s); ^{13}C NMR peaks at δ 19.49 (t), 19.64 (q), 28.55 (2 q), 32.93 (t), 34.03 (s), 39.64 (t), 58.23 (t), 132.90 (s) and 137.43 (s); MS peaks at *m/e* (%): 154 (M, 38), 139 (29), 136 (15), 123 (71), 121 (100), 93 (60), 81 (29), 79 (43), 67 (16), 55 (24) and 43 (20).

Preparation of 2-hydroxymethyl-1,3,3-trimethyl-1,2-cyclohexanediol (4). To a solution of 934 mg of osmium tetroxide in 5 ml of pyridine was added 570 mg of β-cyclogeraniol (*6*) dropwise. After stirring at room temperature for 2 h a solution of 1.80 g of sodium bisulfite in 30 ml of aqueous pyridine (40 %) was added. The reaction mixture was diluted with water, extracted with ethyl acetate, washed with aqueous HCl, dried and evaporated. The residue was chromatographed over silica gel using ethyl acetate-hexane (40:60) as eluent to afford 592 mg of 2-hydroxymethyl-1,3,3-trimethyl-1,2-cyclohexanediol (*4*), which had m.p. 158 – 161 °C (hexane); IR (KBr) bands at 3500 (s) and 3400 (s) cm^{-1}; ^{1}H NMR peaks at δ 0.96 (3 H, s), 1.03 (3 H, s), 1.47 (3 H, s), 3.12 (−O*H*, t, J = 6 Hz), 3.55 (−O*H*, s), 3.65 (−O*H*, s), 3.77 (1 H, dd, J = 6 and 12 Hz) and 4.05 (1 H, dd, J = 6 and 12 Hz) (AB part of an ABX system); ^{13}C NMR peaks at δ 19.44 (t), 24.17 (q), 25.65 (q), 27.30 (q), 36.89 (t), 37.04 (t), 37.33 (s), 64.11 (t), 76.30 (s) and 77.05 (s); MS peaks at *m/e* (%): 170 (M − 18, 2), 157 (13), 139 (15), 111 (26), 86 (100), 71 (28), 69 (32), 55 (26) and 43 (60). The preparation method used requires that the 1,2-diol system in *4* is *cis*. A corresponding *trans*-1,2-diol, having different properties, has been described previously.[7]

Preparation of 2-hydroxymethyl-1,3,3-trimethyl-1,2-cyclohexanediol monotosylate (7). A solution of 510 mg of 2-hydroxymethyl-1,3,3-trimethyl-1,2-cyclohexanediol (*4*) in pyridine was treated with 1 mol excess of *p*-toluenesulfonyl chloride at 0 °C for 24 h. Work up and chromatography over silica gel using ethyl acetate-hexane (40:60) as eluent produced 260 mg of the monotosylate (*7*): IR (film) bands at 3500 (m), 1190 (s), 1175 (s) cm^{-1}; ^1H NMR peaks at δ 0.95 (3 H, s), 1.00 (3 H, s), 1.33 (3 H, s), 2.46 (3 H, s), 4.19 (1 H, d, $J = 10$ Hz) and 4.39 (1 H, d, $J = 10$ Hz) (AB spectrum), 7.38 (2 H, d, $J = 9$ Hz) and 7.82 (2 H, d, $J = 9$ Hz) (AB spectrum).

Preparation of 1,2,3,3-tetramethyl-1,2-cyclohexanediol (9). To a solution of 40 mg of lithium aluminium hydride in ether was added an ethereal solution of 242 mg of the tosylate (*7*) dropwise. The mixture was stirred at room temperature for 1 h. Work-up and chromatography over silica gel using ethyl acetate-hexane (20:80) as eluent furnished 112 mg of 1,2,3,3-tetramethyl-1,2-cyclohexanediol (*9*) as a colourless glass, which had IR (film) bands at 3500 cm^{-1}; ^1H NMR peaks at δ 0.98 (3 H, s), 1.09 (3 H, s), 1.18 (3 H, s) and 1.27 (3 H, s); ^{13}C NMR peaks at δ 18.51 (q), 19.02 (t), 25.74 (q), 25.93 (q), 26.18 (q), 37.01 (t), 37.38 (t), 38.53 (s), 75.16 (s) and 77.78 (s); MS peaks at *m/e* (%): 172 (M, 2), 154 (4), 139 (4), 136 (11), 128 (12), 111 (75), 96 (62), 84 (65), 71 (70), 69 (52) and 43 (100).

Preparation of 3,3-dimethyl-2,7-octanedione (8). To a solution of 93 mg of *9* in 1 ml of acetic acid was added 100 mg of CrO_3 dissolved in 2 ml of aqueous acetic acid (90 %). The mixture was stirred at room temperature for 2 h, diluted with water and extracted with ether, washed with aqueous $NaHCO_3$, dried and evaporated to give 66 mg of 3,3-dimethyl-2,7-octanedione (*8*) [8,9] as a colourless oil, which had IR (film) bands at 1715 (s) and 1705 (s) cm^{-1}; ^1H NMR peaks at δ 1.12 (6 H, s), 2.12 (3 H, s) and 2.13 (3 H, s); ^{13}C NMR peaks at δ 18.99 (C-5), 24.24 (C-9 and C-10), 24.90 (C-1), 29.73 (C-8), 39.18 (C-4), 43.58 (C-6), 47.57 (C-3), 207.76 (C-7) and 212.93 (C-2); MS peaks at *m/e* (%): 170 (M, 1), 152 (1), 127 (8), 109 (43), 86 (28), 71 (18), 69 (70) and 43 (100). Oxidation of the natural product (*1*) using Jones' reagent gave a product indistinguishable from *8* (GC-MS, ^1H NMR).

Preparation of (±)-3,3-dimethyl-7-hydroxy-2-octanone (1). To a cooled (0 °C) solution of 19 mg of *8* in tetrahydrofuran was added 29 mg of lithium tri-*t*-butoxyaluminium hydride. After 10 min the reaction mixture was worked up and chromatographed over silica gel using ethyl acetate-hexane (30:70) as eluent, which gave three fractions. The least polar of these contained 4 mg of unreduced diketone (*8*). The second fraction was purified further by liquid chromatography on columns packed with μ-porasil (Waters) to afford 5.3 mg of (±)-3,3-dimethyl-7-hydroxy-2-octanone (*1*), which was indistinguishable (GC_{RT}, IR, ^1H NMR, ^{13}C NMR and MS) from the new tobacco constituent, and 0.7 mg of 3,3-dimethyl-2-hydroxy-7-octanone (*11*), which had ^1H NMR peaks at δ 0.84 (3 H, s), 0.86 (3 H, s), 1.12 (3 H, d, $J = 6.4$ Hz), 2.14 (3 H, s) and 3.60 (1 H, q, $J = 6.4$ Hz); MS peaks at *m/e* (%): 139 (M − 33, 2), 128 (26), 110 (32), 109 (49), 71 (49), 69 (79) and 43 (100). The most polar fraction contained 5.0 mg of 3,3-dimethyl-2,7-octanediol (*10*), which had MS peaks at *m/e* (%): 141 (M − 33, 2), 129 (8), 112 (22), 111 (20) and 69 (100).

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Analysis of Organochlorine Compounds in Effluents from Bleacheries by Neutron Activation Analysis and Gas Chromatography/Mass Spectrometry

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Effluents from chlorine bleacheries in a sulfite and a sulfate paper plant have been investigated. The total amounts of nonpolar and phenolic organohalogenated compounds have been determined by neutron activation analysis. In the sulfate plant nonpolar (cyclohexane extractable) and phenolic (butyl acetate extractable) chlorinated compounds are present in concentrations of 0.36 and 1.7 ppm, respectively. In the sulfite plant both chlorinated and brominated compounds are present in average concentrations of 0.67 ppm Cl and 0.06 ppm Br for nonpolar compounds and 1.1 ppm Cl and 0.1 ppm Br for phenolic compounds. The occurrence of organobromine compounds in the sulfite plant is due to the use of seawater in the first chlorination step.

The effluents have also been analysed by gas chromatography/mass spectrometry, and a number of halogenated aromatic hydrocarbons have been identified. These include chloro-*p*-methylisopropylbenzene (2 isomers), dichloro-*p*-methylisopropylbenzene (2 isomers), bromo-*p*-methylisopropylbenzene, chloro-*p*-methylisopropenylbenzene (2 isomers) and dichloro-*p*-methylisopropenylbenzene (2 isomers). These compounds are presumably formed by substitution reactions of the parent molecules which are formed by the cooking of the terpenes naturally present in wood.

Production of paper often involves bleaching of the pulp to remove lignin and other organic material which is left in the pulp after the cooking. The most commonly used bleaching agent is chlorine. Acidic chlorination will usually degrade lignin to water or alkali soluble compounds which in turn are extracted by the subsequent alkali treatment.

Several bioassay studies have demonstrated that effluents from chlorine bleach plants contain materials with acute toxic and sublethal effects on different marine species,[1–4] and considerable efforts have been made to identify these compounds. Dence and Ota [5] characterized residues in spent chlorination liquor by spectroscopic titrimetric methods. Collins *et al.*[6] investigated the lignin and carbohydrate residues in bleach effluents by gel chromatography and spectroscopy. More recently, 15 chlorinated phenols, guaiacols and catechols were identified in the effluents from the bleaching and extraction steps utilizing gas chromatography/mass spectrometry (GLC/MS).[7]

Little attention has, however, been paid to the occurrence of nonpolar chlorinated hydrocarbons in the effluent. Harris *et al.*[8] identified chloroform, and more recently Keith [9] identified hexachloroethane (in unbleached kraft paper mill wastewaters). The aim of the present study was to investigate the formation and occurrence of nonpolar, halogenated organic compounds in chlorine bleaching plants. Batch samples from different steps in the bleaching process in plants using the sulfate and the sulfite method were studied. The samples were analysed by neutron activation to determine the total amount of organically bound halogen and by GLC/MS to identify the individual compounds.

EXPERIMENTAL

Sampling. Approximately 5 l batch samples were taken from different steps in the chlorine bleaching plants (see Tables 1 and 2). The samples were stored in glass flasks at 4 °C

until analysed. Samples were taken at 5 different steps in the sulfate plant and 6 different steps in the sulfite plant.

Analysis. The sample volume was adjusted to 4.5 l and KOH was added until pH = 11. Nonpolar organic compounds were extracted twice with 250 ml of cyclohexane using a magnetic stirrer. The samples were acidified to pH 2 – 3 with concentrated HCl and phenolic compounds extracted twice with butyl acetate. Both the cyclohexane and the butyl acetate extracts were concentrated to about 20 ml in a rotating evaporator. The extracts were washed twice with distilled water and dried with magnesium sulfate. The extracts were further concentrated to about 5 ml in a heating block at 30 °C with a slow stream of highly purified nitrogen.

Analysis of the total amount of organic chloro-, bromo- and iodo-compounds, both in the cyclohexane and the butyl acetate extracts, was performed by neutron activation analysis. Neutron irradiation and registration of induced radioactivity was done at the Institute for Atomic Energy, Kjeller, Norway. A complete description of the method used on environmental samples is given elsewhere.[10]

The nonpolar organic compounds in the extracts were further characterized by means of GLC/MS. The instrument used was a Hitachi-Perkin Elmer RMU 6L mass spectrometer equipped with a Perkin Elmer Model 990 gas chromatograph and connected to an AEI stereo DS-50 data system. A 2 m × 3 mm glass column packed with 3 % Dexsil 300 on Gaschrom. Q was used, and the temperature ranged from 70 to 300 °C with a programming rate of 8 °C/min. The carrier gas was He and the flow-rate 17 ml/min. The mass spectra were recorded with 70 eV electron energy and an ion chamber temperature of 250 °C.

For the interpretation of some of the main components comparison was made with a reference compound of *p*-methylisopropylbenzene and its chlorinated and brominated analogs.[11,12]

RESULTS

Neutron activation analysis. The results of the determination of nonpolar (cyclohexane soluble) organohalogenated compounds are given in Tables 1 and 2. Nonpolar organochlorine compounds are present in all samples, organobromine compounds are present in samples from one plant only, and organoiodine compounds are not detected. Assuming that

Table 1. Concentration of organohalogenated compounds (ppm) in the effluent of a sulfate plant chlorine bleachery.

Sample	Cyclohexane extracts			Butyl acetate extracts		
	Cl	Br	I	Cl	Br	I
1. Chlorination	0.5	0.002	0.0002	2.6	<0.003	<0.0003
1. Alkaline extraction	0.9	0.003	0.0002	3.2	<0.004	<0.0006
2. Chlorination	0.3	0.0009	0.0002	0.4	0.003	0.0004
2. Alkaline extraction	0.01	0.0004	0.0001	1.5	0.002	<0.0004
Hypochlorite	0.1	0.0009	0.0006	1.0	0.007	0.00007
Average	0.36	–	–	1.7	–	–
Blank	0.001	<0.0001	<0.0001	0.002	<0.003	<0.00001

Table 2. Concentration of organohalogenated compounds (ppm) in the effluent of a sulfite plant chlorine bleachery.

Sample	Cyclohexane extracts			Butyl acetate extracts		
	Cl	Br	I	Cl	Br	I
1. Chlorination	0.3	0.08	<0.0002	0.6	0.2	<0.0002
2. Chlorination	0.6	0.08	<0.0001	0.02	0.05	<0.0001
Alkaline extraction	0.8	0.06	<0.0002	5.0	0.3	<0.002
1. Hypochlorite	0.8	0.09	<0.0002	0.04	0.003	<0.001
2. Hypochlorite	0.5	0.02	<0.0002	0.5	0.04	0.005
SO_2-treatment	1.0	0.03	<0.0002	0.5	0.02	<0.0001
Average	0.67	0.06	–	1.1	0.1	–
Blank	0.001	<0.0001	<0.0001	0.002	<0.0003	<0.00001

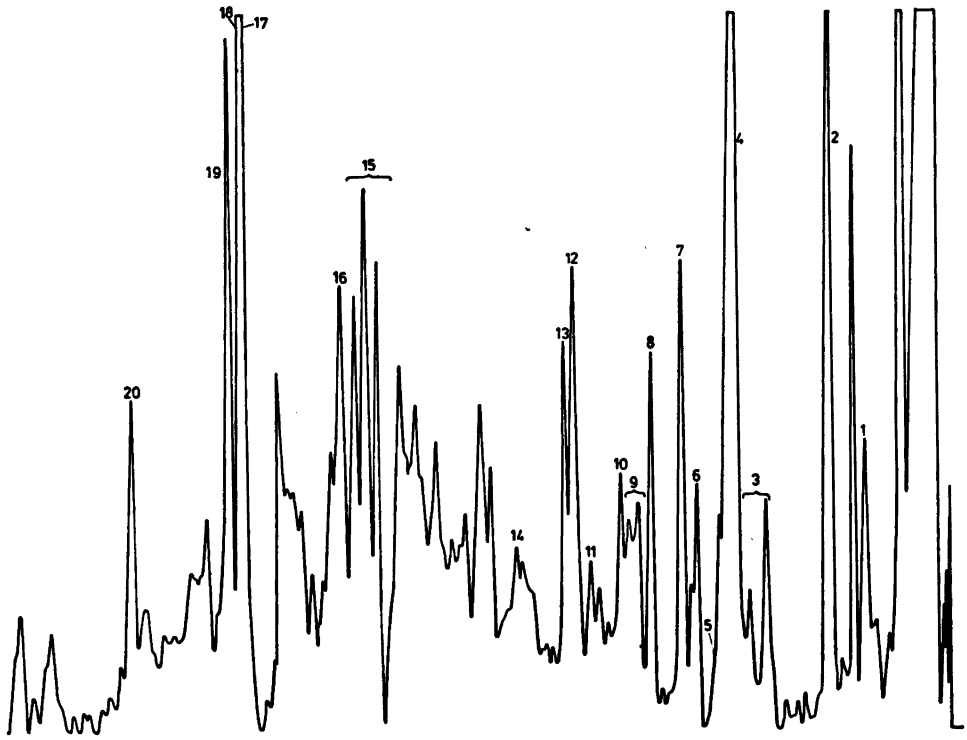


Fig. 1. Gas chromatogram (total ion current) of the cyclohexane extract of the effluent from a sulfite plant chlorine bleachery. The peaks 1 – 20 are interpreted as (1) M = 136 $C_{10}H_{16}$, (2) M = 134 methylisopropylbenzene, (3) M = 152 $C_{10}H_{16}O$, (4) M = 168 chloro-methylisopropylbenzene (2 isomers), (5) M = 134 alkyl(C4)-benzene, (6) M = 166, (7) M = 212 bromo-methylisopropylbenzene, (8) M = 202 dichloro-methylisopropylbenzene, (9) M = 204 sesquiterpene $C_{15}H_{24}$, (10) M = 202 dichloro-methylisopropylbenzene, (11) M = 204 sesquiterpene $C_{15}H_{24}$, (12) M = 202 dimethyl-propyltetralin, (13) M = 200, (14) M = 218, (15) M = 272 diterpene $C_{20}H_{32}$, (16) M = 268, (17) M = 282 diterpene, (18) M = 284 diterpene, (19) M = 314 methyldehydroabietate, (20) M = 328 diterpene.

each step contributes approximately the same amount of process water, an average value is estimated.

In addition, the total amount of organohalogenated phenolic (butyl acetate soluble) compounds is also determined, and the results are given in Tables 1 and 2 for the sulfate and the sulfite plant, respectively.

GC/MS-analysis. The GC/MS technique was used to characterize the individual compounds in the cyclohexane extracts. The GC traces of the samples showed the same compounds to be present in all samples, and only extracts from the first chlorination and alkali-extraction steps were studied by MS. Chromatograms of the two extracts are shown in Figs. 1 and 2, and the identified compounds are listed in the pertinent captions. The molecular structures of the different nonpolar halogenated hydrocarbons are given in Fig. 3.

DISCUSSION

Neutron activation analysis (NAA) is well suited for investigating organohalogenated compounds in the environment. The method is specific and sensitive, and provided that suitable solvents and extraction conditions are chosen, the nonpolar as well as phenolic compounds from the bleacheries may be characterized. The results demonstrate the presence of nonpolar organochlorine compounds in

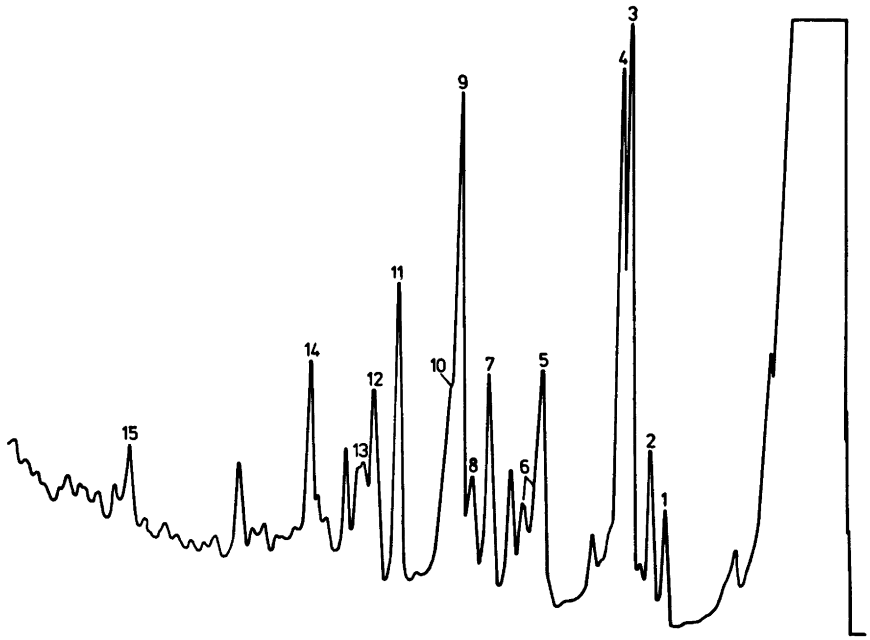


Fig. 2. Gas chromatogram (total ion current) of the cyclohexane extract of the effluent from a sulfate plant chlorine bleachery. The peaks 1 – 15 are interpreted as (1) and (2) M = 152 monoterpene, (3) M = 153 monoterpene, (4) M = 152 camphor, (5) M = 146, (6) terpene, (7) M = 202 dichloro-methylisopropylbenzene, (8) M = 166 chloro-methylpropenylbenzene, (9) M = 146 4-phenyl-3-buten-2-one, (10) M = 202 dichloro-methylisopropylbenzene, (11) M = 170 terpene, (12) M = 200 dichloro-methylisopropenylbenzene, (13) M = 200 dichloro-methylisopropenylbenzene, (14) M = 220 terpene, (15) phthalate.

bleachery effluents from both a sulfate and a sulfite plant. On the average, the concentration of the phenolic compounds is about two

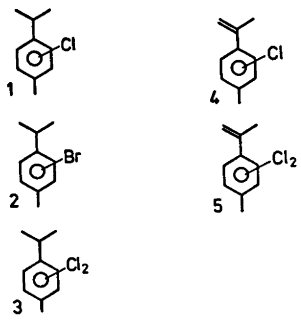


Fig. 3. Halogenated aromatic hydrocarbons identified in effluents from chlorine bleacheries. (1) chloro-methylisopropylbenzene (2 isomers identified), (2) bromo-methylisopropylbenzene, (3) dichloro-methylisopropylbenzene (2 isomers), (4) chloro-methylisopropenylbenzene (2 isomers) and (5) dichloro-methylisopropenylbenzene (2 isomers).

to three times the concentration of the nonpolar compounds.

In the sulfite plant, brominated organic compounds are also present. In this plant the first chlorination step takes place in sea water. Presumably, chlorine will react with the bromide naturally present in sea water (usually about 65 ppm) to give bromine, which in turn may brominate the organic material.

From the data given, the discharge of nonpolar organochlorine compounds may be estimated. A concentration of 1 ppm organically bound chlorine in the effluent from a bleach plant with a water consumption of 20 000 m^3/day will result in a daily discharge of 20 kg (measured as chlorine). This corresponds to about 7 t/year.

The nonpolar halogenated compounds identified in this work are all substituted aromatic hydrocarbons. To the best of our knowledge, no such compounds have been previously identified in bleachery effluents. In general,

halogenated aromatic compounds are of great environmental concern.[13–15] However, little is known about the chemical and biological persistence and the biological effects in the recipient of the specific compounds identified in this work.

As revealed by the GLC/MS-data (Fig. 1), one of the main components in the extracts is unsubstituted methylisopropylbenzene (cymene). This compound is assumed to be the *para* isomer (*p*-cymene) determined by comparison with a reference compound of *p*-methylisopropylbenzene. However, as the mass spectra data given in the literature are quite similar for the three cymene isomers, we could not exclude the *ortho* or *meta* isomer to be present. *p*-Cymene is known to be formed during the cooking process from the terpenes naturally present in the wood and the reaction is catalysed by lignin radicals.[16] It is therefore reasonable to assume that the halogenated *p*-cymenes are formed by substitution reactions on the parent molecule.

It is also interesting to note that chlorinated derivatives of *p*-methylisopropenylbenzene (structure based on the mass spectra and analogy with the methylisopropylbenzenes) are identified only in effluents from the sulfate plant. This suggests that the *p*-methylisopropenylbenzene is formed mainly under the conditions in the sulfate plant and by analogy with *p*-methylisopropylbenzene, that the chlorinated derivatives are formed by substitution reactions on the parent molecule.

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A Peptide Containing Aspartic Acid, Glutamic Acid and Serine in Calf Brain Synaptic Vesicles

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Free amino acids and other amino compounds in calf brain synaptic vesicles were identified and determined by thin-layer chromatography and ion-exchange chromatography. The vesicles contained ten identified amino acids with glutamic acid, aspartic acid, taurine and γ-aminobutyric acid in the highest concentrations, and also cysteic acid (or cysteinesulfinic acid), glutamine, alanine, serine, glycine and lysine. The vesicles also contained certain unknown acid-labile, ninhydrin-positive compounds, one of which was a peptide yielding, after acid hydrolysis, about 40 % aspartic acid, 30 % serine, 15 % glutamic acid, 10 % glycine and possibly some alanine and lysine. The concentration of the peptide in the vesicles was as high as that of all the other amino compounds together.

The vesicles of nerve terminals store transmitter substances, among which acetylcholine and catecholamines are well-known. In addition, eight to nine amino acids have been reported in the vesicles of mammalian cerebral cortex.[1–3] Several peptides have been found in the mammalian central nervous system [4] and Reichelt and Kvamme [5] reported *N*-acetyl-aspartyl-peptides containing various transmitter candidates in the central nervous system, but no one has yet reported these in the vesicles. When analysing free amino acids in calf brain synaptic vesicles we found a low-molecular weight peptide yielding mainly aspartic acid, glutamic acid and serine after acid hydrolysis. This observation is reported in preliminary form elsewhere.[6] Here a complete analysis is given of the amino compounds in calf brain synaptic vesicles.

EXPERIMENTAL

The vesicles were prepared from newly-removed calf brain cortical layer according to the sucrose gradient centrifugation method of Morgan *et al.*[7] In addition to this normal sucrose gradient, we also used a denser gradient containing equal volumes of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0 and 1.2 M sucrose solutions (Fig. 1). When checked under an electron microscope, the isolated vesicles were found to be of good quality and high purity. The yield of the vesicles was about 0.18 mg vesicle protein per 1 g wet weight of the cortex. Data concerning their homogeneity are presented elsewhere.[6] Soluble cytoplasmic impurities were washed off with 2 × 5 ml of 1 mM potassium phosphate buffer (pH 7.5) containing 0.1 mM ethylenediamine tetraacetic acid.

Amino acids were extracted from the vesicles three times using 2 ml of 5 % trichloroacetic acid in water each time, the trichloroacetic acid then being removed by shaking with diethyl ether. The solution was evaporated to dryness in evacuated tubes at 50 °C and the residue taken up in 1 ml of 0.1 N HCl. Amino acids were identified by thin-layer chromatography according to Stahl [8] using 0.25 mm thick Kieselgel G plates. Two-dimensional chromatograms were developed with 70 % ethanol in water and 75 % phenol in water. The dried plates were sprayed with 15 % ninhydrin in 3 M potassium citrate buffer (pH 5.1), and the spots developed for 10 min at 105 °C. The quantitative determination of amino acids was performed using an automatic amino acid analyzer (Hitachi Perkin-Elmer 034 Liquid Chromatograph), employing known standards in ion-exchange chromatography as well as in thin-layer chromatography. Alanine was used as a standard for the unknown compounds. One batch (2 mg) of amino acid extracts was hydrolysed in 6 N HCl for 6 h at 100 °C, and the amino acids in the hydrolysate were determined as above. Quantitative analyses,

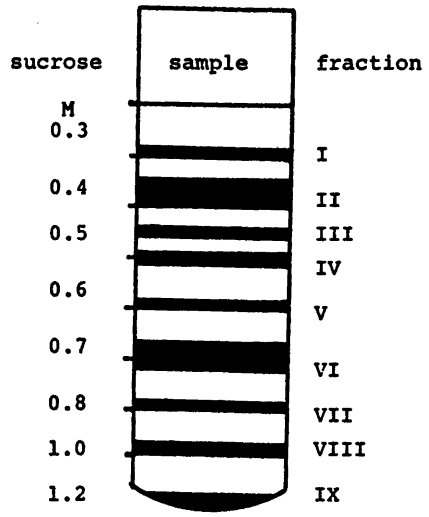


Fig. 1. Diagram of a sucrose gradient used for the fractionation of synaptosomal components, and their subcellular particle fractions after the centrifugation.

in addition to being performed in the vesicle fractions, were also carried out in the sample fraction (Fig. 1) and in the synaptoplasm left after the sedimentation of subcellular particles of hypo-osmotically disrupted synaptosomes.

RESULTS

Ten amino acids were identified in the calf brain synaptic vesicles: cysteic acid (or cysteinesulfinic acid), taurine, aspartic acid, glutamic acid, glutamine, serine, alanine, glycine, γ-aminobutyric acid and lysine (for concentrations, see Fig. 4). In addition, the vesicles contained a high concentration of an unknown ninhydrin-positive compound (Figs. 2, 3, 4). Its concentration in the vesicles (in alanine equivalents) was approximately as high as that of all the other amino compounds together (Fig. 4). It was completely hydrolysed in 6 N HCl (Figs. 2, 4) to a variety of amino acids, mainly aspartic acid, glutamic acid and serine (Fig. 4). The vesicles also contained other unknown acid-labile compounds eluting with the cysteic

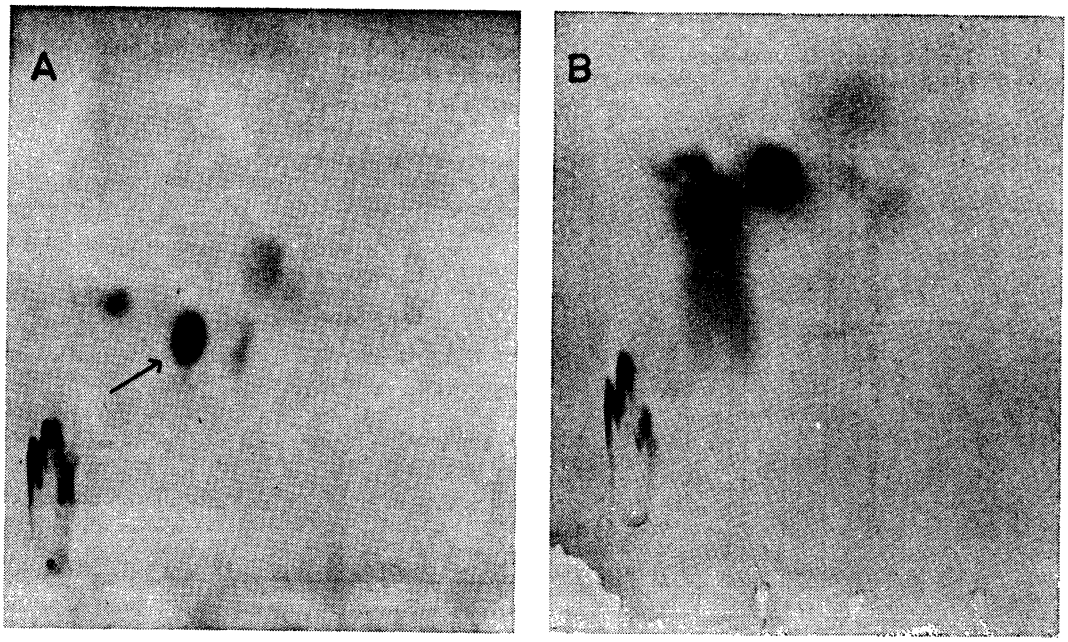


Fig. 2. Photographs of thin-layer chromatograms of unhydrolysed (A) and hydrolysed (B) amino acid samples from calf brain synaptic vesicles on 0.25 mm thick Kieselgel G run with 70 % ethanol (solvent I) and 75 % phenol (solvent II). The dried plates were sprayed with 15 % ninhydrin, and the spots developed for 10 min at 105 °C. The unknown peptide is indicated by the arrow.

acid peak in ion exchange chromatography (Fig. 4). The concentrations of aspartic acid, glutamic acid and serine, and also the amount of glycine, increased significantly after acid hydrolysis, and minute quantities of some other unknown compounds also appeared (Fig. 4).

The unknown compound was concentrated in the main vesicle fraction (fraction II, Fig. 1, Table 1). Use of the dense sucrose gradient enabled the vesicles to be divided into four fractions, the vesicles of which differed in size, being largest in the lowest fraction, but also more seriously contaminated by unidentified

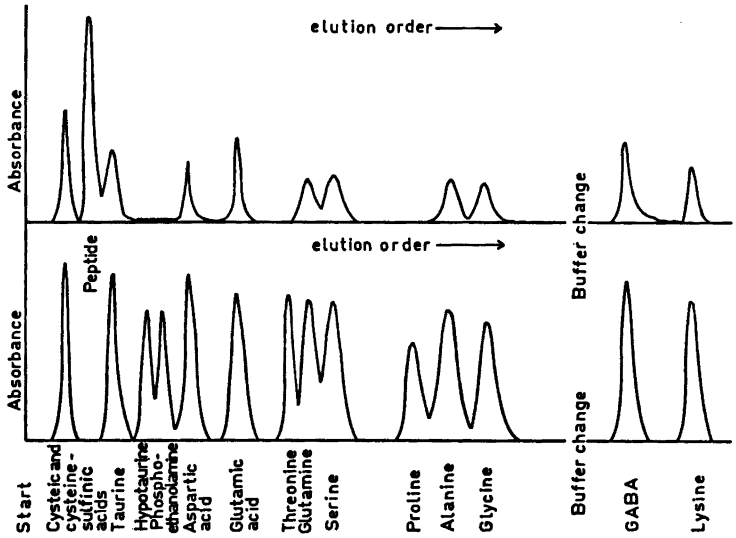


Fig. 3. Ion exchange chromatograms of a standard amino acid mixture (lower diagram) and an unhydrolysed sample from the synaptic vesicles (upper diagram).

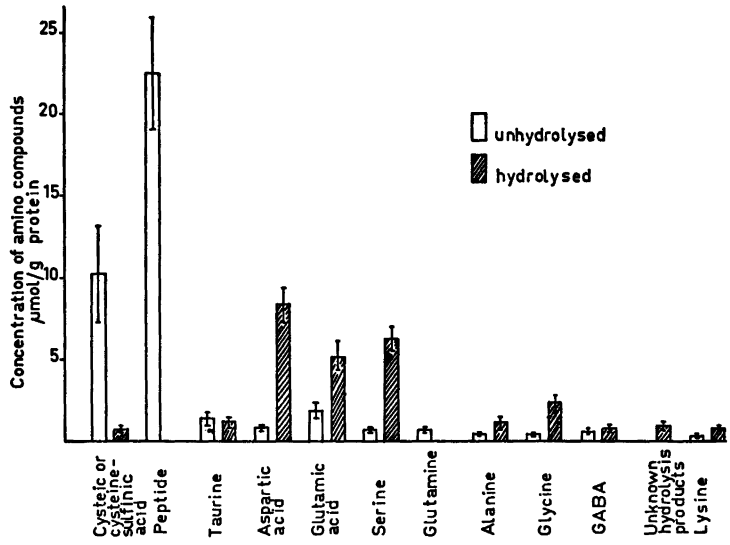


Fig. 4. Quantities (µmol/g vesicle protein) of amino compounds in the vesicles, unhydrolysed and hydrolysed samples. Results (means ± S.E.M. by vertical bars) are given from 8 determinations.

Table 1. Concentrations of amino compounds (μmol/g protein of the fraction, unknown compounds in alanine equivalents) in the various vesicle fractions, the sample fraction and the synaptoplasm of calf brain cortex.

Amino compound	Concentration of amino compounds in fractions I	II	III	IV	Sample fraction	Synapto-plasm
Cysteic acid / Cysteinesulfinic acid	1.0(2)	12.2(24)	0.2(1)	0.3(1)	0.9(1)	2.9(6)
Phosphoethanolamine?	–	–	–	–	–	0.9(3)
Taurine	3.1(6)	2.4(8)	0.3(1)	0.2(1)	52(4)	148(27)
Aspartic acid	3.4(5)	6.1(11)	2.1(3)	0.8(3)	104(9)	185(9)
Glutamic acid	8.2(19)	14.3(30)	2.5(2)	1.1(4)	156(11)	248(12)
Glutamine	3.8(5)	6.1(8)	1.0(2)	0.9(2)	21(2)	48(4)
Alanine	1.4(3)	2.0(4)	0.2(1)	0.3(0)	9.4(3)	29.5(16)
Serine	3.4(6)	5.8(9)	1.0(1)	0.8(2)	6.1(4)	15.7(12)
Glycine	1.0(2)	1.9(3)	0.3(1)	0.2(1)	16.5(14)	36.5(20)
Lysine	0.2(1)	0.9(1)	0.1(1)	0.1(1)	0.4(1)	1.9(4)
γ-Aminobutyric acid	0.4(1)	3.3(6)	0.2(1)	0.1(0)	77(7)	149(8)
Unknown (peptide) / alanine	5.9(11)	30.5(44)	6.8(10)	4.0(8)	18.1(14)	20.2(15)
Unknown / equivalents	–	–	–	–	2.9(9)	24.0(26)
Cysteine	–	–	–	–	0.6(1)	4.8(3)
Methionine	–	–	–	–	1.2(10)	14.0(10)
Leucine	–	–	–	–	6.3(9)	15.2(11)
Isoleucine	–	–	–	–	0.5(4)	2.8(1)
Tyrosine	–	–	–	–	1.1(2)	6.1(3)
Phenylalanine	–	–	–	–	7.9(6)	12.7(10)
Urea	–	–	–	–	–	1.8(2)
Threonine	–	–	–	–	9.8(10)	10.9(9)
Proline	–	–	–	–	0.2(0.3)	3.1(1)
Valine	–	–	–	–	1.8(2)	5.0(4)
Number of experiments	4	5	3	3	4	5

Means (± S.E.M. in parentheses) are given.

arger subcellular particles. Amino acid concentrations were highest in the upper fractions, though their pattern was fairly similar in all fractions (Table 1). These in turn differed considerably from those in the synaptoplasm and sample fractions.

DISCUSSION

The free amino acid composition of the synaptic vesicles presented here agrees fairly well with the figures reported by De Belleroche and Bradford,[1] Mangan and Whittaker [2] and Rassin,[3] our results being significantly lower only in the case of glutamic acid and taurine. Some loss of intravesicular amino acids certainly occurred during the washing of the vesicles. This stage is of importance, however, for eliminating any extravesicular amino acids which have diffused from the sample fraction into the vesicle fraction.

Several small-molecular peptides have been reported in the central nervous system. "Substance P" contains relatively high amounts of the basic amino acids, arginine and lysine, and of leucine.[9,10] A large number of glutamate-peptides (glutathione and other γ-glutamyl-peptides), γ-aminobutyrate-peptides (homocarnosine, homoanserine) and lipo- and phospho-peptides have also been found.[4] An interesting group of central nervous system peptides consists of the *N*-acetylaspartyl-peptides,[4] which have been extensively studied by Reichelt and his associates.[5,11–13] These peptides are of relatively low molecular weight (4 – 6 amino acids). They contain *N*-acetylaspartic acid and several transmitter candidates, some amino acids (including γ-aminobutyric acid and taurine) and also histamine. Two of the most important sequences are *N*-acetyl-aspartyl-seryl-glutamate and *N*-acetyl-aspartyl-glutamyl-seryl-glycine.[12,13] After hydrolysis, these

peptides thus liberate approximately 30 – 40 % aspartic acid, 20 – 30 % serine and glutamic acid and 20 % glycine. The peptide identified in the present paper containing approximately 40 % aspartic acid or *N*-acetylaspartic acid, 30 % serine, 15 % glutamic acid, 10 % glycine and possibly some alanine and lysine after acid hydrolysis, is quite similar to those reported by Reichelt's group. However, there are significant differences regarding the occurrence of histamine, γ-aminobutyric acid and taurine in the peptides demonstrated by Reichelt's group.[11–13] We were unable to identify these in the hydrolysates of our peptide. The mean amino acid composition also shows certain differences between these peptides. The peptide demonstrated in the present work thus does not seem to be identical with the peptides described earlier, although there are some similarities in their chemical structures. Some of the aspartic acid might also be present in the form of free *N*-acetylaspartic acid.[14]

The occurrence of low-molecular peptides in the vesicle fraction is interesting, although their function remains open. Since they contain amino compounds, for which a transmitter role has been proposed, they may function as temporary molecules for storing transmitter substances. The transmitters could then be liberated from them through hydrolysis. This offers a new possibility for terminating the function of transmitter substances in the synaptic region.

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The Crystal and Molecular Structure of Cannabidiol

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The structure of cannabidiol, $C_{21}H_{30}O_2$, has been determined by X-ray methods using 2046 reflections collected by counter methods. The compound crystallizes in the space group $P2_1$ with two molecules in the asymmetric unit. Cell dimensions are: $a=10.644(5)$ Å, $b=10.667(2)$ Å, $c=17.323(5)$ Å, $\beta=95.42(3)°$. The structure model was refined to a conventional R of 0.073. The cyclohexene ring is in the half-chair conformation. The aryl and the isopropenyl substituents are quasi-equatorial and the conformation around the pivot bonds are syn-periplanar relative to the quasi-axial protons. The main difference between the two molecules in the asymmetric unit is in the conformation of the pentyl side-chain. The molecules are kept together mainly by van der Waals forces. There is only one hydrogen bond [2.861(8) Å] which connects the phenolic groups in the two molecules in the asymmetric unit.

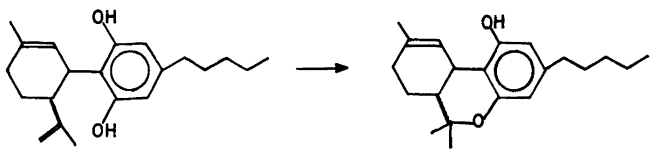
Cannabidiol (CBD, *1*) is the biogenetic precursor of (−)Δ^9-tetrahydrocannabinol (Δ^9-THC, *2*), which is the major psychotomimetic constituent in hashish. Cannabidiolic acid (CBDA) was the first cannabinoid to be studied [1] and is the predominant cannabinoid in nature.[2,3] CBD is psychotomimetically inactive, but it is reported [4] that it will interfere with some of the actions of Δ^9-THC when given in combination. It is also known that CBD potentiates barbiturates [5] and is antibiotic to Gram-positive bacteria *in vitro*.[6]

We have earlier studied the three-dimensional structures of cannabinoids with a pyran ring (Δ^9-THC Acid B,[7] 8-β-hydroxy-Δ^9-THC[8] and cannabinol [9]), and in view of the increasing interest in and importance of cannabinoids which do not possess a pyran ring, we found it of importance to undertake this investigation (see note on p. 811).

EXPERIMENTAL AND STRUCTURE DETERMINATION

A crystal of extreme dimensions 0.24 × 0.38 × 0.44 mm was selected for the crystallographic work. A computer-controlled Syntex P$\bar{1}$ four-circle diffractometer with graphite-monochromatized Mo$K\alpha$ radiation was utilized in the preliminary experiments and the collection of intensity data. The axial solutions (computer program written by R. Sparks, part of the diffractometer program library) from the angular coordinates of fifteen reflections implied a monoclinic system. The systematic absences found in a fast low-angle data collection indicated the space group $P2_1$ or $P2_1/m$ with four molecules in the unit cell. For the optically active compound this uniquely determines the space group to be $P2_1$ with two molecules per asymmetric unit, which will be denoted A and B, respectively. Unit cell dimensions and their standard deviations were determined by a least-squares treatment of the

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I II

angular coordinates of fifteen reflections with 2θ-values between 4 and 16°.

Three-dimensional intensity data were recorded using the $\omega-2\theta$ scanning mode with scan speed variable from 3 to 12° min^{-1} depending on the intensity of the reflection, and a scan area from $2\theta\ (\alpha_1) - 0.7°$ to $2\theta(\alpha_2) + 0.8°$. Background counting time was equal to $0.35 \times$ (scan time) on each side of the scan area. The temperature was kept constant within 2° at 23 °C. The variations in the intensities of three check reflections which were remeasured after every hundred reflections, were random.

The estimated standard deviations were taken as the square root of the total counts with a 2 % addition of the net intensity for experimental uncertainties. Of the 2754 symmetry-independent reflections measured ($2\theta_{max} = 45°$), 2046 had intensities larger than twice their standard deviations. These were regarded as observed reflections, whereas the remaining were excluded from further calculations. The intensities were corrected for Lorentz and polarization effects.[10]

The phase problem could not be solved by the standard MULTAN[11] program assembly. Starting phases were, therefore, generated by the use of magic integers.[12] In the resulting E-map from the statistically best solution from the run with starting phases generated by the use of the four integers 2, 3, 7 and 13, 34 of the 46 nonhydrogen atoms could be located. The other nonhydrogen atoms were found by successive Fourier-refinements. The program used as well as programs subsequently employed, is part of a local (Oslo) assembly of computer programs which is described in Ref. 13. Atomic scattering factors used were those of Doyle and Turner[14] for oxygen and carbon, and those of Stewart *et al.*[15] for hydrogen.

The structure model was refined to a conventional R of 0.13. The introduction of anisotropic temperature factors for all nonhydrogen atoms in the least-squares refinement lowered R to 0.092. Due to the large number of parameters each molecule (A and B, respectively) was refined successively by "full-matrix" refinement. At this point the hydrogen atoms were placed in calculated positions and included in the structure factor calculation with estimated isotropic thermal parameters. The least-squares refinement of all nonhydrogen atomic parameters converged to a weighted R_w of 0.074 and an R of 0.073. The weights used in the refinement were $1/\sigma^2(F_{obs})$. A final difference Fourier synthesis showed only spurious peaks of maximum height 0.4 e $Å^{-3}$.

Atomic parameters for nonhydrogen atoms are given in Table 1. A list of observed and calculated structure factors is available upon request from: Department of Chemistry, University of Oslo, Oslo 3, Norway, as is a list of the parameters used for hydrogen atoms.

CRYSTAL DATA

Cannabidiol, $C_{21}H_{30}O_2$, $M = 314.47$ amu, space group $P2_1$, $a = 10.644(5)$ Å, $b = 10.667(2)$ Å, $c = 17.323(5)$ Å, $\beta = 95.42°(3)$, $V = 1958.2(11)$ $Å^3$, $Z = 4$, $D_{calc} = 1.067$ g cm^{-3}, $F(000) = 688$.

DISCUSSION

The molecular structure and the atomic numbering of the two CBD molecules in the asymmetric unit are shown in Fig. 1, together with a view along the plane of the aromatic ring showing the conformation. Selected dihedral angles are given in Table 2. The absolute configuration was arbitrarily chosen (S,S shown). By chemical synthesis[16] the absolute configuration of the naturally occuring (−)-CBD has been shown to be R,R. A list of bond lengths and bond angles is available from the authors upon request. The estimated standard deviations in bond lengths and bond angles are 0.01 Å and 0.7 − 1.0°, respectively, except for the side-chains where the e.s.d.'s are 0.02 − 0.03 Å and 2 − 3°. Except for some abnormal

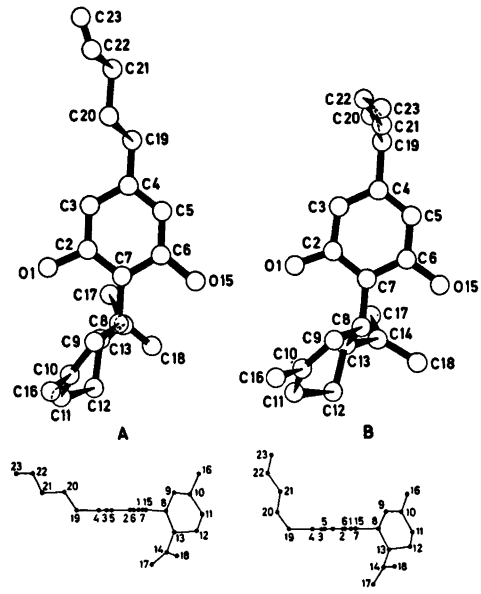


Fig. 1. The molecular structure and the atomic numbering of the two molecules in the asymmetric unit. The upper part of the figure is a view down on the plane of the phenyl ring and the lower part along the plane.

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations for nonhydrogen atoms. The temperature factor is given by $\exp\{-2\pi^2[U_{11}(a^*h)^2+U_{22}(b^*k)^2+U_{33}(c^*l)^2+2U_{12}(a^*b^*hk)+2U_{13}(a^*c^*hl)+2U_{23}(b^*c^*kl)]\}$.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O1A	1.0370(4)	.5666(−)	.8153(3)	.046(3)	.087(4)	.105(4)	−.016(3)	.016(3)	−.028(4)
C2A	.9353(7)	.4824(8)	.8001(4)	.057(5)	.064(6)	.058(5)	−.001(5)	.004(4)	−.008(5)
C3A	.9572(8)	.3771(10)	.7547(5)	.064(6)	.083(7)	.065(6)	.007(6)	.012(4)	−.006(6)
C4A	.8629(8)	.2896(9)	.7403(4)	.054(5)	.074(7)	.055(5)	−.010(5)	−.011(4)	−.004(5)
C5A	.7529(8)	.3075(9)	.7713(5)	.058(6)	.066(7)	.088(6)	−.015(5)	−.024(5)	.004(6)
C6A	.7272(8)	.4153(10)	.8130(5)	.066(6)	.074(7)	.069(6)	−.003(6)	−.011(5)	.006(5)
C7A	.8211(7)	.5091(8)	.8278(4)	.044(5)	.069(6)	.046(5)	−.001(5)	.004(4)	−.002(4)
C8A	.7938(7)	.6291(9)	.8675(4)	.068(5)	.077(7)	.053(5)	−.005(5)	.012(4)	−.017(5)
C9A	.8868(7)	.6452(10)	.9417(5)	.062(6)	.095(8)	.067(6)	.005(6)	.000(5)	−.017(6)
C10A	.9310(8)	.7542(13)	.9667(5)	.081(7)	.103(9)	.066(6)	−.008(7)	.012(5)	−.035(7)
C11A	.8998(10)	.8727(11)	.9252(7)	.103(8)	.086(9)	.121(9)	−.004(7)	.025(7)	−.033(8)
C12A	.7833(10)	.8623(9)	.8666(6)	.125(9)	.055(6)	.119(8)	−.001(6)	.008(7)	−.004(7)
C13A	.7959(7)	.7467(9)	.8157(5)	.074(6)	.068(7)	.080(6)	−.003(5)	.003(5)	−.007(6)
C14A	.6999(10)	.7384(10)	.7455(7)	.1116(8)	.076(7)	.108(9)	.000(7)	−.001(7)	.011(7)
O15A	.6119(5)	.4381(6)	.8404(3)	.052(3)	.103(5)	.107(4)	−.023(4)	.034(3)	−.008(4)
C16A	1.0178(9)	.7587(12)	1.0425(6)	.108(8)	.141(11)	.116(9)	.004(8)	.000(7)	−.055(8)
C17A	.7342(10)	.7184(13)	.6768(7)	.115(8)	.136(11)	.106(8)	−.030(8)	.000(7)	.003(9)
C18A	.5599(9)	.7500(14)	.7603(8)	.062(7)	.175(13)	.243(14)	.030(8)	.021(7)	.019(12)
C19A	.8849(8)	.1737(10)	.6966(5)	.091(7)	.083(8)	.071(6)	.001(6)	−.017(5)	−.003(6)
C20A	.9998(11)	.0963(10)	.7251(6)	.145(10)	.078(8)	.103(8)	.010(7)	.007(7)	−.019(7)
C21A	1.0212(12)	−.0162(12)	.6781(8)	.143(11)	.092(10)	.180(11)	.034(9)	.017(8)	.008(10)
C22A	1.1315(21)	−.0953(18)	.7178(13)	.215(21)	.162(16)	.262(24)	.043(15)	.041(17)	−.068(15)
C23A	1.1811(23)	−.1557(5)	.6821(16)	.222(22)	.795(91)	.266(28)	.244(37)	.070(19)	.041(38)
O1B	.5046(5)	.1922(−)	.6452(3)	.088(4)	.072(4)	.108(5)	.021(3)	.035(4)	−.009(4)
C2B	.4363(7)	.2446(10)	.6990(5)	.058(5)	.079(7)	.073(6)	.000(5)	.008(4)	.003(6)
C3B	.4152(8)	.1715(8)	.7642(5)	.082(6)	.063(7)	.085(6)	−.002(5)	.008(5)	.001(6)
C4B	.3447(8)	.2186(11)	.8214(5)	.067(6)	.093(9)	.071(6)	−.015(6)	.019(5)	.014(6)
C5B	.2973(7)	.3350(10)	.8136(5)	.062(5)	.086(8)	.056(5)	.003(5)	.004(4)	−.006(6)
C6B	.3135(6)	.4076(10)	.7497(5)	.041(4)	.089(8)	.058(5)	−.008(5)	.002(4)	−.005(6)
C7B	.3847(7)	.3624(9)	.6893(4)	.050(4)	.057(6)	.065(5)	−.008(5)	.004(4)	.005(5)
C8B	.4050(6)	.4437(8)	.6196(4)	.048(4)	.084(6)	.057(5)	−.002(5)	.003(4)	.006(5)
C9B	.5422(7)	.4542(8)	.6084(4)	.072(5)	.068(6)	.075(6)	−.012(5)	−.004(4)	.010(5)
C10B	.5971(8)	.4409(9)	.5406(5)	.080(6)	.090(7)	.017(6)	−.004(6)	.031(5)	.007(6)
C11B	.5148(8)	.4233(10)	.4661(4)	.093(6)	.117(9)	.063(5)	.002(7)	.005(6)	.001(6)
C12B	.3751(8)	.4646(9)	.4746(4)	.090(6)	.091(7)	.065(5)	−.002(6)	.004(5)	.009(6)
C13B	.3289(7)	.3974(8)	.5447(4)	.074(5)	.081(7)	.066(5)	.009(5)	.009(11)	.002(5)
C14B	.1849(8)	.4122(12)	.5486(5)	.055(6)	.119(10)	.089(6)	−.010(7)	−.012(5)	.014(7)
O15B	.2647(5)	.5255(6)	.7433(3)	.073(4)	.077(4)	.089(11)	.008(4)	.020(3)	.018(4)
C16B	.7322(8)	.4440(11)	.5381(5)	.076(6)	.152(10)	.099(7)	−.025(7)	.026(5)	.015(7)
C17B	.1122(10)	.3163(14)	.5477(7)	.080(7)	.181(13)	.145(10)	−.020(8)	.002(7)	.007(9)
C18B	.1343(9)	.5412(14)	.5530(7)	.081(8)	.155(12)	.163(11)	.034(8)	.012(7)	.016(10)
C19B	.3264(10)	.1345(11)	.8903(7)	.109(8)	.130(10)	.104(8)	−.019(8)	−.009(7)	.027(8)
C20B	.4289(18)	.1036(16)	.9471(8)	.171(14)	.218(48)	.112(10)	−.038(13)	.005(10)	.060(12)
C21B	.5129(18)	.1874(23)	.9792(9)	.177(14)	.355(27)	.121(13)	−.108(17)	−.058(11)	.102(15)
C22B	.6275(24)	.1438(23)	1.0316(12)	.307(30)	.278(29)	.159(16)	−.150(24)	−.022(17)	.020(16)
C23B	.6909(18)	.2397(28)	1.0661(14)	.159(15)	.312(30)	.278(26)	.029(19)	.012(15)	.121(24)

Table 2. Dihedral angles (°). The angles are positive in a right-hand screw.

Angles (°)	A	B	Angles (°)	A	B
C2–C7–C8–C9	−60.1(9)	−51.4(10)	C2–C7–C8–C13	64.6(9)	73.5(9)
C7–C8–C9–C10	145.2(8)	133.4(9)	C13–C8–C9–C10	18.1(11)	7.5(12)
C8–C9–C10–C11	−2.0(13)	5.1(14)	C9–C10–C11–C12	17.6(13)	17.9(12)
C10–C11–C12–C13	−49.2(11)	−52.6(10)	C11–C12–C13–C8	65.3(9)	65.9(8)
C11–C12–C13–C14	−168.9(9)	−169.1(8)	C12–C13–C8–C9	−48.4(8)	−42.6(9)
C12–C13–C8–C7	−172.9(8)	−167.7(7)	C8–C13–C14–C17	−106.7(12)	−115.4(11)
C12–C13–C14–C17	129.3(12)	120.9(11)	C3–C4–C19–C20	52.2(11)	69.4(15)
C4–C19–C20–C21	−177.8(9)	45.4(22)	C19–C20–C21–C22	−173.6(12)	−172.7(15)
C20–C21–C22–C23	−155.9(35)	−171.8(20)	C8–C13–C14–C18	71.2(11)	64.9(12)
			C12–C13–C14–C18	−52.6(12)	−58.6(13)

Table 3. Deviations from least-squares planes (Å × 10^3). The deviations for those atoms used to define the plane are given in italicized figures.[a]

Atom	A1	B1	Atom	A2	B2	Atom	A3	B3
O1	91	0	C7	−806	−1016	C7	−2520	−2518
C2	*32*	*13*	C8	*16*	*−29*	C8	−1373	−1295
C3	*−11*	*−5*	C9	*−20*	*39*	C9	−1318	−1152
C4	*−20*	*−8*	C10	*−7*	*6*	C10	−200	−18
C5	*27*	*12*	C11	*−2*	*6*	C11	1130	1286
C6	*−6*	*−3*	C12	415	493	C12	1080	1215
C7	*−23*	*−10*	C13	−385	−334	C13	*−4*	*1*
C8	−151	5	C14	−270	−116	C14	*11*	*−2*
O15	−47	20	C16	*13*	*−21*	C17	*−4*	*1*
C19	16	−23				C18	*−4*	*1*

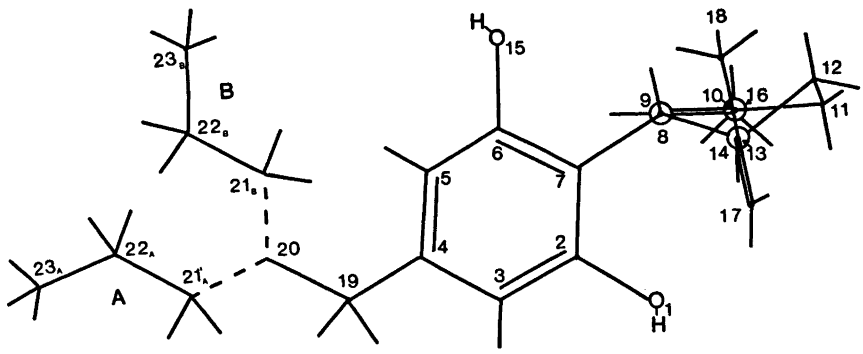
[a] Angle between planes A1 and A2: 80.3°. Angle between planes B1 and B2: 73.4°.

short bonds in the side-chains [C22A–C23A: 1.07(3) Å, and C22B–C23B: 1.34(3) Å] caused by the large thermal vibration, the mean values of bond angles and lengths are normal within their e.s.d.'s, although there are some large differences between molecule A and B.

As seen from Fig. 1, the main difference in conformation between molecule A and molecule B is found in the side-chain. In molecule A the chain is fully extended and all-*anti* while in B the conformation is *gauche* around the C19–C20 bond, with a dihedral angle C4–C19–C20–C21 of 45.4(2.2)°. The mean value of the dihedral angle C3–C4–C19–C20 is 60.9°. The corresponding angle was approximately 90° in both the two Δ^9-THC structures studied.[7,8] The present structure shows that there is no preferred side chain conformation in the cannabinoids but that this will be determined by the packing in a crystal.

The aromatic ring is planar within 0.03 Å in both molecules, but the deviations from the plane for the atoms directly attached to the ring are considerably greater in molecule A than in B (see Table 3, plane A1 and B1).

A comparison of the cyclohexene conformation in CBD and in Δ^9-THC clearly demonstrates the effect of the pyran ring formation. In the present structure the conformation is closer to a true C_2 symmetric half-chair, as observed in cyclohexene,[17] than it was in Δ^9-THC. The mean dihedral angle C10–C11–C12–C13 in CBD is 50.9°, while the value observed in 8β-hydroxy-Δ^9-THC was 37.7°. The deviation from the plane through C8–C9–C10–C11 (plane A2 and B2, Table 4) is +0.41 Å and



−0.39 Å for C12 and C13 in molecule A, respectively, and +0.49 Å and −0.33 Å in molecule B. The corresponding values in 8β-hydroxy-Δ^9-THC were +0.12 and −0.61 Å.

Both the aryl and the isopropenyl groups are quasi-equatorial substituents on the cyclohexene ring, and the conformation around the pivot bonds C7−C8 and C13−C14 are *syn*-clinal. The torsion angle C2−C7−C8−C13 is 64.9(9)° in molecule A and 73.5(9)° in B. The torsion angle C8−C13−C14−C18 is 71.2(9)°. in molecule A and 64.9(10)° in B. This means that the C6−C7 and C14−C17 bonds are *syn*-periplanar relative to the quasi-axial protons on C8 and C13, respectively. This conformation leads to a short contact between the phenolic oxygen O15 and the proton on C8. In molecule A this distance is 2.28 Å and in B 2.34 Å (assuming normal C−H bond distance and angle.)

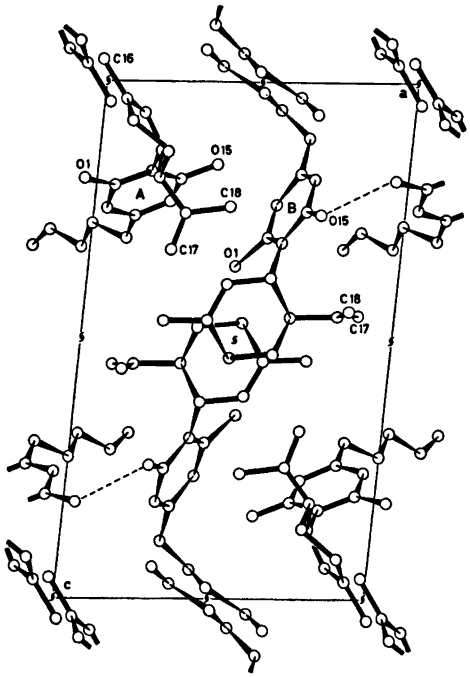


Fig. 2. The molecular packing in the unit cell, as seen down the *b*-axis.

A packing diagram of the crystal is shown in Fig. 2. The molecules are kept together mainly by van der Waals forces. Although there are four free phenolic groups in the asymmetric unit, only one hydrogen bond is formed. This is from the phenolic group O1 in molecule A to the phenolic oxygen O15 in molecule B in the neighbour cell. The O1A···O15B distance is 2.861(8) Å. There are no other *inter*-molecular contacts between nonhydrogen atoms shorter than 3.5 Å.

Note added in proof. An independent X-ray crystallographic structure determination of cannabidiol has been published: Jones, P. G., Falvello, L., Kennard, O., Sheldrick, G. M. and Mechoulam, R. *Acta Crystallogr. B 33* (1977) 3211. The results do not differ significantly.

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Studies on Electrolytic Substitution Reactions. XII. Synthesis of 4-Alkoxy-4′-cyanobiphenyls — a Class of Liquid Crystals — *via* Anodic Cyanation of 4,4′-Dialkoxybiphenyls in Emulsion Systems

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The anodic cyanation of 4,4′-dialkoxybiphenyls has been studied in an emulsified system consisting of dichloromethane/tetrabutylammonium sulfate, substrate and aqueous sodium cyanide. Good yields of 4′-alkoxy-4-cyanobiphenyls (among which an important class of liquid crystals is included) have been obtained, besides minor amounts of products originating from addition of cyanide ion, hydrogen substitution and coupling. The displaced alkoxy group appears as a mixture of the corresponding alcohol and aldehyde, indicating a one-electron process.

The anodic cyanation of aromatic ethers in non-nucleophilic solvents is known to give mainly products originating from substitution of either a ring hydrogen or an alkoxy group. The ratio between these types of products is dependent upon other substituents in the ring. As an example, 1,4-dimethoxybenzene mainly undergoes substitution of a methoxy group whereas 1,3-dimethoxybenzene only gives substitution of ring hydrogens.[1,2] With methanol as solvent, anodic addition of one methoxy and one cyano group across the double bond system occurs in competition with substitution.[3]

We have earlier shown that the use of an emulsified system containing aqueous sodium cyanide, dichloromethane and tetrabutylammonium sulfate is superior to other solvent supporting electrolyte systems used in anodic cyanation reactions.[2,4] The work described in the following report was undertaken in order to demonstrate the synthetic utility of electroorganic methods in general and emulsion electrolysis in particular. The aim of the work was to synthesize 4-alkoxy-4-cyanobiphenyls. Compounds of this class have earlier been synthesized in a five-step reaction sequence, starting from 4-nitrobiphenyl, with an overall yield of 5.5 – 14 %.[5] These compounds belong to a class of substances which can form nematic liquid crystals.

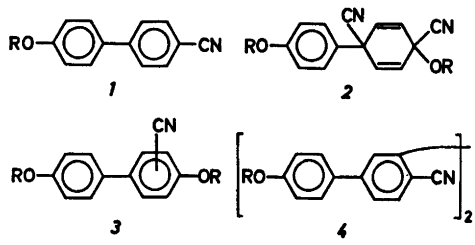
RESULTS

The emulsion electrolyses were performed as described previously,[2,4] using 4,4′-dibutoxy- or 4,4′-dioctyloxybiphenyl as substrates.

4,4′-Dibutoxybiphenyl. Products (*1* – *4*, R = Bu) are shown below and yields are given in Table 1. 4-Butoxy-4′-cyanobiphenyl (*1*) is formed in good material yield without any dependence on current density and degree of conversion (1.8 F/mol gives 86 % conversion of the starting material). The selectivity of the reaction is reasonably good. The ratio between butoxy-substituted product (*1*) and hydrogen-substituted product (*3*) is around 7. This ratio is usually higher for similar systems when the reaction is run in acetonitrile/tetraethylammonium cyanide.[1] Comparison with runs in this system was, however, not possible due to the low solubility of the substrate in acetonitrile.

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Table 1. Anodic cyanation of 4,4′-dibutoxybiphenyl.



Amount of substrate/mmol	Current/ A	Current density/ mA cm^{-2}	Charge/ F mol^{-1}	Yield/% Current	Material[a]	Product (R = butyl)
10	0.15	1.9	1	37	66	*1*
					11	*2*
					9	*3*
10	0.15	1.9	1.8	35	64	*1*
					13	*2*
					8	*3*
					2[b]	*4*
10	0.5	6.25	1.8	29	62	*1*
					16	*2*
					9	*3*

[a] Based on consumed starting material and determined by GLC. [b] Isolated yield.

The yield of the addition product (*2*) increases somewhat with current density and is only little affected by an increase in the degree of conversion. Bis-cyano adducts have not earlier been found in the anodic cyanation of benzene derivatives. An analogous product, 1,2,3,5-tetraphenyl-2,5-dicyano-Δ^3-pyrroline, has been reported from the cyanation of 1,2,3,5-tetraphenylpyrrole.[6] A nitrile-isonitrile adduct was formed in the anodic addition of cyanide ion to 9,10-dialkylanthracenes.[7]

The dimer (*4*), formed in 2 % yield in one experiment, was not sought for in the other experiments given in Table 1 since it could not be analyzed by GLC.

The current yields given in Table 1 are calculated on the basis of a one-electron process. This assumption is discussed further below. The low current yields are probably due to concurrent oxidation of cyanide ion and/or water.

4,4′-Dioctyloxybiphenyl. Yields and products (*1*, R = C_8H_{17}) are given in Table 2. The influence of current density upon the product distribution is similar to that found for 4,4′-dibutoxybiphenyl. The by-products were not isolated and their yields were estimated by assuming that their GLC response factors relative to an internal standard are the same as for the starting material.

The second experiment in Table 2 was run in a divided cell in an argon atmosphere in order to protect the products originating from the alkoxy group from being either reduced at the cathode or oxidized by oxygen from the air. As is seen in Table 2 both octanal and 1-octanol were found in the reaction mixture. The somewhat lower combined yield of these products compared to that of *1*, R = C_8H_{17} is due to the fact that a rather large volume of dichloromethane must be removed by *in vacuo* evaporation before GLC analysis can be performed. During this procedure the more volatile products, octanal and octanol, inevitably are lost to a higher extent than *1*, R = C_8H_{17}.

Structure assignments. The structures of the main products in the reaction were readily assigned from the spectroscopic data given in the experimental part.

Two of the by-products were isolated in amounts large enough for structure determination with spectroscopic methods. The main

Table 2. Anodic cyanation of 4,4′-dioctyloxybiphenyl.

Amount of substrate/mmol	Current/ A	Current density/ mA cm^{-2}	Charge/ F mol^{-1}	Yield/% Current	Material[a]	Product (R = octyl)
5	0.15	1.9	1.8	36	78	*1*
					15	Other products[c]
5[b]	0.15	1.9	1.8	29	76	*1*
					20	[c]
					29	Octanal
					18	Octanol
5	0.50	6.25	1.8	24	70	*1*
					20	[c]

[a] Based on consumed starting material and determined by GLC. [b] Divided cell. [c] According to GLC/MS at least one dicyanooctyloxybiphenyl, one cyanodioctyloxybiphenyl, and one dicyano adduct.

by-product was isolated by column chromatography. MS showed the M^+ peak at *m/e* 350 which is a parent ion equivalent to the addition of two cyano groups to 4,4′-dibutoxybiphenyl. The IR spectrum showed a nitrile band at 2240 cm^{-1}. The ^{1}H NMR spectrum showed two nonequivalent $-O-CH_2-$ triplets, a singlet at δ 6.15 integrating for four protons and a typical pattern of a 1,4-disubstituted benzene at δ 7.1, also integrating for four protons. This shows that the addition occurred over one of the phenyl groups, giving a cyclohexadiene derivative. The singlet at δ 6.15 originates from the olefinic protons and the only possible structure with four olefinic protons is the one given for product *2*. The ^{13}C NMR spectrum showed four signals with high intensity in the olefinic/aromatic region originating from four pairwise equivalent carbon atoms in the aromatic ring and four pairwise equivalent carbon atoms in the cyclohexadiene ring, all carrying a hydrogen substituent. This is in accordance with structure *2*. Whether the cyano groups are situated *cis* or *trans* has not been investigated.

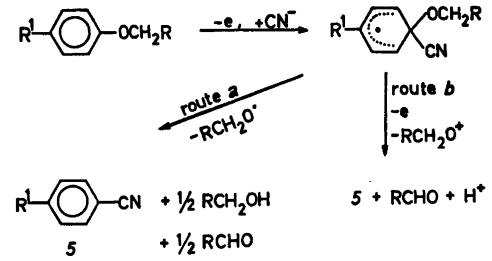
The isolated dimeric product was, according to MS, a dimer of 4-butoxy-4′-cyanobiphenyl. The ^{1}H NMR spectrum showed two groups of signals in the aromatic region, one typical pattern of a 1,4-disubstituted benzene and one multiplet. The multiplet is shifted downfield upon addition of Eu(fod)$_3$, which can bind either to the cyano group or to the oxygen on the butoxy group. By comparing the effect of Eu(fod)$_3$ on 4-methylbenzonitrile and 4-methoxytoluene it was established that the ring protons of 4-methylbenzonitrile were more strongly influenced than the ring protons of 4-methoxytoluene.* This demonstrates that the multiplet in the spectrum of the dimer originates from the rings with the cyano substituent and the bond between the two monomeric units. The ^{13}C NMR spectrum showed ten signals in the aromatic region indicating that the product was one of the two symmetrically coupled isomers. The signals were assigned by comparison between the experimental shifts and shifts calculated from literature data on substituted benzenes.[8] The 3,3′-coupled isomer was the only one giving a good correspondence between calculated and experimental shifts. Hence the isolated dimer was assigned the structure of 4,4‴-dibutoxy-4′,2″-dicyano-1,1′:3′,1″:5″,1‴-quadriphenyl (*4*).

DISCUSSION

It has earlier been postulated [1] but not proved that the substitution of a methoxy group for a cyano group in the anodic cyanation of methoxybenzenes proceeds *via* loss of formaldehyde and a proton from a cyclohexadienyl cation, as exemplified by the formation of 4-methoxybenzonitrile from 1,4-dimethoxybenzene (route *b*, Scheme 1, R′ = CH_3O, R = H).

The results given above show that apart from the expected aldehyde, the corresponding alcohol is also formed in the cyanation of

* This is contrary to what has been found for aliphatic ethers and nitriles with Eu(DPM)$_3$.[9]



Scheme 1.

4,4′-dioctyloxybiphenyl. This result does not fit with the two-electron mechanism depicted in route *b*, Scheme 1. In order to rationalize our results we instead suggest the mechanism shown in route *a*. The initially formed radical cation reacts as usual with cyanide ion to form a cyclohexadienyl radical, which then loses an alkoxy radical with formation of the cyano substitution product. The alkoxy radical undergoes a disproportionation reaction to give alcohol and aldehyde. This means that the conversion of 4,4′-dialkoxybiphenyl to 4-cyano-4′-alkoxybiphenyl is a one-electron process. The fact that more aldehyde than alcohol is formed indicates that the leaving radical to some extent is further oxidized to the cation which gives rise to the excess of aldehyde. A control experiment showed that 1-octanol cannot be oxidized to octanal under the experimental conditions used in the cyanation reaction.

A case where an alkoxy radical is cleaved off from a radical cation has been described by Utley *et al.*[10] When dibenzyl ether was oxidized the radical cation formed was cleaved to give a benzyl cation and a benzyloxy radical. The radical then underwent disproportionation to give the corresponding alcohol and aldehyde.

Finally, it should be noted that displacement of alkoxy by cyano groups in biphenyl derivatives can also be effected photochemically *via* a radical cation mechanism. As an example, photolysis of 4,4′-dimethoxybiphenyl in *t*-BuOH/water (1:3) in the presence of cyanide (0.1 M) gave *1*, R = CH_3 and 4,4′-dicyanobiphenyl in 28 and 15 % yield, respectively.[11]

EXPERIMENTAL

General. IR spectra were recorded with a Perkin-Elmer Model 257 grating spectrometer. ^{1}H NMR spectra were recorded with either a Varian A 60 spectrometer or a Jeol MA 100 spectrometer. ^{13}C NMR spectra were recorded with a Jeol FX 60 spectrometer. Mass spectra were recorded with an LKB 9000 spectrometer coupled to a gas chromatograph. GLC analysis was performed using a Hewlett-Packard 5830 GLC system equipped with either a 1.5 m × 3 mm 3 % OV-1 column or a 2 m × 3 mm OV-17 column.

Chemicals. Solvents, reagents, and supporting electrolytes used in this work were commercially available reagent grade chemicals.

4,4′-Dialkoxybiphenyl. 4,4′-Biphenol (0.01 mol) was dissolved in DMF (100 ml) and pulverized potassium hydroxide (0.03 mol) was added. The mixture was stirred for 10 min before the alkyl bromide (0.03 mol) was added. After stirring for 2.5 h identical amounts of potassium hydroxide and alkyl bromide were added in the same fashion. The mixture was stirred overnight. The white precipitate formed was collected. The mother liquor was diluted with water and yielded a second crop of crystals. The combined crystal fractions were washed with water and dried. The product was recrystallized from ether.

4,4′-Dibutoxybiphenyl. Yield 93 %, m.p. 152–153 °C. ^{1}H NMR: δ 1.0 (6 H, t), 1.7 (8 H, m), 4.0 (4 H, t), 7.25 (8 H, typical pattern of 1,4-disubstituted benzene). MS [*m/e* (% rel. int.)]: 299 (14), 298 (59), 242 (16) 187 (13), 186 (100), 185 (17). (Found: C 80.0; H 8.78. Calc. for $C_{20}H_{26}O_2$: C 80.5; H 8.72).

4,4′-Dioctyloxybiphenyl. Yield 91 %, m.p. 116–117 °C. ^{1}H NMR: δ 0.8–2.0 (30 H, m), 4.0 (4 H, t), 7.2 (8 H, typical pattern of 1,4-disubstituted benzene).

MS [*m/e* (% rel. int.)]: 411 (31), 410 (100), 298 (14), 187 (89), 185 (18). (Found: C 81.8; H 10.2. Calc. for $C_{28}H_{41}O_2$: C 81.9; H 10.31).

Electrolysis equipment. Electrolyses were carried out in a water-jacketed vessel, 800 ml in volume, equipped with a platinum foil anode (80 cm^2) and either a stainless steel rod or a platinum wire cathode. The electrodes were arranged concentrically. An Ultra-Turrax[12] type high-speed stirring device was placed "behind" the cathode. Constant current was maintained with either a Radiak or a Philips power supply.

General electrolysis procedure. Substrate amounts, current and charge passed are given in Tables 1 and 2. The electrolyte consisted of substrate, dichloromethane (230 ml), 1 M sodium cyanide (230 ml) and neutralized tetrabutylammonium hydrogen sulfate (20 mmol) dissolved in water (15 ml). A given amount of charge was passed at constant current. When the electrolysis was started the stirrer had to be turned on for a few seconds and then turned off and left for 30 s and then turned on again. After this procedure the potential drop over the cell increased from around 4 V to 7–15 V. This behavior is probably due to a wetting phenomenon.

Work-up procedure. I. The two phases were allowed to separate and the organic layer was evaporated to dryness. The residue was taken up in ether and the ether solution washed twice with water to remove the supporting electrolyte. Ten percent of the ether solution was used for yield determination by GLC and the remaining 90 % was used for the isolation of products. The remaining starting material was precipitated by cooling the solution in Dry Ice/acetone and filtered off. The mother liquor was then evaporated to dryness and the residue was boiled with pentane leaving an insoluble tarry residue. The pentane extract was reduced in volume and chromatographed on a silica column with pentane/ether (95/5) as eluent.

II. An alternative route to isolate the main product was to cool the pentane solution in Dry Ice/acetone and collect the precipitate formed.

4,4'-Butoxycyanobiphenyl. (*1*, R = Bu). The product was isolated by column chromatography and recrystallized from pentane, yield 37 %, calculated on the basis of unrecovered starting material, m.p. 77 – 78 °C.

^{1}H NMR: δ 1.0 (3 H, t), 1.6 (4 H, m), 4.0 (2 H, t), 7.3 (4 H, typical pattern of a 1,4-disubstituted benzene), 7.7 (4 H, s). The signal at δ 7.7 was shifted downfield and split into the typical pattern of a 1,4-disubstituted benzene upon addition of Eu(fod)$_3$.

^{13}C NMR [15.0 MHz, $CDCl_3$]: 145.0 (C1), 128.2 (C2), 132.3 (C3), 118.9 (C4), 131.0 (C1'), 126.9 (C2'), 114.9 (C3'), 159.7 (C4'), aliphatic carbons 67.7 (C8'), 31.2 (C9'), 19.2 (C10'), 13.8 (C11'). IR: 2220 cm^{-1}. MS [*m/e* (% rel. int.)]: 252 (4), 251 (22), 196 (14), 195 (100). Anal. $C_{17}H_{17}NO$: C, H, N.

1-Butoxy-4-[4-butoxyphenyl]-1,4-dicyano-2,5-cyclohexadiene. (*2*, R = Bu). The substance was isolated by column chromatography and recrystallized from pentane, yield 1 %, m.p. 79 – 80 °C.

^{1}H NMR: δ 0.9 (6 H, m), 1.5 (8 H, m), 3.65 (2 H, t), 3.9 (2 H, t), 6.15 (4 H, s), 7.1 (4 H, typical pattern of a 1,4-disubstituted benzene).

^{13}C NMR [15.0 HMz, $CDCl_3$]: 42.0 (C1), 130.9 (C2), 123.6 (C3), 65.6 (C8), 31.7 (C9), 19.2 (C10,10'), 13.8 (C11,11'), 126.4 (C1'), 127.5 (C2'), 115.2 (C3'), 159.5 (C4'), 67.9 (C8'), 31.2 (C9'), 117.6 (C_{CN}), 116.8 (C_{CN}).

IR: 2240 cm^{-1}. MS [*m/e* (% rel. int.)]: 351 (2), 350 (69), 294 (19), 277 (13), 238 (32), 222 (17), 221 (100), 220 (16), 211 (30), 183 (13).

4,4'-Dibutoxycyanobiphenyl. (*3*, R = Bu). A few mg were isolated by column chromatography and recrystallized from pentane. GLC analysis of this product showed only one peak. M.p. 71 – 72 °C. IR: 2220 cm^{-1}. MS [*m/e* (rel. int. %)]: 324 (12), 323 (45), 267 (23), 220 (13), 212 (14), 211 (100).

4,4'''-Dibutoxy-4',2''-dicyano-1,1':3',1'':5'',1'''-quadriphenyl. (*4*, R = Bu). This dimer was isolated from the tarry residue that was insoluble in pentane. The tarry material was boiled with methanol in which the dimer was insoluble and could be filtered off. Recrystallization from ether gave 100 mg of crystals, m.p. 156 – 157 °C. IR: 220 cm^{-1} (s).

^{1}H NMR: δ 0.9 (6 H, t), 1.6 (8 H, m), 4.0 (4 H, t), 7.3 (8 H, typical pattern of 1,4-disubstituted benzene), 7.8 (6 H, m). The multiplet at 7.8 ppm is shifted downfield upon addition of Eu(fod)$_3$.

^{13}C NMR [15.0 MHz, $CDCl_3$]: δ 130.7 (C1), 126.9 (C2), 115.1 (C3), 160.0 (C4), 145.4 (C1'), 128.6 (C2'), 142.0 (C3'), 118.1 (C4'), 134.0 (C5'), 128.6 (C6'), aliphatic carbons 67.7 (C8), 31.2 (C9), 19.2 (C10), 13.9 (C11).

MS [*m/e* (% rel. int.)]: 502 (8), 501 (29), 500 (78), 445 (6) 444 (16), 389 (29), 388 (100) 387 (16). Anal. $C_{34}H_{32}N_2O_2$: C, H, N.

4-Cyano-4'-octyloxybiphenyl. (*1*, R = C_8H_{17}). The compound was isolated according to work-up procedure II and recrystallized from pentane. The isolated yield from an electrolysis on the 10 mmol scale was 35 %, based on consumed starting material. Anal. $C_{21}H_{25}NO$: C, H, N.

^{1}H NMR: δ 0.82 (3 H, t), 1.3 (12 H, m), 4.0 (2 H, t), 7.0 (2 H, d), 7.5 (2 H, d), 7.65 (4 H, s). The singlet at 7.65 is shifted downfield and split into two doublets upon addition of Eu(fod)$_3$. IR: 2220 cm^{-1} (s). MS: [*m/e* (% rel. int.)]: 308 (6), 307 (19), 196 (16), 195 (100), 166 (5).

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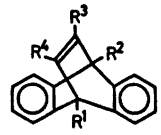
Chlorinated Polycyclic Compounds. III. Preparation and Solvolysis of Some Chloro Derivatives of Dibenzobicyclo[2,2,2]octatriene

TAPIO MIETTINEN

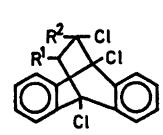
Department of Chemistry, Helsinki University of Technology, SF-02150 Otaniemi, Finland

Chloro derivatives of dibenzobicyclo[2,2,2]octatriene on solvolysis in H_2SO_4-HOAc mixtures gave derivatives of dibenzobicyclo[2,2,2]-octadien-7-ol acetate, dibenzobicyclo[2,2,2]octadien-7-one and dibenzobicyclo[3,2,1]octadien-4-one, the ratio of rearranged to unrearranged product being dependent on H_2SO_4 concentration.

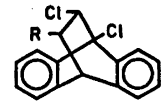
In connection with earlier studies [1] it was shown that the chloro olefin *1f* on hydrolysis in a 60:40 mixture of sulfuric acid and acetic acid gives the rearranged ketone *6c* as the only reaction product. As, however, it was found that in milder acid also the unrearranged hydrolysis product *7d* is formed, the reactions of the chlorides *1a*–*1g* were studied in some more detail. The starting compounds were synthetized by elimination of hydrogen chloride or chlorine from the saturated chlorides. *1f*, *1g* and *1d* were obtained from *2b*, *2c* and *4c*, respectively, by refluxing with 10 % ethanolic potassium hydroxide and *1b* from *5c* with potassium *t*-butoxide in dimethyl sulfoxide. Although *1e* could be prepared from *1f* by hydrogenation and subsequent dehydrochlorination, the reaction with zinc and acetic acid proved a far more convenient method and gave *1e* in a very pure state. Compounds *1a* [2] and *1c* [3] are known. It may be pointed out that the reactivity of the bridge chlorine atoms towards bases is greatly enhanced because of the inductive effect of the bridgehead chlorine atoms. The cleavage of hydrogen chloride from the corresponding 1,4-unsubstituted compounds requires the use of elevated temperatures [4] or a stronger base. [2] Similarly, the dechlorination of the latter has been accomplished with sodium in alcohol [4,5] or ether [6] solvents, zinc being almost ineffective. The saturated chlorides result from different reactions: *2b* from the Diels-Alder reaction of 9,10-dichloroanthracene and *trans*-1,2-dichloroethylene,[7] *2c* from the addition of chlorine to *1f*, *5c* from the reaction of 1-chloro-*endo*-4-hydroxydibenzobicyclo[3,2,1] octadiene with thionyl chloride [1] and *4c* from the Diels-Alder reaction of 9-chloroanthracene and *trans*-1,2-dichloroethylene. The last prep-



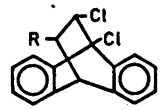
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b	Cl	H	H	H
c	H	H	Cl	Cl
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e	Cl	Cl	H	H
f	Cl	Cl	H	Cl
g	Cl	Cl	Cl	Cl



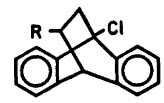
	R^1	R^2
2a	H	H
b	Cl	H
c	Cl	Cl



3a	R = OH
b	R = OAc
c	R = Cl

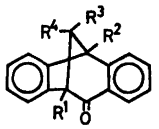


4a	R = OH
b	R = OAc
c	R = Cl

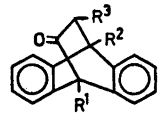


5a	R = OH
b	R = OAc
c	R = Cl

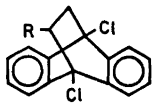
aration was not found to be very convenient, because the yields are only moderate and the purification of the product is rather tedious. However, this method was chosen because the alternative route consisting of addition of chlorine to *1b* gave neither *3c* nor *4c*, but instead, the rearranged chlorides 1-*endo*-4-*syn*-8-trichlorodibenzobicyclo[3,2,1]octadiene and its *exo* epimer.[7] Attempts to synthesize the corresponding chlorides from the alcohols *3a* and *4a* were unsuccessful. Treatment of *3a* with phosphorus pentachloride gave a mixture of the same rearranged trichlorides as above while *4a* gave a mixture of the *anti*-8-isomers.[7] The alcohol *3a* was obtained by reduction of the ketone *7e* by sodium borohydride. This reduction gave only the *cis* chlorohydrin being still more stereoselective than the reduction of the ketone *7c*.[6] The *trans* chlorohydrin *4a* was prepared by acid catalyzed rearrangement of 1-*anti*-8-dichloro-*endo*-4-hydroxydibenzobicyclo[3,2,1]-octadiene [7] followed by hydrolysis of the acetate.



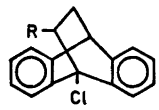
	R^1	R^2	R^3	R^4
6a	H	Cl	H	H
b	Cl	H	H	H
c	Cl	Cl	H	H
d	Cl	Cl	Cl	H
e	Cl	Cl	H	Cl



	R^1	R^2	R^3
7a	H	H	H
b	Cl	H	H
c	H	H	Cl
d	Cl	Cl	H
e	H	Cl	Cl
f	Cl	Cl	Cl



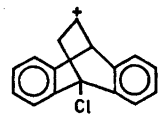
8a	R = OH
b	R = OAc
c	R = H



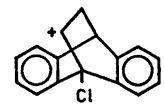
9a	R = OH
b	R = OAc

To study the solvolysis of *1a* – *1g* the chlorides were refluxed with sulfuric acid-acetic acid mixtures of varying concentration. *1d* gave mixtures of the isomeric ketones *6b* and *7b*, the former being the major component. The ratio of rearranged to unrearranged product was found to be dependent on the sulfuric acid concentration but not on the reaction time. On the other hand, the reaction time necessary for complete conversion of the starting material increased rapidly with decreasing acid concentration. *1f* behaved in the same manner (*Fig. 1*) but with the tetrachloride *1g* the formation of the unrearranged ketone *7f* was not observed. Obviously, to obtain *7f*, long reaction times are necessary to allow for lower acidity, for the reaction of *1g* with 30 % sulfuric acid for 96 h gave only 4 % of *6d* and 4 % of *6e* in addition to unchanged starting material. The structures of *7b* and *7d* were confirmed by their spectra and by reduction to the alcohols *9a* and *8a*. The ^{1}H NMR spectra of the alcohols contain typical ABX patterns with coupling constants characteristic of the dibenzobicyclo [2,2,2]octadiene system.[8] The solvolysis of *1a* and *1c* gave the unrearranged ketones *7a* [8,9] and *7c* [6] as the only reaction products. The ketone *6a*, identical with that obtained from *6c* by dechlorination,[1] was formed quantitatively from *1e* and the acetate *5b* [1] was obtained from *1b* as the sole reaction product.

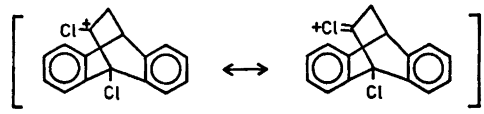
The results show that the features governing the solvolysis are the site of protonation of the double bond and the possible chloro substituent at the bridgehead carbon atom adjacent to the positive charge. The protonation occurs in a direction to give the more stable carbonium



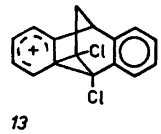
10



11



12



13

ion. In the case of *1b* it is *10* because of the inductive effect of the chlorine atom, but when a chlorine is present in the etheno bridge, the unfavorable inductive effect is outweighed by the possibility of resonance stabilization, as in *12*. Rearranged products are obtained only when the bridgehead carbon atom next to the positive charge is chloro substituted (compounds *1d – 1g*). The intermediate ion *13* [10] has a chlorine atom in the three-membered ring and attack by a nucleophile leads to a ketone *via* an irreversible reaction step. Unlike *12* and *13*, the ions *10* and *11* are not interconvertible.

The fact that the 1,7-dichlorosubstituted compounds give both of the isomeric ketones, can be ascribed to a competition between hydrolysis and rearrangement of the ion *12*. Increased acidity causes a decrease in the H_2O/H_3O^+ ratio and consequently a lower rate of direct hydrolysis (Fig. 1).

EXPERIMENTAL

For general experimental conditions see Ref. 7. Sulfuric acid (95 – 98 %) and acetic acid (99 – 100 %) used for the solvolysis reactions were of commercial quality.

Reaction of 9-chloroanthracene with trans-*1,2-dichloroethylene*. A mixture of 21.2 g (0.1 mol) of 9-chloroanthracene [11] and 291 g (3.0 mol) of *trans*-1,2-dichloroethylene were heated at 210 °C for 24 h in a 1 l stainless steel pressure vessel. Excess dichloroethylene was removed under reduced pressure, the residue dissolved in 300 ml of light petroleum and the solution filtered. After drying the insoluble part weighed 10.5 g and removal of the solvent from the filtrate left 12.5 g of heavy brown oil. TLC and ^1H NMR examination revealed the presence of several components, among which the expected normal adduct *4c* amounted to about 55 %. Separation by preparative TLC (several elutions with light petroleum) gave 6.8 g (22 %) of *trans*-1,7,8-trichlorodibenzobicyclo-[2,2,2]octadiene (*4c*), m.p. 135 °C, δ 4.23 (3 H, m) + 8 Ar-H, *m/e* 308(0.3), 212(100). Other components were not isolated.

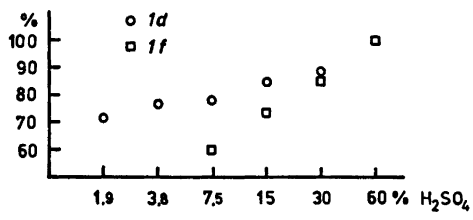


Fig. 1. The percentage of rearranged product as a logarithmic function of H_2SO_4 concentration.

Hydrogenation of 1f. A mixture of 3.0 g of *1f* [1] and 0.4 g of 5 % Pd/C in 50 ml of ethyl acetate was hydrogenated for 5 h under a pressure of 800 mm H_2O over the atmospheric pressure. The solution was filtered and evaporated and the two major components separated by TLC (elution with light petroleum) and purified by crystallization from MeOH. There were obtained 0.7 g (24 %) of 1,4,7-trichlorodibenzobicyclo-[2,2,2]octadiene (*2a*), m.p. 119 °C, δ 2.37 (*cis*-8-H), 2.80 (*trans*-8-H), 4.17 (7-H) + 8 Ar-H, *cis* $J_{7,8} = 9.0$ Hz, *trans* $J_{7,8} = 3.5$ Hz, $J_{8,8} = 12.7$ Hz and 1.6 g (62 %) of 1,4-dichlorodibenzobicyclo[2,2,2]octadiene (*8c*), m.p. 123 °C (lit.[12] 125 – 127 °C), δ 2.17 (4 H, s) + 8 Ar-H.

Elimination of hydrogen chloride from 2a, 2c, 4c *and* 5c. The chloride (0.4 g of *2a* or *2c*) was added to a solution of 1.0 g of KOH in 10 ml of EtOH and the mixture refluxed for 40 min. The cooled mixture was acidified with HCl and extracted twice with ether. The ethereal solution was dried and evaporated. The product was essentially pure in each case. *2a* gave 1,4-dichlorodibenzobicyclo[2,2,2]octatriene (*1e*), m.p. 92 °C, δ 6.72 (2 H, s) + 8 Ar-H and *2c* gave 1,4,7,8-tetrachlorodibenzobicyclo[2,2,2]-octatriene (*1g*), m.p. 200 °C, only Ar-H. Otherwise similarly, but using a reaction time of 160 min, *4c* gave 1,7-dichlorodibenzobicyclo-[2,2,2]octatriene (*1d*), m.p. 95 °C, δ 4.83 (4-H), $J_{4,8} =$ 6.6 Hz, 8-H in the aromatic multiplet. To eliminate HCl from *5c*, 1.0 g of the chloride and 1.0 g of *t*-BuOK were dissolved in 20 ml of DMSO and the mixture stirred for 4 h at room temperature. Water and HCl were added and the reaction product isolated by ether extraction to give 1-chlorodibenzobicyclo[2,2,2]-octatriene (*1b*), m.p. 116 °C, δ 4.90 (4-H), 7- and 8-H in the aromatic multiplet, $J_{4,8} = 6.0$ Hz, $J_{4,7} = 2.0$ Hz. Analytical samples of the unsaturated chlorides were prepared by TLC (elution in light petroleum) and crystallization from EtOH.

Dechlorination of 2b *with zinc and acetic acid*. A mixture of 30 g of *2b*,[7] 20 g of Zn-powder and 200 ml of acetic acid was refluxed for 20 h. The solution was filtered hot, evaporated, the residue dissolved in acetone and filtered through 10 g of silica gel. The acetone was removed and the residue dissolved in 50 ml of 80 % aqueous EtOH, from which the product soon crystallized to yield 18.6 g (78 %) of *1e*.

Addition of chlorine to 1f. A solution of 10 g of *1f* in 100 ml of dry CCl_4 was saturated with chlorine at room temperature. The reaction took place rapidly with evolution of heat. To complete the reaction, the solution was allowed to stand overnight. After removal of the solvent a quantitative yield of 1,4,7,7,8-pentachlorodibenzobicyclo[2,2,2]octadiene (*2c*) was obtained. M.p. 156 °C δ 4.81 (8-H) + 8 Ar-H.

Addition of chlorine to 1b. A solution of 0.1 g of *1b* in 10 ml of dry CCl_4 was saturated with chlorine at 0 °C and the solution allowed to stand for 40 min. The solvent was removed under reduced pressure. According to ^{1}H NMR the residue contained about 85 % of 1-*exo*-4-*syn*-8-trichlorodibenzocyclo[3,2,1]octadiene and 15 % of the *endo* epimer.[7]

Reduction of the ketones 7b, 7d *and* 7e *with sodium borohydride.* A solution of 10 mmol of the ketone (2.55 g of *7b*, 2.89 g of *7d* or *7e* [7]) and 0.38 g (10 mmol) of $NaBH_4$ in 100 ml of EtOH was stirred for 40 min at room temperature. Water and HCl were added and the product isolated by ether extraction. The analytical samples were crystallized from 80 % aqueous EtOH. *7b* gave 1-chlorodibenzobicyclo[2,2,2]octadien-7-ol (*9a*), m.p. 70 °C ν_{max} 3250 cm^{-1}, δ 1.52 (*cis*-8-H), 2.33 (*trans*-8-H), 3.94 (7-H), 4.12 (4-H), 2.05 (OH) + 8 Ar-H, *cis* $J_{7,8}$ = 8.8 Hz, *trans* $J_{7,8}$ = 2.3 Hz, $J_{8,8}$ = 12.5 Hz, $J_{4,8}$ = 2.4 Hz and *7d* gave 1,4-dichlorodibenzobicyclo[2,2,2]octadien-7-ol (*8a*), m.p. 101 °C, ν_{max} 3330 cm^{-1}, δ 1.98 (*cis*-8-H), 2.57 (*trans*-8-H), 3.97 (7-H), 2.10 (OH) + 8 Ar-H *cis* $J_{7,8}$ = 8.8 Hz, *trans* $J_{7,8}$ = 2.8 Hz, $J_{8,8}$ = 12.4 Hz. *7e* gave 1,7-dichlorodibenzobicyclo[2,2,2]-octadien-*cis*-8-ol (*3a*), m.p. 171 °C, ν_{max} 3530 cm^{-1}, δ 4.39 (*trans*-7-H), 4.11 (8-H), 4.33 (4-H), 1.83 (OH) + 8 Ar-H, *cis* $J_{7,8}$ = 7.8 Hz, $J_{4,8}$ = 2.4 Hz. By TLC and ^{1}H NMR examination, the presence of the *trans* isomer was not detected.

Acetylation of the alcohols 9a, 8a *and* 3a. The alcohol *9a* (0.5 g) refluxed for 80 min with a mixture of 10 ml of Ac_2O and 1 ml of pyridine, gave the acetate *9b*, m.p. 151 °C, ν_{max} 1735 cm^{-1}, δ 1.53 (*cis*-8-H), 2.47 (*trans*-8-H), 5.07 (7-H), 4.19 (4-H), 1.89 (OAc) + 8 Ar-H, *cis* $J_{7,8}$ = 8.6 Hz, *trans* $J_{7,8}$ = 2.6 Hz, $J_{8,8}$ = 12.7 Hz, $J_{4,8}$ = 2.5 Hz. Similarly, *8a* gave *8b*, m.p. 143 °C, ν_{max} 1750 cm^{-1}, δ 1.97 (*cis*-8-H), 2.82 (*trans*-8-H), 5.09 (7-H), 1.87 (OAc) + 8 Ar-H, *cis* $J_{7,8}$ = 8.8 Hz, *trans* $J_{7,8}$ = 2.6 Hz, $J_{8,8}$ = 12.5 Hz and *3a* gave *3b*, m.p. 185 °C, ν_{max} 1738 cm^{-1}, δ 4.41 (*trans*-7-H), 5.09 (8-H), 4.30 (4-H), 1.95 (OAc) + 8 Ar-H, *cis* $J_{7,8}$ = 8.2 Hz, $J_{4,8}$ = 2.5 Hz. The analytical samples were crystallized from 80 % aqueous EtOH.

Synthesis of 4a *and* 4b. A solution of 1.0 g of 1-*anti*-8-dichlorodibenzobicyclo[3,2,1]octadien-*endo*-4-ol,[7] 10 g of H_2SO_4 and 40 g of HOAc was refluxed for 20 h. The solution was diluted with water and the product isolated by ether extraction. According to ^{1}H NMR, the reaction yielded almost quantitatively 1,7-dichlorodibenzobicyclo[2,2,2]octadien-*trans*-8-yl acetate (*4b*), m.p. 165 °C, ν_{max} 1737 cm^{-1}, δ 3.98 (*cis*-7-H), 5.05 (8-H), 4.46 (4-H), 1.90 (OAc) + 8 Ar-H, *trans* $J_{7,8}$ = 1.7 Hz, $J_{4,8}$ = 3.0 Hz.

The acetate *4b* (0.5 g) hydrolyzed with 20 ml of 10 % ethanolic KOH for 80 min at room temperature gave the alcohol *4a*, m.p. 131 °C, ν_{max} 3310 cm^{-1}, δ 3.74 (*cis*-7-H), 4.13 (8-H), 4.13 (4-H), 1.81 (OH) + 8 Ar-H, *trans* $J_{7,8}$ = 1.2 Hz. The analytical samples of *4a* and *4b* were crystallized from 80 % aqueous EtOH.

Reactions of 3a *and* 4a *with phosphorus pentachloride.* The alcohol *3a* (0.2 g) was refluxed for 10 min with a mixture of 1.0 g of PCl_5 and 1.0 g of $POCl_3$. Excess reagent was carefully decomposed with water and the product isolated by ether extraction. According to ^{1}H NMR, the product contained 80 % of 1-*exo*-4-*syn*-8-trichlorodibenzobicyclo[3,2,1]octadiene and 20 % of the *endo* epimer. Similar treatment of *4a* gave 95 % of 1-*exo*-4-*anti*-8-trichlorodibenzobicyclo[3,2,1]octadiene and 5 % of the *endo* epimer.[7]

Solvolysis of the unsaturated chlorides 1a – 1g. The general method: A mixture of 1.0 g of the chloride, 6 g of H_2SO_4 and 14 g of HOAc was refluxed for 80 min. The hot reaction mixture was poured into ice water and the product isolated by ether extraction. The yields were estimated from the ^{1}H NMR spectra. Following compounds were obtained (starting material, percentage of products): *1a*, 100 % of *7a*; *1b*, 100 % of *5b*; *1c*, 100 % of *7c*; *1d*, 89 % of *6b* and 11 % of *7b*; *1e*, 90 % of *6a* and 10 % of *1e*; *1f*, 85 % of *6c* and 15 % of *7d*; *1g*, 100 % of *1g*. In the cases where a part of the starting material was recovered, longer reaction times were used. Thus *1e* refluxed for 24 h gave 100 % of *6a*, but *1g* refluxed for 96 h gave only 4 % of *6d* and 4 % of *6e*, in addition to unchanged *1g*. A solution of 1.0 g of *1g*, 12 g of H_2SO_4 and 8 g of HOAc refluxed for 24 h gave 47 % of *6d* and 53 % of *6e*. The solvolysis of *1d* and *1f* was studied in different acid concentrations (Fig. 1.) using reaction times sufficient for complete conversion of the starting material (up to 96 h). Further heating did not affect the product ratios. The analytical samples were prepared by TLC (elution with a 1:1 mixture of benzene and light petroleum) and crystallization from EtOH. The ketones *6a*,[1] *6c*,[1] *6d*,[7] *6e* [7] and the acetate *5b* [1] were identical with the compounds obtained from other reactions and the spectral properties and melting points of the ketones *7a* [8,9] and *7c* [6] were in agreement with the values reported in literature. The ketone *7c* had δ 4.04 (7-H), 4.71 (4-H), 4.55 (1-H) + 8 Ar-H, $J_{1,7}$ = 2.7 Hz. The new compounds had the following properties: 5-chlorodibenzobicyclo[3,2,1]octadien-4-one (*6b*), m.p. 159 °C, ν_{max} 1700 cm^{-1}, δ 3.14 (2 H, d), 4.21 (1 H, tr) + 8 Ar-H, $J_{1,8}$ = 2.4 Hz, *m/e* 254(42), 219(100), 1-chlorodibenzobicyclo[2,2,2]-octadien-7-one (*7b*), m.p. 118 °C, ν_{max} 1725 cm^{-1}, δ 2.39 (2 H, d), 4.45 (1 H, tr) + 8 Ar-H, $J_{4,8}$ = 2.5 Hz, *m/e* 254(0.3), 212(100), 1,4-dichlorodibenzobicyclo[2,2,2]octadien-7-one (*7d*), m.p. 117 °C, ν_{max} 1750 cm^{-1}, δ 2.81 (2 H, s) + 8 Ar-H, *m/e* 288(0.9), 246(100).

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Short Communications

Chemistry of *gem*-Dihalocyclopropanes. XII. Preparation of *gem*-Dibromocyclopropyl Ketones and Alkyl *gem*-Dibromocyclopropanecarboxylates under Phase Transfer Conditions

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In connection with a study aiming to uncover some chemical properties of functionalized *gem*-dibromocyclopropanes it became of interest to react several α,β-unsaturated esters and ketones with bromoform under basic conditions. Such reactions have been carried out according to Seyferth's,[1] Doering-Hoffmann's [2] and Makosza's [3] procedures. Using the phase transfer method Dehmlow prepared several substituted 2,2-dichlorocyclopropanecarboxylic acid esters.[4] We have treated α,β-unsaturated esters and ketones with bromoform using the same method in order to prepare the corresponding *gem*-dibromocyclopropane derivatives; the results of these reactions are reported here.[5]

At the time this work was initiated, the addition of dihalocarbenes to α,β-unsaturated ketones under two-phase conditions had not been reported; however, recently Barlet published the preparation of a number of alkyl-substituted 2-acyl-1,1-dibromocyclopropanes in low to excellent yields.[6]

Most of the reactions were carried out with bromoform, but for comparison some substrates were also reacted with chloroform. Usually a 30 – 100 % excess of haloform, a five-fold excess of base, and a reaction time of 16 h were used; in all reactions triethylbenzylammonium chloride (TEBA) was employed as catalyst. The corresponding *gem*-dihalocyclopropane derivatives were formed in variable yields (Tables 1 and 2). The reactions of mesityl oxide, phorone, and methyl cinnamate with bromoform gave no identifiable products.

Despite the excess of haloform and base used, starting material was isolated unchanged in several of the experiments. The recovered materials of 3,4-dimethyl-3-penten-2-one (*5*) and 2,4,5-trimethyl-4-hexen-3-one (*6*), however, were contaminated, the former with small amounts of 3,4-dimethyl-4-penten-2-one (*32*) and the latter with some 2,4,5-trimethyl-5-hexen-3-one (*33*) as well as traces of 2-hydroxy-2,4,5-trimethyl-4-hexen-3-one (*34*).[7]

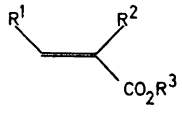
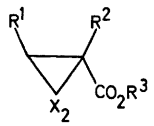
When carvone (*4*) and haloform were exposed to the phase transfer conditions a regioselective reaction took place. Thus, 7,7-dihalo-4-isopropenyl-1-methylbicyclo[4.1.0]heptan-2-one (*10* or *11*) was the only isolable product; no compound arising from addition to the exocyclic double bond was detected. However, from the available data no conclusion can be drawn with respect to the stereospecificity of the reaction.

Compound *11* was also the only product when a mixture of carvone and 2-methyl-2-

Table 1. Alkyl-substituted *gem*-dihalocyclopropyl ketones from α,β-unsaturated ketones and haloform under two-phase conditions.

α,β-Unsaturated ketone	Product	Yield (%)
3-Methyl-3-buten-2-one (*1*)	2-Acetyl-1,1-dibromo-2-methylcyclopropane (*7*)	79
E-3-Methyl-3-penten-2-one (*2*)	*E*-2-Acetyl-1,1-dibromo-2,3-dimethylcyclopropane (*8*)	61
1-Acetylcyclohexene (*3*)	1-Acetyl-7,7-dibromobicyclo[4.1.0]heptane (*9*)	35
Carvone (*4*)	7,7-Dichloro-4-isopropenyl-1-methylbicyclo[4.1.0]heptan-2-one (*10*)	67
	7,7-Dibromo-4-isopropenyl-1-methylbicyclo[4.1.0]heptan-2-one (*11*)	87
3,4-Dimethyl-3-penten-2-one (*5*)	2-Acetyl-1,1-dibromo-2,3,3-trimethylcyclopropane (*12*)	21
2,4,5-Trimethyl-4-hexen-3-one (*6*)	1,1-Dibromo-2-isobutyryl-2,3,3-trimethylcyclopropane (*13*)	30

Table 2. Alkyl *gem*-dihalocyclopropanecarboxylates and other products from the reactions of α,β-unsaturated esters with haloform (CHX_3) under two-phase conditions.

 α,β-Unsaturated ester	X	 Notation	Yield (%)	Other products (% yield)
R^1=H, R^2=R^3=Me (*14*)	Cl	(*20*)	[a]	2,2-Dichloro-1-methylcyclopropanecarboxylic acid (*28*) (60) Methacrylic acid (*29*) (35)
	Br	(*21*)	45 [b]	2,2-Dibromo-1-methylcyclopropanecarboxylic acid (*30*) (42)
R^1=H, R^2=Me, R^3=Et (*15*)	Br	(*22*)	78 [b]	*30* (5)
R^1=H, R^2=Me, R^3=Bu (*16*)	Br	(*23*)	87	
R^1=H, R^2=Ph, R^3=Et (*17*)	Cl	(*24*)	95	
	Br	(*25*)	93	
R^1=R^2=Me, R^3=Et (*18*)	Br	(*26*)	60	
R^1=Ph, R^2=Me, R^3=Et (*19*)	Cl			*E*-2-Methyl-3-phenylpropenoic acid (*31*) (8)
	Br	(*27*)	6	

[a] When an excess of *14* was employed methyl 2,2-dichloro-1-methylcyclopropanecarboxylate (*20*) was formed in 8 % yield. [b] *Cf.* Ref. 12.

butene reacted with bromoform in the usual way, a result that is in accordance with generation of tribromomethylide which subsequently attacks the α,β-unsaturated system prior to formation of dibromocarbene. The intermediate anion reacts further in a fast S_Ni reaction; consistent with this *E*-2-acetyl-1,1-dibromo-2,3-dimethylcyclopropane (*8*), ethyl *E*-2,2-dibromo-1,3-dimethylcyclopropanecarboxylate (*26*) and ethyl *E*-2,2-dibromo-1-methyl-3-phenylcyclopropanecarboxylate (*27*) were formed stereospecifically.

When α,β-unsaturated esters reacted with haloform under phase transfer conditions different types of products were formed (Table 2). In some cases (esters *14* and *19*) the corresponding acids were isolated; this is not surprising since both *14* and *19* were hydrolyzed by aqueous sodium hydroxide in the presence of TEBA (Table 3). However, these acids were not formed when chloroform was replaced by bromoform which indicate that the two-phase system is affected by the haloform.

Table 3. Unsaturated esters hydrolyzed by 50 % aqueous sodium hydroxide in the presence of TEBA.

α,β-Unsaturated ester	Yield of corresponding acid (%)
R^1=H, R^2=R^3=Me (*14*)	90
R^1=H, R^2=Me, R^3=Et (*15*)	60
R^1=H, R^2=Me, R^3=Bu (*16*)	20
R^1=Ph, R^2=Me, R^3=Et (*19*)	20

Similar effects are also attributable to the esters employed. Reactions carried out with bromoform and different methacrylates (*14*–*16*) yielded variable amounts of 2,2-dibromo-1-methylcyclopropanecarboxylic acid (*30*) (Table 2). This acid could be a secondary product, formed by hydrolysis of the esters *21*–*23*, or it could result from addition to initially formed sodium methacrylate. In the former case hydrolysis of these esters, using 50 % aqueous sodium hydroxide as base and TEBA as catalyst, should give acid *30* which turned out to be the case (Table 4).

The latter reaction path is ruled out by experiments. Certainly the esters *14*–*16* were hydrolyzed under the strong basic conditions employed (Table 3), but sodium methacrylate did not give acid *30* when treated with bromoform under phase transfer conditions.

Experimental. General. The apparatus employed have been described elsewhere.[8]

α,β-Unsaturated ketones and esters used were either commercial samples or synthesized as described in the literature.[9–11]

Reactions between haloform and α,β-unsaturated ketones and esters were carried out essentially as described by Dehmlow.[7] The products isolated are recorded in Tables 1 and 2. Spectral data and elemental analyses agreed with the

Table 4. Preparation of 2,2-dibromo-1-methylcyclopropanecarboxylic acid (*30*) from alkyl esters of *30* by 50 % aqueous sodium hydroxide in the presence of TEBA.

Alkyl group	Yield of *30* (%)
Methyl	7
	2 [a]
	1 [b]
Ethyl	40
	2 [b]
Butyl	0

[a] This experiment was carried out in the presence of chloroform. [b] This experiment was run in the presence of bromoform.

Table 5. Physical constants of *gem*-dibromocyclopropane derivatives.

Compound	B.p. (°C/mmHg)	M.p. (°C)
9	80/0.01	44
10	85/0.45	
11	94/0.01	
13	62/0.05	
23	75/0.13	
24	72/0.05	
25		85
26	56/0.10	

assigned structures. Physical constants of the compounds are compiled in Table 5. Ester *27* was isolated as an oil by CC.

Hydrolysis of esters under phase transfer conditions. A mixture of 25 mmol of ester, a 10 % excess of 50 % aqueous sodium hydroxide, 5 – 10 ml of CH_2Cl_2, and 0.1 – 0.3 g of TEBA was stirred vigorously for 12 h at 20 °C. The reaction mixture was acidified and worked up in the usual way.[8]

Reaction of sodium methacrylate with bromoform. A solution of sodium methacrylate was prepared by treating methacrylic acid with 60 % aqueous sodium hydroxide. The reaction was then carried out according to the general procedure.

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A New Synthesis of 3-(3-Carboxy-4-hydroxyphenyl)-L-alanine (3′-Carboxy-L-tyrosine)

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3-(3-Carboxy-4-hydroxyphenyl)-L-alanine (*1*) occurs in various higher plants.[1,2] *1* has been synthesized from L-tyrosine (*2*) *via* 3-(4-hydroxy-3-nitrophenyl)-L-alanine, 3-(3-amino-4-hydroxyphenyl)-L-alanine, and 3-(3-cyano-4-hydroxyphenyl)-L-alanine.[1] However, the yield in the Sandmeyer step has never been satisfactory and the purification from the reaction mixture is difficult. Syntheses of racemic *1* are available.[3,4]

We now report a new, efficient, synthesis of *1* from *2* *via* *N*-acetyl-L-tyrosine (*3*), *N*-acetyl-*O*-methyl-L-tyrosine (*4*), *N*-acetyl-*O*-methyl-L-tyrosine ethyl ester (*5*) and *N*-acetyl-3-(3-formyl-4-methoxyphenyl)-L-alanine ethyl ester (*6*). The step from *5* to *6*, performed with dichloromethyl methyl ether and $TiCl_4$, has recently been described in the literature, although without experimental details.[5] The formylation of *4* with dichloromethyl methyl ether has also recently been described in the literature, although the yield was low.[6,7] Oxidation of *6* to give the desired carboxyl group in 3′-position has after a number of preliminary attempts been accomplished by two different methods. The first, using Ag_2O in NaOH, results in saponification, yielding *N*-acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine (*7*) which is esterified to *N*-acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine diethyl ester (*8*). Demethylation is performed with BCl_3 to give *N*-acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine diethyl-ester(*9*), and acid hydrolysis finally gives *1*. The second oxidation method, using $KMnO_4$, gives *N*-acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine monoethyl ester (*10*) which by demethylation with BCl_3 gives *N*-acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine monoethyl ester (*11*) and by hydrolysis *1*. Both oxidation methods are proceeding with satisfactory yields. All new compounds, including *6*, have been characterized by IR and ^1H NMR spectra, optical rotation and elemental analysis, and mass spectra have been recorded for *7*, *8*, and *10*.

Experimental. M.p.'s and b.p.'s are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer Infracord 337. ^1H NMR spectra were recorded on a JEOL C-60 HL instrument with TMS as internal standard. MS were recorded on an AEI 3074 instrument at 70 eV with an ion source temperature of 200 °C. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. TLC was performed on Silica Gel F_{254} (Merck) with butanol-acetic acid-water (4:1:1) as the mobile phase. Compounds were visualized with UV light, formyl groups with 2,4-dinitrophenylhydrazine [8] and phenol groups with $FeCl_3$ and $K_3Fe(CN)_6$.[9] Microanalyses were performed by Mr. G. Cornali and his staff.

N-Acetyl-O-methyl-L-tyrosine ethyl ester (5). A solution of *4* (prepared in two steps [10] from *2*) (0.20 mol, 47.4 g) and *p*-toluenesulfonic acid monohydrate (3 g) in absolute ethanol (300 ml) and benzene (500 ml) was refluxed for 15 h. Evaporation and crystallization from light petroleum (b.p. 80–110 °C) gave 0.172 mol (45.5 g, 86 %), m.p. 90–92 °C, $[\alpha]_D^{22}$ +22.2° (*c* 0.6, EtOH). TLC: R_F 0.77. Lit. value: M.p. 85–90 °C,[5] 93–94.5 °C, $[\alpha]_D^{25}$ +23.3° (*c* 1.0, EtOH).[6]

N-Acetyl-3-(3-formyl-4-methoxyphenyl)-L-alanine ethyl ester (6). *Cf.* Ref. 5. To a solution of *5* (0.10 mol, 26.5 g) in dry CH_2Cl_2 (450 ml) at −18 °C was added dropwise $TiCl_4$ (74.5 ml) followed by dichloromethyl methyl ether (30 ml). After 2 h the solution was poured on ice (300 g) and HCl (300 ml 3 N). The organic phase was washed with water, saturated $NaHCO_3$, and water, dried over $CaCl_2$ and evaporated to dryness. Recrystallization from light petroleum (b.p. 60–80 °C) gave 0.717 mol (21.0 g, 72 %), m.p. 86–87 °C, $[\alpha]_D^{22}$ +27.2° (*c* 0.6, EtOH), TLC: R_F 0.76. Anal. $C_{15}H_{19}NO_5$: C, H, N. ^1H NMR ($CDCl_3$): δ 1.25 (3 H, t, $COOCH_2CH_3$), 1.95 (3 H, s, $COCH_3$), 3.06 (2 H, d, CH_2), 3.88 (3 H, s, OCH_3), 4.12 (2 H, q, $COOCH_2CH_3$), 4.76 (1 H, sextet, CH), 6.20 (1 H, d, NH), 6.76–7.50 (3 H, m, aromatic protons), 10.33 (1 H, s, CHO). Lit. value:[5–7] M.p. 85 °C, m.p. 100–101 °C, $[\alpha]_D^{25}$ +24.0° (*c* 1.0, H_2O).,

N-Acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine (7). Moist Ag_2O prepared from $AgNO_3$ (20 mmol, 3.4 g) as described in the literature [11] was covered with water (20 ml) and NaOH pellets (3.4 mmol, 1.37 g) were added under vigorous stirring. The mixture was heated to 55 °C and *6* (6.8 mmol, 2.0 g) was added. After 10 min of stirring, the mixture was filtered, and the precipitated Ag washed with water. The filtrate was poured into ice-cold HCl (7.5 ml, conc.). The precipitated white solid was isolated by filtration and dried. Yield, 6.2 mmol (1.74 g, 91 %), m.p. 188 °C (decomp.), $[\alpha]_D^{22}$ +67.1° (*c* 0.6, phosphate buffer pH 7, 0.2 M). TLC: R_F 0.44. Anal. $C_{13}H_{15}NO_6$: C, H, N. ^1H NMR [$(CD_3)_2SO$]: δ 1.80 (3 H, s, $COCH_3$), 2.90 (2 H, d, CH_2), 3.75 (3 H, s, OCH_3), 4–5 (CH, 2COOH), 6.9–7.4 (3 H, m, aromatic protons), 7.90 (1 H, d, NH). MS: Probe temp. 150 °C; (*m/e* (% rel. int.): 281 (0.4, [M]), 264 (0.7, [M−OH]), 250 0.5, [M−OCH_3]), 236 (5.7, [M−COOH]), 222 (59, [M−CH_3CONH_2]), 165 (100, [M−CH_3-CONHCHCOOH]). Oxidation using standard

conditions[11] resulted in a yield of only about 50 %.

N-Acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine diethyl ester (8). To a suspension of *7* (25 mmol, 7.03 g) suspended in absolute ethanol (150 ml) was added $SOCl_2$ (25 mmol, 2.97 g) dropwise under cooling and stirring. After 24 h at room temperature, excess ethanol was removed by evaporation. The crude ester was dissolved in benzene and purified on a column of silica gel (Kiselgel 60, Merck, 70–230 mesh, 83 g, 20 × 360 mm) by elution with benzene (200 ml) and benzene-ethanol (9:1, 200 ml). *8* was obtained as an oil by concentration. $[\alpha]_D^{26}$ +19.7° (*c* 1.0, EtOH), TLC: R_F 0.79. Found: C 61.93; H 6.98; N 3.91. Calc. for $C_{17}H_{23}NO_6$: C 60.52; H 6.87; N 4.15. ^{1}H NMR ($CDCl_3$): δ 1.32 (6 H, q, 2 $COOCH_2CH_3$), 2.02 (3 H, s, $COCH_3$), 3.12 (2 H, d, CH_2), 3.88 (3 H, s, OCH_3), 4.31 (4 H, octet, $2COOCH_2CH_3$), 4.86, (1 H, sextet, CH), 6.10 (1 H, d, NH), 6.8–7.4 (3 H, m, aromatic protons). MS: Probe temp. 70 °C: 337 (0.6, [M]), 292 (22, [M – OC_2H_5]), 278 (100, [M – H_2NCOCH_3]), 264 (2.5, [M – COC_2H_5]), 193 (100, [M – $CH_3CONHCHCOOC_2H_5$]) 141 (264 → 193). The main product of esterification of *7* with *p*-toluenesulfonic acid and ethanol in benzene was *10*.

N-Acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine diethyl ester (9). A solution of *8* (6.0 mmol, 2.20 g) in CH_2Cl_2 (50 ml) was added dropwise at −17 °C into a solution of BCl_3 in CH_2Cl_2 (60 ml containing 60 mmol of BCl_3). After 1 h at −17 °C and 22 h at room temperature, water (100 ml) was added to the solution at −17 °C. After separation of the phases the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with water, $NaHCO_3$-solution, and water, dried over $CaCl_2$ and evaporated to dryness. Crystallization from light petroleum (b.p. 60–80 °C) – benzene (4:1) afforded *9* (3.6 mmol, 1.18 g, 61 %), m.p. 99 °C, $[\alpha]_D^{26}$ +16.8° (*c* 1.0, EtOH), TLC: R_F 0.83. Anal. $C_{16}H_{21}NO_6$: C, H, N, ^{1}H NMR ($CDCl_3$): δ 1.35 (6 H, q, $COOCH_2CH_3$), 2.00 (3 H, s, $COCH_3$), 3.10 (2 H, d, CH_2), 4.28 (4 H, octet, $COOCH_2CH_3$), 4.81 (1 H, q, CH), 5.98 (1 H, d, NH), 6.7–7.4 (3 H, m, aromatic protons).

N-Acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine monoethyl ester (10). To *6* (50 mmol, 14.7 g) in water (150 ml, 70–80 °C) was added a solution of $KMnO_4$ (75 mmol, 11.9 g) in water (200 ml) in a few portions. After 5 min, the solution was filtered and the MnO_2 washed with water. The filtrate was concentrated to 50 ml, cooled and conc. HCl added in excess. Extraction with CH_2Cl_2 with subsequent drying of the organic phase and concentration yielded a yellow oil which afforded *10* by crystallization from ethanol – light petroleum (b.p. 60–80 °C) (1:3). Yield 33.4 mmol (10.3 g, 67 %), m.p. 117–119 °C, $[\alpha]_D^{22}$ +27.0° (*c* 0.6, phosphate buffer pH 7, 0.2 M). TLC: R_F 0.69. Anal. $C_{15}H_{19}NO_6$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.29 (3 H, t, $COOCH_2CH_3$), 2.03 (3 H, s, $COCH_3$), 3.13 (2 H, d, CH_2), 4.05 (3 H, s, OCH_3), 4.23 (2 H, q, $COOCH_2CH_3$), 4.85 (1 H, q, CH), 6.30 (1 H, d, NH), 6.9–7.9 (3 H, m, aromatic protons). MS: Probe temp. < 50 °C: 309 (0.3, [M]), 292 (22, [M – OH]), 276 (0.3, [M – OOH]), 264 (1.1, [M – COOH]), 250 (100, [M – CH_3CONH_2]), 236 (3.2, [M – $COOC_2H_5$]), 165 (77, [M – CH_3-$CONHCHCOOC_2H_5$]).

N-Acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine monoethyl ester (11). *1* (20 mmol, 6.19 g) in CH_2Cl_2 (100 ml) was added dropwise under stirring to a solution of BCl_3 in CH_2Cl_2 (200 ml containing 0.2 mol of BCl_3) at −17 °C. After 30 min water (100 ml) was added. The phases were separated, and the aqueous phase extracted with CH_2Cl_2. The combined organic phases were washed with water, dried over $CaCl_2$ and concentrated to dryness. Yield 5.2 g (88 %), m.p. 68–70 °C, $[\alpha]_D^{26}$ +24.2° (*c* 0.6, phosphate buffer pH 7, 0.2 M), TLC: R_F 0.71. Purification was accomplished by use of a silica gel column as described for *8*. Anal. $C_{14}H_{17}NO_6$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.31 (3 H, t, $COOCH_2CH_3$), 2.09 (3 H, s, $COCH_3$), 3.14 (2 H, d, CH_2), 4.25 (2 H, q, $COOCH_2CH_3$), 4.92 (1 H, q, CH), 6.50 (1 H, d, NH), 6.8–7.7 (3 H, m, aromatic protons).

3-(3-Carboxy-4-hydroxyphenyl)-L-alanine (1) from 9. *9* (0.62 mmol, 200 mg) was refluxed in 4 N HCl (5 ml) for 1 h. After cooling of the solution pH was adjusted to 2.5 with ammonia. After a night in the refrigerator white crystals of *1* were collected. Yield 0.47 mmol (106 mg, 78 %). After recrystallization from water pure *1* was obtained with IR identical with that of authentic material.[1] $[\alpha]_D^{24}$ −30.5° (*c* 0.6, phosphate buffer pH 7, 0.2 M), m.p. 273 °C (decomp.). Lit. values:[1] $[\alpha]_D^{24}$ −29.9° (*c* 0.6, phosphate buffer pH 7, 0.2 M), m.p. 263–264 °C.

1 from *11*. *11* (500 mg) was refluxed in 4N HCl for 1 h. *1* was isolated as described above. Yield, 0.858 mmol (193 mg, 51 %). Recrystallization from water afforded pure *1*, $[\alpha]_D^{26}$ −29.8° (*c* 0.6, phosphate buffer), m.p. 272 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 3.04 (2 H, d, CH_2), 3.93 (1 H, q, CH), 6.6–7.7 (3 H, m, aromatic protons), 9.1 (5 H, broad s, OH, NH_3^+, COOH).

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Chlorophylls. V.* Isolation of Chlorophylls *a* and *b* Using an Improved Two-phase Extraction Method Followed by a Precipitation and a Separation on a Sucrose Column

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Chlorophylls *a* and *b*, each of a high degree of purity, were isolated by means of a method consisting of an improved two-phase extraction method followed by a precipitation of the chlorophylls as aggregates and separation of the precipitated chlorophylls on a sucrose column. The spectroscopic properties of the chlorophylls purified by this means closely matched those previously described for pure chlorophylls. On using the method, 50 g of frozen clover leaves yielded approximately 20 mg of chlorophyll *a* and 3 mg of chlorophyll *b*. The advantage of the method is that the transformation of the chlorophylls into various alteration products can be minimized. The two-phase extraction method followed by precipitation also permits the preparation of large amounts of pure chlorophyll mixture, from which mixtures of pheophytins or pheophorbides *a* and *b* are easily obtained by conventional methods.

The chromatographic techniques employed for the separation and isolation of chloroplast pigments have been extensively reviewed by Holden,[2] Sestak,[3] Strain and Svec,[4,5] and Strain and Sherma.[6,7] Since 1971 several additional reports of investigations on this subject have appeared.[8–14] The inorganic adsorbents (silica gel, Kieselguhr), frequently used in these investigations, are not suitable for the separation of chlorophylls, since the adsorbents cause alteration of these pigments.[5,8,16,17] The reversed-phase partition thinlayer chromatography (RP-TLC), a method originally developed by Egger,[15] appears to be suitable for analytical purposes, but is unsuitable for preparative isolation, since the impregnating triglycerides are eluted from the chromatograms and interfere seriously with subsequent re-chromatography.[11] Hynninen and Ellfolk [14] isolated chlorophylls *a* and *b* by the Martin-Synge distribution (MSD).[18] In the final method developed, chloroplast pigments were extracted from frozen soybean leaves by means of a two-phase extraction method, and thereafter separated in the PBMF-system [light petroleum (8) – benzene (1)/methanol (6.75) – formamide (2.25)] employing a partition apparatus of 100 tubes.

The original two-phase extraction method [14] as performed in a separatory funnel and under an argon atmosphere has since proved to be somewhat cumbersome. In addition, the method developed suffers from the restriction that it requires the availability of a partition apparatus which is rather difficult to prepare. In the present publication, the author reports the combination of an improved two-phase extraction method with a precipitation and a separation of the precipitated chlorophylls on a sucrose column.

EXPERIMENTAL

Extraction of the pigments from plant material. Frozen clover leaves (a mixture of *Trifolium pratense*, *T. hybridum* and *T. repens*) were used as a source of chloroplast pigments. Immediately after harvesting, the leaves were transferred to the dark at a temperature of −30 °C and were stored under these conditions until

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used. The improved two-phase extraction method described as follows was performed rapidly and in dimmed light.

Fifty grams of frozen clover leaves were suspended in a mixture of 400 ml of light petroleum (b.p. 60 – 80 °C) + 300 ml of methanol + 100 ml of formamide in a 2 l decanter. Before suspending the leaves, the solvent mixture was cooled to a temperature below 0 °C in a freezer. The suspension was immediately homogenized by means of an Ultra-Turrax homogenizer (1 min, full speed). The phases were allowed to separate while gently stirring the mixture occasionally. After phase separation, which took about 5 min, the upper phase (*ca.* 300 ml) was decanted off, transferred into a separatory funnel and washed several times with cold (0 °C) CO_2-free distilled water until the chlorophylls aggregated.

Separation of the chlorophylls from carotenoid pigments by precipitation. The suspension containing the aggregated chlorophylls, carotenes and xanthophylls was allowed to stand for 2 h or overnight at – 30 °C in a freezer to make the precipitation of the chlorophylls complete. After this, the precipitated chlorophylls were separated from the suspension by centrifugation (+4 °C, 10 300 *g*, 10 min.) The green-yellow supernatant containing the carotenoids and the possible small amount of pheophytin *a* and chlorophyll *a'* was carefully decanted off and discarded. The precipitate was washed with cold (– 30 °C) light petroleum (b.p. 60 – 80 °C, *ca.* 160 ml). After centrifugation, the wash-liquid was decanted off and the chlorophyll precipitate was dissolved in 160 ml of cold peroxide-free diethyl ether. The solution was evaporated to a volume of *ca.* 10 ml by means of a rotatory evaporator.

Chromatography on sucrose column. The conventional chromatographic procedure on a sucrose column,[4–6] was employed in the separations. Icing sugar (The Finnish Sugar Co., Helsinki, Finland), containing 0.69 % tricalcium phosphate, was used in the separations as provided, with the exception of one separation where the sugar was dried at +80 °C for 1 h before use. The sugar was mixed with the eluent (light petroleum, b.p. 60 – 80 °C, containing 0.5 % 1-propanol) to form a slurry,

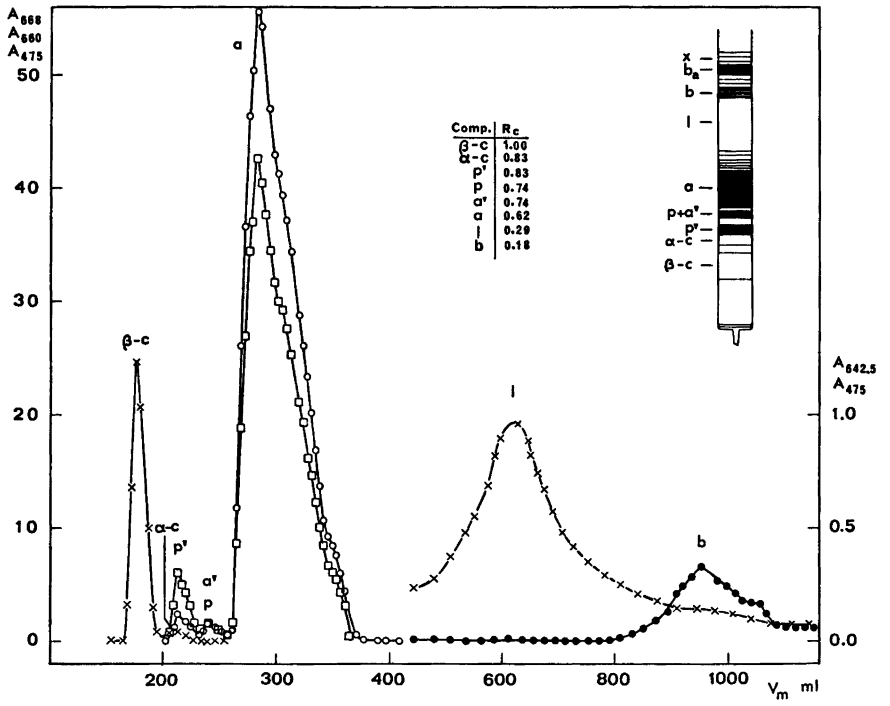


Fig. 1. Separation of chloroplast pigments on a sucrose column. Height of the sucrose layer = $h = 40.0$ cm. Flow rate = $u = 0.7$ ml/min. V_m = effluent volume. × = A_{475}, □ = A_{668}, ○ = A_{660}, and ● = $A_{642.5}$. Components from β-c to a: left-hand absorbance scale; components l and b: right-hand absorbance, scale. β-c = β-carotene, α-c = α-carotene, p' = pheophytin *a'*, p = pheophytin *a*, a' = chlorophyll *a'*. a = chlorophyll *a*, l = lutein, b = chlorophyll *b*, b_a = aggregated chlorophyll *b*, and x = xanthophyll The upper right-hand part of the figure shows the situation before β-c had emerged from the column.

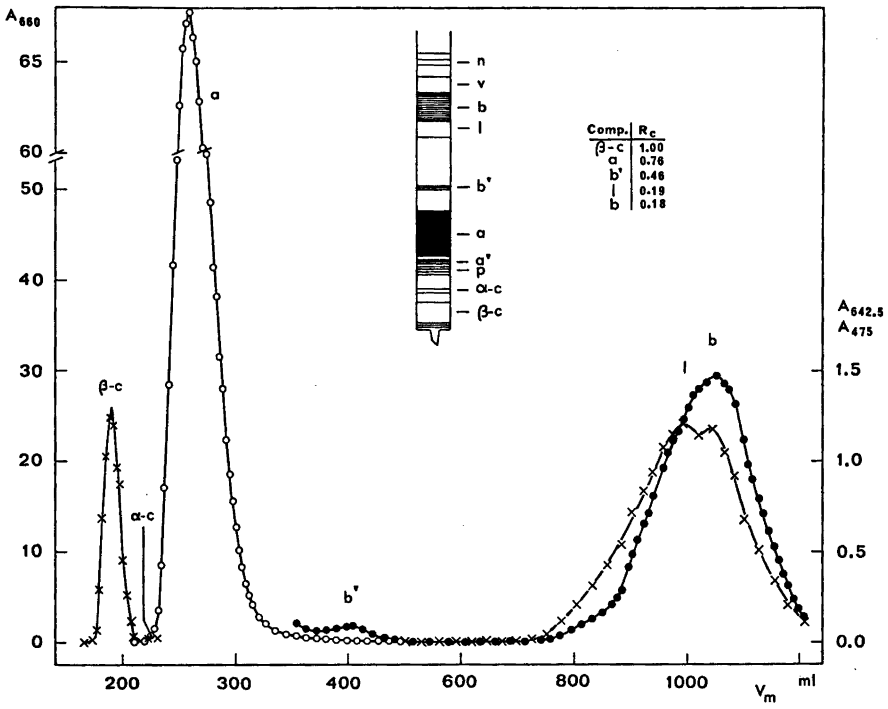


Fig. 2. Separation of chloroplast pigments on a sucrose column. $h = 42.8$ cm. $u = 0.6$ ml/min. × = A_{475}, ○ = A_{660}, and ● = $A_{642.5}$. Components β-c, α-c, and a: left-hand absorbance scale; components b′, l, and b: right-hand absorbance scale. β-c = β-carotene, α-c = α-carotene, a = chlorophyll *a*, b′ = chlorophyll *b*′, l = lutein, b = chlorophyll *b*, v = violaxanthin, and n = neoxanthin. The upper middle part of the figure shows the situation before β-c had emerged from the column.

which was then poured into a glass column having an inside diameter of 3.0 cm and a height of 50 cm. The sugar particles were allowed to settle until no descending movement of the layer was observed.

The pigments in the sample to be fractionated were first made soluble by the following procedure. The light petroleum or diethyl ether dispersion of the mixture of the pigments was evaporated under reduced pressure to near dryness and the residue was dissolved in 4 ml of the eluent. The solution was evaporated under vacuum to near dryness (evaporating was stopped when there were a few drops of the eluent left). The residue was dissolved in 2 to 4 ml of the eluent and the solution was introduced into the top of the column with a pipette. The pigments were allowed to absorb into the sugar layer to form a narrow initial zone and the column was then eluted until chlorophyll *b* had emerged completely (when the flow-rate was 0.6 ml/min, this took about 17 h). β-Carotene was used as a reference compound to facilitate the comparison of different separation results and the identification of the components.

The migration rate of a component was expressed in terms of R_c-values: R_c = the elution volume of β-carotene/the elution volume of the component. If β-carotene was not included in the mixture to be separated, the sugar column was first "calibrated" with a small amount of pure β-carotene before the actual separation was started. The absorbances (A) of the collected fractions were measured at selected wavelengths by means of a Perkin-Elmer 139 UV-VIS spectrophotometer. All chromatographic separations were performed in the dark at a temperature of about +4 °C.

Absorption spectra. The visible absorption spectra of the components were recorded utilizing a Cary Model 118 C spectrophotometer. Measurements were performed directly upon the effluent after the components had emerged from the column as well as after they had been transferred into ethyl ether by means of a Thunberg tube at reduced pressure.

Solvents. Formamide was purified by distillation *in vacuo*.[19] Depending on the purity of the commercial product, 2 to 4 distillations were required to obtain a formamide which reacted

green-yellow to bromothymol blue. This product was used without fractional crystallization. The other solvents used were of reagent grade purity and were employed without further purification.

RESULTS

Separation of chloroplast pigments. Fig. 1 presents the results from the fractionation of the chloroplast pigments obtained by the improved two-phase extraction method. Two yellow pigments, presumably β- and α-carotene, were first eluted from the column. The α-carotene was not completely separated from pheophytin *a'* which appeared as a grey lustrous zone. A small amount of green chlorophyll *a'* + pheophytin *a* was spectroscopically detected between pheophytin *a'* and chlorophyll *a*. Chlorophyll *a* was eluted as a broad zone and shows two small shoulders on the right hand side of its elution curve. These may have resulted from the keto-enol tautomerization [14–20] of the chlorophyll. Another probable reason for these shoulders is the small amount of diethyl ether used to dissolve the pigments in the sample. It was noted that the ether causes broadening and doubling of the zones at the beginning of the fractionation. The doubling effect may result from aggregation caused by the use of diethyl ether. The ether washes the small amount of 1-propanol from the top of the sugar layer whereafter the aggregation of chlorophyll occurs rapidly. This effect was observed clearly in the case of chlorophyll *b* which was divided into two zones. The yellow-green zone (soluble chlorophyll *b*) emerged from the column after the yellow lutein. The other zone (aggregated chlorophyll *b*) remained at the top of the sugar layer and appeared as a dark grey-green zone which was practically immobile. The aggregated chlorophyll *b* probably overlapped the other leaf xanthophylls (violaxanthin and neoxanthin). No chlorophyll *b'* could be spectroscopically detected between chlorophyll *a* and lutein. *Ca.* 30 mg of spectroscopically pure chlorophyll *a* (Table 1; a,b) was obtained from this fractionation ($V_m = 260 - 365$ ml). Chlorophyll *b* (less than 1 mg; $V_m = 850 - 1100$ ml), however, was contaminated by xanthophyll. The molar absorptivities used for the estimation of the amounts were 8.63×10^4 l

Table 1. Spectroscopic properties of the chlorophylls

Compound			Solvent	Peak positions (nm) and peak ratios (R^c) I		II		III		IV		V		S		Ss1		Ss2	
				nm	*R*	nm	*R*	nm	*R*	nm	*R*	nm	*R*	nm	*R*	nm	*R*	nm	*R*
a.	Chl[d] *a*	(1)[a]	Effl[d]	661.0	1.21	615.5	8.02	578.0	15.8	531.0	31.7	(495)[b]	(62.0)	429.0	1.00	411.0	1.54	381.0	2.50
b.	Chl *a*	(1)	Ee	659.0	1.29	613.0	7.97	575.0	12.7	528.5	18.2	(495)	(23.7)	428.0	1.00	411.5	1.47	380.0	2.36
c.	Chl *b*	(1)	Effl	642.5	2.83	596.0	17.0							451.0	1.00	427.0	2.53		
d.	Chl *a*	(2)	Effl	660.5	1.18	614.0	7.67	575.0	14.5	531.0	27.0	(494)	(48.0)	428.5	1.00	411.0	1.48	381.0	2.41
e.	Chl *a*	(2)	Ee	660.0	1.28	613.5	8.33	575.0	15.7	530.0	28.4	(496)	(52.5)	428.0	1.00	410.5	1.53	380.0	2.53
f.	Chl *a*	(3)	Effl	660.0	1.23	614.0	8.03	577.0	15.4	530.5	30.8	(496)	(61.7)	428.5	1.00	410.5	1.53	381.0	2.50
g.	Chl *a*	(3)	Ee	659.0	1.31	613.0	7.80	575.0	16.7	529.0	30.0	(495)	(60.0)	428.0	1.00	409.5	1.58	379.0	2.62
h.	Chl *b*	(3)	Effl	642.0	2.77	594.0	15.3							451.0	1.00	427.0	2.88		
i.	Chl *b*	(3)	Ee	642.5	3.06	592.0	18.1							451.0	1.00	427.0	2.90		

[a] Number in parentheses refers to figure number. [b] Peak positions and ratios stated in parentheses are approximate. [c] R = quotient of absorbance at Soret band divided by absorbance at wavelength indicated; S = Soret band, Ss1 = 1st satellite of Soret band, Ss2 = 2nd satellite of Soret band; [d] Chl = chlorophyll, Ee = diethyl ether, Effl = 0.5 % 1-propanol in light pertoleum, 60 – 80 °C.

mol^{-1} cm^{-1} for chlorophyll *a* and 5.61×10^4 l mol^{-1} cm^{-1} for chlorophyll *b*.[4,21]

The above fractionation was repeated on a fresh pigment extract with the difference that no diethyl ether was used in the sample. The pigments were made soluble in this case using the procedure described in EXPERIMENTAL. The elution curves of chlorophylls *a* and *b* had no shoulders in this case (Fig. 2). Neither could any aggregated chlorophyll *b* be detected at the upper part of the column. Chlorophyll *b* was, however, almost completely overlapped by lutein, and the pheophytins as well as chlorophyll *a'* cannot be distinguished in the effluent curve (they were observed as partly resolved on the column). A small amount of chlorophyll *b'* could be detected in this case between chlorophyll *a* and lutein.

On drying the sugar at +80 °C for 1 h before packing the column, chlorophyll *b* was eluted from the column before lutein and violaxanthin. The resolution of chlorophyll *b* from lutein was, however, poor also in this case.

Separation of the chlorophylls after precipitation from the extract. In the final method developed for the isolation of chlorophylls *a* and *b*, the pigments were first precipitated from the chloroplast extract obtained by the two-phase extraction method (see EXPERIMENTAL) and thereafter separated on a sucrose column. As can be observed from Fig. 3, the separation problem is remarkably simplified on precipitating the chlorophylls. This procedure effectively separates chlorophylls *a* and *b* from the carotenoids, pheophytins and chlorophyll *a'*. None of these pigments could be detected either in the effluent or on the column. Chlorophyll *a* was eluted from the column as a narrow peak. Traces of chlorophyll *b'* were spectroscopically detected at $V_m = 310$ ml (note that the absorbance scale for chlorophyll *b'* and *b* is different from that for chlorophyll *a*). The front part of

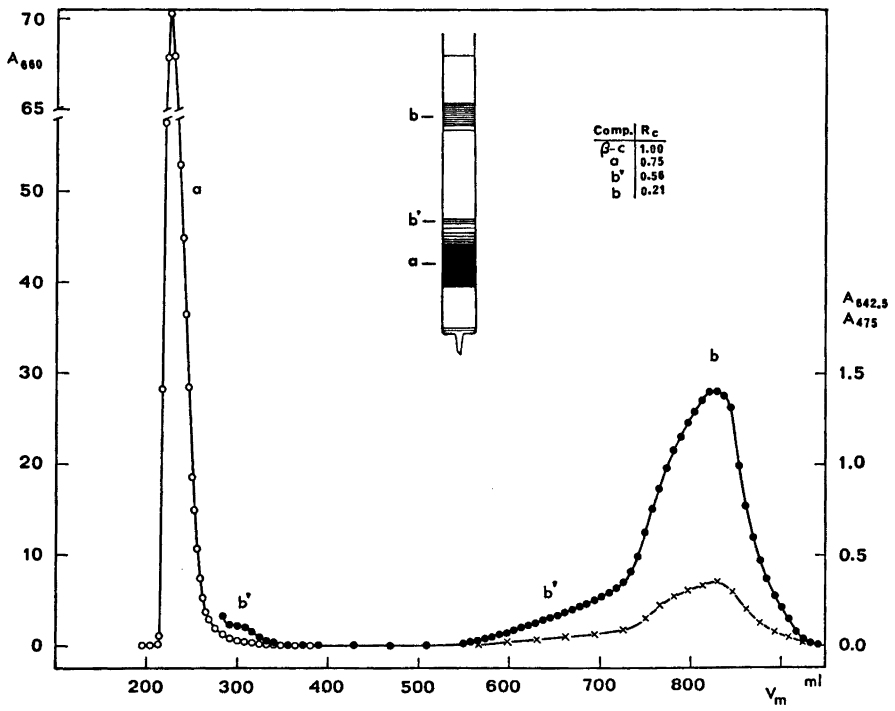


Fig. 3. Separation of chlorophylls on a sucrose column. $h = 40.0$ cm. $u = 0.6$ ml/min. ○ = A_{660}, ● = $A_{642.5}$, and × = A_{475}. Component a: left-hand absorbance scale; components b' and b: right-hand absorbance scale. a = chlorophyll *a*, b' = chlorophyll *b'*, and b = chlorophyll *b*. The upper middle part of the figure shows the situation before a had emerged from the column.

the chlorophyll *b* zone was, however, broadened. This phenomenon indicates that a slow transformation of chlorophyll *b* to chlorophyll *b'* had occurred during the separation. This effect can be minimized by using a shorter column or by removing the sugar layer with chlorophyll *b* adsorbed on it through the upper end of the column and then eluting chlorophyll *b* from the sugar with diethyl ether. The spectroscopic properties of chlorophylls *a* and *b* from the fractionation of Fig. 3 are presented in Table 1; f–i. These properties closely matched those previously [14] reported for chlorophylls *a* and *b* obtained by multiple liquid-liquid partition. Approximately 20 mg of chlorophyll *a* (V_m = 214 – 256 ml) and 3 mg of chlorophyll *b* (V_m = 669 – 901 ml), each of a high degree of purity, were yielded by the fractionation in Fig. 3. Both chlorophyll preparations reacted positively to the phase test.

DISCUSSION

Owing to the susceptibility of the chlorophylls to a number of chemical transformations which yield various alteration products, the isolation of pure chlorophylls is known to be a difficult task.[4] These transformation reactions take place during extraction as well as during fractionation. In 1966 Strain and Svec [4] wrote: "The preparation and isolation of relatively pure chlorophylls..., require care and have been accomplished by only a few investigators". They gave nine references to the investigators capable of doing the isolation.

Among the chemical transformations of the chlorophylls, the "isomerization" to chlorophylls *a'* and *b'*[20,22–24] and the allomerization,[25,26] which is essentially oxidation at C-10 of the isocyclic ring V of chlorophyll, occur very easily in solvents containing methanol, ethanol or 1-propanol. The formation of chlorophylls *a'* and *b'* is known to occur also in pyridine.[20,22] Since these solvents are frequently included in solvent mixtures used for extraction and separation, special care is required to avoid the transformations.

The conventional procedures, in which 80 % acetone or light petroleum – methanol is employed for the extraction of chloroplast pigments, were consistently found to lead to considerable amounts of allomerization or other alteration products.[14] This was explained by the reaction scheme,[14] according to which the allomerization as well as the "isomerization" to chlorophylls *a'* and *b'* are preceded by the enolization reaction. The enolization was supposed to occur in electron donor solvents such as ethyl ether, acetone, pyridine, dioxane, tetrahydrofuran and alcohols in which the chlorophylls occur as monomers. The free enol formed was subsequently presumed to undergo further transformation depending on the polarity of the solvent. In Lewis bases the free enol is probably ionized to the labile enolate anion which is rapidly attacked by oxygen to yield a cyclic peroxide derivative as an intermediate (assuming an addition mechanism [26] for this reaction, one should take into account the possibility that also the free enol may react with $^1O_2$). The peroxide derivative thereafter converts into various oxidation products whose nature depends on the solvent.[26] In solvents possessing relatively weak hydrogen bonding capabilities such as pyridine, tetrahydrofuran and light petroleum containing a small amount of 1-propanol the free enol was assumed to convert to the hydrogen chelate. This was considered to be identical with the chlorophyll' isomer.[20]

On the basis of the foregoing, it is understandable why we so easily get allomerization products or chlorophyll prime isomers on trying to isolate chlorophylls. We have to use polar solvents in the extraction, since on employing nonpolar solvents for this purpose, very little chlorophyll is released from the chloroplasts.[4] Then, however, more or less of the free enol is produced during the extraction. Since water is, as a rule, included in the extracting solvent, the further transformation of the free enol is prevented by the formation of hydrogen bonds between water (or formamide) and the free enol. In subsequent phases of the isolation, the hydrogen bonded water molecule may be stripped off and the free enol may then convert to the enolate anion or the prime isomer.

The reported fact that salts accelerate the allomerization [27] also becomes now comprehesible. On using concentrated salt solutions in the extraction, the salt ions compete with the free enol for water molecules. Consequently, water is removed and the free enol becomes susceptible to transformations. Therefore, con-

centrated salt solutions should not be used in the extraction of the pigments (it has been a common practice in the conventional extraction procedures to add saturated sodium chloride solution in order to transfer the pigments from the polar phase into the light petroleum phase!).

To minimize the enolization of chlorophyll, the extraction procedure should be rapid. This requirement is fullfilled by the two-phase extraction method developed by the present author. In this method, the pigments are loosened from the chloroplasts by the polar lower phase and transferred immediately into the nonpolar upper phase, where the chlorophylls do not undergo alteration. In its improved form, the two-phase extraction method has proved to be very easy and rapid to perform. No argon or nitrogen atmosphere is necessary in this modification owing to its rapidity and the low temperature used.

The precipitation of the chlorophylls as aggregates is an efficient procedure for the separation of the chlorophylls from carotenoids. This separation is required before the chromatography on a sucrose column, since a weak point of the chromatographic procedure is that chlorophyll *b* is overlapped by lutein. This difficulty can be eliminated by extracting the lutein from the light petroleum solution of the pigments with aqueous methanol.[4] However, this method is not recommended by the present author, since, although the allomerization does not occur in aqueous methanol, the enolization does, and when the water molecules are removed, the free enol becomes susceptible to transformation.

The time requirement of the new isolation method is comparable to that of the liquid-liquid partition method.[14] Starting from the packing of the sucrose column, approximately 24 h were needed to obtain pure chlorophylls *a* and *b*.

The scale of the isolation method described can be easily increased. It is possible to prepare large amounts of chlorophyll mixture utilizing the two-phase extraction method followed by precipitation. Mixtures of pheophytins *a* and *b* as well as pheophorbides *a* and *b* are easily obtained from a pure chlorophyll mixture by conventional methods.[14]

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^{13}C NMR and Force Field Calculations on the Barrier to Internal Rotation in and Conformational Equilibria of the Benzenedicarbaldehydes

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The proton coupled ^{13}C NMR spectra of the three benzenedicarbaldehydes have been fully analysed. The barrier to internal rotation of the formyl groups has been obtained from band-shape analysis of the low temperature proton noise-decoupled ^{13}C spectra, resulting in $\Delta G^{\ddagger}$ values of 31.0 ± 0.5 and 28.6 ± 0.5 kJ/mol for 1,3- and 1,4-benzenedicarbaldehyde, respectively. At the lowest temperatures (*ca.* -150 °C) the rotamer equilibria for these two isomers could also be determined. This was not possible for 1,2-benzenedicarbaldehyde where either predominantly one conformation is adopted or the barrier to rotation is lower than for the other compounds. Force field calculations indicate a barrier to internal rotation considerably lower for the 1,2 isomer than for the other two.

In previous work we have determined the formyl rotational barrier in some *para*-substituted benzaldehydes [1a] and both the barrier and conformational equilibria for some *ortho*- and *meta*-substituted benzaldehydes.[1b] As a continuation we have now also studied the three different benzenedicarbaldehyde isomers.*** In the present work the proton coupled carbon-13 spectra have also been studied to find out if the magnitude of the $^{13}C-{}^{1}H$ coupling constants can be useful in estimations of conformational equilibria, in the same way as the proton-proton five-bond coupling has been used.[3] We also report some force field calculations on these compounds,[4] which have been performed in order to confirm the conclusions drawn regarding especially the barrier in the 1,2 isomer.

RESULTS AND DISCUSSION

^{13}C NMR spectra. Carbon-13 spectra have been obtained from 0.5 M Freon solutions at low temperature as well as from concentrated chloroform solutions to determine the $^{13}C-{}^{1}H$ spin coupling constants. For 1,4-benzenedicarbaldehyde the assignment is obvious as there is only one aromatic carbon signal from four equivalent proton-bearing carbons and one from two non-proton-bearing carbons. For 1,3-benzenedicarbaldehyde the assignment of the aromatic ^{13}C resonances was made from the behaviour of the signals at low temperature. This assignment is also in agreement with the expected spin coupling pattern of the various resonances. The 1,2-benzenedicarbaldehyde spectrum showed no temperature dependence and the assignment is thus based on the coupling pattern only. The substituent induced shifts are not used as they are known to be less accurate for *ortho*-substituted benzenes and furthermore the unknown conformation equilibrium makes them even less usable.[1a] The observed chemical shifts are summerized in Table 1. All the $^{13}C-{}^{1}H$ coupling constants have been

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*** It has recently been shown by Lunazzi *et al.* that the torsional barrier in the 1,4 isomer is sufficiently high to be observed by NMR.[2] The activation energy was, however, not determined.

Table 1. Carbon-13 chemical shifts of the benzenedicarbaldehydes in Freon solution at −70 °C. Shift in ppm downfield from TMS.

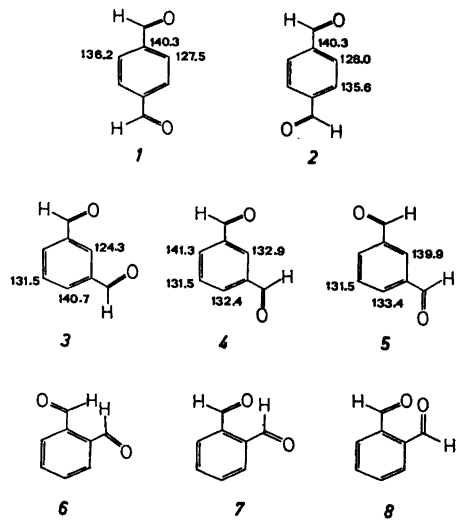
Isomer	C-1	C-2	C-3	C-4	C-5	C-6	CHO
1,2	137.2	137.2	133.1	135.8	135.8	133.1	196.1
1,3	137.9	132.6	137.9	136.4	131.5	136.4	193.8
1,4	141.2	131.7	131.7	141.2	131.7	131.7	201.9

Table 2. $^{13}C-{}^{1}H$ spin coupling constants in the three benzenedicarbaldehydes.

	H-2	H-3	H-4	H-5	H-6	H-7	H-8
1,2-Isomer							
C-1		6.43	−1.07	6.93	0.38	24.85	1.91
C-3		163.03	1.61	7.71	−0.89	0.51	2.10
C-4		0.85	163.83	1.47	7.55	0.00	0.41
1,3-Isomer							
C-1	0.35		−0.58	7.14	0.44	24.94	0.74
C-2	162.85		6.54	−1.37	6.54	2.00	2.00
C-4	6.34		162.75	1.79	7.81	0.00	1.99
C-5	−0.49		0.70	165.11	0.70	0.49	0.49
1,4-Isomer							
C-1	−[a]	−[a]		−[a]	−[a]	24.70	0.00
C-2	163.4	0.97		−1.96	7.16	1.97	0.45

[a] Not determined.

determined from the proton coupled ^{13}C spectra. It was not possible to make a first order analysis since the proton spectra are strongly coupled and thus the computer program UEAITR [5] was used to simulate the ^{13}C spectra. The obtained coupling constants are given in Table 2 and in Fig. 1 the aromatic part of the proton coupled spectrum from 1,2-benzenedicarbaldehyde is shown. As can be seen there is an overlap between the downfield parts of the C-1, C-2 and C-4, C-5 resonances making the analysis of the C-1, C-2 spectrum difficult. In order to get a clear spectrum from the C-1, C-2 carbons we used the $180-\tau-90$ pulse sequence with τ adjusted to get a null signal from the C-4, C-5 resonance, resulting in the spectrum shown in Fig. 2. This procedure worked very well in this case where there was a large difference in the spin-lattice relaxation time for the C-1, C-2 and C-4, C-5 resonances. Our results on the coupling



Scheme 1.

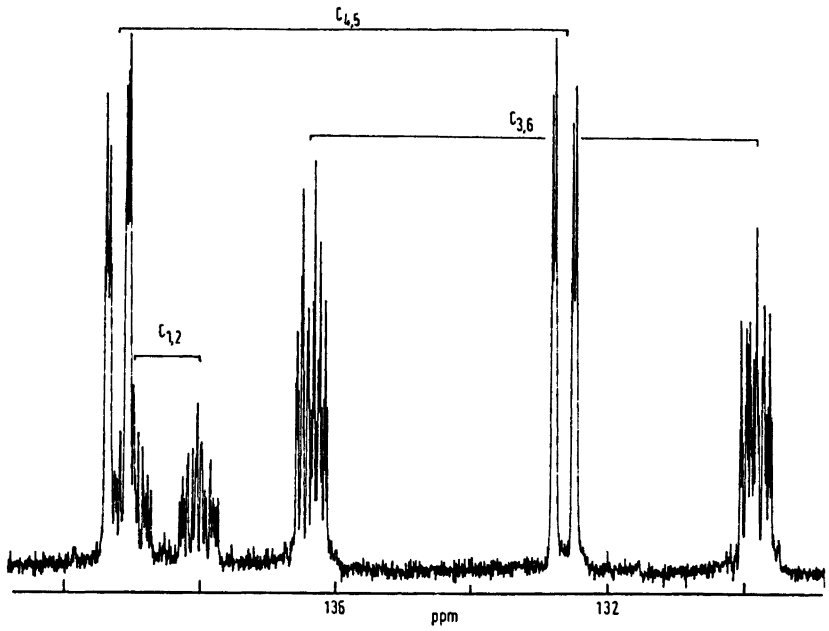


Fig. 1. ^{13}C proton coupled NMR spectrum of the aromatic carbons in 1,2-benzenedicarbaldehyde.

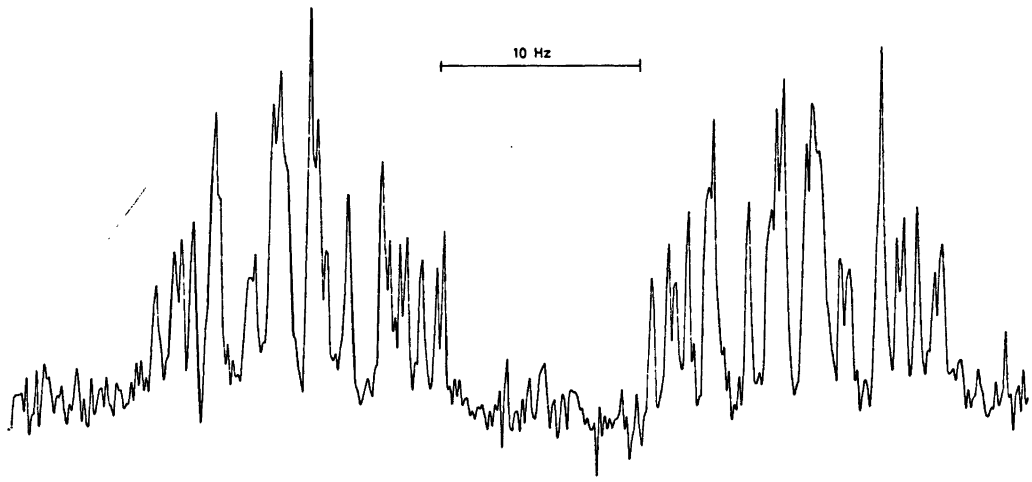


Fig. 2. An expansion of the C-1 resonance in 1,2-benzenedicarbaldehyde, obtained without proton decoupling. The $(T-180-\tau-90)_n$ pulse sequence was used with $T=30$ s and $\tau=3.1$ s to null the signals from the C-4 and C-5 carbons.

constants are in fair agreement with previous fragmentary data.[6,7]

In Scheme 1 the planar or nearly planar conformers of the benzenedicarbaldehydes are shown together with the assignment of the carbon-13 resonances to the various conformers for 1,3 and 1,4 isomers. The shifts for the 1,3 isomer were obtained at -141 °C and those for the 1,4 isomer at -160 °C. The effect from the orientation of the formyl group on the shift of the *meta* carbons is not well-known, but a comparison with *m*-fluorobenzaldehyde in-

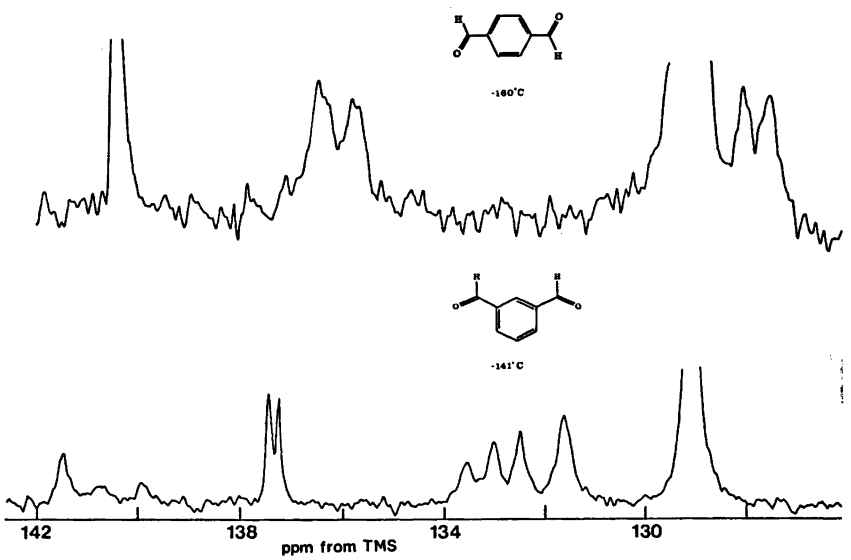


Fig. 3. Parts of the low temperature proton noise-decoupled ^{13}C NMR spectra from 1,3- and 1,4-benzenedicarbaldehyde.

dicates that the assignment should be as given for 1,4-dicarbaldehyde. For the 1,3 isomer a more reliable assignment could be made due to the non-equal populations of the various rotamers, see Fig. 3. For two pairs of signals the assignment is only tentative, the favoured one is, however, the one that is in best agreement with the high temperature chemical shifts (−70 °C). In principle it should be possible to make a complete assignment of the signals in the 1,3 isomer from a bandshape analysis of the spectrum in the temperature region where the shape is temperature dependent due to exchange. This was, however, not possible since the quality of the spectra was not sufficiently high (Fig. 3).

Conformational equilibria. At low temperature where the rotation about the formyl bond is slow on the NMR time scale the population of the various rotamers can be directly obtained from the spectrum. For 1,4-benzenedicarbaldehyde the high temperature signal from the four equivalent carbons gives rise at low temperatures to four signals of almost identical intensity, showing that the populations of the two rotamers are approximately equal. This means that the orientation of a *p*-formyl group has no influence on the orientation of the other formyl group. This is also confirmed by force field calculations, which gave the same energy for the two rotamers when the dipolar energy was neglected. The calculated difference in the dipolar energy for the two planar rotamers, *1* and *2*, was *ca.* 1.2 kJ/mol for an isolated molecule. Since the dipolar energy [8] is strongly dependent on the "effective" dielectric constant, the dipole-dipole interaction should be negligible. (The Freon dielectric constant is estimated to be *ca.* 10 at the temperature in question). Barassin *et al.*[9] have reported the same 50:50 population ratio based on dipole moment measurements, whereas Gore *et al.*[10] suggest that the molecule cannot be planar since their dipole moment and Kerr constant are not in agreement with a mixture of two planar conformations. An SCF-MO calculation,[11] however, also indicates that 1,4-benzenedicarbaldehyde should exist as a 50:50 mixture of the two planar conformations.

The force field calculations on the 1,3 isomer indicate that all three different rotamers (all planar) should be present in solution, with about the same populations (Table 3). As can be seen from Fig. 3 a fairly complicated ^{13}C NMR spectrum was obtained in the aromatic region at low temperature. The assignment

Table 3. Relative energies of the various rotamers as obtained from the force field calculations.

Rotamer[a]	E kJ/mol	E' kJ/mol[b]
1	0.0	20.2
2	0.0	20.2
3	0.0	20.2
4	0.0	20.2
5	0.0	20.2
6	0.0	13.0
7	1.1	13.0
8	7.1	–

[a] See Scheme 1. [b] Energy obtained when the formyl group on C-1 is perpendicular to the benzene ring. The calculated [4] and experimental [13] barrier for benzaldehyde is 20.5 kJ/mol in the gas phase. The gas phase numbers may be corrected for solvation by adding *ca.* 10 kJ/mol, which is the difference in rotational barrier between gas phase and solution.

was, however, straightforward as discussed above and the populations were obtained from the spectrum at −141 °C by means of a total bandshape analysis treating the spectrum as two overlapping four-site cases resulting in the population 0.15, 0.58 and 0.27 for rotamers *3*, *4* and *5*, respectively. The corresponding energy difference between rotamers *3* and *4* is 0.7 kJ/mol with an accuracy of *ca.* ±0.03 estimated from the agreement between experimental and calculated bandshapes. Observe that the *4* isomers are two identical ones, which have been taken into account when calculating the energy difference. These results are in reasonable agreement with previous dipole moment [9] and theoretical data.[11]

As discussed above the ^{13}C NMR spectrum of the 1,2 isomer showed no pronounced temperature dependence. There are two possible explanations to this behaviour, either the rotation of the formyl groups is sufficiently fast, even at the lowest temperatures, to make the observation of signals from individual rotamers impossible and/or the molecule is predominantly in conformer *6* or *8*. (Rotamer *7* should under slow exchange conditions give rise to a more complex spectrum since the C-3 and C-6 carbons are not equivalent.) From measurements on other *ortho*-substituted benzaldehydes [1b] we have observed that they are normally in the *trans* conformation. For *ortho*-Me, however, the population ratio is 50:50 and the barrier is reduced but not below the level of detectability. The force field calculations rule out rotamer *8* as the dominant one, whereas the other two, *6* and *7*, might be present in comparable amounts, (Table 3), which is also indicated by the magnitude of the proton-proton five bond coupling to the aldehyde protons, $J = 0.3$ Hz, and the published dipole moment.[9] The force field calculations also indicate that the formyl groups in the two low energy conformers, *6* and *7*, are twisted 10 to 20° out of the ring plane and the barrier is reduced *ca.* 7 kJ/mol, compared to the other two diformylbenzenes (Table 3). It thus seems probable that the fact that we cannot observe any effect from slow rotation on the ^{13}C spectrum in 1,2-benzenedicarbaldehyde is due to a low torsional barrier, *ca.* 20 kJ/mol.

When we initiated this work we decided to determine all $^{13}C-^{1}H$ coupling constants to investigate the possibility of using them to estimate the orientation of the formyl groups. We found that the three-bond coupling to the formyl proton is *ca.* 2 Hz and the four-bond one *ca.* 0.5 Hz and that these do not change in a systematic way with the conformation of the formyl group. These molecules are, however, not very well suited for this investigation. Both the 1,3 and 1,4 isomers have population ratios close to 50:50 and the 1,2 isomer is probably not planar. None of the coupling constants seem to be as sensitive to the formyl group orientation as the $^{1}H-^{1}H$ five-bond coupling. For a series of *p*-substituted benzaldehydes, however, we found that the magnitude of the two-bond coupling to the formyl proton varies linearly with the free activation energy of the formyl rotation barrier, as shown in Fig. 4.

Torsional barriers. For both the 1,3- and 1,4-dicarbaldehydes the barrier to internal rotation was determined from band shape analysis of low temperature ^{13}C NMR spectra. In these cases each of the signals, equivalent at high temperature, can in principle give rise to three or four different signals at low temperature due to the effect of the "frozen-out" rotation of two formyl groups. The high temperature singlet from the 2-, 3-, 5- and 6-carbons for the 1,4 isomer is split into four signals of almost equal intensity at temperatures below −140 °C

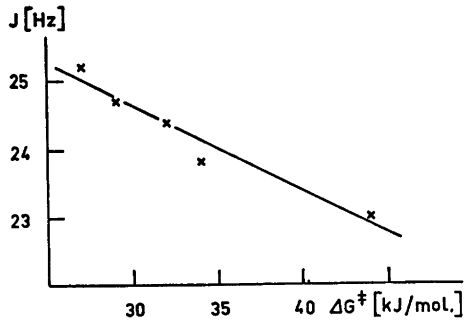


Fig. 4. A plot of the magnitude of the two-bond proton – carbon-13 coupling (C-1 – aldehyde proton) against the activation free energy for the formyl group rotation.

and as can be seen from Fig. 3 the low temperature spectrum from the 1,3 isomer is fairly complex, but is easily interpretable. The signals from the aldehyde carbon and the ring carbons 1 and 3 are little influenced by the orientation of the formyl groups and give rise to narrow doublets. The 2-carbon can give rise to three different signals and the 4- and 6-carbons can give four signals. All these signals are really observed at low temperature (below -135 °C). The remaining signal at 131.7 ppm is from the 5-carbon and never splits but broadens slightly at the lowest temperature. This could in principle be a triplet.

In order to calculate the exchange rates from these spectra it was necessary to treat them as four site exchange cases, and furthermore the 1,3 isomer was treated as two overlapping four site exchange cases. For the 1,4 isomer the band-shape calculations were made on spectra from -108 to -143 °C resulting in $\Delta G^{\ddagger} = 28.6 \pm 0.5$ kJ/mol and for the 1,3 isomer from -98 to -127 °C resulting in $\Delta G^{\ddagger} = 31.0 \pm 0.5$ kJ/mol. For both the compounds $\Delta S^{\ddagger}$ was found to be close to zero and slightly negative ($|\Delta S^{\ddagger}| < 10$ J/mol) in agreement with other formyl group torsional barriers.[1a] The free energy of activation data are also in good agreement with the data for other benzaldehydes and also demonstrate a stronger effect from a *p*-substituent as compared to an *m*-substituent. The force field calculations, however, gave the same activation energy for both the 1,3 and 1,4 isomers.

EXPERIMENTAL

The benzenedicarbaldehydes were commercial products from FLUKA AG (Switzerland) and were recrystallized before use. The Freons were from Hoechst AG (Germany) and were used without purification.

The low temperature spectra were obtained as described before[1b] and to obtain proton coupled spectra the gated decoupling technique was used to retain the Overhauser effect and in one case a $180 - \tau - 90$ pulse sequence was used to nullify the signals from the proton-bearing carbons and in this way isolate the spectrum from the substituted ring carbons. In this latter case we used a pulse delay of 30 s and $\tau = 3.1$ s.

The calculation of the bandshapes of the low temperature spectra were made by means of a four-site exchange program, described elsewhere[13] with the exchange between single lines. For the 1,3 isomer the final bandshape was the sum of two independent four-site cases. The determination of the rate constants was made by means of a visual fitting of the calculated to experimental spectra, which always gave excellent agreement. All calculations were performed on a Univac 1108 computer.

The temperature in the sample was measured with a thermocouple fixed inside the insert as described previously.[1b]

The force field calculations were performed as described by Liljefors and Allinger.[4]

Acknowledgement. We are grateful to the Swedish Natural Science Research Council, le Centre National de la Recherche Scientifique and the Royal Physiografic Society of Lund for financial support.

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Derivatives and Reactions of Glutacondialdehyde. VII. Reaction of the Glutacondialdehyde Anion with Heterocumulenes

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Dedicated to Professor K. A. Jensen on his 70th birthday

Organic isothiocyanates and isoselenocyanates react with the glutacondialdehyde anion (*1*) to give 1-substituted-3-formyl-2(1*H*)-pyridinethiones and -selones, respectively. ^{1}H NMR spectroscopy showed the intervention of a stable intermediate, *i.e.* the 2-(*N*-substituted-thio or selenocarbamoyl)glutacondialdehyde anions (*8*). Subsequent cyclization leading to the products only takes place in the presence of water.

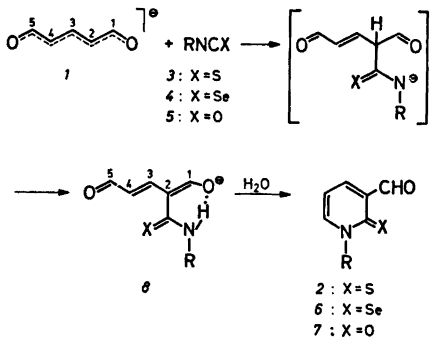
The reaction of *1* with organic isocyanates, at best, gives 1-substituted-3-formyl-2(1*H*)-pyridones in poor yield. The preparation of these compounds is most conveniently effected by desulfuration of the pyridinethiones.

The reactions of *1* can be rationalized in terms of the concept of hard and soft acids and bases.

Derivatives of glutacondialdehyde are bifunctional reagents and, as such, potential precursors for various heterocyclic compounds.[1] In connection with a current investigation [2] of the properties of the glutacondialdehyde anion (*1*) we have studied the reactions with heterocumulenes. Recently, we have shown [3] that 1-substituted-3-formyl-2(1*H*)-pyridinethiones (*2*) are formed from *1* and organic isothiocyanates (*3*). We now report on the course of this reaction and present a study of the reaction of *1* with organic isoseleno- (*4*) and isocyanates (*5*) which leads to 1-substituted-3-formyl-2(1*H*)-pyridineselones (*6*) and -ones (*7*), respectively. The last reaction is not suited for preparative work and alternative routes to *7* from the readily available compounds *2* have been included.*

* The spectroscopic properties of *2* and *6* will be reported in a separate paper.

RESULTS



Scheme 1.

Course of reaction. The time dependent ^{1}H NMR spectrum of the reaction mixture of *1* (potassium salt) and methyl isothiocyanate is shown in Fig. 1. The figure shows the disappearance of the signal from *1* and simultaneous appearance of the signal of a single new species (*8*). The same pattern was observed in the reactions of *1* with phenyl and *t*-butyl isothiocyanate and with *t*-butyl isoselenocyanate. The integral ratio of the well-separated signals from H(1)+H(5) in *8* and from H(2)+H(4) in *1* allows an estimate of the rate of the reaction. The following periods for 50 % conversion have been obtained in this way: Reagent ($t_{50\%}$ min; °C), phenyl isothiocyanate (10; 20), methyl isothiocyanate (97; 50 and 16; 80) and *t*-butyl isothiocyanate (*ca.* 3×10^3; 73). The results show that aryl isothiocyanates are more

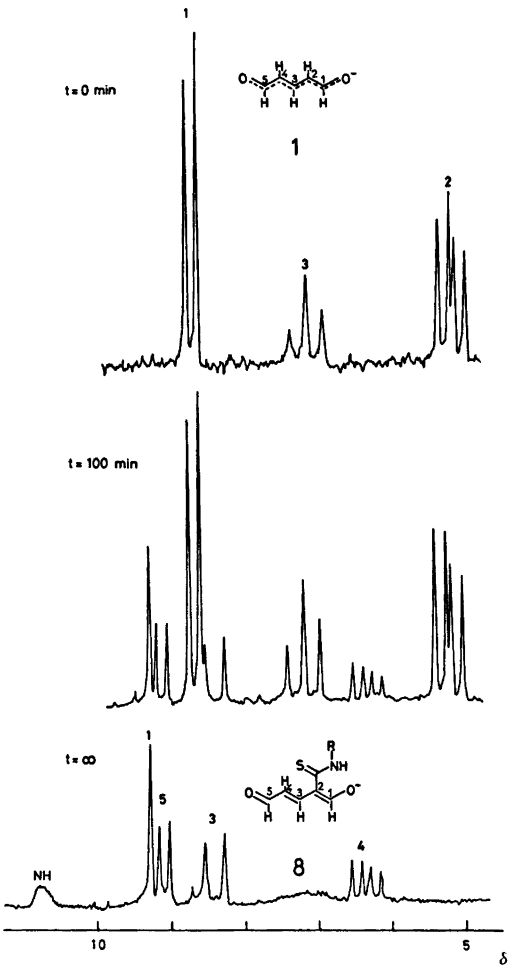


Fig. 1. ^{1}H NMR spectrum of the reaction mixture of *1* and methyl isothiocyanate (DMSO-d_6, 50 °C).

reactive than alkyl isothiocyanates and that the sterically hindered *t*-butyl isothiocyanate is the least reactive species. The same trend was qualitatively observed in the isoselenocyanate series.

Table 1 presents ^{13}C and ^{1}H chemical shifts and H–H coupling constants * for selected compounds *8*. When these values are compared to the corresponding values for the anion *1* and some of its simple derivatives, the structures of which have been established as *all-trans*,[2] this comparison strongly indicates that also the intermediate has the *all-trans* configuration *8*. Additional evidence for this structure is found in the low-field NH-signal ($\delta \sim 12$) indicating a chelated proton. Furthermore, the ^{13}C shift of C(5) is in complete agreement with values reported for α,β-unsaturated aldehydes,[6] suggesting that the formulation *8* is the most reasonable classical representation of the delocalized anion.

Solutions of *8* in dry DMSO stay unchanged for several days at room temperature. This is not surprising since the system *8* is a vinylogue of a push-pull substituted ethylene. The latter systems are reported to form stable, almost nonbasic, mono-anions.[7] However, on addition of water, solutions of *8* undergo a rapid cyclization to give the products, with the exception of the *N*-*t*-butyl derivatives.

The function of water in the cyclization step is almost certainly that of an acid-base active species. The conversion of the stable intermediate *8* into a species which can undergo cyclization requires *trans-cis* isomerization around the C(3) – C(4) bond and a tautomerization disrupting the internal interaction between electrophilic and nucleophilic centers. Proton exchange steps are probably involved in both conversions.

1-Alkyl and aryl-3-formyl-2(1H)pyridineselones (6). The yields of *6* obtained from the reaction of *1* with a series of alkyl and aryl isoselenocyanates are presented in Table 2. Neither these yields nor the reaction conditions differ appreciably from those reported previously for the analogous sulfur compounds.[3] Thus, the aryl isoselenocyanates reacted readily with *1* at room temperature whereas the reaction with alkyl isoselenocyanates required an elevated temperature (*ca.* 80 °C). As in the sulfur series the yield of the *t*-butyl derivative was anomalously low. This feature can be ascribed to a strong repulsive interaction between the bulky *t*-butyl group and the neighbouring chalcogen atom, impeding the cyclization to give *6*. In these terms it is reasonable that the yield of the selenium compound is even lower than that of its thio analogue.

The chemical properties of compounds *6* resemble those of their sulfur analogues. In

* The assignment of the ^{13}C NMR chemical shifts were based on both the decoupled and the undecoupled spectra.

Table 1. ^1H and ^{13}C chemical shifts of the intermediates *8* (DMSO-d_6).

R	H(1)[a]	H(3)	H(4)	H(5)	NH	Other H[b]
Methyl	9.30s	8.40d	6.31dd	9.15d	11.65s	2.82d CH_3
X=S		J=15	J=15, 8	J=8		J=4
Phenyl	9.31s	8.15d	6.35dd	9.20d	13.95s	7.2–7.9 m
X=S		J=15	J=15, 8	J=8		phenyl
t-Butyl	9.15s	8.43d	6.11dd	9.00d	11.80s	1.45s CH_3
X=S		J=15	J=15, 8	J=8		
t-Butyl	9.16s	8.96d	6.00dd	9.13d	12.50s	–
X=Se		J=15	J=15, 8	J=8		
R	C(1)[a]	C(2)	C(3)	C(4)	C(5)	Other C[c]
Methyl	180.8	110.1	161.9	110.5	191.4	214.5 C=S
X=S						30.6 CH_3
Phenyl	180.7	111.2	161.2	112.1	191.8	214.7 C=S
X=S						

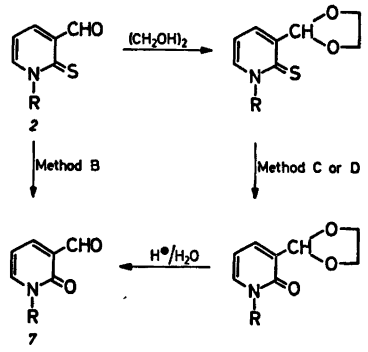
[a] The numbering of the intermediate is given in Scheme 1. [b] δ (ppm from TMS), J in Hz. [c] Hz from TMS.

particular, they are much more inert than is customary of compounds containing a formal selenocarbonyl group *i.e.* they are neither affected by dilute acid or base, nor by atmospheric oxygen at room temperature and they are thermally stable up to at least 150 °C.

1-Alkyl and aryl-3-formyl-2(1H)-pyridones (7). The reaction of *1* with aryl isocyanates afforded compounds *7* in low yields (method A, Table 3). A study of this reaction by ^1H NMR spectroscopy showed the emergence of a complex spectrum which defied analysis in terms of a single intermediate or reaction product. Thus, the low yield of *7* is caused by the fact that the isocyanates, unlike their thio- and selenoanalogues, do not react specifically at C(2) of *1*.

Alternative preparative routes to *7* from *1* *via* the readily accessible compounds *2* were studied (Table 3 and Scheme 2). We have previously prepared *7b* from the corresponding thione by reaction with mercury(II) oxide.[3] However, mercury(II) acetate is normally used [8] and better (although only fair) yields were obtained with this reagent (method B).

The oxidation with alkaline hydrogen peroxide is a general method for the conversion of thiocarbonyl to carbonyl functions.[9] Before this reagent was used in the present investigation, the aldehyde group was protected by 1,3-dioxolane formation with ethylene glycol. Compound *7b* was prepared in a good yield in this way (method C), whereas only traces of the *N*-alkyl compounds, *7c* and *7d*, were observed. Probably oxidation of the *N*-alkyl group takes place under these reaction conditions. The three compounds *7b*–*7d* were obtained in very good yields by oxidation with *m*-chloroperoxybenzoic acid (method D).



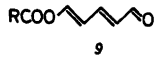
Scheme 2. Method B, $(CH_3CO_2)_2Hg$; C, H_2O_2/OH^-; D, *m*-$ClC_6H_4CO_3^+$. *Cf.* Table 3 for yields.

DISCUSSION

The reactions of ambident anions such as *1* have been subject to several mechanistic interpretations.[10] Of these, the concept of hard and soft acids and bases (HSAB), introduced by Pearson,[4] has the most general applicability. In Klopman's [5] interpretation the hard acid-hard base reactions are dominated by the electrostatic interaction and the soft acid-soft

base reactions by frontier orbital (LUMO-HOMO) interactions between the reaction partners.

The striking difference between the reaction of *1* with *3* and *4* (almost clean formation of *8*) compared with *5* (complex reaction mixture) can be rationalized in terms of the HSAB concept. In the delocalized aldehyde anion *1* the higher charge density is found at the terminal oxygen atoms, whereas the HOMO amplitude is the highest at the carbon atoms,[2,11] C(2) and C(4). Thus, the oxygen centers should be the harder and the carbon centers the softer bases. In accordance with this view acyl chlorides which are considered as relatively hard generalized acids [4] react at the oxygen atom to give the enol esters [2] *9*.



9

The organic isocyanates *5* are harder acids than their thio- (*3*) and seleno (*4*) analogues. This is apparent from the application of Jørgensen's symbiotic principle [12] or by a more explicit consideration of the LUMO of these three reagents. Since this orbital is of the π^* type, the poor π-overlap between carbon and third- and higher-row elements ensures a lowered LUMO energy of the S- and Se- relative to the O-compounds. Consequently, the observations can be rationalized such that the relatively soft acids *3* and *4* react virtually exclusively at the carbon center whereas competing reaction at the oxygen center becomes important for the harder acids *5*.

The enhanced reactivity of aryl isothio- and isoselenocyanates compared with the alkyl compounds may also be explained by the Klopman approach.[5] The in-phase interaction of the XCN-group LUMO with a higher lying unoccupied orbital of the arene substructure lowers the energy of the compound LUMO thus giving a more efficient soft acid.

EXPERIMENTAL

Microanalyses were carried out by Mr. P. Hansen, Microanalytical Department of the University of Copenhagen.

Instrumentation. IR: Perkin Elmer 457. UV: Beckman ACTA III. ^{1}H NMR: Jeol JNM-PMX 60 or Varian T-60. ^{13}C NMR: Jeol X60. M.p.: Büchi apparatus (uncorrected).

1-Substituted-3-formyl-2(1H)-pyridineselones (6). 1-Aryl-3-formyl-2(1H)-pyridineselones. Aryl isoselenocyanate [13] (25 mmol) and the potassium salt of glutacondialdehyde (25 mmol) were dissolved in DMSO (100 ml). The mixture was stirred for 90 min at room temperature. Half-saturated aqueous sodium chloride (200 ml) was added and the solution was cooled to 0 °C with continued stirring. A red precipitate of *2* was isolated by filtration, washed with water and dried. The crude product was dissolved in methylene chloride-light petroleum (1:1), filtered through alumina (Woelm W 200 neutral; 5 g) and then crystallized from the same solvent mixture. 1-Alkyl-3-formyl-2(1*H*)-pyridineselones were prepared by the same procedure; however, the reaction temperature was raised to *ca.* 80 °C.

1-Substituted-3-formyl-2(1H)-pyridones (7). Method A. To *1* (potassium salt, 0.01 mol) in DMSO or DMF (50 ml) at 50 °C was added aryl isocyanate (0.01 mol). After 1 h the dark red reaction mixture was added to water (100 ml). Filtration, continuous extraction with ether (24 h) and concentration *in vacuo* of the extract yielded a crystalline crude product.

Method B. To the appropriate 3-formyl-2-(1*H*)-pyridinethione [3] (0.01 mol) in chloroform (60 ml) was added a solution of mercury(II)

Table 2. 1-Alkyl and aryl-3-formyl-2(1*H*)-pyridineselones (*6*).

R	Yield %	M.p. °C	Analyses (C, H, N and Se)
Methyl	55	148 – 150	C_7H_7NOSe
Isopropyl	47	119 – 121	$C_9H_{11}NOSe$
t-Butyl	2	65 – 68	$C_{10}H_{13}NOSe$
Cyclohexyl	27	164 – 167	$C_{12}H_{15}NOSe$
Methylbenzyl	58	106 – 107	$C_{14}H_{13}NOSe$
Phenyl	55	195 – 197	$C_{12}H_9NOSe$
p-Methoxyphenyl	67	191 – 193	$C_{13}H_{11}NOSe$
p-Chlorophenyl	24	182 – 184	$C_{12}H_8ClNOSe$
2′-Naphthyl	52	217 – 219	$C_{16}H_{11}NOSe$

Table 3. 1-Alkyl and aryl-3-formyl-2(1*H*)-pyridones (*7*).

Compound	R	Method[a] A	B	C	D	M.p. °C	Analyses (C, H and N)
7a	1'-Naphthyl	8	–	–	–	138–141[c]	$C_{16}H_{11}NO_2$
7b	Phenyl	11	46	65	75	155–157[c]	$C_{12}H_9NO_2$
7c	Methylbenzyl	–	37	0[b]	82	132–133[c]	$C_{14}H_{13}NO_2$
7d	Isopropyl	–	–	0[b]	73	115–116[c]	$C_9H_{11}NO_2$

[a] Yields (%). [b] Pure material was not isolated, but *7c* and *7d* were detected by TLC. [c] Recrystallized from methylcyclohexane or a mixture of benzene and methylcyclohexane.

acetate (0.01 mol) in glacial acetic acid (150 ml). The mixture was left for 30 days and then filtered. The filtrate was poured on a mixture of water (500 ml) and chloroform (50 ml). Further extraction with chloroform (100 ml) and concentration *in vacuo* yielded a pale yellow oil. Stirring with 4 M hydrochloric acid (50 ml) for 4 h at room temperature, extraction with chloroform and work-up gave pale yellow crystals.

Method C. To 1-phenyl-3-(2'-dioxolanyl)-2-(1*H*)-pyridinethiones (0.01 mol) in ice-cold 2 M sodium hydroxide (80 ml) was added 50 % hydrogen peroxide (40 ml). The mixture was stirred at room temperature for 21 h. Then unreacted hydrogen peroxide was destroyed by addition of sodium bisulfite. Acidification with 2 M sulfuric acid (80 ml), stirring for 1 h, extraction with chloroform and concentration *in vacuo* yielded pale yellow crystals.

Method D. To an ice-cold solution of 1-substituted-3-(2'-dioxolanyl)-2(1*H*)-pyridinethione (0.01 mol) and sodium hydroxide (0.04 mol) in ethanol (350 ml) was added *m*-chloroperoxybenzoic acid (0.04 mol). When the temperature reached 20–22 °C, the initial red-orange colour suddenly disappeared. Stirring for 1 h at room temperature, addition of a solution of sodium bisulfite, filtration, evaporation, addition of 2 M sodium hydroxide (100 ml), extraction with ethyl acetate or chloroform (2 × 100 ml) and concentration *in vacuo* yielded a pale yellow oil. Hydrochloric acid (4 M, 50 ml) was added and the mixture was stirred for 4 h at room temperature. Extraction with chloroform and concentration *in vacuo* gave pale yellow crystals.

1-Substituted-3-(2'-dioxolanyl)-2(1H)-pyridinethiones. 1-Substituted-3-formyl-2(1*H*)-pyridinethione [3] (0.04 mol), ethylene glycol (0.08 mol) and *p*-toluenesulfonic acid (0.5 g) in benzene (500 ml) were refluxed (with water separator) for 6 h. If the resulting product was not obtained analytically pure, dissolution in benzene, washing with hydrazine hydrate (*ca.* 98 %) and water removed the impurities.

1-R,S-(Methylbenzyl)-3-(2'-dioxolanyl)-2(1H)-pyridinethione. Yield 91 %. Oil, which after standing for *ca.* two months, crystallized. M.p. = 89–91 °C. Anal. $C_{16}H_{17}NSO_2$: C, H, N, S. *1-Isopropyl-3-(2'-dioxolanyl)-2(1H)-pyridinethione.* Yield 62 %. Orange crystals. M.p. = 79–82 °C. Anal. $C_{11}H_{15}NSO_2$: C, H, N, S, *1-Phenyl-3-(2'-dioxolanyl)-2(1H)pyridinethione.* Reported previously.[3]

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Photolysis of 1,2,5-Selenadiazoles. Formation of Nitrile Selenides

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Dedicated to Professor K. A. Jensen on his 70th birthday

UV photolysis of 1,2,5-selenadiazole, dimethyl-1,2,5-selenadiazole, diphenyl-1,2,5-selenadiazole, diphenyl-1,2,4-selenadiazole and 2,1,3-benzoselenadiazole at room temperature produces nitriles and selenium. At low temperatures (20 K, 85 K) all compounds except the almost photostable 2,1,3-benzoselenadiazole photolyse to very labile transients. Identical transients are formed from diphenyl-1,2,5-selenadiazole and diphenyl-1,2,4-selenadiazole and on the basis of the spectroscopic properties, in particular the IR absorption at 2200 cm^{-1} (solid nitrogen), it is identified as benzonitrile selenide, C_6H_5CNSe. Transients from the photolysis of 1,2,5-selenadiazole and dimethyl-1,2,5-selenadiazole have been detected by low temperature UV spectroscopy. These transients are tentatively assigned the structures HCNSe and CH_3CNSe. Mechanistic studies were undertaken in order to determine the character of the excited states involved. At room temperature 1,2,5-selenadiazole, dimethyl-1,2,5-selenadiazole and diphenyl-1,2,4-selenadiazole photolyse *via* the singlet state exclusively, with quantum yields of 0.71 ± 0.05, 0.8 ± 0.1 and 0.085 ± 0.05, respectively. Diphenyl-1,2,5-selenadiazole photolyses both *via* the singlet state (80 %) and the triplet state (20 %) with a total quantum yield of 0.96 ± 0.05.

Benzonitrile sulfide has been generated as a reactive intermediate by thermal and photochemical reactions of a variety of substrates and it has been trapped in 1,3-dipolar cycloaddition reactions with acetylenes, nitriles and maleic anhydride.[1] Furthermore, it has been characterized by low temperature spectroscopic methods.[1c] Acetonitrile sulfide can also be generated thermally [1a,2] and trapped. Since nitrile selenides were unknown a photochemical study of selenadiazoles (Chart 1) was undertaken.

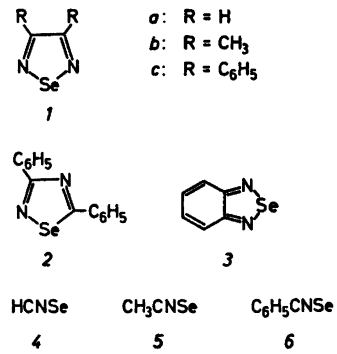


Chart 1.

The photolysis at ambient temperature of systems related to *1a–c*, *2* and *3* has been the subject of several investigations. Photolysis of dimethyl- and diphenyl-1,2,5-thiadiazole gave acetonitrile and benzonitrile, respectively, as well as sulfur.[3] Diphenyl-1,2,5-oxadiazole (diphenylfurazan) was found to give benzonitrile together with products derived from initially formed benzonitrile oxide.[3,4] Nitrile oxides have also been trapped from dimethylfurazan [3] and from benzofurazan.[5] In contrast, diphenyl-1,2,4-oxadiazole photolyses giving products derived from an intermediate nitrene.[6]

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RESULTS

Irradiation of compounds 1a–c *at room temperature.* In a series of experiments $\sim 10^{-4}$ M solutions of *1a–c* in 96 % ethanol were photolysed at 300 ± 20 nm, and the result of photolysis analyzed by UV spectroscopy. Photolysis of substrates *1a* (λ_{max} 285 nm, ε 6300 M^{-1} cm^{-1}) and *1b* (λ_{max} 288 nm, ε 7300 M^{-1} cm^{-1}) only permitted the observation of a continuous absorption due to colloidal selenium. Photolysis of compound *1c* (λ_{max} 317 nm, ε 12 700 M^{-1} cm^{-1}) produced benzonitrile and selenium.

Identical results were obtained with aerated and deoxygenated solutions and no effect of concentration on the product distribution was detected when 10^{-2} M solutions were photolysed with the more intense pyrex-filtered light ($\lambda > 300$ nm). Photolysis of *1a* in 96 % ethanol and analysis by titration with silver nitrate gave an 87 % yield of hydrogen cyanide. Analysis by GLC of the photolysis mixture from *1b* in chloroform showed that the formation of acetonitrile was quantitative. Likewise, comparison by UV spectroscopy of the diluted photolysis mixture from *1c* in 96 % ethanol with a reference solution of benzonitrile showed that only formation of benzonitrile had taken place. Compounds *1a–c* thus undergo cleavage to nitriles and selenium.

$$1a-c \xrightarrow{h\nu,\ 25\ ^\circ C} 2RCN + Se$$

Attempts were made to trap the initially formed benzonitrile selenide (*6*) by irradiation of *1c* in neat dimethyl acetylenedicarboxylate, but without success.

Irradiation of compounds 2 *and* 3 *at room temperature.* 10^{-4} and 10^{-2} M aerated solutions of *2* [λ_{max} 310 nm (sh), ε 5800 M^{-1} cm^{-1}] in 96 % ethanol were irradiated with pyrex-filtered light. This produced only benzonitrile and selenium (UV).

$$2 \xrightarrow{h\nu,\ 25\ ^\circ C} 2C_6H_5CN + Se$$

2,1,3-Benzoselenadiazole (*3*, λ_{max} 332 nm, ε 15 900 M^{-1} cm^{-1}), 4×10^{-3} M in non-degassed 96 % ethanol, photolysed extremely slowly to a mixture of 2,4-hexadienedinitriles as well as selenium (Chart 2) when irradiated with pyrex-filtered light. The ratios of isomers formed were *cis,cis*:*cis,trans*:*trans,trans* = 1.0:2.6:2.0.

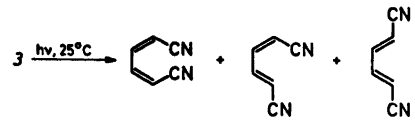


Chart 2.

The three isomeric 2,4-hexadienedinitriles, which are known compounds,[7] were identified by elemental analysis and IR spectroscopy (see Experimental).

Irradiation of *cis,cis*-2,4-hexadienedinitrile in ethanolic solution (λ_{max} 260 nm) through quartz gave a mixture of the isomers *cis,cis*:*cis,trans*:*trans,trans* = 1.0:2.6:1.7. This result indicates that photoequilibration among the isomers is taking place during photolysis of *3*.

Irradiation of compounds 1c *and* 2 *at low temperature.* Irradiation of compound *1c* at low temperature was performed in a variety of media [poly(vinyl chloride) (PVC), diethyl ether, isopentane, ethanol 5:5:2 (EPA), nitrogen] and the reactions followed by spectroscopy. The best UV results were obtained in a PVC film, *ca.* 20 μm thick. Irradiation at 85 K with 300 nm light gave rise to new absorptions at 255 nm (ε 13 000 – 25 000 M^{-1} cm^{-1}), 340 nm

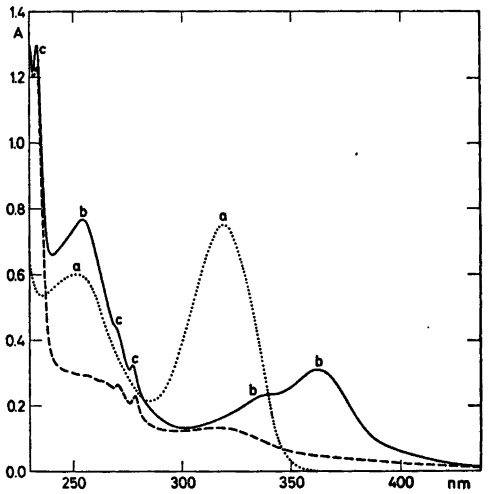


Fig. 1. UV spectra after photolysis at 85 K of diphenyl-1,2,5-selenadiazole (*1c*) in PVC film, 20 μm thick, with 300 ± 20 nm light. a, starting material at 85 K, λ_{max} 320 nm, b, benzonitrile selenide at 255 nm, 340 nm and 362 nm, c, benzonitrile. ---, after heating from 85 K to room temperature. Absorptions from starting material and benzonitrile can be seen.

and 362 nm (ε 5000 – 10 000 M^{-1} cm^{-1}) (Fig. 1). Simultaneously, absorptions from benzonitrile appeared at 233, 270 and 278 nm. Upon prolonged photolysis at 300 nm or on heating above ~240 K the absorption bands at 255, 340 and 362 nm disappeared. Simultaneously, the absorption bands from benzonitrile increased in intensity (Fig. 1). The new absorption bands were assigned to benzonitrile selenide. This assignment is based on the thermal and photochemical properties, on the IR results discussed below and on the results of the following experiment with compound *2*.

When compound *2* was irradiated in PVC under similar experimental conditions, absorptions from the same transient appeared at 258, 325 and 360 nm (ε 5000 – 10 000 M^{-1} cm^{-1}) together with absorptions from benzonitrile at 233 and 277 nm (Fig. 2). Bands from the transient were less distinct in this experiment due to the greater photostability of *2* relative to *1c*.

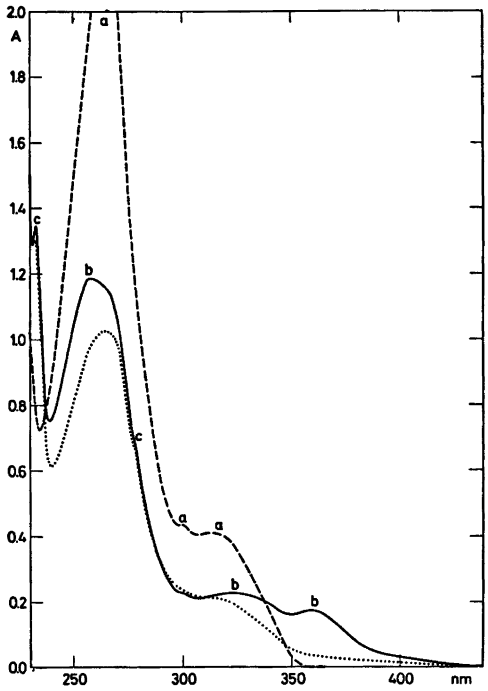
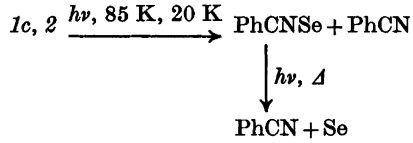


Fig. 2. UV spectra after photolysis at 85 K of diphenyl-1,2,4-selenadiazole (*2*) in PVC film, 20 μm thick, with 300 ± 20 nm light. a, starting material at 85 K, λ_{max} 313 nm, b, benzonitrile selenide at 258 nm and 360 nm, c, benzonitrile. . . . . , after heating from 85 K to room temperature. Absorptions from starting material and benzonitrile can be seen.



By irradiation of *1c* in frozen EPA (diethyl ether, isopentane, ethanol 5:5:2) at 85 K the same spectroscopic feature (Table 1) resulted, although competitive photolysis of the benzonitrile selenide formed made its absorptions less intense. Prolonged irradiation at 300 nm, selective excitation of product absorptions at 360 nm or heating above 110 K caused the benzonitrile selenide bands to disappear with simultaneous enhancement of the absorption bands from benzonitrile. Selenium was precipitated when samples were heated from cryogenic temperatures.

Experiments were also undertaken in nitrogen matrix with the primary aim of obtaining characteristic absorptions of benzonitrile selenide in the infrared. UV photolysis at 300 nm of *1c* after deposition in solid N_2 at 20 K gave valuable data, although competitive photolysis of the benzonitrile selenide so formed remained a serious problem. The UV and IR spectra from these experiments are shown in Figs. 3 and 4. These spectra were recorded from the *same* sample.

The UV spectrum in Fig. 3 shows the two lowest lying transitions of benzonitrile selenide at 356 and 390 nm. The longer wavelength band also appears as a maximum in EPA but as a continuum in PVC (Figs. 1 and 2). The IR spectrum (Fig. 4) consists of two bands in the

Table 1. The UV absorptions of benzonitrile selenide, *6*.

Compound	Medium	Absorption/nm			
1c	PVC	255	340	362	[a]
2	PVC	258	325	360	[a]
1c	EPA	254	325(sh)	357	384
1c	N_2			356	390

[a] The long wavelength absorption cannot be located in PVC since site effects render the absorption bands broad.

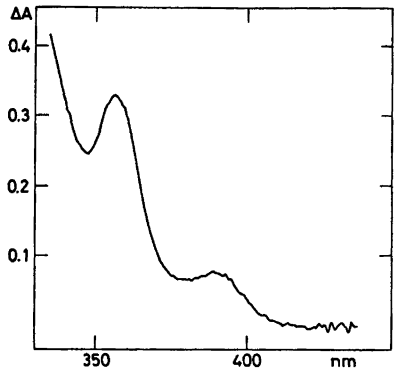


Fig. 3. UV spectrum after photolysis at 20 K of diphenyl-1,2,5-selenadiazole (*1c*) in a nitrogen matrix with 300 ± 10 nm light. The bands at 356 and 390 nm are assigned to benzonitrile selenide.

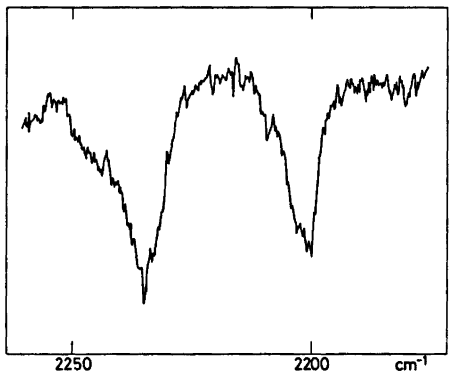


Fig. 4. IR spectrum after photolysis at 20 K of diphenyl-1,2,5-selenadiazole (*1c*) in a nitrogen matrix with 300 ± 10 nm light. The bands are assigned to benzonitrile selenide, 2200 cm^{-1}, and benzonitrile, 2235 cm^{-1}. Intensity in transmission.

2300 – 1900 cm^{-1} region, benzonitrile at 2235 cm^{-1} and benzonitrile selenide at 2200 cm^{-1}. Irradiation of the matrix with pyrex-filtered light caused the absorptions in the UV spectrum and the absorption in the IR spectrum at 2200 cm^{-1} to disappear. Simultaneously, the benzonitrile band was slightly enhanced in intensity.

IR absorptions of benzonitrile selenide were also observed at 85 K when *1c* in a PVC film (0.5 M, 100 μm thick) was irradiated in the long wavelength part of its absorption (λ_{max} ~320 nm) with 330 nm light. However, the maximum intensity of the absorption at 2190 cm^{-1} was only small.

The extremely small quantum yield for photolysis of *3* at 85 K prevented spectroscopic detection of primary products from this species.

Irradiation of compounds 1a – b *at low temperature.* These irradiations were carried out in PVC, EPA and argon. Spectra corresponding to the irradiation products of compounds *1a – b* in PVC with 300 nm light are shown in Figs. 5 and 6. Absorption bands for the photolysis product of compound *1a* were found at 237 nm (ε ~ 18 000 M^{-1} cm^{-1}) and ~255 nm (shoulder) and absorption bands for the photolysis product of *1b* at 233 nm (ε ~ 25 000 M^{-1} cm^{-1}) and ~252 nm (shoulder). These bands disappeared upon prolonged photolysis with pyrex-filtered light ($\lambda > 300$ nm) and upon heating above *ca.* 220 K. No new absorptions resulted (HCN and CH_3CN does not absorb above 200 nm [8]).

The same spectroscopic behaviour was found when *1a* was photolysed in frozen EPA at 85 K with 300 nm light. The spectrum of the product had maximum absorption at 233 nm (ε ~ 19 000 M^{-1} cm^{-1}) and this product disappeared when the matrix was heated above 110 K. No new absorptions resulted. Selenium was precipitated from samples heated from cryogenic temperatures.

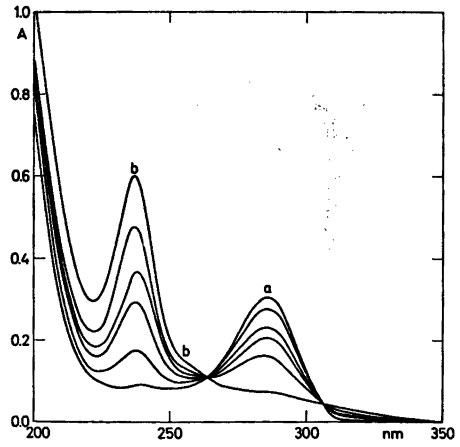


Fig. 5. UV spectra after photolysis at 85 K of 1,2,5-selenadiazole (*1a*) in PVC film, 20 μm thick, with 300 ± 20 nm light. a, starting material at 287 nm, b, photoproduct at 237 and 255 nm.

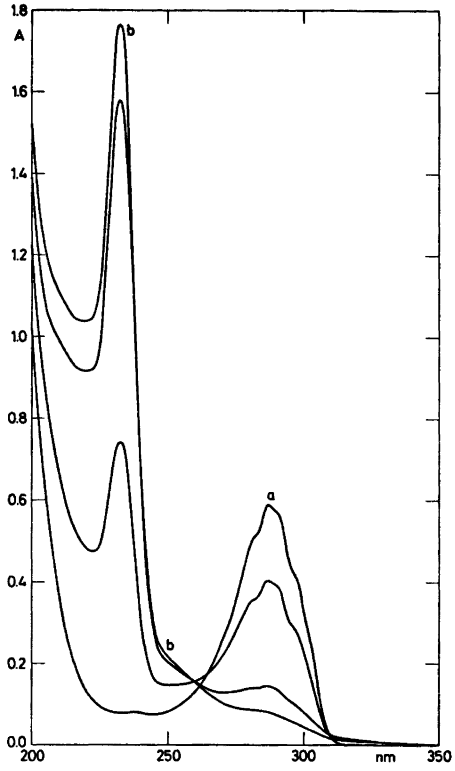


Fig. 6. UV spectra after photolysis at 85 K of dimethyl-1,2,5-selenadiazole (*1b*) in PVC film, 20 μm thick, with 300 ± 20 nm light. a, starting material at 287 nm, b, photoproduct at 233 and 252 nm.

The argon matrix experiments were carried out at 20 K with guest-host ratios of 1/10 000 – 1/1000. The results of these experiments are shown in Fig. 7. The absorption bands for the photolysis products of *1a* and *1b* with maxima at 233.0 and 230.2 nm, respectively, possess vibrational fine-structure * which is not observed in PVC or EPA at 85 K. The products were photolabile and disappeared on further irradiation at 300 nm. This problem of secondary photolysis prevented IR detection of the intermediates and it could not be circumvented by consecutive application and photolysis or by photolysis with plane-polarized light. Thus irradiation of *1a* in argon (1/5000) with 300 nm light produced sharp bands at 2044.8 and 2046.5 cm^{-1} and a weak broad band at 2091 cm^{-1}. The two sharp bands are assigned to the radical CN (2046.0 cm^{-1} in Ar,[9] the splitting of the band is due to site-effects) and the band at 2091 cm^{-1} to HCN-monomer.[10] Additional bands from HCN-oligomers [10b] were observed, especially when a more concentrated gas mixture (1/1000) was irradiated.

Photolysis of compound *1b* in argon (1/5000) at 300 nm led to absorptions from acetonitrile at 2250 and 2252 cm^{-1} (site effect),[11] from the CN radical as a doublet at 2046 cm^{-1},[9] and from hydrogen cyanide monomer at 2090 cm^{-1}.[10] In addition, bands from hydrogen cyanide oligomers were present.[10b]

An interesting detail in these IR experiments with compounds *1a* and *1b* was the occurrence of a green emission when the photolysed matrix was slowly heated. We tentatively assign this emission to chemiluminescence from the reaction $Se + Se \rightarrow Se_2 + h\nu$. Diselenium is known to fluoresce when excited in the gas phase.[12]

IR experiments were also performed with PVC films (100 μm thick). PVC is sufficiently transparent between 2800 and 1400 cm^{-1}. The experiments were carried out at 85 K and at 20 K with UV light of different wavelength (255, 295 and 330 nm). However, the product distribution was independent of the experimental conditions chosen. The only observed products were hydrogen cyanide at 2095 and 2070 cm^{-1} and acetonitrile at 2250 cm^{-1}.

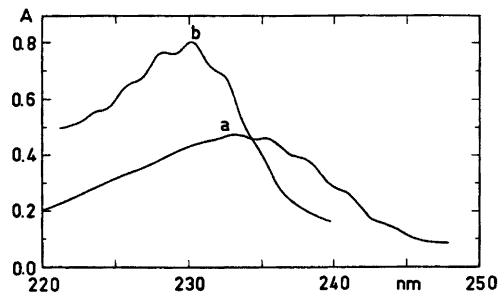


Fig. 7. UV spectra after photolysis at 20 K of 1,2,5-selenadiazole (*1a*), guest-host ratio 1/5000, and of dimethyl-1,2,5-selenadiazole (*1b*), guest-host ratio 1/10 000, in argonmatrices with 300 ± 10 nm light. a, photoproduct from *1a*, λ_{max} 233.0 nm, b, photoproduct from *1b*, λ_{max} 230.2 nm.

* Photoproduct from *1a*, 230.8, 233.0, 235.3, 238.1, 241.2 and 244.4 nm. Photoproduct from *1b*, 223.4, 225.7, 227.9, 230.2, 232.3 and 234.8 nm.

Table 2. Quantum yields of reaction ($\Phi_r°$) and reactive state of selenadiazoles at room temperature.

Compound	$\Phi_r°$	Reactive state, unsensitized
1a	0.71(5) at 285 (12) nm	Singlet
1b	0.8 (1) at 290(8) nm	Singlet
1c	0.96(5) at 317 (8) nm	Singlet + triplet
2	0.085(5) at 310 (10) nm	Singlet

The results of the low-temperature experiments with compounds *1a* and *1b* are summarized below.

$$1a \xrightarrow{h\nu,\ 20\ K} HCN + HCNSe? \xrightarrow{h\nu,\ 20\ K} HCN + CN + Se$$

$$1b \xrightarrow{h\nu,\ 20\ K} CH_3CN + CH_3CNSe? \xrightarrow{h\nu,\ 20\ K} CH_3CN + HCN + CN + Se$$

Quantum yields. Quantum yields for chemical reaction, $\Phi_r°$, were measured in 96 % ethanol at room temperature with samples stirred and deoxygenated with a stream of argon. The wavelength of light used corresponds to that of maximum absorption of the solutions. The measured quantum yields are given in Table 2 (see also Experimental Section). $\Phi_r°$ (*1c*) in heptane, benzene and ethanol were identical within 5 % deviation.

Quenching and sensitization experiments. In order to determine the multiplicity of the excited states involved experiments were carried out with added quenchers (oxygen, $E_S = 23$ kcal/mol [13,*] and piperylene, $E_T = 57-59$ kcal/mol [14a]) and sensitizers (xanthone, $E_T = 74$ kcal/mol,[13] and thioxanthone, $E_T = 65$ kcal/mol [13]).

Triplet sensitized photolysis of compounds *1a* and *1b* was studied in PVC films (100 μm thick) with xanthone (~0.5 M) as sensitizer and with acceptor concentrations of *1a* or *1b* ~0.5 M. Irradiation was carried out at 85 K and at 195 K. Xanthone was excited selectively with 363 nm light (interference filter). Only absorptions from hydrogen cyanide and acetonitrile were detected (IR).

* 1 kcal = 4.184 kJ.

Analogous experiments with compounds *1c* and *2* were performed in poly (methyl methacrylate) (PMMA, 50 μm thick) at room temperature with thioxanthone as sensitizer and with concentrations of donor and acceptor ~0.1 M. When the sensitizer was selectively excited at 395 nm (interference filter), benzonitrile was readily formed (IR, 2220 cm^{-1}) from compound *1c* but sensitized photolysis of *2* did not occur even on prolonged photolysis.

The effect of quenching with piperylene and oxygen was measured relative to deoxygenated samples. The rate of photolysis of selenadiazoles *1a* (in heptane) and *1b* (in benzene) was insensitive to saturation of solutions with oxygen (1 atm) and also insensitive to concentrations of piperylene up to 0.3 M. However, the photoreaction of diphenylselenadiazole, *1c*, (in ethanol), was partly quenched by oxygen ($\Phi_r = 0.33 \pm 0.05$).

The degree of quenching of *1c* in benzene was studied as a function of the concentration of added piperylene. The so-called Stern-Volmer plot is shown in Fig. 8, where the ratio $\Phi_r°/\Phi_r$ is depicted as a function of piperylene concentration. Irradiation was carried out at 320 ± 7 nm. Under these conditions, light absorption by piperylene is negligible. Also shown in Fig. 8 is the insensitivity of $\Phi_r(2)$ to the presence of piperylene. The mechanistic consequences of these experiments are discussed in the next section and summarized in Table 2.

Luminescence of excited states. Compounds *1a–c* did not luminesce in liquid solution at room temperature, but in frozen EPA at 77 K compounds *1b–c* were found to phosphoresce. No fine structure could be seen in the spectra. Compound *1a* did not luminesce at 77 K. Quantum yields of emission were measured relative to compounds with known quantum yields. Phosphorescence lifetimes (EPA, 77 K) were measured using the rotating chopper of the phosphorescence accessories in the emission spectrophotometer (see Experimental Section). Emission data are given in Table 3.

DISCUSSION

Photolysis of compounds 1a–c *and* 2. It is concluded from the low temperature UV spectra in Figs. 1 and 2 that compounds *1c*

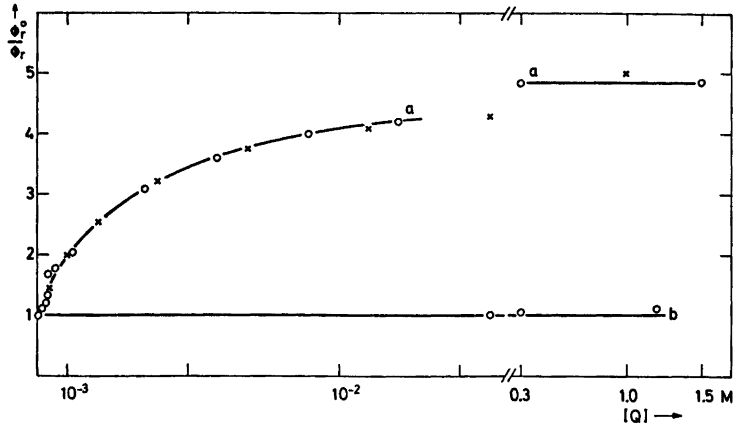


Fig. 8. Plots of Φ_r°/Φ_r against concentration of piperylene in benzene at room temperature. a, diphenyl-1,2,5-selenadiazole (*1c*) (4.0×10^{-4} M in benzene, λ_{ex} 320 ± 7 nm) and b, diphenyl-1,2,4-selenadiazole (*2*) (4.6×10^{-4} M in benzene, λ_{ex} 310 ± 20 nm). ×, calculated values using $a = 4$ and $k_q\tau = 1600$ M^{-1}, see Discussion.

and *2* photolyse to benzonitrile and a common intermediate, which is identified as benzonitrile selenide on the basis of its properties: (i) Photolysis or warming from cryogenic temperatures produces benzonitrile exclusively. (ii) In nitrogen matrix the IR absorption at 2200 cm^{-1} (Fig. 4, 2190 cm^{-1} in PVC) disappeared on photolysis, *and the UV bands of the same sample (Fig. 3) disappeared simultaneously.*

These properties are very similar to the properties of benzonitrile sulfide,[1c] which absorbs at 2185 cm^{-1} in PVC [15] and features a UV spectrum with the same profile as that of benzonitrile selenide shown in Figs. 1–3, except that the maxima of the selenide are red-shifted. Benzonitrile selenide is thermally less stable than the sulfide.[1c] This probably explains why trapping experiments were unsuccessful.

Table 3. Phosphorescence data of selenadiazoles *1b* and *1c* in EPA at 77 K.

Compound	λ_{max}/nm [b]	Φ_p	$\tau_p/10^3$ s
1b [a]	455	0.14(3)	2.5(1)
1c [a]	538	0.33(8)	1.7(1)

[a] Samples of *1b* and *1c* ($\sim 10^{-4}$ M) were excited at 280–290 nm and 310–320 nm, respectively. [b] Spectra were corrected.

A comparison of the two spectra in Fig. 7 shows that 1,2,5-selenadiazole (*1a*) and dimethyl-1,2,5-selenadiazole (*1b*) photolyse to different products. This excludes possible common intermediates, *e.g.* Se_n, CN, HSe and C_2N_2 as candidates for the assignment of the spectra. Since the positions and the intensities of the maxima are only slightly different (Figs. 5, 6 and 7) we assume that the two primary products differ only by the substitution of the hydrogen with a methyl group. Their thermal stabilities are comparable to the stability of benzonitrile selenide. The two intermediates must display weak absorptions in the near ultraviolet since photolysis with 300 nm light caused degradation of all material absorbing in this spectral region. The nitrile selenides HCNSe (*4*) and CH_3CNSe (*5*) are expected to photolyse to nitriles and selenium, neither of which absorbs above 200 nm. This tentative assignment is further supported by the observation of chemiluminescence, believed to originate from dimerization of selenium atoms.

On the other hand photolysis of *1a* as well as *1b* caused formation of CN radicals observed at 2046 cm^{-1}. This result is only consistent with the assumption that HCNSe/HCN and CH_3CNSe/CH_3CN are the sole primary photoproducts of *1a* and *1b* if the two nitrile selenides can produce CN radicals, at least partially, upon irradiation. As mentioned, the analogy to benzonitrile selenide makes us believe, that

extrusion of selenium is the prevailing photolytic pathway. Alternatively, the CN radicals are formed directly from *1a* and *1b* or from primary photoproducts of these with structures different from *4* and *5*. HSeNC and CH_3SeNC could be such precursors, but intermediates with this structure would probably rearrange thermally to selenocyanates, RSeCN. Methyl selenocyanate, a stable compound, was not formed when *1b* was photolysed at room temperature and no absorption from methyl selenocyanate (2152 cm^{-1} [16]) was observed in the IR experiment in PVC.

In conclusion, the origin of the CN radicals remains unknown and the definite assignment of the spectra corresponding to the irradiation products of *1a–b* must await further experiments.

Mechanistic results. The photodegradation of compounds *1a* and *1b* could not be quenched by oxygen or piperylene. Neither the triplet energy (63 kcal/mol) nor the triplet lifetime (2.5 ms at 77 K) of *1b* can be prohibiting factors in triplet energy transfer from *1b* to oxygen or piperylene. Thus we conclude, that the photoactive state of *1b* and probably *1a* too, is a singlet state.

The photoreactions of *1a* and *1b* could be triplet-sensitized by using xanthone, demonstrating the reactivity of the triplet states. But these are apparently not populated on direct excitation at room temperature.

The curvature of the Stern-Volmer plot for quenching of *1c* (Fig. 8) indicates that two photoactive states are involved. At high piperylene concentrations the plot is linear with a slope of zero. These results are interpreted by assuming that *triplet-1c* ($T_1(1c)$) and *singlet-1c* (S_1 (*1c*)) are leading to products but only the triplet state can be quenched.

Excitation:

$$S_0(1c) \xrightarrow{h\nu} S_1(1c)$$

Chemical reaction of excited singlet:

$$S_1(1c) \xrightarrow{k_r^1} 2\mathrm{PhCN} + \mathrm{Se}$$

Internal conversion:

$$S_1(1c) \xrightarrow{k_{ic}} S_0(1c)$$

Intersystem crossing:

$$S_1(1c) \xrightarrow{k_{isc}} T_1(1c)$$

Chemical reaction of triplet:

$$T_1(1c) \xrightarrow{k_r^3} 2\mathrm{PhCN} + \mathrm{Se}$$

Unimolecular decay of triplet:

$$T_1(1c) \xrightarrow{k_d^3} S_0(1c)$$

Bimolecular quenching of triplet:

$$T_1(1c) + \mathrm{Q} \xrightarrow{k_q} S_0(1c) + T_1(\mathrm{Q})$$

If a photoreaction takes place from an excited singlet and triplet state but only the triplet reaction can be quenched, the kinetic expression has the following form:[17]

$$\Phi_r^\circ/\Phi_r = (1+a)(1+k_q\tau[\mathrm{Q}])/(1+a+k_q\tau[\mathrm{Q}])$$

a is the ratio of triplet to singlet reaction and $\tau = 1/(k_r^3 + k_d^3)$.

Since Φ_r° was estimated to be 1.0, all radiationless deactivation must be negligible, *i.e.* $k_{ic} \ll k_{isc} + k_r^1$ and $k_d^3 \ll k_r^3$. Consequently, $\tau \approx 1/k_r^3$. The limiting value of the kinetic expression at high quencher concentrations is

$$\lim_{[\mathrm{Q}]\to\infty} \Phi_r^\circ/\Phi_r = 1 + a = 5 \quad \text{(Fig. 8)}$$

Thus $a = 4 = k_{isc}/k_r^1$. By using $a = 4$ and by simple iteration $k_q\tau$ is calculated to 1600 ± 400 M^{-1}. The simulated values are seen to fit the experimental curve satisfactorily (Fig. 8). The diffusion controlled bimolecular quenching constant depends on structures and on relative triplet energies of donor and acceptor.[14b] Usually, 10^9 $M^{-1}s^{-1} < k_q < 10^{10}$ $M^{-1}s^{-1}$.[14b] In conclusion, the lifetime of $T_1(1c)$ in benzene at room temperature is $1.6 \times 10^{-7}\,s < \tau < 1.6 \times 10^{-6}\,s$.

Since Φ_p (*1c*, 77 K, EPA) was estimated to ~ 0.33 the quantum yield for chemical reaction in EPA at 77 K cannot exceed 0.67. This result and the fact that the triplet lifetime at 77 K in EPA is three orders of magnitude greater than the lifetime at room temperature in benzene solution shows that the ring cleavage from the triplet state is associated with an activation barrier.

The photochemical reaction of diphenyl-1,2,4-selenadiazole (*2*) could not be quenched with piperylene (Fig. 8) and unlike compounds *1a–c* triplet sensitized photolysis using thioxanthone was inefficient. The photoactive state is therefore the excited singlet state.

The difference in energy between S_1 and T_1 of heterocycles [$\Delta E(S_1,T_1)$] is generally >3000 cm^{-1} for $\pi-\pi^*$ states but ≤ 2500 cm^{-1} for $n-\pi^*$ states.[18] $\Delta E(S_1,T_1)$ for *1b–c* was determined as the difference in onset-energy between the UV absorption bands [~310 nm (*1b*) and ~350 nm (*1c*)] and the phosphorescence bands [~390 nm (*1b*) and ~450 nm (*1c*)]. This gave the values $\Delta E(S_1,T_1)$ ~7000 cm^{-1} for *1b* and ~6000 cm^{-1} for *1c* indicating that the lowest states are $\pi-\pi^*$ in character.

Spin forbidden processes are affected by heavy atoms in the molecule and a decrease in triplet lifetime is expected when oxygen, sulfur and selenium analogues are compared.[19]

The measured lifetimes (Table 3) are therefore reasonable for $T_1(\pi-\pi^*)$ states of *1b* and *1c*.

EXPERIMENTAL

Compounds. *1a–c* were prepared according to Ref. 20, *2* according to Ref. 21 and *3* according to Ref. 22.

Polymer films. Poly(methyl methacrylate) (PMMA) was prepared by polymerization of neat monomer initiated by azobis-isobutyronitrile at 60–80 °C. The monomer was carefully purified by distillation through a Vigreux column. Poly(vinyl chloride) (PVC), Corvic D60/13, was used without further purification. Films were prepared using the solvent casting technique.[23]

Photolyses at room temperature. Irradiation of *1a–c*, *2* and *cis,cis*-2,4-hexadienedinitrile was carried out using a Bausch and Lomb SP-200 mercury point source with or without monochromator as specified. (Typical monochromator band width was ~20 nm). In the sensitization experiments selective excitation was ensured by use of interference filters (363 nm and 395 nm). Photolysis of *1c* in dimethyl acetylenedicarboxylate was performed with a Rayonet reator, type RPR-208 with RUL 3000 Å lamps. Compound *3* was irradiated with an internal light source, type Hanovia Q-700, cooled with tap water. Stirring was ensured. Photolysis mixtures were analyzed with either a Unicam SP-800 A spectrophotometer, a Perkin-Elmer 337 grating infrared spectrophotometer or with a Pye Unicam 104 gas chromatograph connected to a Varian Aerograph model 477 electronic integrator. Preparative layer chromatography (PLC) was performed using plates with a 2.5 mm thick layer of silica gel (Merck PF $_{254+366}$). The plates were developed with a mixture of benzene, light petroleum and acetone, 7:7:1. Quantum yields for chemical reaction were measured using the potassium trisoxalatoferrate(III) actinometer.[24] In this case the highly stabilized 150 W Xe/Hg superpressure point source of the fluorescence spectrophotometer (see below) was used. Deoxygenation and stirring were effected with argon.

Photolyses at low temperature. As light source at liquid nitrogen and dry ice temperatures, the Bausch and Lomb mercury source mentioned above was used. The low temperature UV cell, cooled with liquid nitrogen, is described in Ref. 1c. Spectra were recorded on a Cary 14 instrument. The IR cryostat used for irradiation of polymer films down to liq. N_2 temperatures was constructed by the group in Copenhagen. The film is kept between two KBr discs in a copper cavity. The hollow walls of the cavity are filled with liquid nitrogen. High vacuum is maintained around the probe. IR spectra were recorded on the Perkin-Elmer 337.

The matrix experiments at 10 and 20 K were performed using the apparatus in Newcastle which is based on an Air Products Displex CS-202 closed-cycle Helium refrigerator.[25] Irradiations were carried out with a Philips HPK-125 W medium pressure Hg arc and an Applied Photophysics high radiance monochromator (band width ~10 nm). UV spectra were recorded on a Perkin-Elmer 356 spectrophotometer. IR spectra were recorded on a Grubb Parsons Spectromajor spectrophotometer calibrated with gaseous DCl.

Emission spectra. These were obtained on a Perkin-Elmer MPF-3 fluorescence spectrophotometer with corrected spectra and phosphorescence accessories. Lifetimes were calculated from decay curves, stored by means of a Tektronix 549 oscilloscope using a chopper frequency of ~100 Hz. Quantum yields of phosphorescence (Φ_p) at 77 K were measured relative to compounds with known quantum yields. If two solutions absorb the same fraction of light at a given wavelength the ratio of the quantum efficiences can be determined as the ratio of the areas of the corrected emission spectra. These areas were determined by cutting and weighing. The areas of the phosphorescence spectra from each sample were reproducible within 10 %. Quantum yields of the standards were taken from Ref. 26. Φ_p (*1b*) was measured relative to benzoic acid (Φ_p 0.27) and phenanthrene (Φ_p 0.11). Solutions were excited at $280-285\pm10$ nm. Φ_p (*1c*) was measured relative to anthraquinone (Φ_p 0.41) and benzil (Φ_p 0.67). Solutions were excited at $310-317\pm7$ nm.

Photolysis of 1,2,5-selenadiazole in ethanol at room temperature. 1,2,5-selenadiazole (0.058 g) in 96 % ethanol (20 ml) was photolysed through

pyrex until deposition of selenium was complete (24 h). The photolysis mixture was poured into water (50 ml) and titrated potentiometrically with 0.050 M silver nitrate. The yield of hydrogen cyanide was measured to 87 %.

Photolysis of dimethyl-1,2,5-selenadiazole in chloroform at room temperature. Dimethyl-1,2,5-selenadiazole (0.108 g) in chloroform (20 ml) was photolysed through pyrex for 20 h. The photolysis mixture was filtrated and analyzed by GLC (10 % SE-30 on Gaschrom Q 100 – 120 mesh, 40 °C). Comparison with a reference solution of acetonitrile in chloroform showed that acetonitrile had been formed quantitatively. Peak areas were reproducible within 10 %.

Photolysis of diphenyl-1,2,5-selenadiazole in dimethyl acetylenedicarboxylate. Diphenyl-1,2,5-selenadiazole (1.00 g) was suspended in dimethyl acetylenedicarboxylate (10 ml) and photolysed through pyrex for 5 days, after which most of the starting material had disappeared (TLC). The solvent was evaporated *in vacuo* and separated by PLC. Besides unreacted starting material (0.032 g) 3 fractions (0.148 g, 0.036 g and 0.136 g) were isolated and analyzed with 60 MHz NMR. None of the products contained aromatic protons.

Photolysis of 2,1,3-benzoselenadiazole in ethanol at room temperature. 2,1,3-Benzoselenadiazole (1.00 g) was dissolved in 96 % ethanol (1.30 l) and photolysed with the internal light source (see above) until deposition of selenium was complete (7 days). The irradiated mixture was evaporated *in vacuo* and separated by PLC into *cis,cis*-2,4-hexadienedinitrile (0.051 g ~9 %, m.p. 123 – 124 °C (benzene/light petroleum), Ref. 7, 128 °C), *trans,trans*-2,4-hexadienedinitrile (0.100 g ~18 %, m.p. 155 – 157 °C (benzene/light petroleum), Ref. 7, 159 – 160 °C) and *cis,trans*-2,4-hexadienedinitrile (0.130 g ~23 %, m.p. 70 – 71 °C (benzene/light petroleum), Ref. 7, 72 – 73 °C).

Photolysis of cis,cis-2,4-hexadienedinitrile in ethanol at room temperature. cis,cis-2,4-Hexadienedinitrile (0.020 g) was dissolved in 96 % ethanol (4 ml) and irradiated through quartz for 10 h. The photolysis mixture was analyzed by GLC (10 % poly(propylene glycol) on chromosorb W 60 – 80 mesh, 150 °C). By comparison of peak areas in chromatograms of a standard solution containing the three 2,4-hexadienedinitrile isomers the ratio of isomers *cis,cis*:*cis,trans*:*trans,trans* was calculated to 1.0:2.6:1.7. Peak areas were reproducible within 10 %.

Photolysis of diphenyl-1,2,5-selenadiazole with added piperylene. Quenching experiments with piperylene were performed according to the following procedure: Stock solutions of *1c* in benzene (4.0×10^{-4} M) and piperylene (1:25 and 1:250) were prepared. 2.00 ml of the solution of *1c* was transferred to a 3 ml UV cuvette. Piperylene and benzene were then added to give a total volume of 3.00 ml. Prior to and during photolysis (320 ± 7 nm, 5 min) argon was bubbled through the solution. The lamp of the emission spectrograph (see above) was used as light source. Errors due to evaporation of benzene and piperylene were found to be negligible. 1.00 ml of photolysis mixture was then diluted to 3.00 ml and the absorbance (~1) measured with UV spectroscopy. This allowed Φ_r°/Φ_r to be calculated as $\Delta A^\circ/\Delta A$. To ensure total absorption of light and minimize light absorption by colloidal selenium, the amount of conversions did not exceed 15 %.

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Selectivity in the Transfer of Specified Diastereotopic Methyl Groups from a Dimethylsulfonium Ion

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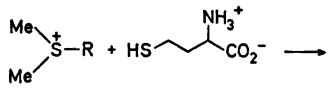
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Dedicated to Professor K. A. Jensen on his 70th birthday

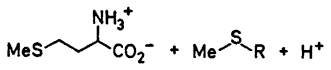
By specific ^{14}C-labelling it is demonstrated that the prochiral methyl groups present in the (*RS*)-dimethyl(1-methylpropyl)sulfonium ion are transferred to an acceptor molecule, the 4-methylbenzenethiolate ion, with greatly different rates. Only to a minute extent, however, is the observed rate difference attributable to the diastereotopic character of the two methyl groups, whereas a surprisingly large composite isotope effect of 1.16 ± 0.02, slightly surpassing the highest previously reported values for primary $^{12}C/^{14}C$-isotope effects, accounts almost entirely for the observed rate difference. This observation, pertaining to transmethylation from a dimethylalkylsulfonium ion, deserves attention and may, in certain cases, be of consequence for biochemical methodology.

As part of the synthetic sequence, the relative configuration of one of the diastereomeric (carboxymethyl)methyl(1-methylpropyl)sulfonium perchlorates has been established. The crystal structure was solved from single crystal X-ray data. *Crystal data:* orthorhombic $P2_12_12_1$, $a = 6.725(1)$, $b = 7.287(1)$, $c = 24.382(2)$, and $Z = 4$. The 166 parameters of the derived structural model were refined *versus* 828 structure factor amplitudes to a linear, unweighted R-value of 0.042. Although the structure is partially disordered, the obtained relative configuration at the two chiral centers of the sulfonium ion is uniquely determined as (R_cS_s), or (S_cR_s).

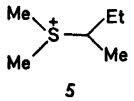
Though unrivalled as a general, biological methylating agent, *S*-adenosyl-L-methionine shares with the naturally occurring dimethylsulfonium compounds, *S*-methylmethionine (*1*) and the thetin (*2*), the capacity to convert homocysteine (*3*) into methionine (*4*) in certain enzyme-catalyzed processes.[1,2] Regardless of the C_1 or C_s-substrate character prevailing in *1* and *2*, respectively, the enzymic methyl transfer reactions are expected to proceed stereospecifically; experimental evidence to this effect, however, is lacking. In contrast to the thetin (*2*), which possesses enantiotopic methyl groups, the methionine derivative (*1*) must, in principle, exhibit selectivity in transfer of its diastereotopic methyl groups to an achiral acceptor even under non-enzymic conditions. We



1, 2 *3*



4



5

Scheme 1. Compound *1*, R = $(CH_2)_2CH(NH_3^+)$-CO_2^-; *2*, R = $(CH_2)_2CO_2^-$.

* To whom correspondence may be addressed.

have attempted to verify this prediction, yet, for experimental reasons, employing the dimethyl(1-methylpropyl)sulfonium ion (*5*) as C_1-donor.[3]

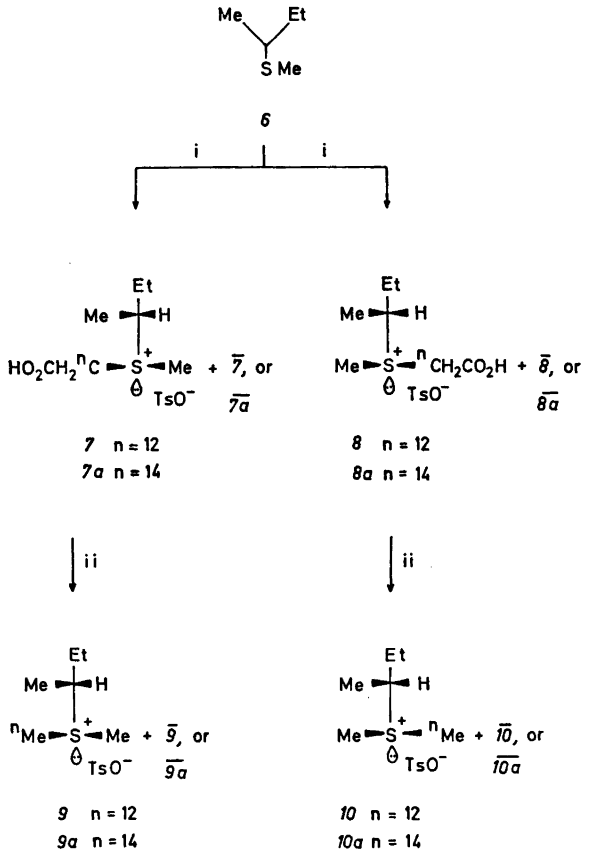
RESULTS

Reactions designed to assess the diastereoselectivity of the transmethylation from *5* to an acceptor must, of course, proceed under conditions mild enough to preclude pyramidal inversion of the sulfonium ions. The 4-methylbenzenethiolate ion admirably meets the requirements as a smoothly reacting acceptor and was employed for this purpose throughout the present work.

The diastereomeric, racemic [*methyl*-^{14}C]-[*methyl*-^{12}C](1-methylpropyl)sulfonium salts, (*9a*,$\overline{9a}$ * and *10a*,$\overline{10a}$), required as donor species, were synthesized as outlined in Scheme 2.

In the non-labelled series, treatment of racemic 2-(methylthio)butane (*6*)[4] with bromoacetic acid afforded a *ca.* 1:1 mixture of the diastereomeric salts (*7*,$\overline{7}$ and *8*,$\overline{8}$) individually recognizable by ^{1}H NMR spectroscopy. Repeated recrystallization of the mixture from acetonitrile yielded a product containing more than 96 % of one of the diastereomeric 4-methylbenzenesulfonates. An X-ray analysis of the corresponding perchlorate, prepared by an alternative route (see Experimental), served to establish its identity as the perchlorate of the (R_cS_s/S_cR_s)-sulfonium ion (*7*,$\overline{7}$).

* The designation $\overline{i}$ denotes the mirror image of *i*, produced in the ratio 1:1.



Scheme 2.

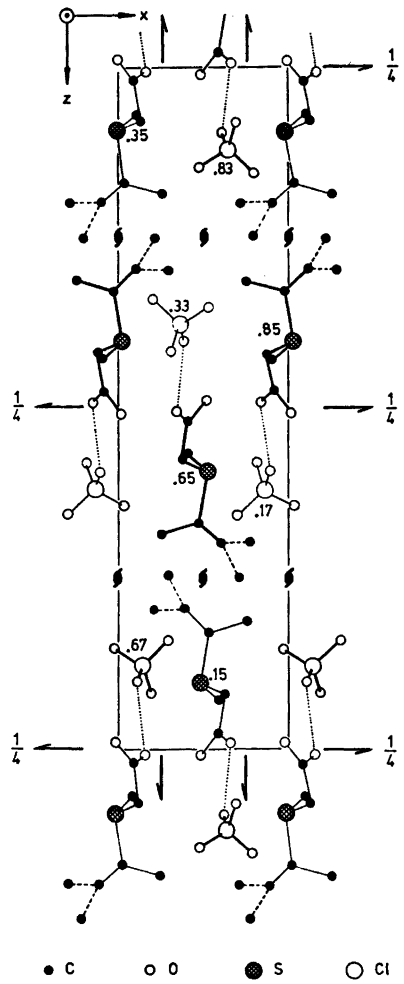


Fig. 1. Molecular packing diagram for the crystal structure of one of the diastereomeric (carboxymethyl)methyl(1-methylpropyl)sulfonium perchlorates. The dotted lines denote the intermolecular hydrogen bonds (O(5)···O(2)), while the dashed lines indicate the covalent bond from C(4) to the alternative positions of C(5). Only nonhydrogen atoms are included. The numbers given are the approximate y-coordinates for the nearest sulfur or chlorine atoms.

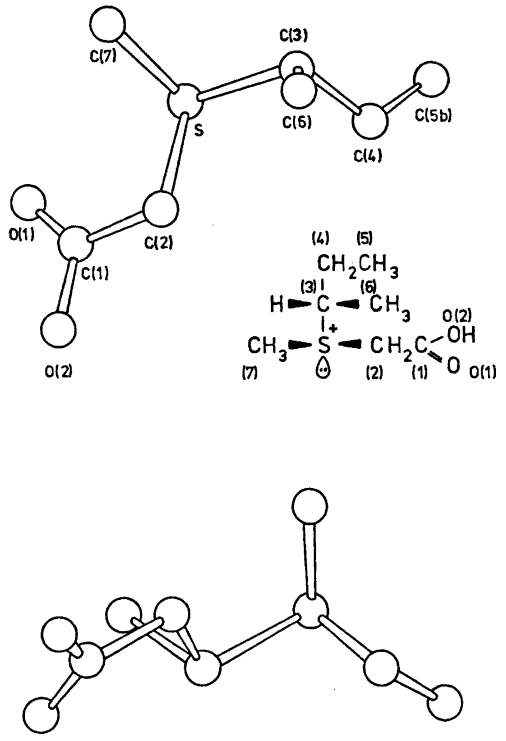


Fig. 2. Molecular geometry of one of the diastereomeric (carboxymethyl)methyl(1-methylpropyl)sulfonium perchlorates, viewed perpendicular and parallel to the plane through the three carbon atoms adjacent to the sulfur atom; hydrogen atoms are not included.

X-Ray structure determination. The molecular packing obtained for the crystal structure of the perchlorate is shown in Fig. 1. The structure can be envisioned as built up by layers of about 12 Å thickness perpendicular to the c direction (*e.g.* the layers $-1/4 < z < 1/4$ and $1/4 < z < 3/4$, respectively). Within these layers the ionic molecules are held together mainly by hydrogen bonds as well as by conventional electrostatic interactions between the ions. Between the layers (across the planes $z = 1/4$ and $z = 3/4$, respectively) there are only relatively weak van der Waals interactions. The lack of any larger intermolecular forces across these layers is most probably of relevance for the observed disorder of the terminal methyl group of the 1-methylpropyl grouping and also for the comparatively large thermal vibrations indicated for this group. Thus, the carbon atom C(5) (*cf.* Fig. 2) was found to be distributed, with almost equal probability, among two different positions labelled C(5a) and C(5b), respectively. Such a disorder appears to be a rather common feature of 1-methylpropyl substituents,[5] possibly caused by the circum-

Table 1. Intermolecular contact distances (Å) less than 3.4 Å between the nonhydrogen atoms.

Symmetry code:

None	x,y,z		
(i)	$-x,1/2+y,1/2-z$	(v)	$1/2-x,-y,-1/2+z$
(ii)	$-1/2+x,1/2-y,-z$	(vi)	$-x,-1/2+y,1/2-z$
(iii)	$1/2-x,-y,-1/2-z$	(vii)	$1-x,-1/2+y,1/2-z$
(iv)	$x,y-1,z$	(viii)	$x-1,y,z$

S···O(5 i)	3.298(6)	O(2)···O(5 v)	2.736(7)
O(1)···C(1 ii)	3.178(8)	C(1)···O(6 vi)	3.202(10)
O(1)···C(2 ii)	3.188(8)	C(2)···O(4 vii)	3.399(11)
O(1)···C(7 ii)	3.245(10)	C(2)···O(6 vi)	3.304(12)
O(1)···O(5 iii)	3.342(7)	C(5a)···O(4 viii)	3.166(19)
O(2)···C(7 iv)	3.228(8)	C(5b)···C(6 viii)	3.288(27)

stance that in one of the C(5) positions, C(5a), the atoms C(5)–C(4)–C(3)–C(6) form an energetically favourable (almost staggered) conformation. In the other position, C(5b), a favourable molecular packing can be achieved for the elongated molecules (*cf.* Fig. 2). Apart from the short O(2)···O(5) contact of 2.74 Å caused by an intermolecular hydrogen bond (Fig. 1), most of the other shorter contacts (*cf.* Table 1) are formed between atoms expected to have opposite atomic charges.

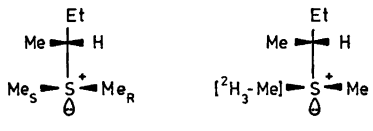
As has been frequently observed in crystals of organic compounds containing perchlorate ions, three [O(3), O(4), and O(6)] of the four oxygen atoms of the perchlorate ions are slightly disordered as judged by their large thermal vibrations. The fourth, not disordered oxygen atom, O(5), is the one involved in the hydrogen bonding to O(2). Apart from the bond distances and bond angles (Table 2) involving the disordered atoms [C(5), O(3), O(4), and O(6)] the general features of the molecular structure agree well with earlier observations on similar compounds.[6] Thus, the sulfur atom and its three adjacent carbon atoms, C(2), C(3), and C(7), form a flattened trigonal pyramid (*cf.* Fig. 2) with the C–S–C bond angles ranging from 102.4 to 104.2° and with the sulfur atom 0.76 Å above the plane through the three carbon atoms. As seen from Fig. 2, the crystal structure determination shows that the configuration of the carboxymethylsulfonium perchlorate building up the crystal is either that of *7* or its enantiomer ($\bar{7}$).

Decarboxylation and transmethylation. Decarboxylation of *7*,$\bar{7}$, or of a mixture of

Table 2. Intramolecular bond distances (Å) and angles (°) between nonhydrogen atoms.

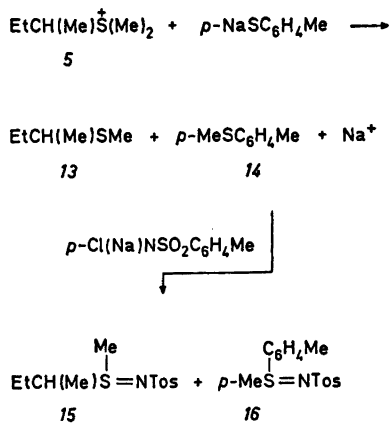
S–C(2)	1.799(6)	C(3)–C(6)	1.510(18)
S–C(3)	1.796(8)	C(4)–C(5a)	1.420(21)
S–C(7)	1.778(8)	C(4)–C(5b)	1.201(27)
O(1)–C(1)	1.198(7)	Cl–O(3)	1.395(9)
O(2)–C(1)	1.328(7)	Cl–O(4)	1.369(9)
C(1)–C(2)	1.500(8)	Cl–O(5)	1.433(5)
C(3)–C(4)	1.528(15)	Cl–O(6)	1.382(7)
C(2)–S–C(3)	104.2(3)	C(4)–C(3)–C(6)	112.6(8)
C(2)–S–C(7)	103.4(3)	C(3)–C(4)–C(5a)	119.2(10)
C(3)–S–C(7)	102.4(4)	C(3)–C(4)–C(5b)	120.0(13)
O(1)–C(1)–O(2)	124.9(6)	O(3)–Cl–O(4)	111.5(5)
O(1)–C(1)–C(2)	124.7(5)	O(3)–Cl–O(5)	108.3(4)
O(2)–C(1)–C(2)	110.4(5)	O(3)–Cl–O(6)	109.6(5)
C(1)–C(2)–S	108.0(4)	O(4)–Cl–O(5)	108.7(5)
S–C(3)–C(4)	108.4(7)	O(4)–Cl–O(6)	108.3(5)
S–C(3)–C(6)	113.6(6)	O(5)–Cl–O(6)	110.4(4)

7,*7̄* and *8*,*8̄*, under closely controlled conditions, yielded the monochiral, racemic sulfonium salt (*11*,*11̄*), the ^{1}H NMR spectrum of which exhibited signals at δ 2.83 and 2.77 (in CD_3CN). These could be attributed to the *pro*-(*R*) and *pro*-(*S*) *S*-methyl-groups of *11* [or, to be sure, the *pro*-(*S*) and *pro*-(*R*) of *11̄*], respectively, by comparison with the spectrum of the trideuteriomethyl analogue (*12*,*12̄*). The latter was produced by exchange of the carboxymethylsulfonium salt (*7*,*7̄*) with D_2O, followed by decarboxylation, performed in $(CD_3)_2CO$ under non-equilibrating conditions; only the signal at δ 2.83 persisted with unchanged intensity.



11 *12*

Repetition of the above sequence with 2-^{14}C-bromoacetic acid yielded a specimen of the salt (*7a*,*7̄a*), which, after recrystallization to constant specific activity, was converted, by decarboxylation, into the (R_cR_s/S_cS_s) salt (*9a*,*9̄a*). Comparison of the ^{1}H NMR spectrum of the product with that of the deuterated salt (*12*) showed it to be contaminated with less than 10 % of the diastereomeric (R_cS_s/S_cR_s)-salt (*10a*,*10̄a*). In boiling acetonitrile, a specimen of the salt underwent pyramidal inversion, resulting in an equilibrium mixture containing nearly equal amounts of the diastereomeric salts (*9a*,*9̄a* and *10a*,*10̄a*). Its composition was established by comparison of its ^{1}H NMR spectrum with that of the deuterio-ion (*12*). The *ca.* 9:1 and 1:1 mixtures were studied separately as donor substrates in the transmethylation reaction. This proceeded smoothly on treating a dimethylformamide solution of the 4-methylbenzenesulfonate of dimethyl(1-methylpropyl)sulfonium ion (*5*) with sodium 4-methylbenzenethiolate, yielding an equimolar mixture of 2-(methylthio)-butane (*13*) and 1-methyl-4-(methylthio)-benzene (*14*). In order to facilitate their quantitative separation, the sulfide mixture was converted into a corresponding mixture of *S*-methyl-*S*-(1-methylpropyl)-*N*-[(4-methylphenyl)sulfonyl]-sulfimide (*15*)[4] and S-methyl-



S-(4-methylphenyl)-*N*-[(4-methylphenyl)sulfonyl)]sulfimide (*16*).[7] These were separated by liquid chromatography, yielding the two known, crystalline components. As expected, the former derivative consisted of two, non-separated racemates, revealed by the appearance of several, slightly anisochronous signals in its ^{1}H NMR spectrum. The same reaction sequence was subsequently applied to (i) the *ca.* 9:1-, and (ii) the *ca.* 1:1-mixture of the diastereomeric, ^{14}C-labelled salts (*9a*, *9̄a* and *10a*, *10̄a*), yielding the radioactive analogues of *15* and *16*, both of which were recrystallized to constant activity. The distribution of radioactivity in the individual specimens can be calculated from the data presented in Table 3. Arithmetical correction of the experimentally derived 'mean values' (see Table 3), obtaining for the two above mixtures of diastereomers, afforded the values 1.14 ± 0.02 and 1.18 ± 0.03, respectively, for the activity ratio $A_{(15)}/A_{(16)}$ pertaining to the homogeneous isomers (*9a*, *9̄a* and *10a*, *10̄a*).

DISCUSSION

As is apparent from Table 3, the prochiral methyl groups of the racemic ion (*9a*, *9̄a*), exhibit considerable selectivity in their reaction with the attacking nucleophile, the 4-methylbenzenethiolate ion. That only a minute fraction of the observed selectivity is attributable to the topologically conditioned, non-identical reaction rates of the two groups becomes apparent, however, when the data are compared with those obtaining for the diastereo-

Table 3. Relative specific activities, $A_{(n)}$ of compounds n, determined on at least three individually weighed samples, each counted at least four times. Specific activities were within the range 0.3 – 3.2 μCi mmol^{-1}.

$A_{(9a,\ \overline{9a})}$ [a]	$A_{(9a,\ \overline{9a})}+A_{(10a,\ \overline{10a})}$ [b]	$A_{(15)}$	$A_{(16)}$	$A_{(15)}/A_{(16)}$	Mean value
1.000 ± 0.020 [c]		0.527 ± 0.012	0.460 ± 0.011	1.147 ± 0.037	1.144 ± 0.023 [d,f]
1.000 ± 0.018		0.529 ± 0.012	0.464 ± 0.005	1.140 ± 0.028	
	1.000 ± 0.012	0.534 ± 0.006	0.455 ± 0.006	1.174 ± 0.020	
	1.000 ± 0.018	0.525 ± 0.006	0.456 ± 0.009	1.152 ± 0.027	1.160 ± 0.015 [e,f]
	1.000 ± 0.016	0.523 ± 0.011	0.453 ± 0.006	1.155 ± 0.028	

[a] With a maximum contribution of 10 % of the activity from (*10a*, $\overline{10a}$). [b] An approximately 1:1 mixture of the two diastereometers. [c] Mean errors were calculated from the observed values and include an allowance for a 1 % contribution from possible impurities. [d] Calculated for homogeneous (*9a*, $\overline{9a}$): 1.14 ± 0.02. [e] Calculated for homogeneous (*10a*, $\overline{10a}$): 1.18 ± 0.03. [f] Mean values calculated from values of $A_{(15)}$ and $A_{(16)}$ without any rounding off.

meric racemate (*10a*, $\overline{10a}$). Evidently, the isotope effect plays a predominant role in determining the observed differences in reaction rates. Put into quantitative terms, the contribution, D, arising from the diastereotopic nature of the two *S*-methyl groups of the dimethylsulfonium ion (*11*) can be expressed as follows:

$$D=\frac{k_R^{11}}{k_S^{11}}\equiv\left[\frac{k_{12}^{9a}\,k_{14}^{10a}}{k_{14}^{9a}\,k_{12}^{10a}}\right]^{\frac{1}{2}}\times\left[\frac{k_{14}^{9a}\,k_R^{11}}{k_S^{11}\,k_{14}^{10a}}\times\frac{k_{12}^{10a}\,k_R^{11}}{k_S^{11}\,k_{12}^{9a}}\right]^{\frac{1}{2}}=\left[\frac{k_{12}^{9a}\,k_{14}^{10a}}{k_{14}^{9a}\,k_{12}^{10a}}\right]^{\frac{1}{2}}\times\left[\frac{I_R\,I_R^*}{I_S\,I_S^*}\right]^{\frac{1}{2}}$$

where k_n^i denotes the rate constant relating to the ^{12}C isotope-labelled methyl group in compound No. $i,\bar{i}$; k_R^{11} and k_S^{11} the rate constants pertaining to the prochiral methyl groups in *11*; and I_R,I_S, respectively I_R^*,I_S^*, the primary and secondary isotope effects of consequence in bond-breaking processes to the diastereotopic methyl groups.*

On the reasonable assumption that $I_R=I_S$ and $I_R^*=I_S^*$, and equalling rate constant ratios with activity ratios, it follows that:

$$D=\left[\frac{k_{12}^{9a}\,k_{14}^{10a}}{k_{14}^{9a}\,k_{12}^{10a}}\right]^{\frac{1}{2}}=\left[\frac{1.14\pm0.02}{1.18\pm0.03}\right]^{\frac{1}{2}}=0.98\pm0.02$$

Similarly, the observed, composite isotope effect, I, can be expressed as:

$$I=\left[\frac{k_{12}^{9a}\,k_{12}^{10a}}{k_{14}^{9a}\,k_{14}^{10a}}\right]^{\frac{1}{2}}\equiv\left[\frac{k_R^{11}\,k_S^{11}}{k_{14}^{10a}\,k_{14}^{9a}}\times\frac{k_{12}^{9a}\,k_{12}^{10a}}{k_R^{11}\,k_S^{11}}\right]^{\frac{1}{2}}=\left[\frac{I_S\,I_R}{I_R^*\,I_S^*}\right]^{\frac{1}{2}}$$

Again, inserting the experimentally derived rate constant ratio, one finds:

$$I=\left[\frac{k_{12}^{9a}\,k_{12}^{10a}}{k_{14}^{9a}\,k_{14}^{10a}}\right]^{\frac{1}{2}}=[(1.14\pm0.02)\times(1.18\pm0.03)]^{\frac{1}{2}}=1.16\pm0.02$$

A small, yet probably significant predilection of the 4-methylbenzenethiolate ion for the *pro*-(*S*) *S*-methyl group of *11*, [or, by the same token, the *pro*-(*R*) *S*-methyl group of $\overline{11}$], thus prevails. No conclusions can be drawn from the present study, however, regarding the magnitude of the diastereoselectivity as a function of the donor substrate, the accepting nucleophile, the solvent *etc.*

The composite isotope effect observed in the present work seems among the largest ever reported for ^{12}C/^{14}C-nuclei. It appears of interest, however, that primary isotope effects of comparable magnitude ($\simeq$1.10 – 1.15) have typically been reported for nucleophilic displacement reactions, most likely akin to that involved in the present transmethylation studies.[9]

There is hope that continued studies within this area, encompassing enzymically catalyzed processes, will further clarify the stereospecificity of transmethylation reactions in-

* Large, inverse α-deuterium secondary isotope effects have been reported in the transfer of methyl groups from *S*-adenosylmethionine to 1-(3,4-dihydroxyphenyl)ethane.[8]

volving dimethylsulfonium ions. For the interpretation of such studies, conducted by ^{14}C-labelling technique, the large isotope effect observed in the present study, may serve as a caveat (see *Added in proof*).

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage microscope. NMR spectra were recorded on a 90 MHz Bruker HX-90E instrument.

Radioactive measurements were carried out by liquid scintillation technique on a Packard Tricarb Scintillation Spectrometer model 3320; the radioactive components (*ca.* 1 mg) were dissolved in methanol (1.00 ml), and a solution (10.00 ml) of scintillation liquid was added.[10] The efficiency of counting was determined for the individual samples by internal calibration.

(Carboxymethyl)methyl(1-methylpropyl)sulfonium perchlorate and 2,4,6-trinitrobenzenesulfonate. A solution of 2-(methylthio)butane [4] (*6*) (50 mmol), bromoacetic acid (75 mmol), and silver perchlorate (50 mmol) in acetonitrile (50 ml) was kept at 65 °C for 16 h. After filtering off the silver bromide (49.7 mmol), the filtrate was concentrated to an oil. Trituration with ether left a crystalline mixture of two diastereomeric sulfonium perchlorates, composed, according to the ^1H NMR spectrum (in CH_3CN), of approximately equal amounts. Since separation by fractional crystallization was unsuccessful, the perchlorate mixture (30 mmol) was dissolved in water (20 ml). A solution of the tetrahydrate of 2,4,6-trinitrobenzenesulfonic acid (30 mmol) in water (30 ml) was added, and the solid collected by filtration. Repeated recrystallizations from water (50 – 75 ml), followed by thermal equilibration of the mother liquors (heating to 90 °C for 10 – 15 min) and renewed recrystallization, gave a specimen (6 mmol) of one of the diastereomeric trinitrobenzenesulfonates, m.p. 172 – 175 °C (dec.), contaminated, according to ^1H NMR spectroscopy (DMSO-d_6), by less than 5 % of the other isomer [*Me*-S$^+$: δ 2.91 (major isomer), 2.84 (minor isomer)]. Anal. $C_{18}H_{17}N_3O_{11}S_2$: C, H, N, S. The salt (2.5 mmol) was stirred with sodium perchlorate monohydrate (2.5 mmol) in nitromethane (10 ml) for 1 h. After centrifugation, and concentration of the supernatant, the residue was recrystallized from acetic acid:ethyl acetate to give an analytical specimen of a perchlorate (~0.8 mmol), again containing less than 5 % of the diastereomeric salt. Anal. $C_7H_{15}ClO_6S$: C, H, Cl, S. This preparation was employed for X-ray analysis.

This virtually homogeneous perchlorate (14 mmol) was dissolved in water, and the solution was slowly passed through a column containing 30 ml of Amberlite IRA-400 ion exchange resin, loaded in its 4-methylbenzenesulfonate form. After concentration *in vacuo* and recrystallization from acetic acid:ethyl acetate (1:3), a crystalline sulfonium 4-methylbenzenesulfonate was obtained (10.6 mmol), containing less than 5 % of the diastereomeric salt and identical with the salt prepared directly, as described below.

X-Ray structure determination. Single crystal X-ray diffraction data for the (carboxymethyl)-methyl(1-methylpropyl)sulfonium perchlorate were collected from a selected crystal with the dimensions $0.22 \times 0.12 \times 0.05$ mm by means of a CAD-4 diffractometer using graphite-monochromatized Mo$K\alpha$-radiation. *Crystal data:* Space-group $P2_12_12_1$, $a = 6.725(1)$, $b = 7.287(1)$, $c = 24.382(2)$ Å and $Z = 4$. The crystal structure was solved by direct methods and refined by conventional least-squares techniques to an unweighted linear R-value of 0.042. Atoms other than hydrogens were allowed to vibrate anisotropically, while the hydrogens were given fixed isotropic values of a size comparable to the vibrations of the attached nonhydrogen atoms. Due to disorder not all of the hydrogens within the 1-methylpropyl group could be located, and some of those located were given fixed positions to avoid divergence during the refinement cycles. In the refinement all of the 828 reflexions, having d-values above 0.76 Å and observed net intensities (not corrected for absorption effects) greater than four times their estimated standard deviations, were used. No attempts were made to determine the absolute configuration. The obtained structural parameters * are given in Tables 4 and 5. The atomic labels used are shown in Fig. 2.

(R_cS_s/S_cR_s)-(Carboxymethyl)methyl(1-methylpropyl)sulfonium 4-methylbenzenesulfonate (7,7). A solution in acetonitrile (5 ml) of bromoacetic acid (5.4 mmol), 2-(methylthio)-butane (5.4 mmol), and silver 4-methylbenzenesulfonate (5.2 mmol) was kept at 50 °C for 20 h. After removal of silver bromide (5.0 mmol), and concentration of the filtrate, the residue was washed with ether (removing about 0.5 mmol of carboxymethyl 4-methylbenzenesulfonate, according to ^1H NMR spectroscopy) and recrystallized to give 4.7 mmol of a 1:1 mixture (^1H NMR in CD_3CN) of the two diastereomeric salts. Five recrystallizations from acetonitrile (4 – 8 ml), combined with thermal equilibration of the mother liquors, produced 2.0 mmol of one of the salts, contaminated with less than 4 % of the other. M.p. 116 – 119 °C (dec.). ^1H NMR (CD_3CN): δ 2.87 (s) (*Me*–S$^+$); [the (R_cR_s/S_cS_s)-isomer: δ 2.80 (s)]. Anal. $C_{14}H_{22}O_5S_2$: C, H, S. The salt proved identical with that produced from the perchlorate submitted for X-ray analysis (*vide supra*), and hence consists of the (R_cS_s/S_cR_s)-sulfonium-4-methylbenzenesulfonate (*7,7*).

* A list of structure factors is available from one of the authors (R.N.) on request.

Table 4. Fractional atomic coordinates for all atoms and isotropic temperature factors for the hydrogens.

	x	*y*	*z*	*U*
S	−0.0173(2)	0.3488(2)	0.0955(1)	
O(1)	−0.0099(8)	0.1847(5)	−0.0103(2)	
O(2)	0.1667(9)	−0.0668(5)	0.0102(2)	
C(1)	0.0845(9)	0.0945(7)	0.0215(3)	
C(2)	0.1300(10)	0.1498(7)	0.0794(2)	
C(3)	0.0272(16)	0.3872(8)	0.1672(3)	
C(4)	−0.1122(22)	0.2630(11)	0.2000(5)	
C(5a)	−0.2075(23)	0.3342(25)	0.2474(7)	
C(5b)	−0.2807(34)	0.3093(30)	0.2082(9)	
C(6)	0.2424(22)	0.3649(14)	0.1836(3)	
C(7)	0.1165(14)	0.5315(8)	0.0643(3)	
Cl	0.3584(2)	0.3269(2)	0.3788(1)	
O(3)	0.2034(14)	0.3191(9)	0.3409(4)	
O(4)	0.5324(13)	0.3814(14)	0.3547(4)	
O(5)	0.3858(9)	0.1473(7)	0.4015(2)	
O(6)	0.3117(13)	0.4511(9)	0.4197(3)	
H(1)	0.136(11)	−0.086(9)	−0.018(3)	5.0
H(2a)	0.292(9)	0.184(8)	0.079(2)	5.0
H(2b)	0.080(9)	0.068(8)	0.108(3)	5.0
H(3)	−0.037(9)	0.527(8)	0.182(2)	5.0
H(6a)	0.28	0.23	0.182	10.0
H(6b)	0.26	0.41	0.224	10.0
H(6c)	0.33	0.44	0.160	10.0
H(7a)	0.269(11)	0.541(8)	0.073(2)	5.0
H(7b)	0.057(9)	0.649(9)	0.080(2)	5.0
H(7c)	0.109(10)	0.516(9)	0.031(3)	5.0

Table 5. Thermal parameters for nonhydrogen atoms. The atoms C(5a) and C(5b) were allowed to vibrate isotropically, the others anisotropically.

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S	48(1)	36(1)	72(1)	1(1)	10(1)	−4(1)
O(1)	67(2)	50(2)	62(2)	4(2)	−12(2)	−1(2)
O(2)	90(4)	39(2)	56(3)	15(2)	−2(3)	−10(2)
C(1)	48(3)	32(2)	58(4)	−7(2)	5(3)	1(2)
C(2)	56(3)	36(2)	47(3)	2(3)	8(3)	2(2)
C(3)	122(7)	45(3)	62(4)	0(4)	34(5)	−6(3)
C(4)	213(15)	54(4)	136(9)	−13(6)	122(10)	−8(5)
C(6)	177(10)	83(6)	60(5)	−16(7)	−20(6)	−5(5)
C(7)	76(5)	36(3)	70(4)	−3(3)	5(4)	3(3)
Cl	57(1)	40(1)	49(1)	−3(1)	−2(1)	5(1)
O(3)	195(8)	81(4)	146(6)	−28(5)	−113(6)	25(4)
O(4)	128(6)	146(8)	199(9)	−39(6)	79(6)	35(6)
O(5)	101(3)	58(2)	74(3)	16(3)	−1(3)	17(2)
O(6)	176(8)	99(5)	98(4)	57(5)	−6(5)	−32(4)
C(5a)	78(4)					
C(5b)	97(6)					

(RS)-*Dimethyl(1-methylpropyl)sulfonium 4-methylbenzenesulfonate(5)* = *(9,$\bar{9}$)* = *(10,$\bar{10}$)*. The individual, *(7,$\bar{7}$)*, or mixed, *(7,$\bar{7}$* and *8,$\bar{8}$)*, diastereomers of the carboxymethylsulfonium 4-methylbenzenesulfonates (1 mmol), were dissolved in anhydrous acetone (2 ml), containing 1.2 mmol of tributylamine. After standing at 50 °C for 10 min, the solutions were cooled, and the residues were washed with ether. The crystalline product (0.98 mmol), which proved homogeneous on ^{1}H NMR spectroscopy (CD_3CN), was recrystallized from acetone:ethyl

acetate to give an analytical specimen (hygroscopic) of (*RS*)-dimethyl(1-methylpropyl)sulfonium 4-methylbenzenesulfonate (*5*) = (*9,9̄*) = (*10,10̄*), m.p. 80 – 82 °C. Anal. $C_{13}H_{22}O_3S_2$: C,H,S. ^{1}H NMR (CD_3CN): δ 2.83 (s) (*pro-(R) Me-S*$^+$) 2.77 (*pro-(S) Me-S*$^+$), as specified in the enantiomer *11*.

Decarboxylation of the corresponding perchlorates proceeded similarly to give the homogeneous, yet non-crystalline dimethyl(1-methylpropyl)sulfonium perchlorate.

Methylation of 2-(methylthio)-butane proceeded readily on keeping it (62 mmol), admixed with methyl 4-methylbenzenesulfonate (60 mmol), at 90 °C for 3 h. Extraction with ether (2 × 40 ml), and recrystallization from acetone:ethyl acetate afforded the pure dimethyl(1-methylpropyl)sulfonium 4-methylbenzenesulfonate(*5*) = (*9,9̄*) = (*10,10̄*).

Methylation of sodium 4-methylbenzenethiolate with (RS)-dimethyl(1-methylpropyl)sulfonium 4-methylbenzenesulfonate. The transmethylation proceeded rapidly in dipolar, aprotic solvents. In a typical run, a solution of the sulfonium salt (1.0 mmol) and the sodium thiolate (1.1 mmol) in dimethylformamide (2 ml) was kept for 1 h at 23 °C, when ^{1}H NMR spectroscopy revealed a quantitative conversion to the sulfides (*13* and *14*).

Sulfimide derivatives and their separation. A 3 N solution of anhydrous Chloramine T in dimethylformamide (3 ml), prepared from the commercial trihydrate by azeotropic distillation with chlorobenzene,[11] was added to the above sulfide mixture. After standing at 23 °C for 1 h, when conversion was complete according to ^{1}H NMR spectroscopy, most solvent was removed (< 55 °C/10 mm), and the residue was taken up in benzene (10 ml). The solution was washed with sodium hydroxide and water, and the benzene was distilled off.

The residue was applied to a silica gel column ('Merck Fertigsäule, Kieselgel 60, Grösse B') and the column was eluted with ethyl acetate, with continuous UV-detection of the effluate. Fraction 1 (30 – 45 min), and fraction 2 (80 – 120 min) were collected separately. The former yielded 0.5 – 0.7 mmol of *S*-methyl-*S*-(4-methylphenyl)-*N*-[(4-methylphenyl)sulfonyl]-sulfimide (*16*), which was recrystallized from benzene:light petroleum, m.p. 125 – 127 °C (Ref. 7, m.p. 125 – 126 °C), ^{1}H NMR ($CDCl_3$): δ 2.82 (s), *Me* – S (Ref. 12, δ 2.81), whereas fraction 2 afforded *S*-methyl-*S*-(1-methylpropyl)-*N*-[(4-methylphenyl)sulfonyl]-sulfimide (*15*), m.p. 80 – 94 °C (Ref. 4, m.p. 79 – 80 °C). The broad melting point range, in connection with doubling of several of the signals in the ^{1}H NMR spectrum, *e.g.* $\Delta\delta$ 0.005 in the δ 2.52 (s) signals (*Me* – S), is indicative of the derivative being a mixture of two diastereomeric racemates.

Deuterium exchange experiments. The virtually homogeneous carboxymethylsulfonium 4-methylbenzenesulfonate (*7,7̄*) (0.4 mmol) was dissolved in D_2O (1 ml) and the solution was concentrated to dryness *in vacuo*. The procedure was repeated, and the crystalline residue was dried *in vacuo* over phosphorus pentoxide. ^{1}H NMR control revealed a content of more than 95 % of the (R_cS_s/S_cR_s)-[*carboxymethyl*-$^2H_3$]methyl(1-methylpropyl)sulfonium 4-methylbenzenesulfonate.

Decarboxylation, carried out in acetone-d_6 (dried over molecular sieve MS 3), as described above, yielded the (R_cR_s/S_cS_s)-[*methyl*-$^1H_3$]-[*methyl*-$^2H_3$] (1-methylpropyl)sulfonium 4-methylbenzenesulfonate (*12,12̄*), containing less than 10 % of the diastereomeric racemate, as estimated from the intensity of the signal at δ 2.77.

Thermal equilibration of the above mixture in acetonitrile-d_3 at 71 °C proceeded as a first order reaction with a rate constant $k_1 = k_{-1}$ of 1.5 × 10^{-4}s.

^{14}C-Labelling experiments. 2-^{14}C-Bromoacetic acid (Amersham Radiochemicals CFA.18) (35 μCi), was employed as an alkylation reagent in experiments identical to those described above, with regard to scale as well as execution. After recrystallization to constant activity, a 40 % yield of the (R_cR_s/S_cS_s)-[*carboxymethyl*-$^{14}C_1$]methyl(1-methylpropyl)sulfonium 4-methylbenzenesulfonate (*7a,7̄a*), containing less than 4 % of the diastereomeric salt, was obtained.

Again, the decarboxylation was carried out precisely as described above. Before recrystallization of the product to constant activity, it was diluted with an equal quantity of non-labelled product.

The transmethylation, derivation, and separation were performed as described.

The combined mother liquors from the recrystallizations of the decarboxylated products in two experimental series were concentrated, redissolved in acetonitrile (10 ml), and heated, in separate experiments, to 74 °C for 2, 4, and 27 h. The specific activity was reduced by 50, 80, and 80 %, respectively, by adding non-labelled material. After recrystallization to constant activity, the three samples were separately taken through the usual sequence of transmethylation, derivation, separation, and radioassay.

Acknowledgements. The authors are grateful to Dr. E. Kelstrup for experimental details as to the performance of ion exchange operations and decarboxylation reactions on sulfonium salts, and to Professor Ian Spenser for helpful discussions.

Added in proof. Methyl group transfer from the ion *9,9̄a* to 1-adamantanethiolate ion to give 1-adamantyl methyl sulfide, characterized as *S*-methyl-*N*-[(4-methylphenyl)sulfonyl]-*S*-(tricyclo[3.3.1.1.^{3,7}]decyl)sulfimide, m.p. 176 – 178 °C [Anal. $C_{18}H_{25}NO_2S_2$:C,H,N,S], proceeded

with very similar selectivity: $D = 0.97 \pm 0.02$, and $I = 1.16 \pm 0.03$.

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Resolution and Absolute Configuration of α-(Benzimidazolyl-2-thio)-propionic Acid

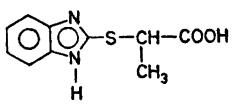
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Dedicated to Professor K. A. Jensen on his 70th birthday

The title compound (*1*) has been prepared and resolved using quinine and quinidine. The enantiomers have $[\alpha]_D^{25} \pm 124°$ (in 96 % ethanol). (*S*)-(−)-α-Bromopropionic acid gave the (+)-form of the title compound. Postulating an S_N2 mechanism, the (+)-form of the title compound should then possess the (*R*)-configuration. The growth-regulating activity is being investigated and some preliminary data are given. The acetic acid derivative has been prepared for comparison.

In the course of current investigations on optically active compounds having growth-regulating effects in higher plants, the title compound (*1*) has been prepared and resolved. It is known that various phenoxy and naphthoxy acids have strong auxin effects while the corresponding arylthio and arylseleno acids are very weak auxins or even anti-auxins. In general there is a great difference between optical isomers.[1–5] It was therefore of interest to study a bicyclic aromatic system containing nitrogen. While the mechanism of the auxin activity is still obscure, it is obvious that an acceptable theory must explain both positive and antagonistic effects and their dependence on structural and sterical factors. The problems connected with the auxin mechanism have also some interest with regard to current discussions on possible deleterious effects of phenoxy acids used as herbicides in agriculture and forestry.



1

The title compound is easily prepared from 2-mercaptobenzimidazole and α-bromopropionic acid in slightly alkaline solution. The corresponding acetic acid has also been prepared for comparison. The acids gave no reaction for the mercapto group, showing that the reaction had proceeded as expected.

The propionic acid derivative could be resolved using quinine and quinidine, giving the enantiomers with $[\alpha]_D^{25} \pm 124°$ in 96 % ethanol. The synthesis was also carried out with partially resolved (*S*)-(−)-α-bromopropionic acid giving a dextrorotatory product. No significant racemisation had taken place. In all probability the reaction follows an S_N2 mechanism with inversion. The (+)-form of the title compound should therefore possess the (*R*)-configuration.

The growth-regulating activity is being investigated by Professor B. Åberg (Agricultural University of Sweden), who has kindly submitted some preliminary results.[6] The (*S*)-(−)-α-(benzimidazolyl-2-thio)-propionic acid is a rather strong anti-auxin. The (*R*)-(+)-form has a much weaker anti-auxin effect; possibly it can be classified as an "intermediate" with a very weak auxin component. This is in full agreement with the configurational assignment.

EXPERIMENTAL (in collaboration with Kerstin Stadell)

The rotations have been measured in 96 % ethanol. The analyses were carried out by the Analytical Division of the Institute.

(±)-α-(Benzimidazolyl-2-thio)-propionic acid. 2-Mercaptobenzimidazole (12.0 g, 0.08 mol) [7] was dissolved with sodium hydroxide (3.2 g, 0.08 mol) in 500 ml of water and α-bromopropionic acid (12.2 g, 0.08 mol) neutralised with sodium hydrogen carbonate in 50 ml of water was added. After 1 h the solution was acidified with hydrochloric acid to pH 3. The separated acid was filtered off after standing for 2–3 h and washed with water. Yield, 15.5 g (87 %), after recrystallisation from 320 ml of 35 % ethanol, 13.1 g (73 %). Colourless needles with m.p. 166–167 °C. Anal. $C_{10}H_{10}N_2O_2S$: C, H, N, S. Equiv.wt. Found: 222.0. Calc. 222.3.

Preliminary experiments on resolution. Salts of common alkaloids were prepared on a 0.001 mol scale in dilute ethanol. The quinine salt gave the acid with $[\alpha]_D^{25}$ −115° while the acid from the quinidine salt had $[\alpha]_D^{25}$ +107°. Strychnine and cinchonidine gave acid with very low rotation. The cinchonine salt was obtained in a very low yield and the brucine salt failed to crystallise.

(−)-α-(Benzimidazolyl-2-thio)-propionic acid. Racemic acid (26.6 g, 0.12 mol) was dissolved in 350 ml of 96 % ethanol and quinine (38.9 g, 0.12 mol) in 100 ml of the same solvent were added. After standing overnight, the salt (34.9 g) was filtered off: $[\alpha]_D^{25}$ of the acid −112°. Since the salt was only sparingly soluble, it was divided into two portions of 17 g each. The first portion was recrystallised as shown in Table 1.

The second portion of the salt was treated in the same way. Systematic fractionation of the salt from the mother liquors gave a third crop. The total yield of salt containing acid of maximum activity was 21.5 g. It was decomposed with excess aqueous ammonia, the quinine extracted with chloroform and the remaining solution acidified with hydrochloric acid to pH 3. The crude product (7.4 g) was recrystallised from 96 % ethanol, yielding 6.7 g of colourless needles with m.p. 176–177 °C: Equiv.wt. Found: 222.2. Calc. for $C_{10}H_{10}N_2O_2S$, 222.3. $[\alpha]_D^{25}$ −124°; $[M]_D^{25}$ −276° (*c* 0.522, 96 % ethanol).

(+)-α-(Benzimidazolyl-2-thio)-propionic acid. The various mother liquors from the quinine salt yielded 11.2 g of acid having $[\alpha]_D^{25}$ +119°. This acid (0.05 mol) was dissolved with quinidine (16.2 g, 0.05 mol) in 350 ml of ethanol and 50 ml of water were added. After evaporation to half the volume, the solution yielded 9.7 g of salt containing acid with $[\alpha]_D^{25}$ +123°. Recrystallisation from 80 ml of 75 % ethanol gave 6.9 g salt with acid of $[\alpha]_D^{25}$ +124°. Systematic fractionation of the salt from the mother liquors gave a further 13.8 g salt with acid of maximum activity. The acid was isolated and recrystallised as described for the enantiomer. The salt (19.0 g) gave 6.5 g of pure acid, m.p. 176–177 °C. Equiv.wt. Found: 222.1. Calc. for $C_{10}H_{10}N_2O_2S$: 222.3. $[\alpha]_D^{25}$ +124°; $[M]_D^{25}$ +276° (*c* 0.488, 96 % ethanol).

Table 1. Recrystallisation of the quinine salt from dilute ethanol to constant activity of the acid.

Recryst.	Ethanol/ml/	Water/ml	Salt obtained/g	$[\alpha]_D^{25}$/°
1	380	190	11.8	−119
2	330	170	8.7	−123
3	230	120	6.6	−124
4	190	90	5.2	−124

Synthesis from (−)-α-bromopropionic acid. Since the preparation of optically pure acid is a very tedious process, a partially resolved acid was used. It was prepared as described by Ramberg [8] and had α_D^{25} −19.3° (homogeneous, 1 dm) which means 40 % of active acid. The reaction was carried out as described above. The crude product had $[\alpha]_D^{25}$ +54°, which increased on recrystallisation from 60 % ethanol. Equiv.wt. Found: 221.9. Calc. for $C_{10}H_{10}N_2O_2S$: 222.3. $[\alpha]_D^{25}$ +64° (*c* 0.863, 96 % ethanol).

The crude product thus contained 44 % and the recrystallised compound 52 % of active acid. The results show that no significant racemisation had taken place and that the proportion of the less soluble active acid had increased during the operations.

Benzimidazolyl-2-thio-acetic acid. 2-Mercaptobenzimidazole (4.5 g, 0.03 mol) was dissolved with sodium hydroxide (1.3 g, 0.033 mol) in 100 ml of water and chloroacetic acid (3.1 g, 0.032 mol) neutralised with potassium hydrogen carbonate in 30 ml of water was added. After 2 days the solution was acidified with hydrochloric acid to pH 3. Yield of crude product, 6.2 g (92 %). 2.0 g recrystallised from 200 ml of 60 % ethanol gave 1.5 g of colourless needles, m.p. 202 °C (dec.). Anal. $C_9H_8N_2O_2S$: C, H, N, S. Equiv.wt. Found: 208.3. Calc.: 208.2.

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Structure and Reactions of the $C_6H_7N^{+\cdot}$ and $C_6H_6N^+$ Ions Formed from Phenylurethane on Electron Impact*

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Dedicated to Professor K. A. Jensen on his 70th birthday

The $C_6H_7N^{+\cdot}$ fragment ions from $C_6H_5NHCOOC_2H_5$ and $C_6H_5NHCSOC_2H_5$ are formed with an aniline-like structure despite indications to the contrary in literature spectra of deuterium labeled phenylurethanes. An earlier suggestion that pivalanilide likewise generates $C_6H_7N^{+\cdot}$ ions with an aniline-like structure has been confirmed. $C_6H_5NH^+$ ions formed by elimination of $C_2H_5OCO^{\cdot}$ from phenylurethane do not undergo extensive hydrogen scrambling prior to decomposition, in contrast to $NH_2C_6H_4^+$ ions.

A number of recent studies have established that the abundant $C_6H_7N^{+\cdot}$ fragment ions formed by elimination of small neutral molecules from the molecular ions of acetanilides,[1–4] higher anilides,[5] and formanilides [6] are formed with an aniline-like structure. The published mass spectra of phenylurethane (ethyl *N*-phenylcarbamate) [7,8] and higher esters of *N*-phenylcarbamic acid show that these compounds likewise give rise to abundant $C_6H_7N^{+\cdot}$ fragment ions, and *a priori* it would appear reasonable to assume that the initial structure of these ions is also aniline-like. However, a comparison of the mass spectra of phenylurethane and some labeled analogs ($C_6H_5NHCOOCH_2CD_3$ and $C_6H_5NHCOOC_2D_5$)[7,8] with the spectra of acetanilide and acetanilide-d_3 ($C_6H_5NHCOCD_3$) [2] does not lend immediate support to this assumption. For acetanilide, ketene elimination from the molecular ions gives rise to *m/e* 93 ions ($C_6H_7N^{+\cdot}$); these decompose by loss of HNC, leading to *m/e* 66 ions which again eliminate H$^{\cdot}$ with formation of *m/e* 65 ions. In the spectrum of acetanilide-d_3, the corresponding peaks are *m/e* 94 ($C_6H_6DN^{+\cdot}$), *m/e* 67 and *m/e* 66 (elimination of HNC and DNC from *m/e* 94), and *m/e* 66, 65 (H/D loss from *m/e* 67 and 66). The spectrum of phenylurethane is almost identical to that of acetanilide in the *m/e* 60 – 100 region, showing peaks at *m/e* 93, 66 and 65. However, the published spectra [7,8] of the d_3 and d_5 analogs of phenylurethane show the expected shift of *m/e* 93 to 94, but the abundant *m/e* 65 peak is *not* shifted to *m/e* 66. One possible explanation would be that the D-atom transferred from the ethyl group in the reaction leading to $C_6H_6DN^{+\cdot}$ (*m/e* 94) is completely lost in the subsequent fragmentation (*i.e.*, exclusive loss of DNC), in which case the decomposing $C_6H_7H^{+\cdot}$ ions of phenylurethane would be structurally different from the corresponding ions formed from the aniline derivatives previously examined.[1–6]

In order to resolve this problem, we have re-examined the mass spectra of phenylurethane and some labeled analogs hereof.

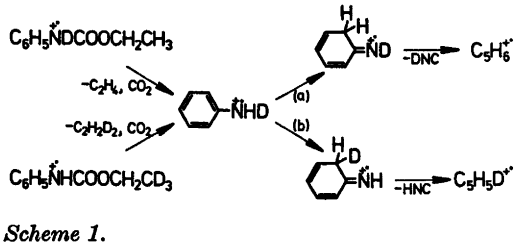
$C_6H_5NHCOOCH_2CH_3$	$C_6H_5NDCOOCH_2CH_3$
1	*1-d*$_1$
$C_6H_5NHCOOCH_2CD_3$	$C_6D_5NHCOOCH_2CH_3$
1-d$_3$	*1-d*$_5$

* A preliminary account of this work was presented at the VIIth Triennial Mass Spectrometry Conference, Firenze, Italy, Aug. 30 – Sept. 3, 1976 (*Adv. Mass Spectrom. 7. In press*).

As shown previously,[1,2,5,6] the course of the hydrogen migration leading to $C_6H_7N^{+\cdot}$ ions may be established through a study of the decomposition of metastable $C_6H_6DN^{+\cdot}$ ions. These ions were in the present case formed by expulsion of $C_2H_4+CO_2$ from *1-d*$_1$ or of $C_2H_2D_2+CO_2$ from *1-d*$_2$ (Scheme 1). In the field-free region between the electric and magnetic sectors these ions decompose by elimination in both cases of HNC and DNC in equal amounts, as shown by the relative areas of the corresponding metastable peaks:

Precursor	*1-d*$_1$	*1-d*$_2$	*1-d*$_5$	*2-d*$_1$	*7-d*$_1$
m_{HNC}^*/m_{DNC}^*	1.0	1.05	4.8	1.0	1.25

This shows that the rearranged hydrogen atom (from the terminal CH_3) has become chemically equivalent to the hydrogen originally on nitrogen prior to the subsequent decomposition by HNC (DNC) elimination, *i.e.*, that the hydrogen transfer must occur to *nitrogen*; transfer of the migrating hydrogen atom to the ring would not allow (only) these two hydrogen atoms to become equivalent. This then strongly suggests that the $C_6H_7N^{+\cdot}$ is formed with an aniline-like structure.



Scheme 1.

To complement this result, the mass spectra of the sulfur analogs, *2* and *3*, of phenylurethane were studied.

$C_6H_5NHCSOC_2H_5$ *2* $\qquad$ $C_6H_5NDCSOC_2H_5$ *2-d*$_1$

$C_6H_5NHCSSC_2H_5$ *3*

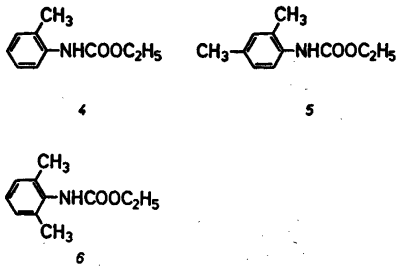
However, *3* does not decompose to give abundant $C_6H_7N^{+\cdot}$ ions; instead $C_6H_5S^+$ ions are formed, after migration of the phenyl group from N to S as previously described for similar systems.[9] The mono-thio compound, *2*, decomposes very much like *1*; in particular, the course of the hydrogen rearrangement leading to formation of $C_6H_7N^{+\cdot}$ is not affected by the exchange of oxygen for sulfur. The metastable $C_6H_6DN^{+\cdot}$ ions formed from *2-d*$_1$ eliminate HNC and DNC in equal amounts (see above), showing again that the rearranged hydrogen atom has become equivalent to the hydrogen on the nitrogen atom prior to HNC (DNC) loss.

It has previously been shown[1] for aniline molecular ions that limited interchange occurs between the hydrogen atoms of the $-NH_2$ group and the *ortho* hydrogen atoms (reversibility of paths *a* and *b* in Scheme 1). For $C_6H_5NHD^{+\cdot}$ ions this should increase the probability for loss of HNC relative to DNC. At the same time, there is an isotope effect on the hydrogen transfer step that precedes HNC elimination, which favors retention of D on nitrogen (path *a* in Scheme 1) and hence should cause preferential loss of DNC. Elimination of HNC and DNC in equal amounts from the $C_6H_6DN^{+\cdot}$ ions of *1-d*$_1$ *1-d*$_3$, and *2-d*$_1$ shows that the two effects in these cases, as for acetanilides[1,3] and formanilides,[6] are of the same order of magnitude. In the decomposition of $C_6D_5NH_2^{+\cdot}$ ions the two effects should reinforce each other, leading to significant DNC loss, which is indeed observed for the $C_6H_2D_5N^{+\cdot}$ ions formed from *1-d*$_5$ (see above).

Previous studies of the decomposition of acetanilide[10] and pivalanilide[11] (see also below) have shown that the reaction leading to the $C_6H_7N^{+\cdot}$ fragment ions is not inhibited by the presence of *ortho* substituents. The relative abundances of the corresponding ion ($Q=M-C_2H_4-CO_2$) and its daughter ions ($Q'=Q-H^{\cdot}$; and $Q''=Q-CH_3^{\cdot}$) in the mass spectra of the tolyl- and xylylurethanes *4*–*6* show that methyl groups in one or both *ortho* positions do not inhibit the elimination of $C_2H_4+CO_2$:

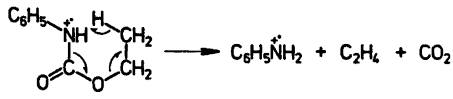
Compound	*1*	*4*	*5*	*6*
M/base peak	72	83	100	78
Q/M	1.4	0.5	0.4	0.3
(Q+Q′+Q″)/M	2.0	2.0	1.5	1.9

The absence of steric hindrance for the hydrogen transfer reaction corroborates the conclusion that hydrogen transfer occurs to nitrogen rather than the aromatic ring.



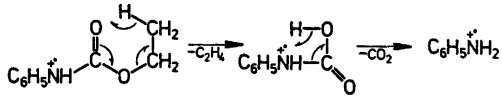
4 5 6

It is not clear whether the formation of $C_6H_7N^+$ from *1* occurs as a one-step elimination of $C_2H_4 + CO_2$ with hydrogen transfer to nitrogen in a six-membered cyclic transition state, as depicted in Scheme 2, or if the reaction occurs stepwise, with initial transfer of hydrogen



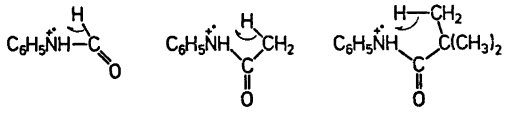
Scheme 2.

to oxygen in a six-membered cyclic transition state and elimination of C_2H_4 (a McLafferty rearrangement), followed by transfer of the same hydrogen from oxygen to nitrogen in a four-membered cyclic transition state with loss of CO_2, as shown in Scheme 3.

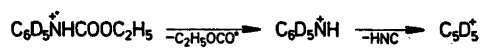


Scheme 3.

A comparison with the processes leading to formation of $C_6H_7N^+$ ions from other aniline derivatives does not provide a clue to the size of the transition state, inasmuch as the hydrogen transfer in formanilides takes place in a three-membered cyclic transition state, while the transition state for acetanilides is four-membered, and for pivalanilides the hydrogen atom is transferred in a five-membered cyclic transition state.



A number of investigations [2,12] of the decomposition of aniline and simple aniline derivatives have provided strong evidence that many even-electron $C_6H_6N^+$ ions undergo extensive scrambling of both hydrogen and carbon atoms prior to decomposition. However, this appears not to be the case for the $C_6H_5NH^+$ ions formed from phenylurethanes by loss of the C_2H_5OCO radical. In the spectrum of *1-d*$_5$, the metastable peak for HNC elimination from $C_6D_5NH^+$ is about five times as intense as that for DNC elimination, indicating a high degree of retention of the amino hydrogen on nitrogen even for the metastable ions.



Scheme 4.

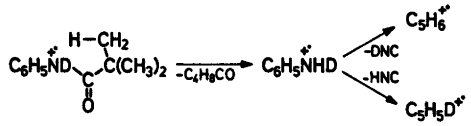
In the spectrum of *1-d*$_1$, the metastable peak for DNC elimination from $C_6H_5ND^+$ is more intense than that for HNC loss, confirming the preferential retention of D on nitrogen. A quantitative measure of the ratio of DNC to HNC loss is in this case precluded by the presence of $C_6H_5NH_2^{+\cdot}$ ions (same nominal mass as $C_6H_5ND^+$) that also eliminate HNC. A similar specificity has been observed for the $C_6H_5NH^+$ ions formed from phenylhydrazines.[13]

These observations provide the reason why *m/e* 65 is not partially shifted to *m/e* 66 in the spectra of $C_6H_5NHCOOCH_2CD_3$ and $C_6H_5NHCOOC_2D_5$.[7,8] Apparently, most $C_5H_5^+$ ions (*m/e* 65) are formed by fragmentation without scrambling of the $C_6H_5NH^+$ ions generated by loss of $C_2H_5OCO^{\cdot}$ from the molecular ions and will hence not incorporate any of the atoms originating in the $-NHCOOC_2H_5$ group (*cf.* Scheme 4).

The reason for the differing behaviour with respect to scrambling of the $C_6H_6N^+$ ions described in the literature [2,12] and those of the present study may well be that most of the previously examined $C_6H_6N^+$ ions have been generated as $NH_2C_6H_4^+$ (*e.g.*, by loss of NO_2 from nitroaniline), whereas the initial structure of the $C_6H_6N^+$ ions from phenylurethanes will be $C_6H_5NH^+$. Apparently, for $NH_2C_6H_4^+$ the energy of activation necessary to reach the reacting configuration for HNC loss is higher than that needed for scrambling of the hydrogen

atoms, while the reverse relationship exists for $C_6H_5NH^+$.

The conclusion that the $C_6H_7N^{+\cdot}$ ion formed by loss of C_4H_8CO from pivalanilide (*7*) has an aniline-like structure was based on the observation [11] that the *ortho* bromine atoms of 2,4,6-tribromopivalanilide do not inhibit the elimination of C_4H_8CO. To confirm this conclusion we have studied the mass spectrum of pivalanilide-*N*-d_1 (*7*-d_1). This compound decomposes with formation of abundant $C_6H_6DN^{+\cdot}$ ions, which subsequently fragment by loss of either HNC or DNC.



Scheme 5.

Again, the metastable peaks for HNC and DNC loss are of similar intensity (see above), supporting the conclusion that the $C_6H_7N^{+\cdot}$ fragment ions are formed *via* hydrogen migration to nitrogen. The ratio of the metastable peak intensities (1.25) is, however, sufficiently different from that observed for other aniline derivatives to leave open the possibility that more than one mechanism is operating.

EXPERIMENTAL

The mass spectra were recorded by Ms E. Wolff-Jensen with an AEI MS-902 mass spectrometer operating with an ion source temperature of 100 °C, using the direct insertion probe. Phenylurethanes *1*, *1*-d_5, *4*, *5*, and *6* were prepared from ethyl chloroformate and the appropriate aniline;[14] *1*-d_3 was obtained by addition of 2,2,2-trideuterioethanol to phenyl isocyanate.[15] The sulfur analogs, *2* and *3*, were prepared by previously described methods.[16,17] Pivalanilide, *7*, was prepared from aniline and pivaloyl chloride. The *N*-d_1 compounds, *1*-d_1, *2*-d_1, and *7*-d_1 were obtained by repeated recrystallizations of the unlabeled compounds from 10 % CH_3COOD in D_2O; back exchange in the inlet system of the mass spectrometer was minimized by introduction of D_2O prior to introduction of the sample. The isotopic purity of the labeled compounds (determined from low-voltage mass spectra) were as follows: *1*-d_1: 84 % d_1; *1*-d_3: 96 % d_3, 4 % d_2; *1*-d_5: 96 % d_5, 4 % d_4; *2*-d_1: 89 % d_1; *7*-d_1: 61 % d_1.

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The Crystal Structure and the Conformation of 1,3,5-Triacetoxyhexahydro-1,3,5-triazine

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Dedicated to Professor K. A. Jensen on his 70th birthday

The crystal structure of acetylated "triformoxime" has been studied. The proposed molecular structure 1,3,5-triacetoxyhexahydro-1,3,5-triazine was definitely proved. The monoclinic crystal structure, space group $C2/c$, was determined from three-dimensional Mo$K\alpha$ X-ray diffraction data. The structure was solved by direct methods and refined by full-matrix least-squares techniques to an R-value of 0.043. In the crystal structure the heterocyclic ring has a flattened chair conformation with the three substituents in axial positions. The average ring dimensions are: C–N = 1.458 Å, ∠CNC = 111.4° and ∠NCN = 118.2°. The geometry of the hexahydro-1,3,5-triazine ring is discussed in relation to the ideal conformation proposed.[7] In the O-acylated hydroxylamine moiety the arrangement of the C=O and the O–N bonds is *cisoid*. The average C=O bond is 1.189 Å. Only few short *inter*-molecular contacts are formed, but a number of polar attractions between carbonyl oxygen atoms and hydrogen atoms contribute to the stabilization of the crystal structure.

The molecular structure of acetylated "triformoxime" has been studied by Jensen and Holm.[1] Their results strongly support the proposed formula 1,3,5-triacetoxyhexahydro-1,3,5-triazine, but they suggested that a crystal structure determination of the compound should be carried out with the purpose of obtaining an unequivocal confirmation of the molecular structure.

A preliminary crystallographic study proved, that the proposed molecular structure was correct. It also gave the unexpected result that in the crystalline state all the three substituents on the chair-formed ring are placed in axial positions. Furthermore it was found, that the N-acetoxy groups are approximately planar with a *cisoid* arrangement of the C=O and N–O bonds. Jensen and Holm[1] measured the dipole moment of the compound in dioxan to 3.25 D. This result implies that such conformers as described above can only contribute to a certain extent to the conformational equilibrium in solution. In order to get a better understanding of the molecular conformation adopted in the crystal structure a new set of X-ray data was collected, and a detailed refinement of the crystal structure was carried out.

EXPERIMENTAL

The title compound was prepared according to directions by Jensen and Holm.[1] The crystals used for the X-ray experiments were grown by slowly evaporating a benzene solution at room temperature. Colourless, prismatic crystals were obtained.

Preliminary film investigations revealed monoclinic symmetry. Systematically absent reflections are hkl when $h+k$ odd and $h0l$ when l odd, the space group is thus Cc or $C2/c$. The unit-cell parameters were determined by a least-squares fit to the diffractometer measured 2θ angles for 27 reflections (see CRYSTAL DATA). The crystal density was measured

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by flotation in a chlorobenzene/bromobenzene mixture.

Three-dimensional diffraction data were measured at room temperature on a NONIUS three-circle automatic diffractometer using graphite monochromated Mo$K\alpha$ radiation (λ = 0.71069 Å). The ω-scan technique with a scan speed of 1.2° min^{-1} was employed. Background counts were taken for half the scanning time at each of the scan range limits. All the data were measured from a single crystal with approximate dimensions $0.3 \times 0.4 \times 0.4$ mm. The crystal was sealed in a glass capillary and oriented with the c axis parallel to the ϕ axis of the goniostat.

Due to instrumental difficulties the one standard reflection sampled every 50 intensity measurements showed two distinct intensity levels over the period of data collection. The data set was therefore divided into two parts each given a scale factor evaluated from the relative intensities of the standard. Of the 2772 unique reflections measured in the range $2.9 \leq \theta \leq 31.0°$, 1666 had net intensities greater than $3.0\sigma(I)$, where $\sigma(I)$ is the standard deviation from counting statistics. These were regarded as observed reflections and used in the refinement procedure. Lorentz and polarization corrections were applied, but no absorption corrections were made.

Structure solution and refinements. Normalized structure-factor amplitudes were calculated, and statistical tests indicated the presence of centres of symmetry; the space group was thus assumed to be $C2/c$. Phases were determined statistically for 117 reflections ($|E| \geq 1.5$) following the modified symbolic addition procedure used in the program system *X-RAY 72*.[2] An E map constructed with these phases gave the positions of 16 non-hydrogen atoms of the molecule. The two remaining non-hydrogen atoms were localized by the use of successive Fourier refinements. Individual atomic parameters of this model were refined, first with isotropic and then anisotropic thermal parameters using the full-matrix least-squares method. The quantity minimized was $\sum(w|F_o| - |F_c|)^2$ where weights were taken as unity. After the refinement had converged, the six methylene hydrogen atoms were clearly located from a difference electron density map, whereas the map only showed diffuse clouds of electron density in the three areas where the methyl hydrogen atoms were expected to be situated. Refinement was continued varying two scale factors, the positional and anisotropic thermal parameters of the non-hydrogen atoms and the positional parameters of the six methylene hydrogen atoms (the thermal parameters of the hydrogen atoms were fixed at

Table 1. Final positional and thermal ($\times 10^3$ Å^2) parameters. The standard deviations are given in parentheses. The temperature factor is of the form: $\exp\{-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23})\}$.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N1	.4520(1)	.3985(1)	.4520(2)	4.69(11)	3.60(10)	5.24(11)	−.11(8)	1.16(8)	−.81(8)
C2	.4424(1)	.4009(2)	.5812(2)	4.64(13)	3.93(12)	4.61(13)	−.18(10)	.35(10)	−.97(10)
N3	.3724(1)	.3613(1)	.6041(2)	4.95(11)	3.63(10)	4.32(10)	−.22(8)	.57(8)	−.66(8)
C4	.3090(1)	.4013(2)	.5107(2)	4.49(13)	4.75(14)	4.74(13)	.31(10)	1.08(10)	−.53(10)
N5	.3128(1)	.4018(1)	.3780(2)	4.92(11)	4.15(10)	4.51(11)	.18(8)	.66(8)	−.21(8)
C6	.3859(1)	.4356(2)	.3625(2)	5.77(14)	4.00(13)	4.79(14)	.14(11)	1.24(11)	.04(11)
O11	.4658(1)	.2909(1)	.4228(2)	5.06(9)	3.74(9)	6.38(10)	−.07(7)	1.22(7)	−1.33(7)
O12	.5831(1)	.3419(2)	.4259(2)	5.53(11)	6.42(13)	9.51(15)	.40(9)	2.97(10)	.97(10)
C11	.5379(1)	.2755(2)	.4205(2)	5.48(15)	5.24(15)	3.78(12)	.62(12)	.95(10)	−.25(10)
C12	.5532(2)	.1628(2)	.4116(3)	8.03(19)	5.92(17)	6.94(18)	1.77(14)	1.45(15)	−1.41(14)
O31	.3701(1)	.2489(1)	.5865(1)	5.75(10)	3.85(8)	4.29(9)	−.32(7)	.39(7)	−.37(7)
O32	.4235(1)	.2352(2)	.7936(2)	8.36(14)	7.21(13)	4.39(10)	−1.73(10)	.12(9)	.48(9)
C31	.3976(1)	.1960(2)	.6946(2)	3.83(12)	5.15(14)	5.39(15)	−.35(10)	.94(10)	.57(12)
C32	.3898(2)	.0833(2)	.6692(3)	7.08(19)	4.99(16)	8.85(21)	.25(13)	.59(15)	.63(14)
O51	.3011(1)	.2953(1)	.3328(1)	5.68(10)	4.65(9)	4.18(9)	−.21(7)	.20(7)	−.58(7)
O52	.2423(1)	.3651(2)	.1465(2)	8.51(14)	8.25(15)	4.37(10)	−.04(11)	−.09(9)	.36(10)
C51	.2621(1)	.2904(2)	.2099(2)	4.65(13)	7.14(17)	3.70(12)	−.63(12)	1.01(10)	−.73(12)
C52	.2494(2)	.1804(2)	.1717(3)	6.68(17)	7.52(20)	6.00(16)	−1.15(14)	1.57(13)	−2.33(14)
H21	.483(1)	.364(2)	.630(2)	5.0					
H41	.263(1)	.366(2)	.517(2)	5.0					
H61	.390(1)	.420(2)	.279(2)	5.0					
H22	.445(1)	.474(2)	.604(2)	5.0					
H42	.305(1)	.477(2)	.533(2)	5.0					
H62	.386(1)	.509(2)	.377(2)	5.0					

isotropic values with $U=0.05$ Å^2). After two cycles nine strong and five weak reflections which were considered to be affected by extinction and/or other factors were removed from the data set. A new difference synthesis again showed only diffuse electron density in the areas where the methyl hydrogen atoms were expected to be positioned. In the subsequent calculations the hydrogen atoms of each methyl group were introduced as two fixed sets each consisting of three hydrogen atoms with site occupational factors of 0.5 and isotropic temperature factors of $U=0.09$ Å^2. The C–H distances were taken as 1.0 Å, the HCH angles as 109.5°, and the torsion angle O=C–C–H was arbitrarily fixed as 0° in one set and as 60° in the other. The least-squares refinement was completed with the introduction of a weighting scheme of the form $w=1/\{1+[(|F_o|-19)/18]^2\}$. On the last cycle of least-squares refinement the values of maximum and average shift/error were 0.05 and 0.01, respectively. The final conventional R value is 0.043 and $R_w=0.052$ for 1652 unique reflections. A final difference synthesis showed no peaks or depressions greater than 0.2 e Å^{-3}. Table 1 lists the final positional and thermal parameters for the non-hydrogen atoms and six methylene hydrogen atoms. The terminal set of structure factors listed with the observed data are available by request.

The X-ray atomic scattering factors used were those of Cromer and Mann [3] for O, N and C, and of Stewart, Davidson and Simpson [4] for H. The computations were performed on an IBM 370/165 computer. The programs used were a local version of the *NRC Picker Data Reduction Program*,[5] the program system *X-RAY 72*,[2] and the drawing program *ORTEP II*.[6]

CRYSTAL DATA

1,3,5-Triacetoxyhexahydro-1,3,5-triazine, $C_9H_{15}N_3O_6$, M = 261.2, m.p. 133 °C.
Space group *C2/c* (No. 15);
$a=18.448(4)$ Å, $b=12.991(3)$ Å, $c=10.860(8)$ Å,
$\beta=103.31(3)°$, $V=2517$ Å^3,
$Z=8$, $D_m=1.37$ g cm^{-3}, $D_x=1.378$ g cm^{-3},
$F(000)=1104$, $\mu(\mathrm{Mo}K\alpha)=1.25$ cm^{-1}.

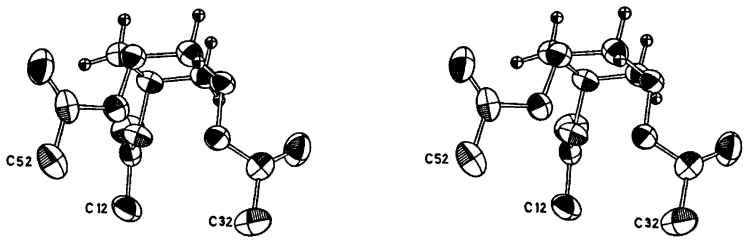


Fig. 1. Stereo view of the molecule.

Table 2. Bond lengths (Å) and valency angles (°) with estimated standard deviations in parentheses. The numbering of the atoms is indicated in Fig. 2.

N1–C2	1.454(3)	N1–O11	1.461(2)	C11–O12	1.189(3)	C2–H21	0.94(2)
C2–N3	1.464(3)	N3–O31	1.464(2)	C31–O32	1.185(3)	C4–H41	0.98(3)
N3–C4	1.455(3)	N5–O51	1.460(2)	C51–O52	1.193(3)	C6–H61	0.95(3)
C4–N5	1.459(3)	O11–C11	1.351(3)	C11–C12	1.491(4)	C2–H22	0.97(3)
N5–C6	1.463(3)	O31–C31	1.352(3)	C31–C32	1.483(4)	C4–H42	1.02(3)
C6–N1	1.454(3)	O51–C51	1.364(3)	C51–C52	1.483(4)	C6–H62	0.96(3)

C6–N1–C2	111.6(2)	N1–O11–C11	111.4(2)	H21–C2–N1	106(2)
N1–C2–N3	117.7(2)	N3–O31–C31	113.1(2)	H21–C2–N3	110(2)
C2–N3–C4	110.9(2)	N5–O51–C51	112.1(2)	H41–C4–N3	111(1)
N3–C4–N5	118.5(2)	O11–C11–O12	125.2(2)	H41–C4–N5	108(1)
C4–N5–C6	111.7(2)	O31–C31–O32	124.4(2)	H61–C6–N1	109(1)
N5–C6–N1	118.6(2)	O51–C51–O52	123.4(2)	H61–C6–N5	109(1)
C2–N1–O11	107.2(2)	O11–C11–C12	110.3(2)	H22–C2–N1	105(1)
C6–N1–O11	109.2(2)	O31–C31–C32	109.5(2)	H22–C2–N3	106(1)
C2–N3–O31	108.9(2)	O51–C51–C52	109.4(2)	H42–C4–N3	105(1)
C4–N3–O31	105.4(2)	O12–C11–C12	124.5(3)	H42–C4–N5	105(1)
C4–N5–O51	106.9(2)	O32–C31–C32	126.1(2)	H62–C6–N1	104(1)
C6–N5–O51	108.1(2)	O52–C51–C52	127.3(2)	H62–C6–N5	104(1)
H21–C2–H22	111(2)	H41–C4–H42	108(2)	H61–C6–H62	112(2)

RESULTS AND DISCUSSION

Molecular constitution. A stereo view of a single molecule as found in the crystal structure is shown in Fig. 1. The bond lengths and valency angles are given in Table 2. These results definitely prove the molecular formula 1,3,5-triacetoxyhexahydro-1,3,5-triazine advocated by Jensen and Holm [1] for triacetylated "triformoxime".

Average molecular dimensions. The lengths of the chemically equivalent bonds are found to be almost identical with one another in the crystal structure determination. A good a-

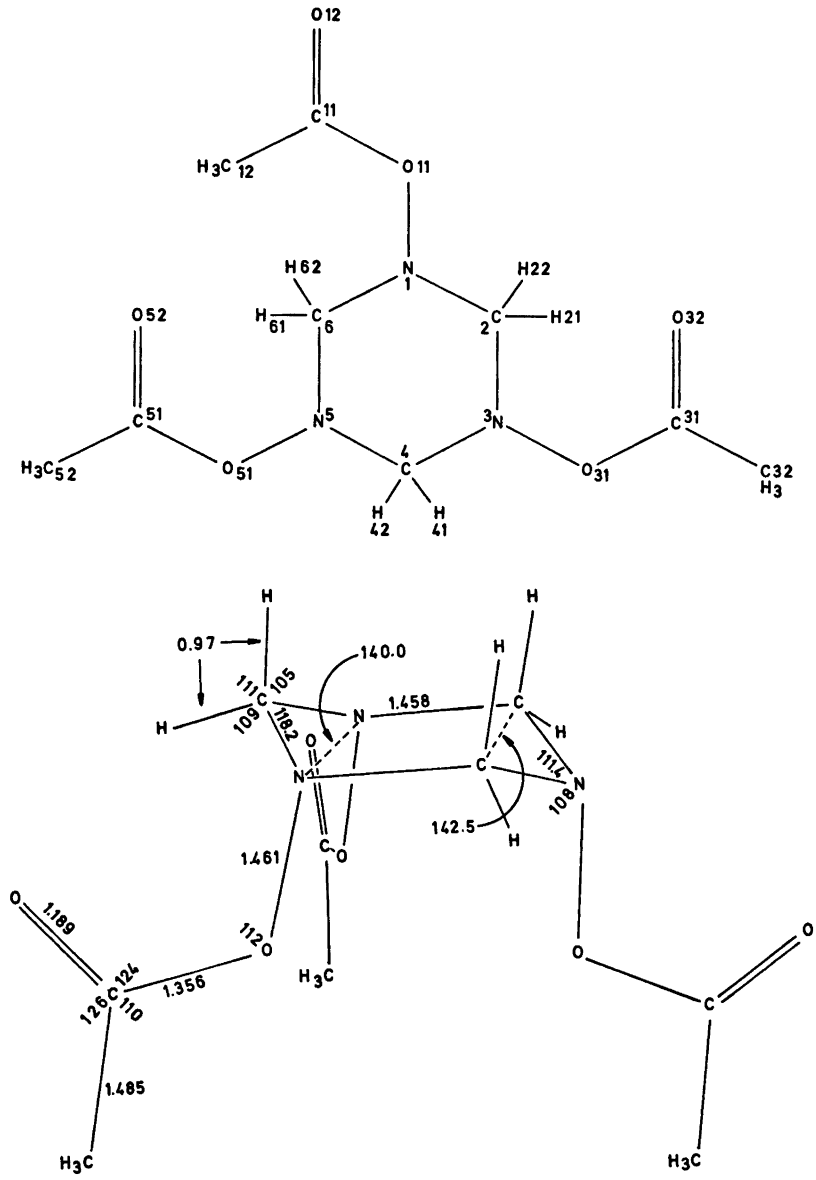


Fig. 2. (a) Numbering of the atoms; the even-numbered methylene hydrogen atoms are axial. (b) Average dimensions of the molecule (in Å, resp.°).

greement is also observed between chemically equivalent bond angles, except the six CNO angles, which deviate up to 2.2° from the mean value. The conformation of the heterocyclic ring is not quite regular as indicated by deviations of the individual torsion angles up to 2.6° from the average value 43.7°. These torsion angles and the exocyclic CNO angles are, however, likely to be appreciably influenced by the packing of the molecules in the crystal, and the variations found are not considered to reflect any difference inherent in the molecular structure.

In the following discussion reference is made to an average molecule, which is defined by the average values of the chemically equivalent bond distances and valency angles. By this definition the average conformation of the heterocyclic ring is defined. The conformation is given either as the average torsion angle or as the average values for the two dihedral angles CNC−CCNN and NCN−NNCC. The average dimensions of the molecule are given in Fig. 2, in which the numbering of the atoms used in the description is also indicated.

The hexahydro-1,3,5-triazine ring. Blackburne *et al.*[7] have calculated the ring-conformation with minimum strain energy for a number of six-membered, saturated rings. In calculating this "ideal conformation" for hexahydro-1,3,5-triazine an invariant C−N distance of 1.47 Å was assumed, and the angle between the lone pair and the exocyclic bond was taken to be 113° and bisected by the CNC plane.

The crystal structures of two 1,3,5-trisubstituted hexahydro-1,3,5-triazines have previously been reported, *viz.* that of the triacetyl derivative, "TRAT"[8] and that of the trinitro derivative, "RDX".[9] The average dimensions of the rings found in these two structures together with those of the present molecule and of the "ideal conformation" are given in Table 3.

In the TRAT molecule the three bonds from each ring-nitrogen atom are nearly coplanar owing to the amide character of the nitrogen atoms. The molecule has a pseudo threefold symmetry. From Table 3 it can be seen that the conformation of the ring is only slightly distorted from that of the ideal one. This is in accordance with the facts that the acetyl groups do not sterically interfere with one another, and that there are no strong *inter*-molecular interactions.

In the RDX molecule a pseudo mirror plane is found. The substituents are arranged with one nitro group in a distorted equatorial position and the other two nitro groups in distorted axial positions. The ring is considerably distorted compared to the ideal conformation, see Table 3. The CNC angle is expanded about 3°, and though the average magnitude of the NCN angles is as in the ideal ring, the individual values vary considerably. This irregularity is reflected in the large fluctuations of the torsion angles. The strong *intra*-molecular interaction between the oxygen atoms of the nitro groups and the methylene groups of the ring [9] is presumably the main cause of the ring distortion, though some rather short *inter*-molecular contacts are also found in the crystal structure.

The heterocyclic ring in the present structure is in a flattened chair conformation with an average torsion angle of 43.7°. The C−N bond distances agree well with those found in TRAT (X-ray diffraction study) and in RDX (neutron diffraction study). All the N-substituents are in axial positions. The conformation of the average ring as compared to the "ideal conformation" is characterized by a large expansion

Table 3. Conformation of some hexahydro-1,3,5-triazine rings. The average values of some chemically equivalent angles and bond lengths are indicated with the maximum deviation of the individual values in square brackets.

Compound	C−N (Å)	Angle C−N−C	Angle N−C−N	Torsion angle	Dihedral angles (°) CNC−CCNN	NCN−NNCC
"Ideal ring"[7]	(1.47)	111.5	109.2	57.7	127.2	128.3
Present structure	1.458[.006]	111.4[.5]	118.2[.5]	43.7[2.6]	142.5[1.7]	140.0[1.7]
TRAT[8]	1.455[.012]	111.1[.4]	110.4[.9]	51.4[1.5]		
RDX[9]	1.454[.014]	114.8[.3]	109.3[2.4]	47.6[5.7]		

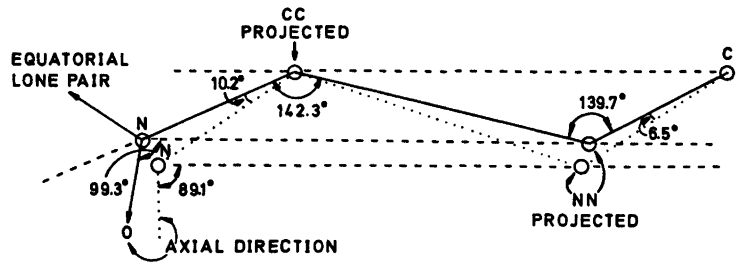


Fig. 3. Section through a superposition of the ideal conformation of the hexahydro-1,3,5-triazine ring (dotted lines) and a conformation derived from the ideal one by expanding the angle N – C – N to 118.2° (full lines). *Cf.* the observed average values of the dihedral angles given in Table 3.

of the NCN angle, whereas the CNC angle is unchanged. This conformation can be derived from the ideal geometry by rotating each of the three nitrogen atoms through an angle of 10.2° outwards about an axis connecting the adjacent carbon atoms, see Fig. 3. Presuming the same angular distribution of the valencies and the lone pair of the nitrogen atoms as in the ideal ring, calculations give a corresponding CNO angle = 108.2° and for an N – O bond = 1.46 Å an $O_{axial}\cdots O_{axial}$ distance of 2.91 Å, *i.e.* slightly longer than twice the van der Waals radius of oxygen. These values are very close to the average values found in the crystal structure determination, *viz.* the CNO angle 108° and $O_{axial}\cdots O_{axial} = 2.87$ Å (the measured values are O11···O31 = 2.83, O11···O51 = 2.97, and O31···O51 = 2.82 Å). The average conformation of the hexahydro-1,3,5-triazine ring found in the present crystal structure thus represents a distortion of the ideal ring, which just allows the accommodation of three axial oxygen atoms attached to the nitrogen atoms.

The N*-acetoxy groups.* The conformations of the *N*-acetoxy groups are given by the respective torsion angles N – O – C = O and C_{ring} – N – O – C (Table 4). The *N*-acetoxy groups at N5 and N3 are approximately planar, whereas the *N*1-acetoxy group deviates more from planarity. The three *N*-acetoxy groups are unsymmetrically oriented with respect to the hexahydrotriazine ring; the best plane through the *N*5-acetoxy group points roughly at atom N3, and the corresponding N3-plane is directed towards N5, whereas the best plane through the *N*1-acetoxy group is directed towards the central part of the ring, *cf.* Fig. 1.

Table 4. Selected torsion angles (°).

C11 – O11 – N1 – C2	∓106.4(2)
C11 – O11 – N1 – C6	±132.5(2)
O12 – C11 – O11 – N1	∓ 8.7(3)
C31 – O31 – N3 – C2	± 91.8(2)
C31 – O31 – N3 – C4	∓149.2(2)
O32 – C31 – O31 – N3	∓ 2.3(3)
C51 – O51 – N5 – C6	∓ 95.0(2)
C51 – O51 – N5 – C4	±144.6(2)
O52 – C51 – O51 – N5	± 2.3(3)

The average N – O distance is identical to the N – O distance 1.458(5) Å found in *O*-carbamoylhydroxylamine.[10] The remaining dimensions of the *N*-acetoxy group and the *N*-carbamoyloxy group are not comparable. The average *O*-acyl dimensions found in the present structure determination agree with the average results of a crystal structure study of four *N*,*O*-dibenzoylhydroxylamines (having methyl-, chloro- or no substituents in the benzene rings).[11] The *cisoid* position of the C = O and the O – N bond found in the present structure is also found in the *N*-acyloxy groups of these four *N*,*O*-dibenzoylhydroxylamines [11] as well as in some *O*-acyloximes.[12,13]

The molecular packing. The packing of the molecules may be described as a layer structure in which all hydrogen atoms are situated near the surfaces of the layers. The layers are running parallel to the *ac*-plane, their cross-section being about $\frac{1}{2}b$. In Fig. 4 two such layers are shown. The axial hydrogen atoms are sticking out of the layers, whereas the acetoxy groups are situated in the interior.

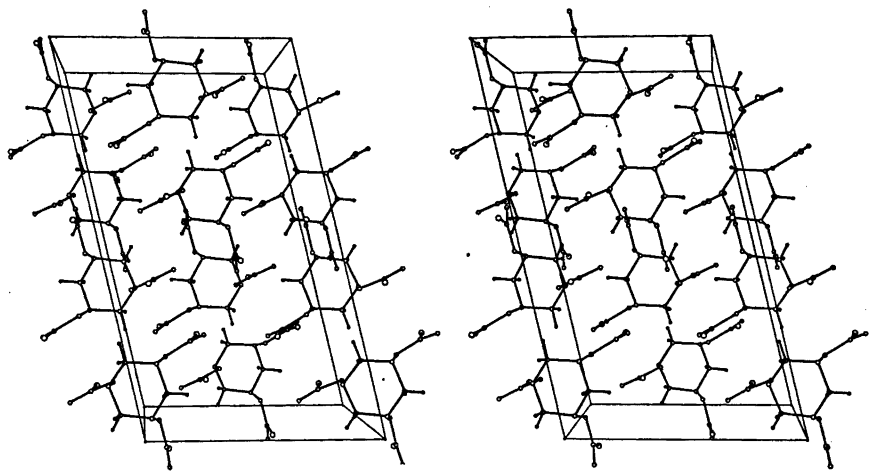


Fig. 4. Stereo diagram illustrating the molecular packing. The view is down the *b* axis, *a* ↑ and *c* →

Table 5. Inter-molecular distances, non H−non H < 3.5 Å, non H−$H_{methylene}$ < 3.0 Å. The distances are given between a molecule with the parameters *x,y,z* as in Table 1 and molecules related by symmetry operations $\bar{1}$ (in 0,0,0), *2* (in $0,y,\frac{1}{4}$) or *c* (in *x*,0,*z*) combined with translations along the axial directions.

Symmetry operation + translation	Non H−non H distances < 3.5 Å		Non H−$H_{methylene}$ < 3.0 Å	
Inter-molecular distances within a layer				
2 + 1,0,1	O32···O11	3.36	O32···H21	2.40
	O32···C11	3.07		
	O32···O12	3.37		
	O32···C12	3.27		
	O32···C31	3.31		
	O32···O32	3.18		
	O32···C2	3.32		
$\bar{1}+\frac{1}{2},\frac{1}{2},1$	C51···C31	3.35	O31···H41	2.86
	O32···C51	3.43		
	O32···C52	3.47		
2 + 1,0,0			O12···H61	2.59
Inter-molecular distances between neighbouring layers				
$\bar{1}$ + 1,1,1	N1···N1	3.20	N1···H22	2.70
	N1···C2	3.31	O12···H62	2.84
	O12···C2	3.35	O12···H22	2.44
$c+0,1,\bar{1}$			O52···H42	2.77

The shortest *inter*-molecular distances are given in Table 5. There are no short contacts between the molecules. The most prominent *inter*-molecular interactions are formed between two molecules placed in the same layer and related by a two-fold axis (the equivalent positions *x,y,z* and $1-x,y,3/2-z$ are seen in Fig. 4). It is inferred, that these contacts are largely responsible for the conformation adopted by the acetoxy groups in the crystal structure. Furthermore several *inter*-molecular carbonyl oxygen−hydrogen distances in the range 2.4−2.8 Å should be mentioned. These distances involve methylene hydrogen atoms as indicated

in Table 5, and also methyl hydrogen atoms; the precise values of the latter distances cannot be given owing to the conformational disorder of the methyl groups. The corresponding many, albeit weak, polar attractions may contribute significantly to stabilize the crystal structure.

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Energetics of Electrode Reactions. III.[1] The Dependence of the Magnitude of Substituent Effects upon the Absolute Potential of Electrode Processes

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Dedicated to Professor K. A. Jensen on his 70th birthday

Linear correlations were obtained from plots of electrode potentials *vs.* σ_p for the oxidation of substituted anthracenes, the reduction of methyl substituted anthracenes as well as the reduction of methyl substituted naphthalenes. A relationship was found by which the absolute potential for an electrode reaction involving alternant aromatic hydrocarbons can be estimated from the measured potential difference ($\Delta E_{1,2}$) for two different electrode processes involving parent aromatic compounds and the slopes of E *vs.* σp (ϱ_1 and ϱ_2) for the two series of substituted alternant aromatic hydrocarbons. The

$$E^1_{abs} = \Delta E_{1,2}/(1-\varrho_2/\varrho_1)$$

equation results from the observation that the magnitudes of the slopes, ϱ_1 and ϱ_2, were found to depend upon the magnitudes of E_1 and E_2 measured on an arbitrary scale. The assumption that a zero substituent effect ($\varrho = 0$) would imply zero energy of the parent alternant aromatic hydrocarbon electrode process, allowed for the scaling of a plot of ϱ *vs.* E to pass through the origin and thus convert the arbitrary scale of electrode potentials to the absolute scale. The absolute potential for the oxidation of anthracene was estimated to be equal to 5.42 V while the absolute potentials for the oxidation of the anthracene and naphthalene anion radicals were estimated to be equal to 2.08 and 1.56 V. respectively.

Linear free energy relationships are often found in comparisons of rates and equilibria of reactions belonging to a closely related series and such relationships have had a profound effect upon the development of modern organic chemistry.[2] A number of linear free energy relationships have been found upon examination of oxidation and reduction potentials of organic compounds.[3] Electrode potentials, measured on an arbitrary scale, can be substituted into the form of the Hammett equation [2] which applies to equilibria (1) to give (2) where E_x is the potential for the substituted compound and

$$\log K_x/K_0 = \varrho\sigma_x \quad (1)$$

$$E_x - E_0 = \varrho\sigma_x \quad (2)$$

E_0 is that for the parent in the series.

We have found that redox reactions of alternant aromatic hydrocarbons (AAH) and substituted AAH constitute a family of reaction series which give linear plots of electrode potentials *vs.* σ constants. The electrode reaction of the unsubstituted compound is the parent reaction for each series. That is, each AAH can belong to two different electrode reaction series, one series being the oxidation and the other the reduction of the compounds. The various series of reactions investigated in this study, written in the direction of oxidation are illustrated in eqns. (3)–(5) where the symbols AN and NA refer to anthracenes and naphthalenes, respectively. The oxidation of

Table 1. Oxidation potentials of 9- and 9,10-substituted anthracenes.

Substituents	$\sum\sigma_p$ [a]	Acetonitrile [b]	Dichloromethane [b]
9,10-Dimethoxy	−0.54	0.56	0.65
9-Ethoxy-10-ethyl	−0.42	0.60	0.67
9,10-Dimethyl	−0.34	0.65	0.72
9,10-Diethyl	−0.30	0.67	0.73
9-Methyl	−0.17	0.79	0.82
Unsubstituted	0.00	— [c]	0.91
9-Bromo	+0.23	0.94	1.01
9,10-Dibromo	+0.46	1.02	1.10
9,10-Dichloro	+0.46	1.01	1.09
9-Nitro	+0.78	1.20	1.29
Slope (mV)		473	485
Correlation coefficient		0.994	0.997

[a] Values taken from Ref. 2. [b] All potentials reported in this study are formal potentials in V *vs.* SCE measured at the potential midway between the oxidation and reduction peaks from reversible cyclic voltammograms. Supporting electrolyte: Bu_4NBF_4 (0.1 M). [c] Oxidation irreversible due to the reaction of the cation radical with acetonitrile.[4]

naphthalenes, eqn. (6), is not suitable for linear free energy

$$AN \rightleftharpoons AN^+ + e^- \quad (3)$$

$$AN^- \rightleftharpoons AN + e^- \quad (4)$$

$$NA^- \rightleftharpoons NA + e^- \quad (5)$$

$$NA \rightleftharpoons NA^+ + e^- \quad (6)$$

studies since naphthalene cation radicals are very reactive and it has not been possible to measure reversible electrode potentials for this series.

Plots of E *vs.* σ_p for reaction series (3) – (5) were observed to be linear. Early in this investigation, we noted that the slopes of the correlation lines, *i.e.* ϱ values, varied over a wide range and appeared to be dependent upon the relative potentials for the parent reactions in the series. In this paper, we present the results of a detailed investigation of the Hammet plots of reactions (3) – (5) which lead to the conclusion that a direct relationship exists between ϱ and the potential of the parent electrode processes.

RESULTS

Reversible oxidation potentials for several 9- and 9,10-substituted anthracenes along with those for the unsubstituted compound in both acetonitrile and dichloromethane are summarized in Table 1. The potentials were measured from cyclic voltammograms under conditions where the cation radicals were stable on the time scale of cyclic voltammetry. The latter was accomplished by making voltammetric measurement on solutions containing suspended neutral alumina, a technique which has recently been used to advantage in observing reactive intermediates generated at electrodes.[5] The potentials measured in dichloromethane are on the average about 80 mV more positive than those in acetonitrile. This potential shift is most likely connected with the reference electrode since both the oxidation and reduction potentials of anthracenes and related compounds have been shown to be nearly independent of the solvent.[6–8] The slopes given at the end of the table are the least squares values from plots of E *vs.* $\sum\sigma_p$.

Both oxidation and reduction potentials for a number of anthracenes substituted only with methyl groups are tabulated in Table 2. Here again, only reversible potentials are reported and they were obtained in the manner described previously for the oxidation of substituted anthracenes.

The reduction potentials for a large number of methyl substituted naphthalenes [9] and anthracenes [10] have been reported by Klemm.

Table 2. Redox potentials of methyl substituted anthracenes.

Substituents	Oxidation [a]	Reduction [b]
Unsubstituted	1.340	−2.035
2-Methyl	1.278	−2.076
9-Methyl	1.224	−2.042
2,3-Dimethyl	1.210	−2.128
2,6-Dimethyl	1.201	−2.117
2,7-Dimethyl	1.189	−2.121
9,10-Dimethyl	1.125	−2.044
2,3,9-Trimethyl	1.088	−2.129
2,6,9-Trimethyl	1.090	−2.121

[a] Potential in V *vs.* SCE measured midway between the oxidation and reduction peaks of reversible cyclic voltammograms. Solvent: dichloromethane, supporting electrolyte: Bu_4NBF_4 (0.1 M). [b] Potential in V *vs.* SCE measured midway between the oxidation and reduction peaks of reversible cyclic voltammograms. Solvent: dimethylformamide, supporting electrolyte: Bu_4NBF_4 (0.1 M).

In the latter studies, the effect of methyl substitution was observed to depend upon the position of the substituent. For naphthalene there are two unique positions, 1 and 2, for mono substitution, while for anthracene there are three, 1, 2 and 9. Thus, in order to account for the effect of methyl substitution it was necessary to average the effect over two positions for naphthalenes and over three positions for anthracenes. In Table 3, the averaged reduction potentials for methyl substituted naphthalenes [9] and anthracenes [10] measured in 75 % dioxane-water are tabulated along with the averaged values for the reduction of methyl anthracenes in anhydrous dimethylformamide (DMF) and the oxidation potentials for the same compounds in dichloromethane, the latter two sets of potentials were derived from the data in Table 2. The naphthalene potentials were averaged from two monomethyl values, ten dimethyl values and four trimethyl values. In the case of the anthracenes, potentials are from three monomethyl values, four dimethyl values, two trimethyl and two tetramethyl values.

The slopes recorded at the bottom of Table 3 refer to plots of the averaged potentials *vs.* $\sum\sigma_p$. It is of particular interest to note that the slopes for the reductions of the anthracenes in anhydrous DMF and in 75 % dioxane-water are identical at 185 mV. We certainly do not claim that the precision in the measurements and the linear relationships are so high so as to expect equal slopes for the two sets of experiments. We do believe, however, that it is significant that the slopes are equal or nearly so in that it shows the absence or near absence of a solvent effect. The latter also indicated that reduction potentials of AAH measured in aqueous dioxane compare very favorably with those in anhydrous media where the processes are known to be reversible. Exactly the same conclusion was recently arrived at in comparing Bergmans [11] potentials for AAH reduction in aqueous dioxane with reversible potentials measured in anhydrous

Table 3. The effect of methyl substitution on redox potentials of anthracene and naphthalene.

Number of methyls	$\sum\sigma_p$	$NA \rightarrow NA^-$ Diox−H_2O [a]	$AN \rightarrow AN^-$ Diox−H_2O [b]	$AN \rightarrow AN^-$ DMF [c]	$AN \rightarrow AN^+$ Dichloromethane [c]
0	0.00	−2.437	−1.924	−2.035	1.340
1	0.17	−2.459	−1.944	−2.059	1.246
2	0.34	−2.484	−1.987	−2.103	1.181
3	0.51	−2.516	−2.005	−2.125	1.089
4	0.68	–	−2.049	–	–
6	1.02	–	−2.109	–	–
Slope (mV) [d]		138	185	185	481
Correlation coefficient		0.999	0.996	0.991	0.998

[a] Potentials averaged from Ref. 7. [b] Potentials averaged from Ref. 8. [c] Potentials averaged from Table 2. [d] All slopes are positive when referring to the reaction series in the oxidation direction.

media.[12] These observations are reasonable in view of the fact that the protonation of the anion radicals of both naphthalene and anthracene have been observed to be very slow in aqueous DMF, second order rate constants of 4.6×10^{-3} and 2.0×10^{-4} M^{-1} s^{-1}, respectively.[13] Thus, if the protonation rate constants are of the same order of magnitude in 75 % dioxane-water the measured potentials in that medium would be expected to be very close to the reversible values.

The slopes reported in the tables are values obtained from the least squares procedure. The slope resulting from the reduction potentials of naphthalenes in dioxane-water, was derived neglecting the point with $\sum\sigma_p = -0.51$ which deviates significantly from the line passing through the other three points. Including the latter point changes the slope from 138 to 154 mV and the correlation coefficient from 0.999 to 0.996.

DISCUSSION

The Hammet ϱ value represents the relative sensitivity of a series of rates or equilibria to substituent effects.[2] Both reaction series (3) and (4) have identical or nearly identical ϱ values in two different solvents which show that the substituent effects for AAH redox reactions are nearly solvent independent. Relative potentials for these processes change little with solvent indicating that the solvation energy differences between the ions and the neutral compounds are also nearly independent of the solvent.[6] The only variable changing in going from one series to the next appears to be the electrode potential of the reaction of the parent in the series. A plot of ϱ for series (3)–(5) *vs.* the parent AAH redox potential on a relative scale gives a good linear correlation. This indicates that a unique value of ϱ is associated with each and every value of the electrode potential, on every reference scale, for the reaction of the parent AAH in the series.

The discussion in the previous paragraph has a very important consequence. Since the value of ϱ is directly proportional to the free energy of the parent electrode process, it is reasonable to assume that ϱ equals zero when the free energy of the standard reaction in the series equals zero. This corresponds to the assumption that a plot of E, on the absolute scale, *vs.* ϱ passes through the origin. Given this additional reference point, eqn. (7) can

Table 4. Absolute potential for the oxidation of anthracene calculated from correlation slopes.

	AN+ (MeCN)[a] 9,10 pos.	AN+ (CH_2Cl_2)[b] 9,10 pos.	AN+ (CH_2Cl_2)[c] methyl	AN^- (DMF)[d] methyl	AN^- (diox)[e] methyl	NA^- (diox)[f] methyl
AN+ (acetonitrile)[a] 9,10 positions		–	–	5.48	5.48	5.45
AN+ (dichloromethane)[b] 9,10 positions	–		–	5.40	5.40	5.40
AN+ (dichloromethane)[c] methyl substituents	–	–		5.43	5.43	5.41
AN^- (dimethylformamide)[d] methyl substituents	5.48	5.40	5.43		–	5.39
AN^- (dioxane-water)[e] methyl substituents	5.48	5.40	5.43	–		5.39
NA^- (dioxane-water)[f] methyl substituents	5.45	5.40	5.41	5.39	5.39	

ϱ = [a] 473, [b] 485, [c] 481, [d] 185, [e] 185, and [f] 138 mV.

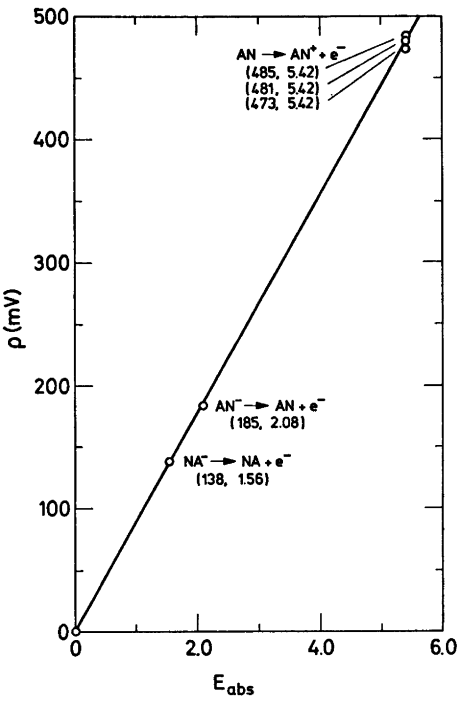


Fig. 1. Plot of ϱ *vs.* E_{abs} for AAH redox reactions. Correlation coefficient: 0.9998.

readily be derived.* The absolute potential for the standard reaction in series 1 is equal to the measured potential difference between the two standard reactions ($\Delta E_{1,2}$) divided by 1.0 minus the ratio of ϱ values for series 2 and 1.

$$E^1_{abs} = \Delta E_{1,2}/(1 - \varrho_2/\varrho_1) \quad (7)$$

The absolute potential for the oxidation of anthracene was calculated from eqn. (7) using all possible combinations of the data and the values obtained are gathered in Table 4. The average value is 5.42 ± 0.02 V and the largest deviation from the average is equal to only 0.06 V. The excellent linear correlation of all of the data, passing through the origin is illustrated by the plot of ϱ *vs.* absolute electrode potential in Fig. 1.

* For two electrode potentials, we have two equations $E^1_{abs} = m\varrho_1$ and $E^2_{abs} = m\varrho_2$, where m is the slope of the potentials *vs.* ϱ line, which passes through the origin. Substracting the second equation from the first and rearranging gives: $E/m\varrho_1 = 1 - \varrho_2/\varrho_1$, which after the appropriate substitution gives eqn. 7.

There is no need to emphasize the importance of the determination of an absolute electrode potential. Once that one such potential is available, the absolute values of all measured reversible potentials will be known. This in turn allows for the evaluation of the energies of single ions in solution, the goal of a large volume of research in physical chemistry.

In order to place the present work in context, we can refer to some recent work on attempts to calculate the absolute potential of the hydrogen electrode. Calculation of the latter ultimately involves the chemical free energy for the hydration of the proton ($\mu_{H^+H_2O,^o}$).[14] Two approaches have been taken, both involving approximate non-thermodynamic procedures, to evaluate the hydration energy of the proton. In the first, conventional hydration energies referred to an arbitrary scale are plotted against a suitable function of the ionic radius based on models and finally extrapolation to radius equal to zero gives the chemical free energy of hydration of the proton. The second approach [15] makes use of the real free energy of hydration of the proton ($\alpha_{H^+H_2O,^o}$), which is a measureable quantity, and equation (8). The drawback to this method is that $\varkappa$,

$$\alpha_{H^+H_2O,^o} = \mu_{H^+H_2O,^o} + F\varkappa^{H_2O} \quad (8)$$

the surface potential of water, cannot be measured and must be estimated. Values for the absolute potential of the hydrogen electrode equal to 4.63 [16] and 4.813 [17] using the first procedure while values of 4.31 [14] and 4.78 [15] have been reported using the second approach.

It can be seen from the preceding paragraph that a great deal of uncertainty exists in the absolute potential of the hydrogen electrode. In comparison, the variation in the calculated values of the absolute potential for the oxidation of anthracene listed in Table 4 are indeed small. However, at this point we must stress, in spite of the excellent agreement of the values in Table 4, that the amount of data is too limited to place a high level of confidence on 5.42 ± 0.02 V as the absolute potential of the anthracene-anthracene cation radical half reaction.

In the previous paper of this series another approach was taken to arrive at the values of absolute potentials of AAH redox reactions.[1b] The sums of the solvation energies of an AAH^+ and the corresponding AAH^- were calculated

according to eqn. (9). The derivation of eqn. (9) did not require the value of the potential of a reference electrode since E°_{sum} is the difference in potential for the oxidation and reduction

$$2(\Delta G^\circ_{solv})^\pm = (\Delta G^\circ_{solv})^- + (\Delta G^\circ_{solv})^+ = E^\circ_{sum} + EA - IP \quad (9)$$

of an AAH. By making a single assumption, *i.e.* that the solvation energies of AAH^- $(\Delta G^\circ_{solv})^-$ and AAH^+ $(\Delta G^\circ_{solv})^+$ are equal, the oxidation and reduction potentials of AAH could be referred to an absolute energy scale. Thus, eqn. (10) gives the absolute potential for the oxidation of an AAH for which $(\Delta G^\circ_{solv})^\pm$ is known from eqn. (9). For AN, EA has been determined to be equal to 0.55 eV [18] and the photoelectron ionization potential has been reported to be equal to 7.47 eV.[19] Since the latter IP value is for the vertical ionization of AN, it is somewhat higher than the adiabatic value, needed for eqn. (9). The calculated vertical IP values reported by Dewar [20] are on the average 0.17 eV higher than the corresponding adiabatic values. Therefore, a reasonable estimate of the adiabatic IP for AN can be arrived at by subtracting 0.17 eV from the photoelectron value resulting in an estimate of 7.30 eV. Thus, we arrive at a value of -1.71 V for $(\Delta G^\circ_{solv})^\pm$ according to (9).* The absolute potential for the oxidation of anthracene can then be estimated from eqn. (10) to be equal to 5.59 V. It has previously been proposed that although the

$$E_{abs} = IP + (\Delta G^\circ_{solv})^+ \quad (10)$$

chemical solvation energies of AAH^+ and AAH^- should be equal, the real free energies of solvation should differ by twice the surface potential of the solvent. The value of χ for acetonitrile was estimated to be equal to -0.10 ± 0.06 V.[21] Taking the latter into account, values of -1.81 and -1.61 V, respectively, are obtained for $(\Delta G^\circ_{solv})^+$ and $(\Delta G^\circ_{solv})^-$. This value for the solvation energy of AAH^+ leads to a value of 5.49 for the absolute potential of the anthracene-anthracene cation radical redox couple according to eqn. (10).

The agreement between E_{abs} for the oxidation of anthracene from Table 4 and the value estimated from eqn. (9) and eqn. (10) is remarkable in view of the large discrepancies which arise from the different approaches in estimating the absolute potential of the hydrogen electrode. The method employing eqns. (9) and (10) might be considered a more conventional approach than the use of linear free energy relationships as in this paper. However, the uncertainty in adiabatic IP values, experimental error in measuring EA values, the uncertainty in the value of surface potentials, as well as the assumption that the solvation energies of AAH^- and AAH^+ are equal allows for the possibility of much greater error by the former procedure. On the other hand, the fact that the two methods give almost identical values for the absolute potential for the oxidation of anthracene suggests that the Hammett ϱ *vs.* absolute potential relationship is indeed legitimate.

In conclusion, we once again emphasize the completely empirical manner in which the data in Table 4 were arrived at. One assumption is involved in this treatment, *i.e.* a ϱ value of zero corresponds to a free energy change of zero for the standard electrode process. Stated still another way, the assumption is that since there is only one value of ϱ for each value of E_{abs} of the standard electrode process, the value of E_{abs} is zero when ϱ is zero. One might inquire as to what further data might be obtained to strengthen the arguments presented here and make the absolute potential scale so defined more precise. Obviously, more experimental points would be highly desirable. If a series of substituted tetracenes (TE) were available, predicted absolute potentials for the oxidation and reduction of TE are 4.95 and 2.55 V, respectively, the gap between the oxidation and reduction of anthracene in Fig. 1 would be filled and these extra points would greatly increase the reliability of the treatment. However, when one considers the structure of TE it becomes obvious that to obtain the necessary methyl substituted compound would be an enormous exercise in organic synthesis. Just considering the mono and disubstituted derivatives we find that there are three possible of the former and twelve of the latter. In spite of the complexity of the tetracene series and in view of the importance of the definition of the absolute potential scale, work is planned on that series.

* A somewhat larger value, -1.97 V, was obtained using calculated vertical IP values.[1b]

EXPERIMENTAL

The equipment used for voltammetric measurements has been described earlier.[22] Acetonitrile (Fluka, *purum*) and dimethylformamide (Fluka, *puriss.*) were purified as recommended by Moe.[23] Dichloromethane (Merck, *pro analysi*) was used as obtained All solvents were predried by passing through neutral alumina (ICN Pharmaceuticals W 200) prior to use. Supporting electrolyte was in all cases Bu_4NBF_4 prepared from Bu_4NHSO_4 (Hässle, Göteborg, Sweden) and HBF_4 (50 % aqueous solution, Riedel-de Haën AG). 9-Substituted and 9,10-disubstituted anthracenes were reagent grade and recrystallized from ethanol or acetic acid. Methyl substituted anthracenes [10] were not further purified. Voltammetry was conducted under nitrogen in the presence of neutral alumina. This technique has been described in detail previously.[5a] The temperature was controlled to $20.0 \pm 0.1°$.

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Reaction of a β-Oxo-α-chlorosulfenyl Chloride with Nucleophiles. Formation and Reactions of Thione *S*-Dimethoxides and *S*-Imides

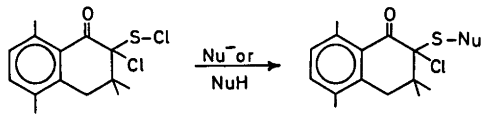
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Dedicated to Professor K. A. Jensen on his 70th birthday

The α-chloro-β-oxosulfenyl chloride *1* reacts with methoxide, acetate, cyanide, aniline, trimethylaniline, and dimethylamine, to give the *S*-substituted compounds *2a–f*, respectively. Excess methoxide gives the *S*-dimethoxythione *5*, which rearranges in acids to the *S,C*-dimethoxy compound *6a*. Reaction of *1* with excess *tert*-butylamine gives the thione-*S*-imide *3*, which reacts with methanol or with acetic acid to give the α-methoxy- and the α-acetoxysulfenamides *4a* and *4b*, respectively. Reaction of *1* with dipivaloylmethane anion gives *7*, a dimer of the thioketone formed by reductive elimination of chlorine from *1*.

The reactions of α-chlorosulfenyl chlorides with nucleophiles have lately been studied in several laboratories and shown to involve substitution at sulfur.[1–3] These studies have here been extended to include the now readily available β-oxo-α-chlorosulfenyl chloride *1*.[4]



1 *2*

a Nu = OCH_3 *d* NuH = $C_6H_5NH_2$

b Nu = $OCOCH_3$ *e* NuH =

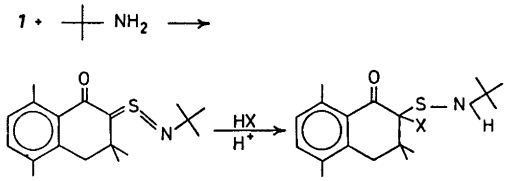
c Nu = CN *f* NuH = $(CH_3)_2NH$

Compound *1* gave the monosubstituted compounds *2a–f* when treated with molar amounts of the anions (*2a–c*) or with two equivalents of the amines (2d–f). Under these conditions no disubstitution was observed. The stability of the anilides was unexpected, partly on account of observations on similar compounds,[1] partly because of the reported ability of the amides from primary amines to eliminate HCl and give the colored thione *S*-imides.[1] The transient deep red color[5] observed when the anilides came in contact with, *e.g.*, aqueous sodium hydroxide might be due to this reaction. The trimethylanilide *2e* was prepared in a vain attempt to characterize the corresponding sterically hindered but apparently labile thione imide.

In contrast, the *tert*-butyl substituted thione imide *3* was stable.[1] The structure may be formulated as a carbonyl stabilized ylide:

$$\overline{O}-C=C-\overset{+}{S}=N- \longleftrightarrow O=C-C=S=N-$$

but the reactions of the imide with methanol (acid catalyzed) and with acetic acid to give the α-methoxy- and α-acetoxysulfenylamides *4a* and *4b*, respectively, bear witness to the thion character of *3*. Crystallographic data on *3* show the O–C–C–S–N system to be planar and form a CSN angle of 110° with the nitrogen *trans* to oxygen. The C–S and S–N bond

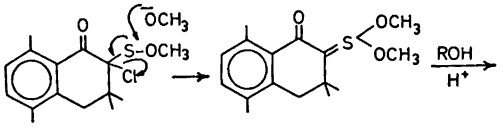


3 *a* X = OCH_3 *4*

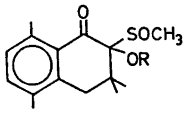
b X = $OCOCH_3$

lengths are 1.65 and 1.56 Å, respectively. Details will be published elsewhere.[6]

The reaction of the α-chlorosulfenyl chloride *1* (or the sulfenic ester *2a*) with methanolic sodium methoxide in excess did not result in direct substitution of chlorine at the carbon atom. Rather, the methoxide attacked the dicoordinate sulfur in a conjugate, S_N2'-like manner to give the tricoordinate "sulfine ketal" *5*. As in the case of the thione imide *3*, the thione dimethoxide *5* may be formulated as an α-oxo ylide. Compounds exhibiting the structural feature of an *S*-dialkoxylated thione as in *5* have been described, but apparently in all cases the sulfur and the two oxygens form part of fused and aromatically resonance stabilized ring systems.[7–10] Compound *5* lacks this stabilizing effect, and slight decomposition takes place even at −19 °C *in vacuo*.



5



6a R = CH_3
6b R = CD_3

A solution of the *S,S*-dimethoxide in carbon tetrachloride rearranges to the *S,C*-dimethoxide *6a* in the presence of *p*-toluenesulfonic acid. If methanol-d_4 is present during the rearrangement $^2H_3$-methoxy is incorporated to give substantial amounts of *6b*. This suggests a monomethoxysulfonium intermediate:

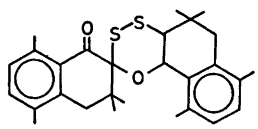
$$>C=\overset{+}{S}-OCH_3 + CD_3OD \rightarrow CD_3O-C-S-OCH_3 + D^+$$

again reflecting the thione character of the C–S bond as in the imide *3*.

Attempts to prepare ylides of the type >C=S=C<, in analogy with the preparation of thione imide *3* described above, by reacting sulfenyl chloride *1* with carbon nucleophiles such as the anion of dipivaloylmethane failed.[11] The attack apparently took place at the *S*-chlorine of *1* to give an α-oxothioketone, which subsequently dimerized to *7*:

$$Cl-C-S-Cl + \equiv\overline{C} \rightarrow \overline{Cl} + \equiv CCl + >C=S \rightarrow 7$$

A similar dimerization has been published.[12] A more recent and detailed work on dimerization of heterocyclic α-oxothioketones including theoretical considerations shows another (4+2)-cycloaddition type of this species. Under extrusion of sulfur 1,3-oxathiols are formed.[13]



7

The structures proposed for the isolated compounds are in accordance with the ^{13}C NMR, ^1H NMR, and mass spectral data given in Experimental. However, spectral evidence as to which of the two chlorine atoms has been substituted is not conclusive. Thus, the loss of HSCl in the MS of the anilides *2e* and *2f* might even suggest substitution of Cl at C2 and not at S. This is not reasonable, however, considering the high reactivity of sulfenyl chlorides. The mass spectral decomposition pattern of the anilides may be rationalized by assuming loss of HCl to give the thione imide (*m/e* 323 in *2d*) and subsequent loss of S. The latter reaction must involve a rearrangement, and evidence is furnished from the MS of the *N-tert*-butyl thione imide *3* which exhibits loss of SH. A thiaziridine intermediate may explain the latter reaction.[1,2,14]

EXPERIMENTAL

Mass spectra were recorded on a Perkin-Elmer 270 instrument; IP 70 eV. ^1H NMR were recorded on a Varian 360 instrument at 60 MHz, and ^{13}C NMR on a Bruker WH-90 at 22.63 MHz with broad band noise ^1H-decoupling. Long relaxation times are indicated by a superscript a and the concentrations were *ca.* 20 % in deuteriochloroform.

2-Chloro-3,4-dihydro-2-methoxysulfenyl-3,3,5,-8-tetramethyl-1-(2H)-naphthalenone 2a. The sulfenyl chloride (*1*)[4] (606 mg) was suspended in ether (6 ml) and sodium methoxide in methanol (2 mmol in 2 ml) was added with stirring. After 2 min at 22 °C the reaction mixture was poured

onto ice, the aqueous phase extracted with ether, dried (K_2CO_3) and concentrated *in vacuo* to give colorless crystals. Yield 573 mg, 96 %, m.p. 58 – 61 °C. Recrystallization from hexane gave m.p. 64 – 65 °C. Anal. $C_{15}H_{19}ClO_2S$: C, H, Cl, S. MS [*m/e* (% rel. int.)]: 298 (33, M), 262 (16, M – HCl), 235 (44, M – $SOCH_3$), 231 (42, M – HCl – OCH_3), 199 (54), 177 (67), 146 (100). ^{1}H NMR: δ 1.18 and 1.35 (aliphatic methyl), 2.25 and 2.61 (aromatic methyl), 2.92 (broadened s, CH_2), 3.68 (methoxy), 7.04 and 7.18 (aromatic proton, *J* 8.0 Hz). ^{13}C NMR: δ 188.5[a] (C1), 98,2 (C2), 42.6[a] (C3), 41.6 (C4), 24.8 and 26.5 (aliphatic methyl), 19.4 and 22.9 (aromatic methyl), 129.0[a], 130.8, 133.9[a], 134.5, 138.9[a], 140.0[a] (aromatic carbon), 68.0 (methoxy).

2-Acetoxysulfenyl-2-chloro-3,4-dihydro-3,3,5,8-tetramethyl-1(2H)-naphthalenone 2b. The sulfenyl chloride (*1*, 303 mg) in chloroform (1 ml) and sodium acetate (1.15 mmol in 4 ml of methanol) was stirred for 2 min. Addition of CCl_4 and work-up as above gave the acetate *2b*. Yield 240 mg (73 %), m.p. 67 – 71 °C. Recrystallization from a mixture of hexane (1 ml) and toluene (0.2 ml) gave colorless crystals, m.p. 72 – 74 °C. Anal. $C_{16}H_{19}ClO_3S$: C, H, Cl, S. MS [*m/e* (% rel. int.)]: 326 (0.4, M), 286 (13), 284 (35, [M – $H_2C=C=O$]), 238 (30), 236 (92), 221 (100), 146 (38).

^{1}H NMR: δ 1.23, 1.45 (aliphatic methyl), 2.03 (acetoxy), 2.23, 2.56 (aromatic methyl), 2.93 (methylene), 7.08, 7.25 (2H, *J* 7.8 Hz). ^{13}C NMR: δ 188.4[a] (C1), 94.7[a] (C2), 42.7[a] (C3), 41.4 (C4), 25.0, 26.3 (aliphatic methyl), 19.4, 22.7 (aromatic methyl), 20.1, 168.9[a] (acetate), 128.4[a], 130.4, 133.9[a], 137.7, 138.8[a], 140.3[a] (aromatic).

2-Chloro-3,4-dihydro-3,3,5,8-tetramethyl-2-thiocyanato-1-(2H)-naphthalenone 2c. To a solution of the sulfenyl chloride (*1*, 606 mg) in chloroform (2 ml, 23 °C) was rapidly added first ethanol (8 ml) and then a solution of KCN (240 mg) in ethanol (2 ml) and water (2 ml). The mixture was stirred for 1 min, CCl_4 (10 ml) was added. Work-up as above and crystallization from hexane (5 ml) gave colorless crystals. Yield 434 mg (74 %), m.p. 82 – 84 °C. Anal. $C_{15}H_{16}ClNOS$: C, H, Cl, N, S. MS [*m/e* (% rel. int.)]: 293 (2, M), 235 (96, M – NCS), 199 (21), 146 (100). ^{1}H NMR: δ 1.30, 1.45 (aliphatic methyl), 2.25, 2.55 (aromatic methyl), 2.75, 3.07 (methylene, *J* 18.8 Hz), 7.05, 7.24 (2H, *J* 8 Hz). ^{13}C NMR: δ 187.6[a] (C1), 90.0[a] (C2), 44.0[a] (C3), 40.8 (C4), 24.4, 25.1 (aliphatic methyl), 19.3, 22.3 (aromatic methyl), 126.5[a], 130.6, 134.3[a], 135.6, 138.4[a], 140.7[a] (aromatic), 109.7[a] (thiocyanato).

Sulfenamides (2d, e, *and* f). A solution of the sulfenyl chloride (*1*, 2 mmol) and the amine (4.4 mmol) in ethanol (10 ml) was stirred for 3 min. CCl_4 and ice was added. Work-up as above gave the amides.

Anilide 2d, yield 611 mg (85 %), m.p. 115 – 116 °C. Recrystallization from hexane (5 ml) and toluene (1 ml), m.p. 115 – 116 °C. Anal. $C_{20}H_{22}ClNOS$: C, H, Cl, N, S. MS [*m/e* (% rel. int.)]: 359 (18, M), 323 (5, M – HCl), 291 (100, M – S – HCl). 248 (43). ^{1}H NMR: δ 1.10, 1.47 (aliphatic methyl), 2.06, 2.23 (aromatic methyl), 2.84, 3.16 (methylene, *J* 18 Hz), 4.90 (NH), 6.60 – 7.35 (7 H, m). ^{13}C NMR: δ 188.0[a] (C1), 97.3[a] (C2), 41.8[a] (C3), 40.9 (C4), 24.9, 26.1 (aliphatic methyl), 19.5, 22.8 (aromatic methyl), 115.1, 120.5, 128.7, 130.3, 133.6[a], 134.3, 138.4[a], 140.9[a], 145.8[a] (aromatic).

Trimethylanilide 2e, yield 245 mg from hexane (6 ml) (61 %), m.p. 135 – 137 °C. Recrystallization and treatment with carbon gave m.p. 138 – 140 °C. Anal. $C_{23}H_{28}ClNOS$: C, H, Cl, N. S. MS [*m/e* (% rel. int.)]: 401 (3, M), 365 (3, M – HCl), 333 (59, M – HCl – S), 290 (55), 146 (100). ^{1}H NMR: δ 1.04, 1.42 (aliphatic methyl), 1.70, 2.15, 2.20 (aromatic methyl), 2.06 (aromatic methyl, *ortho* to NH), 2.75, 3.04 (methylene, *J* 17 Hz), 4.73 (NH), 6.60 (mesityl), 6.87, 7.20 (2H, *J* 8 Hz). ^{13}C NMR: δ 188.6[a] (C1), 97.1[a] (C2), 41.6[a] (C3), 41.0 (C4), 24.7, 25.9 (aliphatic methyl), 19.3, 21.7, 20.4 (aromatic methyl), 18.9 (aromatic methyl, *ortho* to NH), 128.3[a], 129.3, 129.6, 129.9, 132.1[a], 133.0[a], 133.8, 138.0[a], 138.8[a], 140.7[a] (aromatic).

Dimethylamide 2f was prepared as above from aqueous dimethylamine (1 ml 40 %). The reaction time was *ca.* 30 s. Yield 507 mg (81 %), m.p. 105 – 107 °C. Recrystallization from light petroleum (4 ml) gave m.p. 106 – 107 °C. Anal. $C_{16}H_{22}ClNOS$: C, H, Cl, N. S. MS [*m/e* (% rel. int.)]: 311 (37, M), 276 (1, M – Cl), 268 (2), 235 [52, M – $SN(CH_3)_2$],[1] 199 (100). ^{1}H NMR: δ 1.05, 1.39 (aliphatic methyl), 2.23, 2.60 (aromatic methyl), 2.66 (dimethylamino), 2.76, 3.04 (methylene, *J* 8 Hz), 7.03, 7.19 (aromatic, *J* 17.6 Hz). ^{13}C NMR: δ 189.9[a] (C1), 98.9[a] (C2), 41.9[a] (C3), 41.2 (C4), 24.5, 26.1 (aliphatic methyl), 19.5, 23.1 (aromatic methyl), 129.3[a], 130.3, 133.8[a], 134.0, 138.7[a], 139.5[a] (aromatic), 49.1 (dimethylamino).

No homogeneous compounds were isolated by reaction of *1* with 1-butylamine or with ammonia.[1]

3,4-Dihydro-3,3,5,8-tetramethyl-2-thioxo-1(2H)-naphthalenone, S-tert-butylimide 3. The sulfenyl chloride (*1*, 606 mg) dissolved in chloroform (2 ml) and ethanol (10 ml) was cooled to 5 °C and *tert*-butylamine (0.8 ml) was added rapidly with stirring. The reaction time was 1 min. Work-up as above and crystallization from light petroleum (12 ml) at – 15 °C gave orange-crystals of *3*. Yield 442 mg (73 %), m.p. 119 – 122 °C. Recrystallizations from hexane gave m.p. 121 – 123 °C (dec.). Anal. $C_{18}H_{25}NOS$: C, H, N, S. MS [*m/e* (% rel. int.)]: 303 (11, M), 270 (8, M – SH), 247 (10, M – C_4H_8), 231 (13), 230 (14), 201 (100), 199 (20). ^{1}H NMR: δ 1.40 (*tert*-butyl), 1.50 (aliphatic methyl), 2.28, 2.53 (aromatic methyl), 2.70 (methylene), 6.98, 7.14 (aromatic, *J* 8 Hz). ^{13}C NMR: δ 187.2[a] (C1), 152.4[a] (C2), 40.5[a] (C3), 41.3 (C4), 23.7 (aliphatic methyl), 19.2, 22.6 (aromatic methyl), 31.5, 62.5[a] (*tert*-butyl), 129.7, 131.4[a] 132.6[a], 133.1, 136.4[a], 139.0[a] (aromatic).

2-tert-Butylamidosulfenyl-3,4-dihydro-2-methoxy-3,3,5,8-tetramethyl-1(2H)-naphthalenone 4a. A solution of ylide *3* (303 mg) in chloroform (1 ml) was allowed to react with methanol (5 ml) and *p*-toluenesulfonic acid (1.5 mg) at 22 °C for 30 s. Addition of carbon tetrachloride and work-up as above gave colorless crystals. Yield 334 mg, m.p. 97–103 °C. Recrystallizations from hexane gave m.p. 102–104 °C. Anal. $C_{19}H_{29}NO_2S$: C, H, N, S. MS [*m/e* (% rel. int.)]: 335 (2, M), 231 (41, M–$SNHC_4H_9$), 199 (100). ^{1}H NMR: δ 0.72 (*tert*-butyl), 1.03, 1.16 (aliphatic methyl), 2.23, 2.56 (aromatic methyl), 2.62, 3.32 (methylene, *J* 17.4 Hz), 3.78 (methoxy), 6.95, 7.12 (aromatic, *J* 7.6 Hz). ^{13}C NMR: δ 196.9[a] (C1), 100.4[a] (C2), 41.6[a] (C3), 42.7 (C4), 23.8, 24.7 (aliphatic methyl), 19.4, 22.3 (aromatic methyl), 28.7, 54.3[a] (*tert*-butyl), 54.8 (methoxy), 129.6, 131.8[a], 133.1[a], 133.7, 138.2[a], 139.4[a] (aromatic).

The same product is obtained by the slow addition of *tert*-butylamine to the sulfenyl chloride (*1*) in methanol.

2-tert-Butylamidosulfenyl-3,4-dihydro-2-acetoxy-3,3,5,8-tetramethyl-1(2H)-naphthalenone (4b) was prepared from the ylide *3* (303 mg) dissolved in chloroform (1 ml) and carbon tetrachloride (4 ml) by addition of acetic acid (0.2 ml) and immediate work-up as above. Crystallization from hexane (2 ml) gave colorless crystals. Yield 281 mg (77 %), m.p. 88–91 °C. Recrystallizations from hexane gave m.p. 92–94 °C (dec.). Anal. $C_{20}H_{29}NO_3S$: C, H, N, S. MS [*m/e* (% rel. int.)]: 363 (4, M), 303 (1, M–HOAc), 259 (6, M–$SNHC_4H_9$), 217 (100). ^{1}H NMR: δ 0.58 (*tert*-butyl), 1.07, 1.23 (aliphatic methyl), 2.23, 2.52 (aromatic methyl), 2.23 (acetyl; if the acetate is dissolved in carbon tetrachloride, the resonance is shifted 0.04 ppm upfield from the high field aromatic methyl group at δ 2.22), 2.71, 3.41 (methylene, *J* 17.4 Hz), 3.35 (NH, exchangeable), 6.99, 7.15 (aromatic, *J* 8.0 Hz). ^{13}C NMR: δ 192.0[a] (C1), 101.2[a] (C2), 41.4[a] (C3), 42.1 (C4), 23.9, 24.5 (aliphatic methyl), 19.3, 22.1 (aromatic methyl), 28.6, 52.9[a] (*tert*-butyl), 21.8, 169.8[a] (acetyl), 129.5, 131.3[a], 133.2, 133.7[a], 138.5[a], 138.8[a] (aromatic).

3,4-Dihydro-3,3,5,8-tetramethyl-2-thioxo-1(2H)-naphthalenone, S-*dimethoxide*, 5. The sulfenyl chloride (*1*, 606 mg) was stirred with methanolic sodium methoxide (from 400 mg of sodium and 20 ml of methanol) at 23 °C for 2 min. Addition of ice and work-up as above gave colorless crystals, which were washed with hexane at 0 °C. Yield 422 mg (71 %), m.p. 65–67 °C, gas evolution and smell of formaldehyde. Recrystallization from hexane (0.5 ml) and CCl_4 (0.5 ml) gave m.p. 66–68 °C, not stable at room temperature in the atmosphere, but only slightly decomposed (m.p. 60–68 °C) when kept for 9 months at –19 °C *in vacuo*. Anal. $C_{16}H_{22}O_3S$: C, H, S. MS [*m/e* (% rel. int.)]: 294 (15 M), 279 (25, M–CH_3), 264 (25, M–CH_3O), 231 (83), 201 (100), 146 (55). ^{1}H NMR: δ 1.29 (2 aliphatic methyl groups), 2.24, 2.60 (aromatic methyl), 2.66 (methylene), 3.71 (2 methoxy), 6.95, 7.07 (aromatic, *J* 8 Hz). ^{13}C NMR [CCl_4, 10 % C_6D_6]: δ 187.6[a] (C1), 93.4[a] (C2), 36.8[a] (C3), 43.2 (C4), 28,7 (aliphatic methyl), 19.4, 22.2 (aromatic methyl), 129.4, 131.6, 131.6[a], 132.8[a], 136.1[a], 138.1[a] (aromatic), 54.9 (methoxy, *J* 147 Hz).

2-Methoxy-2-methoxysulfenyl-3,3,5,8-tetramethyl-3,4-dihydro-1(2H)-naphthalenone 6a. A solution of the *S*-dimethoxy compound *5* (67 mg) in CCl_4 (0.4 ml) and methanol (0.1 ml) was mixed with *p*-toluenesulfonic acid (0.03 ml of a 0.1 M solution in $HCCl_3$). Addition of ice and work-up as above gave a yellow oil. Anal. $C_{16}H_{22}O_3S$: C, H, S. MS [*m/e* (% rel. int.)]: 294 (0.4, M), 264 (0.5, M–CH_2O), 231 (57, M–$SOCH_3$), 199 (100). ^{1}H NMR: δ 1.14, 1.20 (aliphatic methyl), 2.28, 2.53 (aromatic methyl), 2.28 (methylene), 3.36, 3.66 (methoxy), 7.05, 7.17 (aromatic, *J* 7.6 Hz). ^{13}C NMR [CCl_4, *ca.* 10 % C_6D_6]: δ 198.8[a] (C1), 102.7[a] (C2), 42.2[a] (C3), 42.0 (C4), 24.0, 24.8 (aliphatic methyl), 19.22, 22.0 (aromatic methyl), 129.5, 130.1[a], 133.5[a], 133.7, 140.0[a] (aromatic), 54.3 (*C*-methoxy), 65.4 (*S*-methoxy).

The corresponding 2-trideuteriomethoxy compound *6b* was prepared by using CD_3OD instead of methanol in the above experiment. MS [*m/e* (%rel. int.)]: 297 (0.6, M), 265 (5, M–CD_3O), 234 (48, M–$SOCH_3$), 199 (100). ^{1}H NMR: as for *6a*; the resonance at δ 3.66 was *ca.* 10 % of that of *6a*. Also ^{13}C NMR showed the same resonance for the two compounds. The peak at δ 54.4 of *6b* had only *ca.* 20 % of the intensity of that of *6a* and was broadened.

Dimer of 3,3,5,8-tetramethyl-2-thiono-1-(2H)-naphthalenone 7. A solution of dipivaloylmethane (180 mg) and sodium ethoxide (23 mg of sodium) in ethanol (3 ml) was added to the α-chlorosulfenyl chloride (*1*, 303 mg) in chloroform (1 ml) at room temperature. Sodium ethoxide (1 ml 1 M) was added dropwise. Work-up as above gave yellow crystals. Yield 183 mg (79 %), m.p. 180–184 °C. Two recrystallizations from toluene (1 ml) gave m.p. 189–190 °C. $C_{28}H_{32}O_2S_2$: C, H, S. MS [*m/e* (% rel. int.)]: 464 (0.3, M of dimer), 232 (100, M of monomer), 217 (50, monomer–CH_3), 199 (40), 146 (97). ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.95, 1.21, 1.25, 1.48 (aliphatic methyl), 2.23, 2.24, 2.55, 2.58 (aromatic methyl), 2.59, 2.78 (methylene, *J* 15.0 Hz), 2.83, 3.11 (methylene, *J* 17.7 Hz), 7.07 (2 H, s), 7.15, 7.30 (2 H, *J* 8 Hz). ^{13}C NMR δ 19.3, 19.7, 21.1, 23.7, 24.5, 25.7, 26.2 (methyl), 36.7[a], 41.4[a] (tertiary carbon), 40.9, 42.5 (methylene), 128.9, 129.7, 130.7[a], 132.2[a], 133.4, 134.9[a], 138.0[a], 139.2[a] (aromatic), 95.8[a] (O–C–S), 115.1[a], 148.0[a] (vinylic), 188.4[a] (C=O).

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Conformational Analysis. Part XVII. A Simple Application of the Karplus Equation to Study the Preferred Conformations of the Ethyl Group in Some Alkylsubstituted 4-Ethyl-1,3-oxathiolanes

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The ^{1}H NMR spectra of 4-ethyl-1,3-oxathiolane and several alkylsubstituted derivatives were recorded and analysed using an iterative LAME program. From the Karplus equation, $J = J_0 \cos^2 \phi$, and the observed coupling constants (J_{46} and $J_{46'}$, where 6 and 6′ are the methylene protons of the ethyl group) the J_0 values were determined and used to estimate the relative amounts of the *gauche*(S), *gauche*(C5) and *trans*(H4) ethyl rotamers. In 5 methyl derivatives the *gauche*(C5) rotamer is practically nonexistent due to the "trans-annular" Me – Me interaction. In general the *gauche*(S) form is 1.7 ± 0.2 kJ mol^{-1} more stable than the *gauche* (C5) form and 3.0 ± 0.4 kJ mol^{-1} more stable than the *trans*(H4) rotamer.

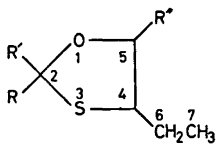
Despite the fact that the conformations of alkylsubstituted saturated heterocycles have been recently studied rather intensively [1] the conformational behaviour of alkyl groups has received much less attention. The ethyl rotation has been studied in the case of some alkylcyclohexanes,[2] 1,3-dioxanes,[3,4] 1,3-dioxolanes [3] and 1,3-dithianes.[5] In the heterocyclic compounds the ethyl group has always been at position 2.

In the present work we will describe some results dealing with the rotameric preferences of the ethyl group in 4-ethyl-1,3-oxathiolane and its alkyl derivatives.

RESULTS AND DISCUSSION

Analysis of the ^{1}H NMR spectra

Configurational assignments (Table 1) were based on the magnitudes of proton-proton coupling constants and chemical shifts for the ring protons which were compared with those of correspondingly substituted 4-methyl-1,3-oxathiolanes.[6–8]



	R	R′	R″
1	H	H	H
2	Me	H	H
3	Me	Me	H
4	Bu^t	Me	H
5	Me	Bu^t	H
6	H	H	Me
7	H	Me	Me
8	Me	Me	Me

The studied spectra fall into two groups of which the one forms an $ABCDEF_3$ system (*1* – *5*) and the other an M_3ABCDE_3 system (*6* – *8*).

The spectrum of 4-ethyl-1,3-oxathiolane (*1*) and that of *trans*-4-ethyl-5-methyl-1,3-oxathiolane (*6*) were completely analysed at 60 MHz and the former also at 300 MHz. The chemical shifts of H6 and H6′ for compounds *2* – *5* and *7* – *8* were taken from spectra of the above compounds due to the overlap of their signals with those of the alkyl groups at position 2.

The analyses were carried out using an iterative LAME program [9] on a DEC-10 computer.

Rotameric behaviour of the 4-ethyl group

The 300 MHz spectrum of 4-ethyl-1,3-oxathiolane (*1*) gives clearly distinct methylene proton signals for the ethyl group in good

Table 1. Chemical shifts (δ) of different protons in ppm from TMS for the studied compounds in CCl_4.

1,3-Oxathiolane	H(2)	H(4)	H(5)	H(5′)	H(6)	H(6′)	Me(7)	Me(5)	R(2)
1 4-Et- (300 MHz)	4.85 4.76	3.34	3.88	3.65	1.67	1.55	0.99		
4-Et- (60 MHz)	4.87 4.78	3.36	3.90	3.67	1.67	1.55	0.99		
2 *cis*-2-Me-4-Et-	5.11	3.35	3.98	3.73			0.95		1.54
3 2,2-Me_2-4-Et-		3.40	4.06	3.78			0.96		1.59,1.55
4 *r*-2-Bu^t-2-Me-*cis*-4-Et-		3.28	4.08	3.91					1.53,1.04[a]
5 *r*-2-Bu^t-2-Me-*trans*-4-Et-		3.31	4.24	3.59					1.55,1.02[a]
6 *trans*-5-Me-4-Et-	4.90 4.82	2.95	3.62		1.78	1.49	1.05	1.31	
7 *r*-2,*c*-5-Me_2-*t*-4-Et-	5.21	3.03	3.54				1.01	1.33	1.52
8 2,2,5-Me_3-*trans*-4-Et-		3.07	3.83				0.98	1.29	1.59

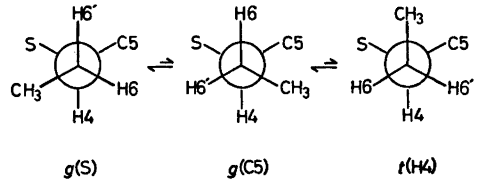
[a] δBu^t.

Table 2. Coupling constants (J/Hz) for the studied compounds.

1,3-Oxathiolane	J_{45}	$J_{45'}$	J_{46}	$J_{46'}$	$J_{55'}$	$J_{66'}$	J_{7Me}	J_{2R}
1 4-Et- (300 MHz) [a]	5.72	4.82	5.90	8.33	−9.03	−13.66	7.35	5.38
4-Et- (60 MHz) [b]	5.64	4.82	5.88	8.29	−8.96	−13.42	7.30	5.13
2 *cis*-2-Me-4-Et- [c]	5.35	2.18	5.93	8.08	−9.15		7.03	5.13
3 2,2-Me_2-4-Et- [c]	5.31	5.20	5.93	8.17	−9.14		7.16	
4 *r*-2-Bu^t-2-Me-*cis*-4-Et- [c]	5.27	3.13	6.14	8.66	−9.37		7.20	
5 *r*-2-Bu^t-2-Me-*trans*-4-Et- [c]	5.54	10.01	5.78	7.63	−9.21		7.45	
6 *trans*-5-Me-4-Et- [d]		7.28	4.13	9.50	6.02 [f]	−13.12	7.26	5.45
7 *r*-2,*c*-5-Me_2-*t*-4-Et- [e]		8.58	3.62	9.19	5.99 [f]		7.08	5.89
8 2,2,5-Me_3-*trans*-4-Et- [e]		8.94	3.52	9.51	6.01 [f]		7.29	

Standard deviation better than [a] 0.027, [b] 0.050, [c] 0.140, [d] 0.063 and [e] 0.059. [f] J_{5Me}.

agreement with the postulation that the anisochronism of these protons is enhanced by the restricted rotation of this group. The same situation prevails, of course, in the other compounds under study. We have pointed out earlier [7] that, *e.g.*, *cis*-2-methyl-4-ethyl-1,3-oxathiolane (*2*) exists predominantly in a C5 envelope (*cf.* the value of $J_{45'}$) where the ethyl group is axial. In this orientation the methyl-in-the-ring rotamer (*trans* in Scheme 1) is so crowded that it may be excluded on steric grounds. Compounds *1* and *3*–*5* include 31, 36, 11 and 93 % of the ring conformation where the ethyl group is equatorial as evident from the values of $J_{45'}$ (model values J_{ee} 2.2 and J_{aa} 10.6 Hz).[6,7] Hence even in compounds *1*, *3* and *4* the contribution of the *trans*(H4) rotamer (Scheme 1) is rather small as shown below.



Scheme 1.

From the two *gauche* rotamers, *gauche*(S) – the methyl end on the sulfur side – and *gauche*(C5) – the methyl end on the C5 side, both include one *trans* coupling [$J_{46'}(t)$ and $J_{46}(t)$, respectively] and one gauche coupling [$J_{46}(g)$ and $J_{46'}(g)$, respectively] whereas the *trans*(H4) form has two *gauche* type couplings.

To solve the relative amounts of the *gauche* rotamers one has to know the values of J_g and

J_t, separately. That is why we adopted the Karplus equation [10] in the form

$$J = J_0 \cos^2 \phi \quad (1)$$

where the magnitude of the J_0 value depends on the nature of the H – C – C – H fragment [11,12] and ϕ is the dihedral angle between the vicinal protons. In the case of the *gauche*(S) rotamers of 4-ethyl-1,3-oxathiolane and its alkyl derivatives it is reasonable to suppose that the ethyl group has an almost ideal trigonal projection symmetry. In other words, for these rotamers:

$$J_t = J_0 \cos^2 180° = J_0 \quad (2)$$

$$J_g = J_0 \cos^2 60° = 1/4 J_0 \quad (3)$$

Several iterative data fittings led, however, to the conclusion that for *gauche*(C5) $\phi_t \sim 185°$ and $\phi_g \sim 65°$ and for *trans*(H4) $\phi_{46'} \sim 70°$ and $\phi_{46} \sim 50°$ (*cf.* Scheme 1). Accordingly, the vicinal *trans* coupling constants are clearly larger than the vicinal *gauche* coupling constants.[12–14]

On the other hand, the methyl-sulfur interaction in the *gauche*(S) rotamer (Scheme 1) should be about half of the conformational energy of an axial methyl group at C5 in the 1,3-dithiane ring for which values from 3.8 [15] to 4.9 [1] kJ mol^{-1} have been reported. Correspondingly, the methyl-methyl interaction [1] in the *gauche*(C5) rotamer should be roughly 3.4 – 3.8 kJ mol^{-1} and hence the free energy difference between the two *gauche* forms should favour the *gauche*(S) rotamer by 1.0 – 1.9 kJ mol^{-1}. Therefore $J_{46'}$ must be larger than J_{46} (Scheme 1). The preceding postulate is close to 1.7 ± 0.2 kJ mol^{-1}, observed for compounds *1* – *5* (Table 3). It is reasonable to suggest that the *trans*(H4) rotamer must be significantly less favoured although this arrangement certainly does not exclude the presence of the *trans*(H4) rotamer in all cases.[12,16] In the light of the above discussion the *gauche*(S) form is at least 3 – 4 kJ mol^{-1} more stable than the *trans*(H4) rotamer. Hence compounds *6* – *8* cannot contain more than about 20 – 25 % of the *trans*(H4) rotamer. Consequently, we may write

$$J_{46'} = x_{g(S)} J_0 + x_{g(C5)} J_0 \cos^2 65° + x_{t(H4)} J_0 \cos^2 70° \quad (4)$$

$$J_{46} = x_{g(S)} 1/4 J_0 + x_{g(C5)} J_0 \cos^2 185° + x_{t(H4)} J_0 \cos^2 50° \quad (5)$$

Since the *cis*-2-Me-4-Et-derivative *2* exists exclusively in a 4-axial C5 envelope [7] it does not contain the *trans*(H4) rotamer at all. Hence

$$\begin{aligned} J_{46'} &= 0.821 x_{g(S)} J_0 + 0.179 J_0 = 8.08 \\ J_{46} &= -0.742 x_{g(S)} J_0 + 0.992 J_0 = 5.93 \end{aligned} \quad (6)$$

which results in $x_{g(S)} = 0.64$, $x_{g(C5)} = 0.36$ and $J_0 = 11.5$ Hz. From the latter the values of the

Table 3. J_0 values (Hz), the relative amounts of ring conformations and ethyl rotamers, the values of $J_{46'}$ and J_{46} corrected for *gauche*(C5) ⇌ *gauche*(S) (compounds *1* – *5*) and *trans*(H4) ⇌ *gauche*(S) equilibria (compounds *6* – *8*), the values of the rotameric equilibrium constants K = *gauche*(S)/*gauche*(C5) (compounds *1* – *5*) and K = *gauche*(S)/*trans*(H4) (compounds *6* – *8*), and the respective free energy differences for the studied 4-Et-1,3-oxathiolanes at 306 K.

Compound	J_0	x_{4e}	x_{4a}	$x_{t(H4)}$	$J_{46'}$	J_{46}	$x_{g(S)}$	$x_{g(C5)}$ or $x_{t(H4)}$	K[d,e]
1	12.1 [a,b]	0.31	0.69	0.05	8.69	5.95	0.66	0.34	1.9
2	11.5 [a,b]	–	1.00	–	8.08	5.93	0.64	0.36	1.8
3	12.1 [a,b]	0.36	0.64	0.06	8.60	5.99	0.65	0.35	1.9
4	12.1 [a,b]	0.11	0.89	0.02	8.81	6.16	0.67	0.33	2.0
5	12.1 [a,b]	0.93	0.07	0.17	8.91	5.94	0.68	0.32	2.1
6	11.5,12.1 [a,b]	0.76[c]	0.24[c]	0.15	9.74	3.56	0.79	0.21	3.8
7	11.5,12.1 [a,b]	0.95[c]	0.05[c]	0.26	9.21	3.50	0.73	0.27	2.7
8	12.1 [a,b]	1.00[c]	–	0.24	9.51	3.52	0.76	0.24	3.2

[a] For the *gauche*(S) rotamer $J_t = 12.1$ and $J_g = 3.0$ Hz, except *2* for which $J_t = 11.5$ and $J_g = 2.9$ Hz. [b] For the *gauche*(C5) rotamer $J_t = 11.4$ and $J_g = 2.05$ Hz in all cases. Correspondingly, for the *trans*(H4) rotamer $J_{46'} = 1.4$ and $J_{46} = 5.0$ Hz. [c] $x_{4e} = x_{4e5e}$ and $x_{4a} = x_{4a5a}$. [d] K = *gauche*(S)/*gauche*(C5) = 1.94 ± 0.15 and $-\Delta G^\ominus$/kJ mol^{-1} = 1.7 ± 0.2. [e] K = *gauche*(S)/*trans*(H4) = 3.2 ± 0.5 and $-\Delta G^\ominus$/kJ mol^{-1} = 3.0 ± 0.4.

different coupling constants are obtained (Scheme 1 and Tables 2 and 3).

Similarly, the 2,2,5-tri-Me-*trans*-4-Et-derivative *8* is exclusively in the 4e5e ring conformation [8] and hence due to the "trans-annular" methyl-methyl interaction does not include the *gauche*(C5) rotamer at all. Accordingly,

$$J_{46'} = 0.883x_{g(S)}J_0 + 0.117J_0$$
$$J_{46} = -0.163x_{g(S)}J_0 + 0.413J_0 \quad (7)$$

which results in $x_{g(S)} = 0.76$, $x_{g(C5)} = 0.24$ and $J_0 = 12.1$ Hz. From them the values of the different coupling constants are again obtained.

The small difference in the J_0 values for *2* and the other compounds -11.5 *vs.* $12.1-$ is easily understood since from compounds *1*–*5* only *2* has a biased ring conformation [7] and compounds *6*–*8* have an extra methyl substituent at C5.

From another study [8] we know, however, that the predominance of the diequatorial 4e5e conformation decreases when passing from *8* to *6*. Use of the equation

$$trans\text{-}4,5\text{: } J_{obs} = xJ_{4a5a} + (1-x)J_{4e5e} \quad (8)$$

where $J_{aa} = 8.9$ and $J_{ee} = 2.0$ Hz,[8] gives the mol fractions of the 4,5-diequatorial (x_e) and 4,5-diaxial (x_a) conformations of *6* and *7* (Table 3). In the diaxial ring conformations of *6* and *7* the amounts of the *gauche*(C5) and *gauche*(S) rotamers are roughly identical with those existing in compounds *1*–*5*. Using the results in Tables 2 and 3 and the postulate above the amounts of the *gauche*(S) and *trans*(H4) conformations in the 4e5e forms of *6* and *7* (Table 3) can be estimated. In all cases the free energy difference between the *gauche*(S) and *trans*(H4) rotamers of the 4e5e ring conformations is practically equal – *i.e.* 3.0 ± 0.4 kJ mol^{-1}.

The results for *2* and *8* were then employed to correct the rotamer equilibria of *1* and *3*–*5* for the contribution of the *trans*(H4) form. The corrected values of the $J_{46'}$ and J_{46} coupling constants were used to estimate the proportions of the *gauche* rotamers (Eqn. 6) and after one iterative cycle a good convergence was reached (Table 3).

Finally we would like to emphasize that our results clearly point out that a double-*gauche* interaction is not necessarily sufficient to exclude a rotameric contribution of this type.[12]

EXPERIMENTAL

4-Ethyl-1,3-oxathiolane and its alkyl derivatives were obtained as by-products in the preparation of some alkyl-substituted 1,3-oxathianes from mixtures of 1,3- and 1,2-mercaptoalcohols.[17,18]

The spectra were obtained for 15 % (v/v) solutions in CCl_4 on a Perkin-Elmer R-10 60 MHz NMR spectrometer at 306 K. For 4-ethyl-1,3-oxathiolane a 300 MHz spectrum was recorded at 293 K on a Varian HR-300 spectrometer at the State University of Gent, Belgium.

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Conformational Analysis. Part XLIII. ^{13}C Chemical Shifts and Coupling Constants as Proof of the Nonplanarity of the 2-Oxo-1,3-dioxolane Ring[1,2]

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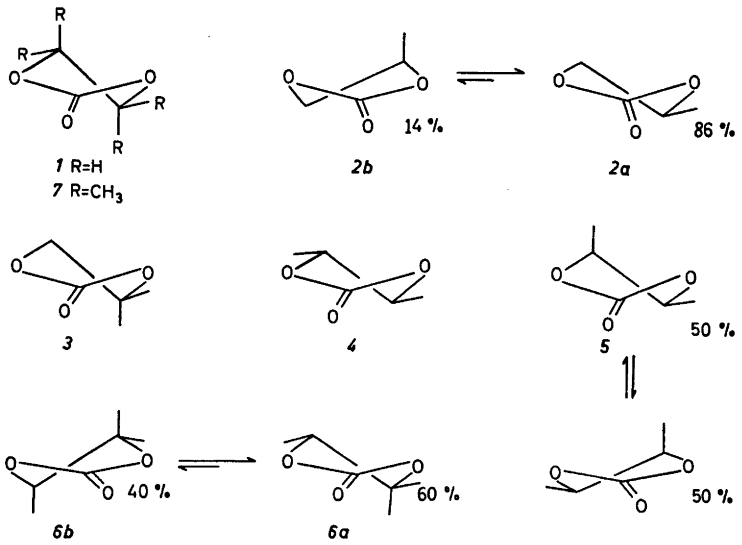
The ^{13}C chemical shifts and coupling constants for 2-oxo-1,3-dioxolane and its methyl-substituted derivatives are reported. Substituent effects on the chemical shifts are derived and their type and magnitude discussed with respect to the ring geometry and other conformational factors. The results are best explained with a half-chair conformation where the bond joining C4 and C5 bisects the CO_3 plane.

It is well documented [3–7] that for a series of compounds with similar conformations

$$\delta C(x) = \delta_p C(x) + \sum SE(x) \qquad (1)$$

where $\delta C(x)$ is the chemical shift of the xth carbon atom in any given derivative, $\delta_p C(x)$ the chemical shift of the same carbon atom in the parent compound and $\sum SE(x)$ the sum of the different substituent effects influencing it. Keeping this in mind ^{13}C chemical shifts may be helpful in removing the controversy still existing with respect to the ring conformation of 2-oxo-1,3-dioxolanes.[8–18]

Brown [8] pointed out by the X-ray method that the crystalline 2-oxo-1,3-dioxolane (*1*) is not planar but the line joining C4 to C5 makes



Scheme 1.

Table 1. ^{13}C chemical shifts (δ) for the 2-oxo-1,3-dioxolanes studied.

Compound	Substitution	C2	C4	C5	C(Me)
1	Nil	156.07	65.10	65.10	–
2	4-Me	155.22	73.75	70.82	19.36
3	4,4-Me_2	154.64	81.80	75.37	25.92
4	*trans*-4,5-Me_2	154.77	80.05	80.05	18.26
5	*cis*-4,5-Me_2	154.77	76.21	76.21	14.29
6	4,4,5-Me_3	154.18	84.21	81.48	25.66(4), 20.92(4), 14.49(5)
7	4,4,5,5-Me_4	153.92	86.09	86.09	22.35
8	1,3-Dioxolane [3]	95.23	64.81	64.81	–

an angle of 20° with the plane containing the CO_3 group. Angell [10] and Simon and Heintz [11] studied the same compound in the liquid state by IR and Raman spectroscopy and stated their results with a planar structure and C_{2v} symmetry. Finegold [12] assumed the 4-methyl-2-oxo-1,3-dioxolane ring (*2*) planar though he did not refer to earlier reports. Paasivirta [13,14] studied the ^1H NMR spectra of some chlorinated 2-oxo-1,3-dioxolanes and interpreted his results with a half-chair structure. Pethrick *et al.*[15] concluded from NMR, IR and ultrasonic absorption measurements that *1*, *2* and 4-chloromethyl-2-oxo-1,3-dioxolane have similar ring conformations though they do not mention any shape. Pethrick [15,16] also refers to a microwave measurement [17b] which is nonexistent since Arbusov studied [17a] dipole moments instead. Wang *et al.*,[18] however, studied the microwave spectrum of *1* in the gaseous state and their results were best explained with a nonplanar ring and C_2 symmetry.[3]

In the present work we determine the ^{13}C chemical shifts for 2-oxo-1,3-dioxolane (*1*) and its methyl-substituted derivatives *2* – *7* and from them the substituent effects on the ring carbon atoms. In some cases we also determine the values of the various coupling constants for further clarification of the actual shape of these molecules.

EXPERIMENTAL

2-Oxo-1,3-dioxolane (*1*) and its methyl derivatives *2* – *7* were prepared conventionally [19] and proved to be identical with authentic samples by IR, NMR and physical constants.[19–21]

The ^{13}C NMR spectra were recorded on a JEOL FX-60 FT NMR spectrometer for 14 – 15 % (w/w) $CDCl_3$ solutions at 298 K. The chemical shifts (δ) and coupling constants are given in Table 1.

Two sets of 6 to 8 simultaneous equations (eqn. 1) with unknown SE's were solved on a DEC-10 computer by the use of a multiple linear regression analysis.[22] The conformationally inhomogeneous compounds (*2*, *5* and *6*) were treated as being composed of appropriate mol fractions of the two conformers (Scheme 1). These mol fractions were obtained in an iterative manner to find out the best fit for the substituent effect correlations (Table 2).

Table 2. Substituent effects on the ^{13}C chemical shifts of methyl-substituted 2-oxo-1,3-dioxolanes.

Substitution	Substituent effect on the ring carbon atom C2	C4[a]
4e-Me	-0.88 ± 0.06	8.66 ± 0.01
4a-Me	-0.59 ± 0.09	8.66 ± 0.01
5e-Me	-0.88 ± 0.06	5.72 ± 0.01
5a-Me	-0.59 ± 0.09	5.72 ± 0.01
4,4-Me_2	–	-0.61 ± 0.01
5,5-Me_2	–	-1.18 ± 0.01
4e5e-Me_2	0.44 ± 0.12	0.57 ± 0.01
4e5a-Me_2	0.15 ± 0.09	-1.35 ± 0.04
4a5e-Me_2	0.15 ± 0.09	-5.20 ± 0.04
Standard deviation of fit in ppm:	0.07	0.01
Chemical shift range:	2.15	20.99

[a] Due to the symmetry of the 2-oxo-1,3-dioxolane ring substituent effects on C5 are equal to those on C4 if 4 and 5 are interchanged in the substitution column.

$$
\begin{array}{lllllll}
8.65 = & SE(4) & & & & & \\
5.72 = & & SE(5) & & & & \\
16.70 = & 2SE(4) & & +SE(4,4) & & & \\
10.27 = & & 2SE(5) & & +SE(5,5) & & \\
14.95 = & SE(4) & +\ SE(5) & & & +\ SE(4e5e) & \\
11.11 = & SE(4) & +\ SE(5) & & & & +0.5SE(4e5a)\ +0.5SE(4a5e) \\
19.11 = & 2SE(4) & +\ SE(5) & +SE(4,4) & & +0.6SE(4e5e) & +0.4SE(4e5a)\ +0.6SE(4a5e) \\
16.38 = & SE(4) & +2SE(5) & & +SE(5,5) & +0.6SE(4e5e) & +0.6SE(4e5a)\ +0.4SE(4a5e) \\
20.99 = & 2SE(4) & +2SE(5) & +SE(4,4) & +SE(5,5) & +\ SE(4e5e) & +\ SE(4e5a)\ +\ SE(4a5e)
\end{array}
$$

Eqns. 2.

RESULTS AND DISCUSSION

Substituent effects on C4 and C5

If the 2-oxo-1,3-dioxolane ring has the half-chair conformation [8] in solution the methyl substituents should have more or less equatorial and/or axial character. It was relatively easy to determine that the best fit for the set of equations (2) was obtained when SE(4e) = SE(4a), SE(5e) = SE(5a) but SE(4e5e) + SE(4a5a) = 0, SE(4e5a) + SE(4a5e).

Due to symmetry *cis*-4,5-dimethyl-2-oxo-1,3-dioxolane (*5*) includes equal amounts of the 4e5a and 4a5e conformations (Scheme 1). The $\Delta\delta$ values of 4,4,5-trimethyl derivative (*6*) in comparison with those of *5* and *7* led to the conclusion that it is not conformationally homogeneous but includes both 4e4a5e (*6a*) and 4a4e5a (*6b*) forms. By an iterative method it was found that the best fit for eqn. 2 was obtained when *6* consisted of 60 % of 4e4a5e (*6a*) form. At this stage one may argue that the *trans*-4,5-dimethyl derivative (*4*) also consists somewhat of the diaxial conformation. However, this compound can easily if compared with *6*, minimize the 4e5e *gauche* interaction without existing appreciably in the 4a5a conformation. At any rate the amount of the diaxial form is clearly less than 10 %.

Eqns. 2 which assume that the 2-oxo-1,3-dioxolane ring has the half-chair conformation led to an excellent fit between the observed and calculated chemical shifts. This is, of course, partly due to the limited number of the methyl-substituted derivatives when compared with the amount of different substituent effects (Table 2). However, we consider our results confirmatory to the report of Brown [8] who presented a half-chair structure for the 2-oxo-1,3-dioxolane ring in the crystalline state.

From the conformational equilibrium of *6* we can further estimate the conformational energy of the axial methyl group if we assume that the *gauche* interactions between the methyl groups have the normal value, 3.6 kJ mol^{-1}. At 298 K $-\Delta G^{\circ}$ (4a-Me) = $3.6 + RT \ln K$ ($K = 1.5$) = 4.6 ± 0.5 kJ mol^{-1}. From this result we estimate that *2* is actually an 86:14 mixture of the equatorial (*2a*) and axial (*2b*) forms which result we need when deriving the substituent effects on C2.

$$
\begin{array}{lllll}
-0.85 = & 0.86SE(4e) & +0.14SE(4a) & & \\
-1.43 = & SE(4e) & +\ SE(4a) & & \\
-1.30 = & 2.00SE(4e) & & +\ SE(4e5e) & \\
-1.30 = & SE(4e) & +\ SE(4a) & & +\ SE(4e5a) \\
-1.89 = & 1.60SE(4e) & +1.40SE(4a) & +0.60SE(4e5e) & +\ SE(4e5a) \\
-2.15 = & 2.00SE(4e) & +2.00SE(4a) & +\ SE(4e5e) & +2SE(4e5a)
\end{array}
$$

Eqns. 3.

Substituent effects on C2

Substitution of the 2-oxo-1,3-dioxolane ring does not have much influence on the chemical shift of C2. However, these influences are again clearly dependent on the spatial arrangement of the C4 – C5 fragment of the molecule (*cf.* Tables 1 and 2). After some trials eqns. 3 were found to give an excellent fit between the observed and calculated chemical shifts of C2.

This result again supports the view that the predominant conformation of the 2-oxo-1,3-dioxolane ring in solution is a half-chair form similar to that in the crystalline state. The chemical shifts of C2 are normal for carbonate carbons whereas that of C4 and C5 in *1* is only slightly different from the value determined [3] for the same carbon atoms in the 1,3-dioxolane ring (Table 1) in agreement with the postulate that both rings are nonplanar and resemble

Table 3. One-bond, geminal and vicinal coupling constants.

Compound	J_{CH}/Hz	J_{CCH}/Hz	J_{COCH}/Hz
1	156.2		
2	156.2 (4)	3.9	2.9
	154.3 (5)	5.5	
	127.9 (CH_3)	4.4	
4	153.3 (4,5) [a]	4.4	2.4
	127.9 (CH_3)	3.9	
5	154.3 (4,5) [b]	4.4	2.4
	127.9 (CH_3)	1.95	

[a] J_{CHax}. [b] $0.5J_{CHax}+0.5J_{CHeq}$. Thus $J_{CHeq}=155.3$ Hz.

each other in the C(4)–C(5) range. This conclusion is verified by the fact that an oxo substituent at position 5 of the 1,3-oxathiolane ring changes the chemical shift of C2 by 4.22 ppm and that of C4 by 1.88 ppm as a consequence of the conformational change from a favoured C(5) envelope form to an S(3) envelope form.[23]

Coupling constants

Further evidence for the half-chair structure can be obtained from the values of the various coupling constants (Table 3). The one-bond, J_{CH}, couplings already point out, *e.g.*, that *4* and *5* have the ee and ea configurations, respectively, since $J_{CHax}=153.3$ Hz and $J_{CHeq}=155.3$ Hz in agreement with other related observations in this laboratory.[23] The same situation is revealed even more strikingly from the geminal J_{CCH} coupling constants between $CH_3(4)$ and $H(4)$ since for *4* $J_{CCHax}=3.9$ Hz and for *5* $J_{CCHeq}=2(J_{CCH}^{obs}-0.5J_{CCHax})=0$.

Conclusion

As a result of our analysis of the chemical shifts and coupling constants of 2-oxo-1,3-dioxolane *1* and its methyl derivatives *2*–*7* we conclude that the ring exists predominantly in a half-chair conformation where the C4–C5 bond bisects the CO_3 plane. This result is in close agreement with the X-ray [8] and microwave [18] results as well as with the postulates of Paasivirta,[13,14] Arbusov [17a] and Pethrick *et al.*[15,16] However, our results do not lend any support to those reports [10–12] which suggest a planar ring in 2-oxo-1,3-dioxolane *1*. Finally we were able to show that the equatorial-axial preference for a methyl group in the studied compounds is 4.6 ± 0.5 kJ mol^{-1} at 298 K.

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Reactions of 2,4,5-Trimethyl-4-hexen-3-one and 2,4,5-Trimethyl-5-hexen-3-one with Base in the Presence of Molecular Oxygen

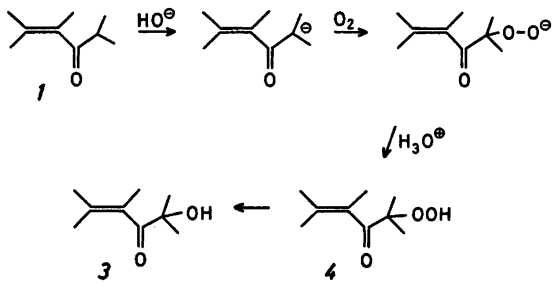
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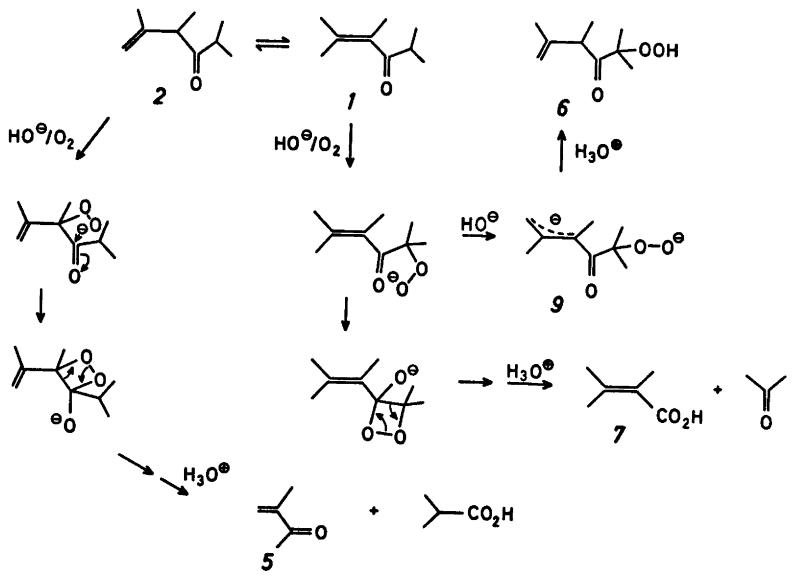
When the title compounds reacted with base under phase transfer conditions with access to air 2,4,5-trimethyl-2-hydroperoxy-4-hexen-3-one (*4*) was formed in 15 – 20 % yield. A complex reaction mixture was obtained when a similar reaction was carried out under pure oxygen, the main products being *4*, 2,4,5-trimethyl-2-hydroperoxy-5-hexen-3-one (*6*), and 2,3-dimethyl-2-butenoic acid (*7*).

When a mixture of 2,4,5-trimethyl-4-hexen-3-one (*1*) and bromoform was treated with 50 % aqueous sodium hydroxide in the presence of triethylbenzylammonium chloride (TEBA) small amounts of 2,4,5-trimethyl-5-hexen-3-one (*2*) and traces of a hydroxy enone (*3*) were isolated in addition to unreacted starting material and 1,1-dibromo-2-isobutyryl-2,3,3-trimethylcyclopropane.[1] Based on limited spectroscopic data it was assumed that the hydroxy enone was 2-hydroxy-2,4,5-trimethyl-4-hexen-3-one which under basic conditions can be formed from *1* as outlined in Scheme 1. To test this hypothesis a study of the reactions of *1* and *2* with base under phase transfer conditions was carried out.

The ketone *1* dissolved in benzene was stirred with a three-fold excess of 50 % aqueous sodium hydroxide in the presence of TEBA and under an atmosphere of air. According to GC analyses the resulting reaction mixture consisted of three products in a ratio of 8:72:20. These were separated by preparative GC and analyzed by IR and NMR spectroscopy. The main product was unreacted ketone whereas the smallest amount present turned out to be enone *2* which is formed from *1* by base-catalyzed isomerization. The IR spectrum of the third compound shows two symmetrical bands at 1365 and 1385 cm^{-1} characteristic of geminal methyl groups, a strong absorption at 1680 cm^{-1} characteristic of an α,β-unsaturated ketone, and a broad band in the 3300 – 3600 cm^{-1} region due to a hydroxyl group. Its NMR spectrum shows a singlet at δ 1.30, three partly overlapping broad singlets at 1.62, 1.73 and 1.80, and a broad singlet at 3.18 with a peak area ratio of 6:3:3:3:1, respectively. Finally, iodine was formed immediately when the compound was treated with an aqueous solution of potas-



Scheme 1.



Scheme 2.

sium iodide. These results are all in accord with the structure 2,4,5-trimethyl-2-hydroperoxy-4-hexen-3-one (*4*) which is confirmed by the ^{13}C NMR spectrum. As expected *4* was not formed when the reaction was carried out under pure nitrogen.

On storage the hydroperoxy ketone *4* decomposed to the corresponding hydroxy compound 2-hydroxy-2,4,5-trimethyl-4-hexen-3-one whose spectroscopic properties were practically identical to those of by-product *3*, facts supporting the rationale sketched in Scheme 1.

Reaction of 2,4,5-trimethyl-5-hexen-3-one (*2*) with base under identical conditions in the presence of air afforded a mixture of compounds *1*, *2*, and *4* in a ratio of 78:7:15, respectively. In the absence of TEBA, however, neither of the products *1* and *4* were formed. TEBA therefore catalyzes the generation of the intermediate anion and thus the formation of ketone *1* and hydroperoxy ketone *4*.

In order to improve the yield of *4* a mixture of ketones *1* and *2* was treated with aqueous sodium hydroxide under an atmosphere of oxygen. As expected the hydroperoxy ketone was formed in higher yield but in addition the number of products increased from three to at least twelve. Ten of these were isolated by preparative GC and on the bases of spectroscopic and chemical evidence and comparison with authentic samples their structures were established. In addition to small amounts of acetone and 3-methyl-3-buten-2-one (*5*) the following products were formed (relative yield of the higher-boiling products): 2,4,5-trimethyl-5-hexen-3-one (*2*) (1 %), 2,4,5-trimethyl-4-hexen-3-one (*1*) (6 %), acetic acid (10 %), 2,4,5-trimethyl-2-hydroperoxy-5-hexen-3-one (*6*) (13 %), isobutyric acid (7 %), 2,4,5-trimethyl-2-hydroperoxy-4-hexen-3-one (*4*) (44 %), 2,3-dimethyl-2-butenoic acid (*7*) (16 %), and 2,4-dihydroxy-2,4,5-trimethyl-5-hexen-3-one (*8*) (3 %). In accordance with an accepted mechanism for the reaction of an enolate anion with molecular oxygen [2-4] the hydroperoxy ketones and most of the cleavage products can be rationalized as outlined in Schemes 1 and 2. However, an alternative pathway of fragmentation involving nucleophilic addition of hydroxide anions to the carbonyl groups of *4* and *6* followed by intramolecular rearrangements [5] also accomodates the results. Acetic acid probably originates from acetone which forms some of this acid when exposed to the same reaction conditions in a separate experiment. The cleavage of acetone probably takes place by

a reaction similar to those outlined in Scheme 2; however, no formaldehyde or formic acid was detected.

The precursor of dihydroxy ketone *8*, however, is still uncertain. Analogous to the formation of *3* (Scheme 1) one may imagine *8* resulting from decomposition of the dihydroperoxy compound [6,7] formed by reaction of dianion *9* with molecular oxygen.

EXPERIMENTAL

General. The apparatus employed have been described elsewhere.[8]

Starting materials. 2,4,5-Trimethyl-4-hexen-3-one (*1*) and 2,4,5-trimethyl-5-hexen-3-one (*2*) were prepared as described by Colonge and Mostafavi [9] and separated by distillation through an efficient spinning band column.

Reaction of ketones with base; general procedure. A mixture of 3 mmol of ketone, 0.2 g of triethylbenzylammonium chloride (TEBA), 9 mmol of 50 % aqueous sodium hydroxide, and 8 ml of benzene was stirred vigorously at room temperature for 16 h. Hydrochloric acid (10 mmol) was added and the products were extracted with ether; the combined organic fractions were dried ($MgSO_4$). Evaporation of the solvent left a pale yellow residue which was analyzed by GC prior to distillation.

The following reactions were carried out according to this procedure.

Reaction of 2,4,5-trimethyl-4-hexen-3-one (1) with base under an atmosphere of air. GC analyses (20 % SE 30, 100 – 120 °C) revealed three compounds in a ratio of 8:72:20 and these were separated by preparative GC (same conditions). The product formed in lowest yield had physical properties identical with those of 2,4,5-trimethyl-5-hexen-3-one (*2*) whereas the predominant compound was unreacted *1*.

The third product, b.p. 93 °C/9 mmHg, was assigned the structure 2,4,5-trimethyl-2-hydroperoxy-4-hexen-3-one (*4*). ^{13}C NMR [15.0 MHz, CCl_4]: δ 16.1 (CH_3), 19.6 (CH_3), 22.6 (CH_3), 27.4 (2 CH_3), 76.5 (1 C, tertiary), 129.2 (1 C, olefinic), 131.8 (1 C, olefinic), 212.6 (C=O).

Storage of *4* gave 2-hydroxy-2,4,5-trimethyl-4-hexen-3-one (*3*). The latter compound gave no iodine when shaken with an aqueous solution of potassium iodide. Its IR and NMR spectra were almost identical to those of *4*. MS [IP 70 eV; *m/e* (% rel. int.)]: 141 (1, [M – CH_3]), 138 (3, [M – H_2O]), 98 (49, [M – acetone]), 97 (85, [M – C_3H_7O]), 83 (7, [98 – CH_3]), 69 (73, [97 – CO]), 59 (100, [$(CH_3)_2C=OH^+$]), 41 (72, [69 – ethylene]), 39 (17, [41 – H_2]). Found: C 68.74; H 10.07. Calc. for $C_9H_{16}O_2$: C 69.19; H 10.32.

Reaction of 1 with base under pure nitrogen. When the reaction was carried out under an atmosphere of nitrogen *1* was transformed to a mixture of *1* and *2* in a ratio of 90:10, respectively, according to GC analyses (20 % SE 30, 115 °C).

Reaction of 2,4,5-trimethyl-5-hexen-3-one (2) with base under an atmosphere of air gave a reaction mixture consisting of *1*, *2* and *4* in a ratio of 78:7:15, respectively, according to GC analyses (conditions as above).

Reaction of a mixture of 1 and 2 with base under pure oxygen. When 4.2 g of a 1:1 mixture of ketones *1* and *2* reacted with base under an atmosphere of oxygen, distillation afforded, besides 0.2 g of a lower-boiling fraction, 3.85 g of a complex mixture of products, b.p. 68 – 93 °C/9 mmHg. The lower-boiling fraction contained small amounts of acetone and 3-methyl-3-buten-2-one (*5*) according to spectroscopic examinations. GC analyses (10 % PEG 4000, 140 – 150 °C) revealed that the higher-boiling fraction consisted of eight products and these were separated by preparative GC (same conditions). Their structures were assigned as listed below in order of increasing retention time (relative yield in parenthesis).

2 (1 %). *1* (6 %). Acetic acid (10 %).

2,4,5-Trimethyl-2-hydroperoxy-5-hexen-3-one (*6*) (13 %) which immediately oxidized iodide to iodine. IR (CCl_4): 3510 (w), 3080 (w), 1710 (s), 1645 (m), 1388 (s), 1368 (s), 1165 (s), 908 (s) cm^{-1}. ^1H NMR (60 MHz, CCl_4): δ 1.25 (3 H, d, *J* 7 Hz), 1.38 (3 H, s), 1.41 (3 H, s), 1.80 (3 H, m), 3.78 (1 H, q, *J* 7 Hz), 4.66 (1 H, broad s), 4.87 (2 H, m). ^{13}C NMR [15.0 MHz, CCl_4]: δ 16.8 (CH_3), 20.0 (CH_3), 26.2 (CH_3), 26.9 (CH_3), 46.8 (1 C, tertiary), 76.7 (1 C, tertiary), 113.1 (1 C, olefinic), 144.0 (1 C, olefinic), 213.0 (C=O).

Isobutyric acid (7 %). *4* (44 %).

2,3-Dimethyl-2-butenoic acid (*7*) (16 %), m.p. 68 – 69 °C from CCl_4 (lit.[10] m.p. 70 – 71 °C).

2,4-Dihydroxy-2,4,5-trimethyl-5-hexen-3-one (*8*) (3 %) which did not oxidize iodide. IR (CCl_4): 3590 (w), 3495 (m), 1722 (s), 1642 (m), 1385 (s); 1370 (s), 1195 (s), 1145 (s), 1120 (s), 1030 (m), 960 (w), 910 (m) cm^{-1}. ^1H NMR (60 MHz, CCl_4): δ 1.37 (3 H, s), 1.40 (6 H, s), 1.75 (3 H, m), 4.35 (2 H, broad s), 4.95 (2 H, m). ^{13}C NMR [15.0 MHz, CCl_4]: δ 18.4 (CH_3), 24.4 (CH_3), 28.2 (2 CH_3), 79.6 (1 C, tertiary), 82.7 (1 C, tertiary), 110.8 (1 C, olefinic), 148.1 (1 C, olefinic); the carbonyl carbon was not detected due to noise. MS [IP 70 eV; *m/e* (% rel. int.)]: 157 (1, [M – CH_3]), 114 (13, [M – acetone]), 113 (4, [M – C_3H_7O]), 86 (47), 85 (39), 59 (100, [$(CH_3)_2C=OH^+$]), 43 (91).

Reaction of acetone with base under pure oxygen. A reaction carried out with acetone according to the general procedure gave an 8 % yield of acetic acid.

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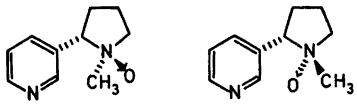
Short Communications

Stereochemistry of the Oxidation of Nicotine to its 1′-*N*-Oxides. The Action of Tungstate(VI) and Molybdate(VI)

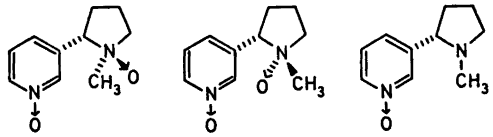
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The stereochemistry of *N*-alkylation reactions in heterocyclic systems has been extensively investigated, but less interest has been paid to the corresponding oxidations to amine *N*-oxides.[1–9] Treatment of nicotine with 10 % aqueous hydrogen peroxide mainly brings about oxygenation at the aliphatic nitrogen atom, and a mixture of 1′*R*,2′*S* (*cis*) (*1*) and 1′*S*,2′*S* (*trans*) isomers (*2*) in the ratio 1:2.3 is obtained.[4] Oxidation with *m*-chloroperbenzoic acid gives the same compounds in the ratio 1:2.[5] The isomers *1* and *2* have been separated by preparative paper chromatography [5,6] and by fractional precipitation with ammonium reineckate, $NH_4[Cr(NH_3)_2(SCN)_4]$.[6]



1 *2*



3 *4* *5*

For a study of the stereospecificity of some *N*-oxide degradation reactions, *1* and *2* were needed in stereochemically pure forms. To facilitate the isolation of *1*, it was desirable to start with a mixture which contained a higher proportion of *1* than the preparations described above. Nicotine has been shown by NMR spectroscopy to consist of about 90 % of the *trans* form (with the methyl and pyridyl groups on different sides of the five-membered ring).[10] A ratio of the isomers *1*:*2* of 0.5 thus means that the *N*-oxidising reagent (hydrogen peroxide or *m*-chloroperbenzoic acid) reacts almost five times faster with the *cis* form than with the *trans* form. We anticipated that sterically more demanding reagents should be even more effective in discriminating between the *cis* and *trans* forms of nicotine. This proved to be the case; for instance, peroxotungstates [11] or peroxomolybdates,[11] obtained *in situ* from aqueous hydrogen peroxide and alkali tungstates(VI) or molybdates(VI), gave mixtures containing more *1* than *2* (Table 1). An analogous compound, MoO_5.HMPA.H_2O,* easily obtained in a crystalline state,[12] oxidised nicotine in dioxane solution (22 °C, 18 h) to a mixture of *1* and *2* in a ratio of 1.5. Peroxotungstates have previously been used for the oxidation of amines,[13,14] but the stereochemical characteristics of the reaction have never been exploited.

The addition of molybdate or, in particular, tungstate not only affected the stereochemistry of the *N*-oxidation reaction but also furnished a more reactive reagent. This is evident from the ^{1}H NMR [4] and HPLC * analyses of the products of the experiments referred to in

* HMPA hexamethylphosphoric amide. HPLC high performance liquid chromatography.

Table 1. Influence of molybdate(VI) and tungstate(VI) on the stereochemistry of oxidation of nicotine to its 1′-*N*-oxides *1* and *2*.[a]

Added salt	Mol equiv. added [b]	Ratio *1*:*2* [c]
K_2MoO_4	0	0.3
	0.05	1.1
	0.20	2.0
	0.80	2.2
Na_2WO_4	0	0.3
	0.05	1.8
	0.20	2.4
	0.80	2.7

[a] Reaction mixtures contained hydrogen peroxide (≈8 mol equivalents, 11 % solution). [b] Nicotine = 1. [c] Obtained by triangulation of HPLC peaks.

Table 1. Only 3 % of the nicotine was oxidised when hydrogen peroxide was the sole reagent. Addition of 0.05 mol equivalents of sodium tungstate raised this figure to 40 %. At the same time, however, oxidation at the less reactive pyridyl nitrogen atom became more important. Only traces of the di-*N*-oxides *3* and *4* were formed in the absence of salts, but with 0.80 mol equivalents of sodium tungstate present, *3* and *4* together amounted to approximately one fourth of the oxidised material. The *N*-oxide *5* could also be detected. In all experiments, the ratio of *3:4* was lower than the ratio of *1:2*. A similar result was obtained in the essentially quantitative oxidation[15] of a mixture of mono-*N*-oxides (*1:2* = 1.5) with *m*-chloroperbenzoic acid into di-*N*-oxides (*3:4* = 0.8).

It is reasonable to assume that *3* is thermodynamically less stable than *4*, and the most likely explanation of the low ratios of *3:4* in the above and previous[4] reactions is that a decomposition of *3* takes place in the presence of peracid, possibly followed by reoxidation to *4*. Support for this view comes from an experiment in which a solution of *3*, *4* and *m*-chloroperbenzoic acid (0.5 mol equiv.) in chloroform was left at 22 °C. After 20 h, the ratio of *3:4* had decreased from 11.6 to 6.7, and after five days to 2.8, as found by HPLC. No similar effects could be observed when nicotine or benzoic acid were used instead of the peracid, either alone or in combination. The latter experiments rule out a transoxygenation reaction in which the unshared electron pair of the amine attacks the oxygen atom of a protonated *N*-oxide to give a new N – O bond and a simultaneous rupture of the old one. An analogous reaction probably occurs in the deoxygenation of amine oxides with triphenylphosphine.[16] In the reaction of this phosphine with a mixture of *1* and *2*, a high preference (87 %) for reaction with *1* was observed.

The *N*-oxidation method using *tert*-butyl hydroperoxide and bis(2,4-pentanedionato)-vanadium(IV) oxide in refluxing *tert*-butyl alcohol (40 min)[17] gave a ratio of *1:2* = 1.1.

In the large-scale preparation of *1*, the oxidation was carried out using 0.05 mol equivalents of sodium tungstate (0 °C, 2 h) and the products were then separated by chromatography on alumina. For the preparation of *2*, it proved advantageous to oxidise with *m*-chloroperbenzoic acid according to the literature conditions[15] to obtain a ratio of *1:2* = 0.18.

Experimental. HPLC was performed on a LiChrosorb ALOX T column (10 μ, 10 × 250 mm, Altex) mounted to an LDC pump and UV monitor (254 nm). Solvent: diethyl ether – methanol – water (10:10:1); flow rate: 2.5 ml/min. The retention times were: nicotine, 5.6 min; *5*, 6.2; *2*, 7.3; *1*, 8.8; *4*, 21.5; *3*, 50 min. Column chromatography (≈1 g) was carried out on basic alumina (Merck) (4 × 40 cm) with diethyl ether – methanol – conc. aqueous ammonia (100:25:2) as solvent. NMR spectra were recorded on a JEOL JNM-FX 100 instrument. The samples were dissolved in D_2O and sodium 3-(trimethylsilyl)propanesulfonate was used as reference. The *cis:trans* ratios were determined by integration over the N-CH_3 signals; the separation of these signals from others was best for acidic solutions. Similar ratios were obtained from HPLC assuming equal responses of stereoisomers *1* and *2* (or *3* and *4*) to the UV detector. On the other hand, *3* and *4* (and presumably *5* as well) gave much better responses than did *1* and *2*. This is to be expected since the extinction coefficient of pyridine-*N*-oxide at 255 nm is approximately 3.4 times that of pyridine.[18]

Oxidation with peroxomolybdates or peroxotungstates. To an ice-cooled and stirred solution of nicotine (0.20 g, 1.23 mmol) and the tungstate or molybdate in water (2.5 g), ice-cooled aqueous hydrogen peroxide (1.0 g, 35 %, 10.3 mmol) was added. After 1 h, an aliquot (0.5 – 1 ml) was taken out and rapidly subjected to ion exchange on a Dowex 1X4 column (chloride form, 5 × 0.5 cm) to remove the W and Mo-containing anions. The yellow (tungstate) or brown (molybdate) colours were thus removed. The eluate was then treated with an excess of manganese dioxide (0 °C) to destroy excess hydrogen peroxide. When the evolution of O_2 had ceased (≈5 min), the solution was filtered. For the HPLC examination, the solution was diluted with the solvent system used, and for the NMR investigation, the solution was acidified (pH ≈ 4) and the water evaporated below 30 °C. The residue was then dissolved in D_2O with or without added NaOD.

Preparation of 1. The oxidation was performed as described above using 0.05 mol equivalent of sodium tungstate, but the reaction time was extended to 2 h. Starting with 1 g of nicotine, 0.1 g of *1* (containing less than 2 % of *2*) was obtained after the column chromatography described above.

Preparation of 2. Nicotine (1.60 g) was oxidized as described.[15] The *N*-oxides were extracted with 1 M hydrochloric acid (2 × 10 ml) and the water was then evaporated under reduced pressure (25 °C). The residue was subjected to ion exchange on a Dowex 1X4 (OH^-) column with methanol as solvent. ^1H NMR and HPLC of the product showed a ratio of *1:2* = 0.18. After the column chromatography described above, 0.2 g of *2* (containing less than 2 % of *1*) was obtained.

Reactions with triphenylphosphine. The starting solutions contained *1* (0.084 mmol) and *2* (0.046 mmol) in acetic acid (1 ml). Different amounts of triphenylphosphine (0, 0.024, 0.045, and 0.062 mmol) were added and the four reaction mixtures were then heated (60 °C, 18 h). Aliquots (≈0.5 ml) were taken out, cooled and neutralised with conc. aqueous ammonia. After filtration through a column of basic alumina (0.5 × 1.5 cm), each sample was

examined by HPLC as described above. Peak areas were determined by triangulation. Results (mmol PPh_3, ratio of *1:2*): 0, 1.76 (2 % of nicotine was formed); 0.024, 1.34; 0.045, 0.96; 0.062, 0.69. These values correspond to an 87 % preference for reaction with *1*.

Acknowledgements. We thank Mrs. Lena Liedgren for running NMR spectra. This work has been supported by the Swedish Tobacco Company and by grants from Stiftelsen Bengt Lundqvists Minne (to L.L.).

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Homogeneous Electron Transfer Reactions. Increased Selectivity in Reduction of Some Disulfones Using Indirect Electrolysis

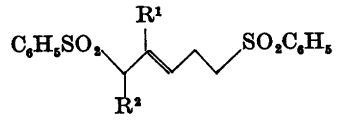
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Electrocatalytic reductions in organic electrochemistry have recently been the subject of several papers.[1–11] Anion radicals or dianions are generated electrochemically, and these species transfer electrons to the substrate. Quite frequently such a homogeneous electron transfer occurs without consumption of the catalyst, whereas in other cases, especially when the substrate is an aliphatic halide,[1] the catalyst concentration decreases due to a coupling reaction.

Indirect electrolysis has certain advantages and disadvantages compared to direct electrolysis. Among the advantages of indirect electrolysis is the relative independence of cell-design. In an ordinary laboratory cell the current distribution at the working electrodes is not uniform and the effective potential is therefore not the same over the whole electrode. It is thus difficult to reduce selectively one of two electroactive groups when the difference in reduction potential between the groups is less than 0.15 V. Other advantages are that complications with inhibitions occurring during macroelectrolysis may be overcome, lower electrode potentials may be employed, and higher current densities may be used especially towards the end of the experiment, compared to direct electrolysis.

In the present investigation a comparison is made with regard to the selectivity of direct and indirect electrochemical reduction of some compounds XRY where the difference in peak potential between the groups X and Y is 0.1 – 0.15 V; X and Y are here both benzenesulfonyl groups, X being allylic, whereas Y is not activated. The following compounds were included: 2-Methyl-1,5-bis(benzenesulfonyl) pent-2-ene(*1*), 2-ethyl-1,5-bis(benzenesulfonyl)-pent-2-ene (*2*), and 2,6-dimethyl-5,9-bis(benzenesulfonyl)-nona-2,6-diene (*3*).



1, $R^1 = CH_3$, $R^2 = H$; *2*, $R^1 = C_2H_5$, $R^2 = H$; *3*, $R^1 = CH_3$, $R^2 = CH_2CH=C(CH_3)_2$.

examined by HPLC as described above. Peak areas were determined by triangulation. Results (mmol PPh_3, ratio of *1:2*): 0, 1.76 (2 % of nicotine was formed); 0.024, 1.34; 0.045, 0.96; 0.062, 0.69. These values correspond to an 87 % preference for reaction with *1*.

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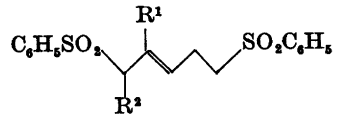
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In *1*, *2*, and *3* the two electrophores ($C_6H_5SO_2CHR^2CR^1=CH-$ and $C_6H_5SO_2^-$) are separated by two sp^3-hybridized C-atoms, so the molecule can to a first approximation be regarded as having two isolated electrophores. In the scheme below A is a compound forming a stable anion radical, whereas XRY is the disulphone. $A^{-\cdot}$ transfers electrons to XRY in a homogeneous reaction.

$$A + e^- \rightleftharpoons A^{-\cdot} \quad (1)$$

$$A^{-\cdot} + YRX \underset{}{\overset{K_x(K_y)}{\rightleftharpoons}} A + YRX^{-\cdot}(XRY^{-\cdot}) \quad (2)$$

$$YRX^{-\cdot}(XRY^{-\cdot}) \xrightarrow{k_x(k_y)} YR^{\cdot} + X^-(XR^{\cdot} + Y^-) \quad (3)$$

$$A^{-\cdot} + YR^{\cdot}(XR^{\cdot}) \xrightarrow{\text{fast}} A + YR^-(XR^-) \quad (4)$$

The peak potentials of *1* and *2* in dimethylformamide (DMF) containing tetrabutylammonium perchlorate (TBAP) are -2.05 and -2.20 V *vs.* SCE for the first and second peak, respectively; for *3* the corresponding values are -2.09 and -2.22 V. In DMF containing phenol the peak potentials are shifted less than 0.02 V, but the peak currents are higher than in aprotic medium due to the fact that in aprotic medium the disulfones themselves act as proton donors and their anions are not electroactive.

It has previously been shown [12] that allylic benzenesulfonyl groups are cleaved preferentially to unactivated ones, so $K_xk_x \gg K_yk_y$. The A-compound should thus be chosen in such a way that it has a suitable reduction potential; K_xk_x should have a reasonable magnitude whereas K_yk_y should be negligible. As the X and Y are practically isolated electrophores the A compounds can be screened by using suitable allylic and unactivated benzenesulfones as RX and RY compounds.

9,10-Diphenylanthracene ($E_p = -1.83$ V *vs.* SCE) is reduced at too positive a potential for the present systems, as the rate of reaction with the disulfones is too low. Pyrene ($E_p = -2.04$ V) has too negative a reduction potential as it exchanges electrons both with allylic and unactivated benzenesulfones. Anthracene (*4*) ($E_p = -1.92$ V) is a suitable choice, which is illustrated in Fig. 1 where the $i-E$ curves of *4* in the presence of increasing concentrations of *2* is shown. Fig. 1 shows that the second reduction peak of XRY behaves normally and gives no indication of a fast electron transfer from $A^{-\cdot}$.

It has furthermore previously been found [3] that allylic and benzylic radicals do not couple with $4^{-\cdot}$, but are reduced by electron transfer from $4^{-\cdot}$. The indirect reduction of the disulfones is thus not complicated by removal of the electron transfer reagent in a coupling reaction.

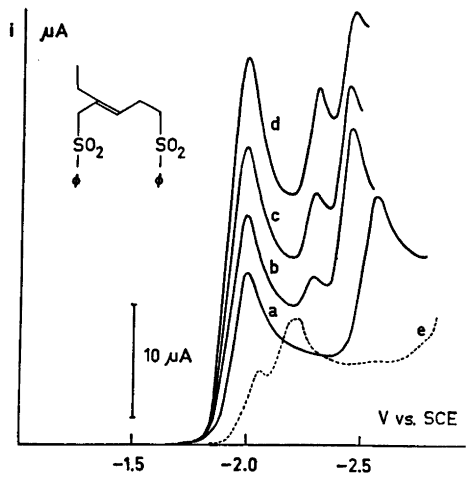


Fig. 1. Voltammetric curves at a stationary mercury electrode of anthracene in the presence of *2*. a: Anthracene (6.5×10^{-3} M); b, c, and d: a+*2*(2.3, 5.1, and 7.6×10^{-3} M, respectively). e: *2* without anthracene. Medium, DMF/1.4×10^{-1} M TBAP. Sweep-rate 10 mV s^{-1}.

In coulometric experiments, using direct electrolysis with the potential of the mercury pool electrode close to the first peak potential of the disulfone (-2.10 V), n values from 2.35 to 2.7 were found, and voltammetric curves obtained before and after electrolysis showed a decrease of the height of the second peak. In contrast to that indirect electrolysis, using *4* as A-compound at a cathode potential of -1.95 V, gave $n = 2.0 \pm 0.1$ F mol^{-1}; the electrolysis was stopped when the blue colour of $4^{-\cdot}$ appeared, and a negligible decrease of the peak-height of the second peak was observed.

The differences in selectivity was furthermore tested by preparative experiments. Direct electrolysis of *1*, *2*, and *3* at -2.10 V *vs.* SCE consumed 3.4 – 3.8 F mol^{-1}; analytical determination of $C_6H_5SO_2^-$ by anodic polarography showed that both benzenesulfonyl groups had been cleaved.

Indirect preparative electrolysis of *1*, *2*, and *3* using *4* as electron transferring agent permitted a selective cleavage of the allylic benzenesulfonyl group. In the reduction of *2* and *3* this was proved by polarography of the product which was shown to be an unactivated benzenesulfonyl compound without any vinylic or allylic benzenesulfonyl groups. From the indirect reduction of *1* 2-methyl-5-benzenesulfonyl-2-pentene (*5*) and a small amount of 2-methyl-5-benzenesulfonyl-1-pentene (*6*) was isolated in good yield. Both isomers (*5* and *6*) would be expected as the allylic anion formed

in (4) could accept a proton at either C-1 or C-3.

The experiments thus show that in certain cases by suitable choice of electron-transferring agent a higher selectivity may be obtained using indirect rather than direct electrolysis, at least when the cell geometry is not perfect.

Experimental. For the coulometric measurements was used a Tacussel cell (RMO5 vessel) with a 10 cm^2 mercury pool electrode. The cell for macroelectrolysis was that used previously.[1,2]

Chemicals. The disulfones were a gift from Professor M. Julia, Paris. DMF was obtained from Merck and distilled once *in vacuo*; tetrabutylammonium perchlorate (TBAP) was delivered by Fluka.

Coulometry. In the direct electrolysis the disulfones ($C \sim 3$ mM) were reduced at a mercury pool electrode in DMF/0.15 M TBAP at the potential of the first voltammetric peak, $E = -2.1$ V *vs* SCE. Voltammograms were measured with the microelectrode directly in the catholyte before and after the electrolysis; the decrease of the second peak was taken as a measure of the extent of cleavage of the second sulfone group. In the coulometric experiment using indirect electrolysis the concentration of anthracene was about 0.5 mM; the experiment was stopped when the blue colour of $4^{-\cdot}$ appeared.

Preparative electrolysis. 1 (0.5 g) was reduced in 70 ml DMF/0.1 M tetrabutylammonium iodide (TBAI) at -2.0 V *vs.* SCE in the presence of *4* (0.2 g). The blue colour of $4^{-\cdot}$ appeared after 2.0 F mol^{-1}, and the electrolysis was stopped. The catholyte was worked up by diluting the catholyte with water and extracting the products with diethyl ether; evaporation of the solvent left a residue which was separated into hydrocarbons and sulfones on a column of silica using a 9:1 mixture of light petroleum and ethyl acetate as eluent. Isolated were 2-methyl-5-benzenesulfonyl-2-pentene *5* (60 % yield) and minor amounts of the 1-pentene isomer *6*.

2 and *3* (0.2 g) were reduced similarly in the presence of *4* (0.05 g). After a similar work-up the fractions containing the benzenesulfones were analyzed by polarography. The voltammograms showed only one peak at -2.20 V *vs.* SCE indicating the presence of an unactivated benzenesulfonyl group and the absence of vinylic and allylic benzenesulfonyl groups.[13]

When *1* was reduced electrolytically at -2.10 V *vs.* SCE in DMF/0.1 M TBAP the reduction consumed 2.6 F mol^{-1}, and a complex mixture of starting material and products was obtained; in the mixture the presence of *5* and *6* (about 10 %) was detected by ^1H NMR spectroscopic analysis.

2 and *3* were reduced similarly and after consumption of 3.4 – 3.8 F mol^{-1} the catholyte was analyzed for $C_6H_5SO_2^-$ by anodic voltammetry.

Acknowledgement. We thank Professor M. Julia, Paris for providing samples of the disulfones.

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Effect of Salt on the Kinetics of Butyrylcholinesterase

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It has previously been reported that the presence of common salts, *e.g.*, NaCl, affects the activity of both acetylcholinesterase (EC 3.1.1.7) and butyrylcholinesterase (EC 3.1.1.8)[1] and also causes a change in the relationship of activity to substrate concentration, particularly for the former enzyme.[2] Recent studies on the effect of NaCl on the kinetics of acetylcholinesterase (from electric eel and bovine erythrocytes) [3,4] demonstrated that these kinetics deviate from the hyperbolic Michaelis-Menten equation at low ionic strength, but not in the presence of high concentrations of NaCl.

In the present paper we describe the effect of different salts on the kinetics of butyrylcholinesterase, particularly with respect to the non-hyperbolic kinetics of the hyrolysis of butyrylthiocholine and thiophenyl acetate demonstrated recently.[5,6]

A partly purified butyrylcholinesterase preparation from Organon (Holland) was used without further purification. Butyrylcholinesterase activity was determined at 30 ± 0.1 °C according to a modified Ellman procedure [7,8] on a Beckman 25 spectrophotometer with a Beckman recorder. The enzyme was incubated 1 – 2 h at 30 °C in 50 mM tris-HCl buffer, pH 7.4, containing 0.25 mM DTNB * and the modifier. The reaction mixture consisted of 2.9 – 3.0 ml of the enzyme solution and 0.1 – 0.005 ml of the substrate solution. The protein concentration in the cuvette was 0.03 mg/ml.

The ratio of the intercept on the abscissa and the intercept extrapolated from high substrate concentrations on the same axis, visualized in a $v/(v/S)$ plot in Fig. 1, was used as a measure of the non-linear kinetic behaviour. These intercept values, I_1 and I_2, respectively, were obtained graphically from the reciprocal slope (when $1/S \rightarrow \infty$) of the Lineweaver-Burk plot $(1/v)/(1/S)$ and from the reciprocal intercept on the ordinate (extrapolated from the slope at high S of the Woolf-Hanes plot $(S/v)/S$, respectively. All values of slopes and intercepts were calculated by linear regression on a Compucorp 327 Scientist.

The intercept ratios, summarized in Table 1, were calculated for the enzymatic hydrolysis of butyrylthiocholine in the presence and absence of different concentrations of salt.

It is obvious that the monovalent cations Na^+ and K^+ produced very similar monotonic decreases in non-linearity up to a concentration of 4 M salt, where the ratio became less than one. In kinetic terms this phenomenon is described as a change from negative to positive co-operativity in the hydrolysis of butyrylthiocholine (Fig. 2). The divalent cation Mg^{2+} did not change these kinetics in the same manner, even though the initial decrease in non-linearity in the presence of Mg^{2+} was faster than that seen with corresponding concentrations of Na^+ and K^+.

The use of salt concentrations as high as 4 M must be accompanied by controls designed to determine whether any irreversible effects or large conformational changes due to a changed

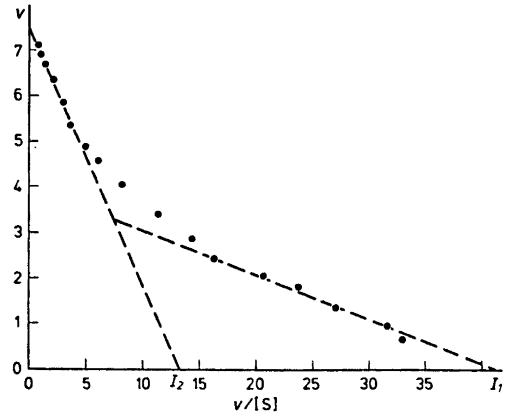


Fig. 1. The EAH plot of butyrylcholinesterase activity with butyrylthiocholine as substrate in the absence of salt. For further details see the text.

* Abbreviations: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EAH, Eadie-Augustinsson-Hoffstee plot or $v/(v/S)$; S, substrate concentration.

Table 1. Non-linearity index (I_1/I_2) in dependence of the salt concentration.

Salt conc./M	I_1/I_2 NaCl	KCl	$MgCl_2$[a]
0	2.9	2.9	2.9
0.02	–	–	2.1
0.2	2.3	–	1.3
1	1.7	1.6	–
2	1.3	1.4	–
3.4	1.0	–	–
4	0.47	0.98	1.5

[a] The concentration values of $MgCl_2$ are expressed as 2 × M.

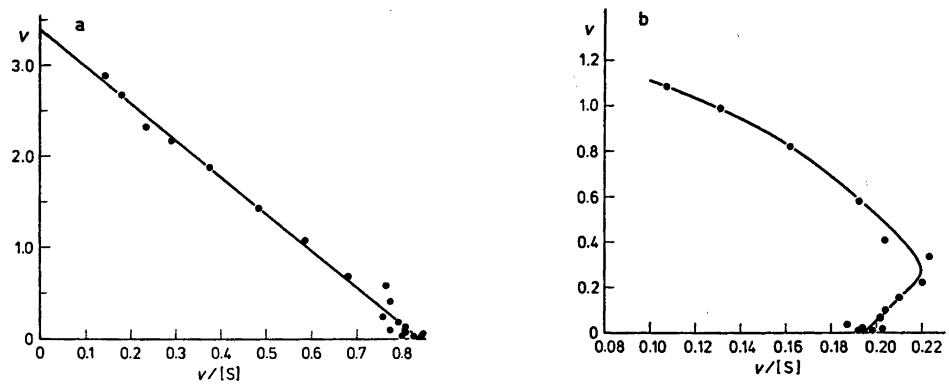


Fig. 2. The EAH plot of butyrylcholinesterase activity with butyrylthiocholine as substrate. (a) Catalysis in the presence of 3.4 M NaCl. The correlation coefficient was 0.995. (b) Catalysis in the presence of 4 M NaCl.

hydratisation of the enzyme, *e.g.* association or dissociation of subunits, did occur. No such irreversible effects could be observed, not even at the highest ionic strength used. After incubating the enzyme for 24 h in the presence of salt, its activity was not changed. Upon dilution to a moderate ionic strength (0.2 M) the specific activity was the same as for the non-incubated enzyme at the same salt concentration.

That the tetrameric nature and the shape of the enzyme are preserved in NaCl at concentrations as high as 2.5 M has been established previously.[9]

One indication that no gross conformational change occurs is the observation that in the presence of 4 M NaCl the enzymatic hydrolysis of thiophenyl acetate was changed very little. The maximum reaction rate was almost identical to that obtained in the absence of salt. At thiophenyl acetate concentrations as small as 0.025 mM the rate in the presence of salt was decreased by only 30 % compared to that in the absence of salt. With butyrylthiocholine as substrate the highest rate was reduced by 80 %; and with 0.025 mM butyrylthiocholine the rate was 0.6 % of the rate in the absence of salt.

This difference in kinetic behaviour might be interpreted in terms of hydrophobic interactions. As the salt concentration increases, the thiocholine moiety of the substrate might be transferred from the charged part of the "anionic site" to a more hydrophobic region.[10,11] Such a hydrophobic effect on the hydrolysis rate would be expected to be more pronounced with butyrylthiocholine than with thiophenyl acetate, of which the thiophenyl part would be expected to be already situated in a hydrophobic region.

It is possible to analyze the shapes of the graphs in Figs. 1 and 2a in terms of a co-operative or non-co-operative type of action of the butyrylcholinesterase upon butyrylthiocholine. However, the plot in Fig. 2b, which illustrates the catalysis of butyrylthiocholine in the presence of 4 M NaCl, is *not* in agreement with a non-co-operative system. In other words, the positive co-operativity observed excludes the possibility of independent binding (or catalytic) sites.

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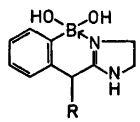
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(N^3-*B*)-2-[1-(*o*-Dihydroxyboryl-phenyl)-2-phenylethyl]-2-imidazoline and its Reactions with Vicinal Dihydroxy Compounds

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Aromatic boronic acids with neighboring amine groups capable of coordinating internally to boron form an interesting group of compounds.[1,2] The facile change of the coordination state of the boron atom shows up in the catalytic properties of these compounds. This facility should also have consequences in the complexation abilities of boronic acids. It is well-known that borate anion forms cyclic esters with vicinal dihydroxy compounds. However, in aqueous solution borate is not a predominant species until at rather high pH values. It can be anticipated that the boronic acids which maintain the tetracoordination at low pH values are better esterifying agents for vicinal dihydroxy compounds than boric acid.

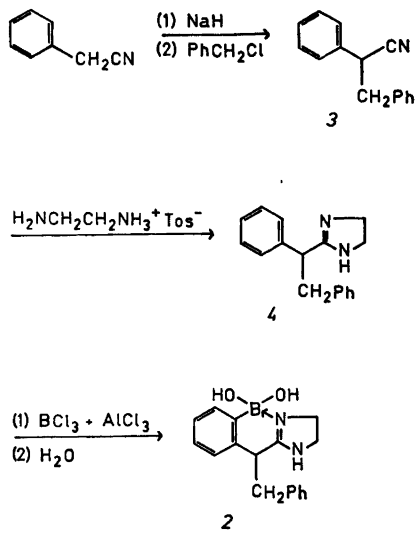


1 R = H

2 R = $PhCH_2$

Compound *1*, (N^3-*B*)-2-(*o*-dihydroxyborylbenzyl)-2-imidazoline, has been synthesized previously [2] and showed complexing properties with pyrocatechol derivatives but not with α-hydroxy acids. It was anticipated that the insertion of large substituents into the molecule might favorably modify the solubilities of the complexes. The benzyl derivative of *1*, (N^3-*B*)-2-[1-(*o*-dihydroxyborylphenyl)-2-phenylethyl]-2-imidazoline (*2*) was synthesized starting from benzyl cyanide, which was benzylated, the resulting 2,3-diphenyl-propionitrile (*3*) was allowed to react with 2-aminoethyl-ammonium tosylate according to the general procedure of Oxley and Short [3] to give 2-(1,2-diphenylethyl)-2-imidazoline (*4*). Compound *2* or actually its hydrochloride was obtained from *4* by a reaction with boron trichloride and aluminium chloride in boiling xylene and hydrolyzing the product.

The hydrochloride of compound *2* is a white crystalline solid with moderate solubility in water. Its aqueous solution is quite acidic, the pK_a determined from the half-neutralization point being *ca.* 3.0. The pK_a values of imidazolines are generally within the range from 10 to 11.[4] The only reasonable explanation for this large deviation is the ringchain equilibrium where the cyclic form (*2*) is essentially neutral.

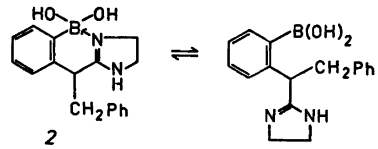


3

4

2

The same kind of tautomerism has been proposed previously [1] on the spectral basis for 2-(*o*-dihydroxyborylphenyl)-benzimidazole.



2

When mixing equal volumes of *ca.* 0.01 M aqueous solution of the hydrochloride of compound *2* and *ca.* 5 % solutions of dihydroxy compounds, a precipitate was formed with glycolic, lactic, 2-hydroxyisobutyric, malic, tartaric, citric, glucuronic, quinic, mucic, saccharic, salicylic, and oxalic acids, and with pyrocatechol. No precipitate was formed with 3-hydroxypropionic, 3-hydroxybutyric, *p*-hydroxybenzoic, and malonic acids, nor with 1,2-ethanediol, 1,2-propanediol, glycerol, mannitol, and 2,3-dimethyl-2,3-butanediol. The results show that α-hydroxy acids are generally precipitated whereas β-hydroxy acids and glycols are not. The exceptions are salicylic acid and pyrocatechol. The potentiality of the reactions as an analytical test is obvious. In the case of tartaric acid opalescence was observed even on mixing *ca.* 10^{-4} M solution of the acid with the organoboron reagent. This observation is leading to a microanalytical assay of tartaric acid which will be published separately.

Experimental. 2,3-Diphenylpropionitrile (3). To a suspension of 27 g of sodium hydride in 450 ml of ether was added dropwise with stirring 127 g (1.08 mol) of freshly distilled benzyl cyanide. After 4 h of stirring under reflux the mixture was cooled to room temperature and 63 g (0.5 mol) of benzyl chloride

was added dropwise as fast as possible without excessively vigorous reaction. After 12 h of stirring ether was evaporated and 450 ml of benzene added. The benzene solution was washed successively with 1 M hydrochloric acid, saturated solution of sodium carbonate and water. The solution was dried with Na_2SO_4, benzene evaporated and the residue distilled under reduced pressure. The fraction boiling at *ca.* 120 – 140 °C/2 mmHg solidified and was recrystallized from ethanol to give 67 g (65 %) of 2,3-diphenylpropionitrile, m.p. 55.5 – 56.5 °C (lit. 46 °C,[5] 58 °C[6]).

2-(1,2-Diphenylethyl)-2-imidazoline (4). A mixture of 2,3-diphenylpropionitrile (31.1 g, 0.15 mol) and 2-aminoethylammonium tosylate [3] (34.9 g, 0.15 mol) was heated at 203 – 205 °C until no more ammonia was evolved (*ca.* 2 h). The resulting solid was dissolved in 700 ml of boiling water and 6 g of sodium hydroxide dissolved in 25 ml of water was added. The oily precipitate solidified at 5 °C. It was dried and recrystallized from a mixture of toluene and light petroleum yielding 26.3 g (70 %) of white needles, m.p. 113 – 115 °C. The mass spectrum showed the parent peak at *m/e* 250.

2-[1-(o-Dihydroxyborylphenyl)-2-phenylethyl]-2-imidazoline (2). To a solution of 12 g (0.1 mol) of boron trichloride in 120 ml of ice-cold xylene 20 g (0.1 mol) of finely ground compound *4* was added. The mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. Finely ground anhydrous aluminium chloride (12.8 g, 0.1 mol) was added and the mixture was refluxed with stirring under a slow nitrogen flow for 20 h. To the cooled solution 65 ml of 2 M hydrochloric acid was cautiously added. Stirring was continued until the sticky mass dissolved. Xylene was decanted off and *ca.* 300 ml of acetone was added to the water layer. The lower layer was separated and allowed to stay overnight in a refrigerator. The precipitate was washed with acetone, dried under reduced pressure and recrystallized from a mixture of 50 ml acetonitrile and 6 ml of water. The yield was 10 g (38 %) of white crystals with no definite melting or decomposition point. Anal. $C_{17}H_{19}BN_2O_2$.HCl:B. Neutralization equivalent, obs. 333.5, calc. 330.5.

No parent peak could be found in the mass spectrum of the compound *2*. A pyrocatechol derivative could be easily prepared as in the case of analogous boron compounds.[2] A thick white precipitate which formed when mixing aqueous solutions of the hydrochloride and pyrocatechol was filtered, washed with water and dried in a vacuum desiccator. The mass spectrum showed the parent peak at *m/e* 368.

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A Stereoselectively Formed Dimer of 6-Benzoyloxy-6-phenylfulvene

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Fulvenes are known to take part in Diels-Alder reactions and other cycloadditions, both as dienes and dienophiles.[1,2] As dienophiles they can react both as 2π and 6π systems.[3] There are, however, few examples of Diels-Alder dimerisations of fulvenes.[2] The loss of the resonance energy in the fulvenes [2] makes these reactions energetically less favourable than the well-known dimerisation of cyclopentadiene. If substituted with different groups at the exocyclic carbon atom, a fulvene could either give a mixture of dimers or stereoselectively give only one dimer in a Diels-Alder reaction. In this paper the formation of such a dimer of 6-benzoyloxy-6-phenylfulvene is reported.

Results and discussion. A cool diethyl ether solution of 6-benzoyloxy-6-phenylfulvene [4] slowly deposits a white precipitate. Analysis and MS show that this precipitate has the same composition as the fulvene. The compound was found to contain one single isomer, whose structure was elucidated by its ^{1}H NMR spectrum (Scheme 1). The spectrum showed the 1/1 ratio of olefinic to tertiary protons as expected for a normal Diels-Alder dimer. Decoupling experiments gave the chemical shifts and coupling constants for the eight protons in the tricyclo [5.2.1.$0^{2,6}$]decadiene ring system. The coupling constants $J_{1,2}$ and $J_{6,7}$ (4.2 Hz) show that the dimer has an *endo* configuration.[5] The remaining structural problem, the configuration at the exocyclic double bonds, was solved by means of a shift reagent. The chemical shifts for the eight protons increased on addition of a shift reagent (Eu fod_3) to the fulvene dimer. A rigorous treatment of the problem is very complex and was not carried out. However, the following qualitative argument should be satisfactory. One can hardly explain the induced shifts without assuming that both ester groups coordinate with the europium salt. Rotation around the single bond between

was added dropwise as fast as possible without excessively vigorous reaction. After 12 h of stirring ether was evaporated and 450 ml of benzene added. The benzene solution was washed successively with 1 M hydrochloric acid, saturated solution of sodium carbonate and water. The solution was dried with Na_2SO_4, benzene evaporated and the residue distilled under reduced pressure. The fraction boiling at *ca.* 120 – 140 °C/2 mmHg solidified and was recrystallized from ethanol to give 67 g (65 %) of 2,3-diphenylpropionitrile, m.p. 55.5 – 56.5 °C (lit. 46 °C,[5] 58 °C[6]).

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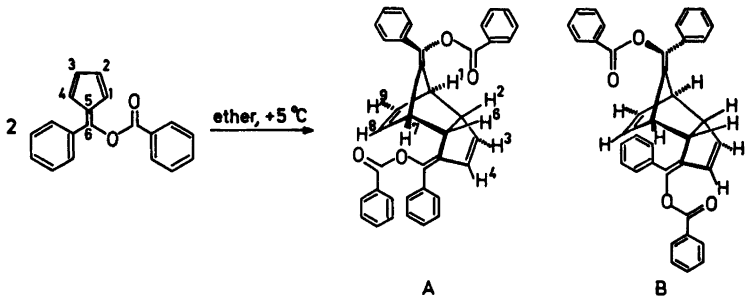
A Stereoselectively Formed Dimer of 6-Benzoyloxy-6-phenylfulvene

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Fulvenes are known to take part in Diels-Alder reactions and other cycloadditions, both as dienes and dienophiles.[1,2] As dienophiles they can react both as 2π and 6π systems.[3] There are, however, few examples of Diels-Alder dimerisations of fulvenes.[2] The loss of the resonance energy in the fulvenes [2] makes these reactions energetically less favourable than the well-known dimerisation of cyclopentadiene. If substituted with different groups at the exocyclic carbon atom, a fulvene could either give a mixture of dimers or stereoselectively give only one dimer in a Diels-Alder reaction. In this paper the formation of such a dimer of 6-benzoyloxy-6-phenylfulvene is reported.

Results and discussion. A cool diethyl ether solution of 6-benzoyloxy-6-phenylfulvene [4] slowly deposits a white precipitate. Analysis and MS show that this precipitate has the same composition as the fulvene. The compound was found to contain one single isomer, whose structure was elucidated by its ^{1}H NMR spectrum (Scheme 1). The spectrum showed the 1/1 ratio of olefinic to tertiary protons as expected for a normal Diels-Alder dimer. Decoupling experiments gave the chemical shifts and coupling constants for the eight protons in the tricyclo [5.2.1.$0^{2,6}$]decadiene ring system. The coupling constants $J_{1,2}$ and $J_{6,7}$ (4.2 Hz) show that the dimer has an *endo* configuration.[5] The remaining structural problem, the configuration at the exocyclic double bonds, was solved by means of a shift reagent. The chemical shifts for the eight protons increased on addition of a shift reagent (Eu fod_3) to the fulvene dimer. A rigorous treatment of the problem is very complex and was not carried out. However, the following qualitative argument should be satisfactory. One can hardly explain the induced shifts without assuming that both ester groups coordinate with the europium salt. Rotation around the single bond between



Scheme 1.

the ester groups and the tricyclic skeleton is essentially free and should decrease the importance of the angle-dependent term [6] on the induced chemical shift. The distance-dependent term [6] should dominate. H-1 and H-7 show the largest induced shifts. Thus the ester groups should be close to these protons as in isomer A, Scheme 1. The induced chemical shifts of the other protons are also consistent with this simple picture.

The selective formation of only one of the many possible dimers of 6-benzoyloxy-6-phenylfulvene could be reasonably well rationalized by frontier orbital arguments. The *endo* selectivity is readily rationalized and well documented in most Diels-Alder reactions, although cycloadditions with fulvenes frequently give *exo* products in thermodynamically controlled reversible reactions.[2,3] The periselectivity, *i.e.* the selective reactivity of the dienophile as a 2π system, using one of the double bonds in the ring, is less readily explained. The lowest unoccupied molecular orbital (LUMO) in fulvenes has a large coefficient at the exocyclic carbon, where the reaction should take place.[3] However, in most cases electrocyclic reactions do not occur at the exocyclic carbon but with one of the double bonds in the ring. Neglecting the possibility of reactions at the exocyclic carbon, one can understand the regiospecificity of the dimerisation. The perturbation of the LUMO and HOMO in the fulvene, caused by the unsymmetrical substitution pattern, should have opposite effects on the coefficients in the LUMO and HOMO. Thus, the new bonds should form between carbons 1 and 4′ or 1′ and 4, resulting in the dimers *A* and *B* (Scheme 1), respectively. Isomer *B* is less likely than isomer *A* for steric reasons. The interaction between one of the phenyl rings and carbons and protons 7 and 8 is substantial in isomer *B*.

Experimental. 6-Benzoyloxy-6-phenylfulvene,[4] prepared from cyclopentadienylcopper tributylphosphine and benzoyl chloride, was kept in the refrigerator (+5 °C) as an ether solution (initially *ca.* 5 %). An almost white precipitate (15–25 %) slowly formed during several weeks, while the red solution turned brown. Thin layer chromatography showed no other major product. Recrystallisation from ethanol or acetic acid gave white crystals (m.p. 155–157 °C). Analysis: C, H. IR (KBr): 1725 (s), 1601 (m), 1495 (m), 1450 (m), 1260 (s), 1218 (m), 1092 (s), 1070 (m), 780 (m), 768 (m), 712 (s), 704 (s), 697 (s) cm^{-1}. UV (ethanol): λ_{max} 283 nm ($\varepsilon = 24\,500$) and 230 (45 000). MS (70 eV): *m/e* 274 (M/2+, 5 %), 106 (8), 105 (100), 77 (38) and 51 (9). The dimer is cleaved on attempted sublimation. ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.22 (m), 8.14 (m), 7.71–7.43 (m), 7.36–7.27 (m), 7.15 (m) and 7.05 (m), aromatic protons, 6.51 (d of d, H-4), 6.22 (d of d, H-8), 6.01 (d of d, H-9), 5.95 (d of d, H-3), 3.86 (m, H-7), 3.77 (m, H-6), 3.60 (m, H-2) and 3.41 (m, H-1). The following coupling constants were obtained, mainly from decoupling experiments: $J_{1,2}$ 4.2 Hz, $J_{1,8}$ 0.8, $J_{1,9}$ 3.0, $J_{2,3}$ 2.6, $J_{2,4}$ 1.6, $J_{2,6}$ 6.0, $J_{3,4}$ 5.8, $J_{6,7}$ 4.2, $J_{7,8}$ 3.0, $J_{7,9}$ 0.5 and $J_{8,9}$ 6.0. On addition of Eu(fod)$_3$ linear induced shifts of the protons were observed. A fivefold excess (by weight) of the shift reagent gave the following induced shifts (ppm): H-1 = 0.80, H-2 0.36, H-3 0.06, H-4 0.19, H-6 0.57 H-7 0.70, H-8 0,32 and H-9 0.16.

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N-Quaternary Compounds. Part XLV.[1] Selective *N*-Vinylation of Pyridine-2-thiones by Decarboxylative Ring-opening of Intermediate 3-Carboxydihydrothiazolo[3,2-*a*]pyridinium Derivatives

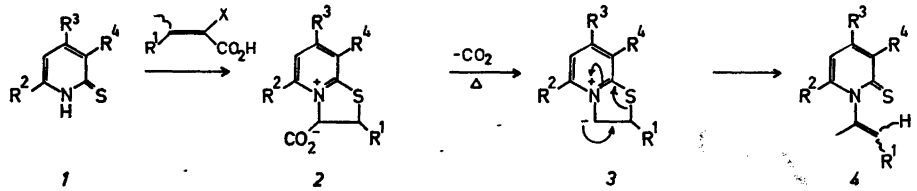
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The direct synthesis of *N*-vinyl derivatives from pyridine-2-thiones by alkylative procedures is difficult to achieve because of preferential attack by electrophilic carbon on the nucleophilic sulfur atom. *S*,*N*-Disubstitution, however, proceeds readily. Hence dihydrothiazolo[3,2-*a*]pyridinium derivatives can be used as intermediates for the *N*-vinylation reaction. Ring-opening of the dihydrothiazolo ring requires the generation of a carbanionic centre in the 3-position which is promoted by the inductive stabilisation from the quaternary nitrogen atom. Preparative decarboxylation at C-3 seemed a likely method for selective generation of a carbanionic centre at C-3 by analogy to the mass spectral behaviour of 3-carboxydihydrothiazolo[3,2-*a*]pyridinium derivatives which yield volatile *N*-vinyl derivatives.[2]

The required intermediate 3-carboxydihydrothiazolo[3,2-*a*]pyridinium derivatives *2* are readily available for instance by Michael-type addition of pyridine-2-thiones *1* to α-halogeno-α,β-unsaturated carbonyl derivatives with subsequent cyclisation.[3–5] Compound *2* can be further modified before ring-opening by electrophilic substitutions if the pyridinium ring contains a hydroxy group.[6,7]

The decarboxylative ring-opening of *2* was carried out under anhydrous conditions after grinding *2* together with quartz sand before heating at 150 – 180 °C at reduced pressure. When the carboxylates *2* were available as hydrobromides, these were ground together with anhydrous potassium carbonate before pyrolysis; the product *4* was collected by sublimation. In the hydroxy substituted derivatives (*2c* – *2i*) the decarboxylation may be accompanied by formation of pyridiniumolates *5* through prototropic shift. The betaines *5* may cosublime but are much less volatile than their *N*-vinyl isomers and the sublimation of the former is generally suppressed by operating at relatively high pressure (15 mmHg). The reaction time for small samples is of the order 45 min; the isopropyl derivative *2i* constitutes an exception in that the sublimate had to be collected after 15 min since further heating in this case led to formation of both the *N*- and *S*-vinyl isomers. Presumably two opposing effects are in operation. The isopropyl group causes sterically accelerated decarboxylation in comparison with the other 5-substituents, but also a sterically retarded ring-opening to the *N*-vinyl derivative because of the interaction between the isopropyl group and the *N*-vinyl group during formation. Hence a relatively high concentration



1 *2* *3* *4* *5*

	R^1	R^2	R^3	R^4
a	H	H	H	H
b	H	Me	H	H
c	H	H	H	OH
d	H	Me	H	OH
e	Me	H	H	OH
f	H	Me	p-ClPh	OH
g	H	Br	Br	OH
h	Me	Me	H	OH
i	H	iPr	H	OH

of the betaine *5* is obtained. Proton abstraction from the latter by base may occur from either C-2 or C-3 leading to the formation of the *S*-vinyl or the *N*-vinyl isomer, respectively.[8]

In protic medium decarboxylation leads exclusively to the betaine *5* as demonstrated by the formation of *5i* on heating the isopropyl derivative *2i* in aqueous dimethylformamide.

The ring opening is not stereoselective in that both the *trans* and the *cis* isomers are formed from 2-substituted derivatives *2*; the formation of the *trans* isomer is favoured. Using 100 MHz NMR and irradiating at the frequency for the methyl protons in the major isomer of *4e*, revealed a vinylic coupling J 14 Hz, and hence the major isomer is assigned the *trans* configuration.

Experimental. 3-Carboxydihydrothiazolo[3,2-a]pyridinium derivatives 2 were available from other work.[3-7]

N-Vinylpyridine-2-thiones 4. The dihydrothiazolo[3,2-*a*]pyridinium-3-carboxylate *2* was ground together with about three times its weight of quartz sand and the mixture heated at 150–180 °C/2–15 mmHg. The optimum temperature and pressure within these intervals vary with the compound. In those cases where the pyridinium derivatives were HBr-salts the latter were ground together with three times their weight of anhydrous potassium carbonate and the mixture pyrolysed as above. The *N*-vinyl derivative formed is sublimed. A little decarboxylated dihydrothiazolo[3,2-*a*]pyridinium derivative (*5*) may cosublime but is readily removed on recrystallisation from an alcoholic or aqueous alcoholic solution. The time of heating depends on the reaction scale; the normal time was 45 min for small samples (< 1 g) and up to 4 h for larger samples (10 g). In the case of the isopropyl derivative *2i* the heating was stopped after 15 min in order to avoid extensive cosublimation of the *S*-vinyl isomer of *4*. The yields were generally of the order 50 % except for the dibromo derivative *2g* where the yield of isolated *N*-vinyl product was *ca.* 10 %. Data for the compounds are given below.

N-Vinylpyridine-2-thione[9] 4a. M.p. 72 °C. Anal. C_7H_7NS: C, H. ^{1}H NMR ($CDCl_3$): δ 5.3 (J 8.5 Hz) and 5.4 (J 16 Hz) (CH_2, J_{gem} 2 Hz), 7.8 (CH), 6.5–7.9 (Pyr.).

N-Propenylpyridine-2-thione 4b. The product ratio *trans: cis* was 3:1. ^{1}H NMR ($CDCl_3$): δ 1.7 (Me *cis*) and 1.9 (Me *trans*) ($J_{Me-H\beta}$ 7 and $J_{Me-H\alpha}$ 1.5 Hz for both isomers), 5.9 (Hβ-*trans*, $J_{H\alpha-\beta}$ 14 Hz); extensive overlapping in *cis* isomer.

N-Vinyl-3-hydroxypyridine-2-thione 4c. M.p. 82–84 °C (EtOH: H_2O). Anal. C_7H_7NOS: C, H. ^{1}H NMR($CDCl$): δ 5.3 (J 8.5 Hz) and 5.5 (J 16 Hz) (CH_2, J_{gem} 2 Hz), 7.9 (CH), 6.6–7.0 and 7.5–7.8 (Pyr.). UV (EtOH, log ε): 378 (3.97), 274 (sh, 3.80), 261 (3.81) nm.

N-Vinyl-3-hydroxy-6-methylpyridine-2-thione 4d. M.p. 114–115 °C (EtOH: H_2O), Anal. C_8H_9NOS: C, H. ^{1}H NMR($CDCl_3$): δ 2.4 (Me), 5.3 (J 16 Hz) and 5.7 J 8 Hz) (CH_2, J_{gem} 1.5 Hz), 6.9 (CH), 6.5 and 6.9 (Pyr., AB, J 8 Hz). UV (EtOH, log ε): 367 (4.09), 275 (3.76) nm.

N-Propenyl-3-hydroxypyridine-2-thione 4e. The product ratio *trans:cis* was 2:1; (M.p. 88 °C) (EtOH: H_2O), Anal. C_8H_9NOS: C, H. ^{1}H NMR ($CDCl_3$): δ 1.7 (Me *cis*), 1.9 (Me *trans*), 5.9 (Hβ-*trans*, $J_{Me-H\beta}$ 7, $J_{H\alpha-\beta}$ 14 Hz), 7.5 (Hα-*trans*, $J_{Me-H\alpha}$ 1.5 Hz); extensive overlapping in *cis*-isomer. UV (EtOH, log ε): 372 (3.96), 277 (sh, 3.78), 260 (3.84) nm.

N-Vinyl-3-hydroxy-4-p-chlorophenyl-6-methylpyridine-2-thione 4f. M.p. 167 °C (MeOH: EtOAc). Anal. $C_{14}H_{12}ClNOS$: C, H. ^{1}H NMR ($CDCl_3$): δ 2.4 (Me), 5.7 (J 8 Hz) and 5.3 (J 16 Hz) (CH_2, J_{gem} 1.5 Hz), 6.8 (CH), 7.5 and 7.7 (Ph, J 8 Hz), 6.7 (Pyr.).

N-Vinyl-3-hydroxy-4,6-dibromopyridine-2-thione 4g. M.p. 134 °C (MeOH). Anal. $C_7H_5Br_2NOS$: C, H. ^{1}H NMR ($CDCl_3$): δ 5.7 (J 8 Hz) and 5.3 (J 16 Hz) (CH_2, J_{gem} 1.5 Hz), 6.7 (CH), 7.2 (Pyr.).

N-Propenyl-3-hydroxy-6-methylpyridine-2-thione 4h. The product ratio *trans:cis* was 1:1 (M.p. 78 °C) (EtOH: H_2O). ^{1}H NMR ($CDCl_3$): δ 1.5 and 2.0 (*Me*-CH), 2.3 and 2.35 (*Me*-Pyr.), 5.4–6.2 (H), 6.5–6.9 (Pyr.ad H). UV (EtOH, log ε): 370 (4.05), 275 (3.72) nm.

N-Vinyl-3-hydroxy-6-isopropylpyridine-2-thione 4i. M.p. 72 °C (EtOH: H_2O). Anal. $C_{10}H_{13}NOS$: C, H. ^{1}H NMR ($CDCl_3$): 1.2 and 3.3 (iPr), 5.3 (J 16 Hz) and 5.7 (J 8 Hz) (CH_2, J_{gem} 1 Hz), 6.8 (CH), 6.6 and 7.0 (Pyr., AB, J 8 Hz). UV (EtOH, log ε): 372 (4.08), 277 (3.70) nm.

5-Isopropyl-8-hydroxydihydrothiazolo[3,2-a]pyridinium bromide 2i. A solution of 3-carboxy-5-isopropyl-8-hydroxydihydrothiazolo[3,2-*a*]-pyridium bromide[3] (150 mg) in DMF (5 ml) and water (1 ml) was heated at 100 °C for 4 h. The solvent was then removed under reduced pressure and the residue triturated with ether. The product thus obtained was chromatographically homogeneous; yield 120 mg (92 %), m.p. 292 °C (decomp.) (iPrOH). Anal.: $C_{10}H_{14}NOS.HBr$: C, H. ^{1}H NMR (TFA), δ 1.4 and 3.3 (iPr), 3.8 (CH_2-S), 5.1 (CH_2-N) 7.2 and 7.7 (Pyr., J 8.5 Hz).

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N-Quaternary Compounds. Part XLVI.[1] Contrasting Regioselectivities in Bromination and Chlorination of the Dihydrothiazolo[3,2-*a*]pyridinium-8-olate Systems

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Simple pyridinium systems resist electrophilic substitution. A 6- or 8-amino substituent in dihydrothiazolo[3,2-*a*]pyridinium derivatives does not introduce sufficient activation for such substitution.[2] A hydroxy substituent in the 8-position, however, activates the system for ready substitution in the 7-position.[3] With a strong electron withdrawing substituent attached to the pyridine ring, the dihydrothiazolo[3,2-*a*]pyridinium ion undergoes electrophilic substitution as a pseudo-base in which case the new substituent enters a pyridine *beta* position, *viz.* at C-6 or at C-8.[2]

Without a 5-substituent in the 8-hydroxy derivative, which presumably reacts as the olate *1*, electrophilic substitution may occur in either the 5- or the 7-position and finally in both positions. Bromination with one equivalent of bromine reagent gave at room temperature mixtures of mono- and dibromo products. Preparatively, the dibromo derivative *4a* is available by the use of two equivalents of bromine in methanol. A 3-carboxy group does not interfere with the bromination at C-5 and *1b* gives *4b*. At −70 °C in methanol, however, the 7-bromo derivative *3a* was selectively formed on very slow addition of one equivalent of bromine. The assignment of the 7-bromo structure to the regioisomer formed, rests on the ^{1}H NMR coupling J 6.5 Hz which corresponds to coupling between vicinal α,β-protons in the pyridine ring. The monochloro derivative obtained by direct substitution, on the other hand, has coupling J 8.5 Hz which corresponds to coupling between vicinal β,γ-protons in pyridines, and hence this isomer is assigned the 5-chloro structure *6*. Further confirmation for the structural assignments follows from the nucleophilic displacement of the bromine in *3a* with chlorine, as discussed below, in which case the coupling J *6.5* Hz is less than for the monochloro product from the direct substitution, and hence the product after bromine displacement must be the 7-chloro derivative *9*.

Chlorinating agents such as *N*-chlorosuccinimide or chlorine led to oxidation of *1* and sulfoxide formation.[4] A good reagent for selective chlorination in the pyridine ring, however, was found to be sulfuryl chloride in dimethylformamide (DMF) at low temperature (−40 to −50 °C). Once the 5-chloro derivative *6* had been formed, further chlorination was difficult to effect without oxidation of the sulfur atom. Nitration of *6* in the 7-position, however, readily occurs using a combination of nitric acid and sulfuric acid. A nitro group situated in an activated position in an electron deficient system can be displaced by nucleophiles.[5] In this case it was found that the nucleophilic substitution to yield the dichloro derivative *8* could be achieved by heating *7* with zinc chloride in 3 N HCl. Alternatively, *8* can be obtained by nucleophilic displacement of both bromine atoms in *4a* with chlorine atoms by heating *4a* with sodium chloride in DMF. In a similar manner the 7-chloro derivative *9* was prepared from its bromo analogue *3a*.

The regioselective bromination in the 7-position is not unexpected in view of numerous literature reports which rationalise preferential vicinal substitution to a strong electron donating substituent, by initial coordination with the electrophile. Rationalisation of the regioselective chlorination in the 5-position, seems less obvious. The chlorine is introduced as an electrophile, and not as a nucleophile by addition to an intermediate pyridinium species, since no bromine was incorporated when the reaction was run in the presence of lithium bromide. The actual chlorinating reagent may well be a relatively bulky complex between sulfuryl chloride and DMF which may for steric reasons prefer the *para* position to the oxygen function.

Experimental. 7-Bromodihydrothiazolo[3,2-a]pyridinium-8-olate HBr-salt 3a. Bromine (4.8 g, 0.03 mol) in methanol (70 ml) at −70 °C was added dropwise over 10 h to a solution of dihydrothiazolo[3,2-*a*]pyridinium-8-olate HBr-salt (7.0 g, 0.03 mol) in methanol (600 ml) at −70 °C. The solution was then allowed to reach room temperature. The residual material after evaporation of the solvent was recrystallised thrice from small volumes of water; yield 3.0 g (32 %), m.p. 245 °C (decomp). Anal. $C_7H_6BrNOS.HBr$: C,H. ^{1}H NMR (60 MHz, TFA): δ 3.9 (CH_2-S), 5.2 (CH_2-S), 7.7 and 8.1 (Pyr., J 6.5 Hz).

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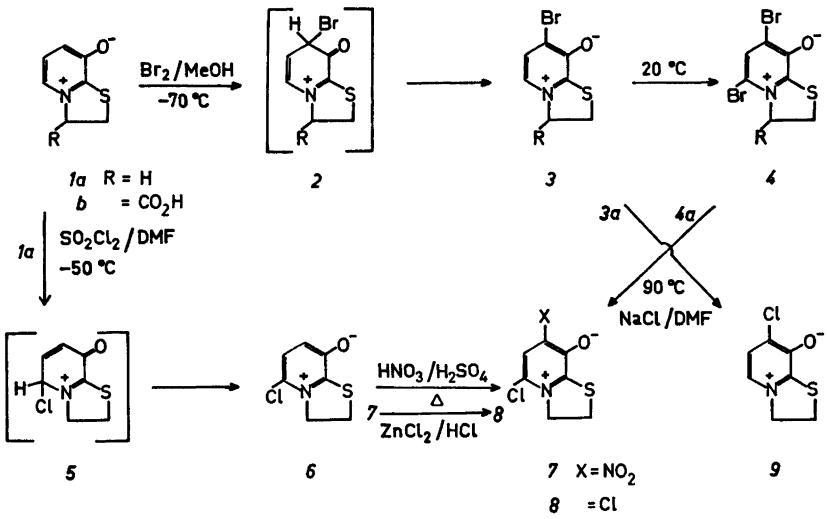
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Experimental. 7-Bromodihydrothiazolo[3,2-a]pyridinium-8-olate HBr-salt 3a. Bromine (4.8 g, 0.03 mol) in methanol (70 ml) at −70 °C was added dropwise over 10 h to a solution of dihydrothiazolo[3,2-*a*]pyridinium-8-olate HBr-salt (7.0 g, 0.03 mol) in methanol (600 ml) at −70 °C. The solution was then allowed to reach room temperature. The residual material after evaporation of the solvent was recrystallised thrice from small volumes of water; yield 3.0 g (32 %), m.p. 245 °C (decomp). Anal. $C_7H_6BrNOS.HBr$: C,H. ^{1}H NMR (60 MHz, TFA): δ 3.9 (CH_2-S), 5.2 (CH_2-S), 7.7 and 8.1 (Pyr., J 6.5 Hz).



5,7-Dibromodihydrothiazolo[3,2-a]pyridinium-8-olate HBr-salt 4a. Bromine (16.0 g, 0.10 mol) in methanol (100 ml) was added dropwise at room temperature to a solution of dihydrothiazolo[3,2-*a*]pyridinium-8-olate HBr-salt (11.9 g, 0.05 mol) in methanol (200 ml). The precipitated product was collected the next day; yield 12.0 g (66 %), m.p. 280 °C (decomp.) (aq. HBr). Anal. $C_7H_5Br_2NOS.HBr$: C, H. ^1H NMR (60 MHz, TFA): δ 4.0 (CH_2-S), 5.4 (CH_2-N), 8.0 (H-6).

5,7-Dibromo-8-hydroxydihydrothiazolo[3,2-a]pyridinium-3-carboxylate 4b. Bromine (5.6 g, 0.04 mol) was added dropwise to a solution of 8-hydroxydihydrothiazolo[3,2-*a*]pyridinium-3-carboxylate (4.2 g, 0.015 mol) in water (300 ml). The decolourised solution was concentrated to a small volume and the solid precipitate recrystallised from water; yield 2.5 g (47 %), m.p. 220 °C (decomp.). Anal. $C_8H_5Br_2NO_3S$: C, H. ^1H NMR (60 MHz, TFA): δ 4.3 (CH_2, m.), 6.5 (H-3), 8.0 (H-6).

5-Chlorodihydrothiazolo[3,2-a]pyridinium-8-olate HCl-salt 6. Dihydrothiazolo[3,2-a]pyridinium-8-olate (7.6 g, 0.05 mol) was dissolved in DMF (500 ml) and the solution cooled to −50 °C. Sulfuryl chloride (6 ml, 0.07 mol) was then added dropwise to this solution at −40 to −50 °C with stirring. When the addition was completed, the reaction mixture was slowly allowed to reach room temperature. The reaction mixture was then evaporated at reduced pressure and the residue triturated with a little ethanol before recrystallisation from a small volume of water; yield 7.4 g (75 %), m.p. 270 °C (decomp). Anal. $C_7H_6ClNOS.HCl$: C, H. ^1H NMR (60 MHz, TFA): δ 3.9 (CH_2-S), 5.3 (CH_2-N)7.4 and 7.7 (Pyr., J 8.5 Hz).

The betaine can be obtained by passage of an aqueous solution over a strong cation exchanger (Amberlite. IR-120, H^+), washing with water until the washings no longer contain Cl^- and finally elution with 0.6 N aq. NH_3.

5-Chloro-7-nitrodihydrothiazolo[3,2-a]pyridinium-8-olate 7. 5-Chlorodihydrothiazolo[3,2-*a*]-pyridinium-8-olate (5.9 g, 0.03 mol) was dissolved in acetic acid (100 ml) and a solution of fuming nitric acid (5 ml) and concentrated sulfuric acid (4 ml) in acetic acid (50 ml) added dropwise with stirring at room temperature. The reddish precipitate of the title compound was collected and recrystallised from ethanol; yield 4.3 g (60 %), m.p. 180 – 190 °C (decomp.). Anal. $C_7H_5ClN_2O_3S$: C, H. ^1H NMR (60 MHz, TFA): δ 4.1 (CH_2-S), 5.5 (CH_2-N), 1.9 (H-6).

5,7-Dichlorodihydrothiazolo[3,2-a]pyridinium-8-olate 8. (a) 5,7-Dibromodihydrothiazolo[3,2-*a*]pyridinium-8-olate HBr-salt in DMF was heated as below with sodium chloride at 90 °C for 4 h and the product worked up in the same way; yield 55 %, m.p. 275 °C (decomp.) (H_2O). Anal. for HBr-salt $C_7H_5Cl_2NOS.HBr$: C, H. ^1H NMR (60 MHz, TFA): δ 4.0 (CH_2-S), 5.3 (CH_2-N), 7.6 (H-6).

(b) A solution of 5-chloro-7-nitrodihydrothiazolo[3,2-*a*]pyridinium-8-olate (1.1 g, 0.005 mol) and zinc chloride (2.0 g) in 3 N HCl (50 ml) was heated under reflux for 3 days. The solution was then evaporated, the residue dissolved in water and the solution passed through a cation exchange column (IR-120 Amberlite, H^+). Elution with water removed the chloride ions before the title compound was eluated with 0.6 N aq. NH_3. Evaporation of the NH_3-eluates left the betaine; yield 78 %, m.p. 220 – 222 °C (decomp.).

7-Chlorodihydrothiazolo[3,2-a]pyridinium-8-olate HBr-salt 9. A solution of 7-bromodihydrothiazolo[3,2-*a*]pyridinium-8-olate as HBr-salt (2.0 g, 0.006 mol) in DMF (175 ml) at 90 °C was saturated with sodium chloride and kept

at this temperature for 4 h. The reaction mixture was then allowed to reach room temperature and the precipitated salt removed before the filtrate was evaporated to dryness. The residue was triturated with ether and recrystallised from a small volume of water; yield 0.9 g (56 %), m.p. >270 °C (decomp.). Anal. $C_7H_8ClNOS.HBr$: C, H. ^1H NMR (60 MHz, TFA): δ 3.9 (CH_2-S), 5.2 (CH_2-N), 7.6 and 8.2 (Pyr., J 6.5 Hz).

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Isoabienol, the Principal Diterpene Alcohol in *Pinus sylvestris* Needles

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The abienols, labdane diterpenoids with conjugated double bonds in the side chain, are widely distributed in the firs. To our knowledge there is only one isolation of isoabienol reported, namely from the oleoresin of *Abies sibirica* Ldb. (Siberian fir).[1] However, it has later been shown, that this natural product was actually impure *trans*-abienol.[2]

The present paper reports the finding of isoabienol as the principal diterpene alcohol in needles of *Pinus sylvestris* L. (Scots pine). In an earlier paper the major diterpene alcohol from technical foliage of Scots pine has erroneously been characterized as abienol.[3]

The isolated compound had m.p. 68 – 68.5 °C, $[\alpha]_D^{20}$ −6.4 ($CHCl_3$), λ_{max}(EtOH) 227 nm (log ε 4.26) ν_{max}(KBr) 3320, 2920, 1600, 1463, 1390, 890 cm^{-1}.

Hydrogenation yielded a product identical with tetrahydroabienol (a quasiracemic compound [4]) obtained from the hydrogenation of *cis*-abienol from the oleoresin of Norway spruce, (^{13}C NMR, MS, GLC), suggesting a normal labdane skeleton.

The mass spectrum of compound *1* [IP 70 eV; *m/e* (rel. int.)]: 290 (2, M), 275 (3, M − CH_3), 272 (5, M − H_2O), 257 (13, M − H_2O − CH_3), 191 (100, M − H_2O − side chain) indicated the presence of a bis-unsaturated side chain and the λ_{max} value led us to suggest conjugation.

The ^1H NMR spectrum showed signals from five vinylic protons. Three of the protons formed an AMX-system at 270 MHz ($CDCl_3$): δ 6.38 (H-14, dd, J 10.6 and 17.6 Hz), 5.34 (H-15, dd, J 17.6 and 0.7 Hz), 5.09 (H-15, dd, J 10.6 and 0.7 Hz). The two vinylic protons at C-16 gave a rather broad singlet at δ 5.05. Three separate methyl singlets were shown, one from the C-18 and C-19 methyls at δ 0.80, one from the C-17 methyl at δ 1.16 and one from the C-20 methyl at δ 0.88. The allylic protons at C-12 gave a symmetrical multiplet centered at δ 2.32.

The low field part of the noise decoupled ^{13}C NMR spectrum ($CDCl_3$) showed four signals due to the olefinic carbons in the side chain. Two of the signals appeared as triplets in the off-resonance decoupled spectrum confirming the presence of two terminal vinylic methylene groups. In analogy to the spectrum of myrcene,[6] the triplet at δ 113.5 was assigned to C-16 and the other one at δ 115.6 to C-15. In the off-resonance decoupled spectrum the C-14 signal

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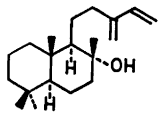
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at δ 138.8 and C-13 at δ 147.5 appeared as a doublet and singlet, respectively.

The above analytical and spectroscopic data for the compound are consistent with the structure *1*.



1

The melting point and UV and MS data were in close agreement with those reported for isoabienol synthesized from sclareol.[3,5]

Experimental. The gas chromatographic analysis was performed with a Varian 2400 gas chromatograph equipped with a packed glass column (1 % XE-60, 110 – 180 °C, 4 °C/min). Mass spectra were recorded with a GLC-MS instrument model LKB-9000 and the same column and conditions as above. IR spectra were obtained with a Perkin-Elmer model 257 spectrometer. ^{1}H NMR spectra were recorded on a Bruker WH 270 spectrometer operating at 270 MHz and ^{13}C NMR spectra on a JEOL FX-60 spectrometer at 15.03 MHz. TLC separations were carried out on precoated plates with silica as adsorbent. Petroleum ether:Et_2O:AcOH (50:50:1, v/v) was used as solvent.

Extraction and isolation. Fresh pine needles were defiberized in liquid N_2 with an Ultra-Turrax atomizer. The ground and freeze-dried material (50 g) was extracted in a Soxhlet apparatus with light petroleum (40 – 60 °C) for 5 h. The extract was treated with aqueous $NaHCO_3$ (5 %) to remove free fatty and resin acids. The solvent was evaporated from the neutral fraction at reduced pressure and the residue was dissolved in a minimum amount of Et_2O. The neutrals were quantitatively applicated on TLC silica layers and a main fraction separated at R_F 0.53 – 0.56. The diterpene alcohol was eluted from the silica and recrystallized 3 times from pentane. The yield was 370 mg.

GLC analysis of total neutrals gave an isoabienol content of 0.8 % of the needle dry weight and about 90 % of the diterpene alcohols.

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