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EFFECTS OF SELECTED REACTION CONDITIONS AND STRUCTURAL VARIATIONS ON THE DISTRIBUTION OF 2-(DIETHYLAMINO)ETHYL GROUPS ON D-GLUCOPYRANOSYL RESIDUES OF 2-(DIETHYLAMINO)-ETHYLATED CELLULOSE, STARCH, AND THE ANOMERS OF METHYL 4,6-*O*-BENZYLIDENE-D-GLUCOPYRANOSIDE

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INTRODUCTION

Our ability to control the disposition of substituents and crosslinks in substituted cotton is dependent on a detailed understanding of the chemical and physical aspects of the chemical reactions involved. The reactivities of the C-2, C-3, and C-6 hydroxyl groups of the D-glucopyranosyl residue are clearly dependent on the class and specific nature of the attacking reagent: the size of the reagent molecule may be a significant factor; the conditions of reaction employed are expected to be important; and the glucosidic structure appears to play a role. The present study attempts to assess some of these factors in a preliminary way.

The reactions of cellulose for which the reactivities of the hydroxyl groups have been studied fall into two classes: equilibrium-controlled reactions (*e.g.*, Michael addition reactions) and rate-controlled reactions (*e.g.*, Williamson syntheses). In general, reaction at the 2-hydroxyl group in cellulose predominates (or is essentially equivalent to that at the 6-hydroxyl group) in the Williamson reactions[1-6], whereas reaction at the 6-hydroxyl group predominates in the Michael reactions[7,8]. The predominance of reaction at the 2-hydroxyl group in Williamson reactions has been attributed to the acidic nature of this hydroxyl group[3,4,9], and the low reactivity at the 3-hydroxyl group has been considered to be a consequence of hydrogen bonding between this hydroxyl group and the ring-oxygen atom of a neighboring D-glucopyranosyl residue[10,11].

Wolfrom and co-workers reported the preparation of the sodio derivatives of methyl α-D-glucopyranoside and of regenerated cellulose, in each of which the sodium was attached almost exclusively at the 2-hydroxyl group; this was determined by reacting the sodio derivative with methyl iodide, followed by characterization of the methylated product[4,9]. Later, Lenz[3] repeated this work and found, by the use of more-modern techniques, that considerable reaction also occurs at the 3- and 6-hydroxyl groups.

Lenz[3] has pointed out that the preferential reaction at the 2-hydroxyl group decreases as the size of the reacting molecule increases. This consideration suggests

that steric factors play an important role in these reactions, tritylation and *p*-toluenesulfonation being the extreme examples[5].

Croon and Lindberg[2] found that reaction occurs predominantly at the 2-hydroxyl group when cotton cellulose is methylated with methyl sulfate and 18.5% sodium hydroxide solution, 35% sodium hydroxide solution, or a quaternary ammonium hydroxide solution. The relative reaction-rates for the hydroxyl groups on C-2, C-3, and C-6 are about the same under the three conditions. These authors concluded that the distribution of substituents is determined only by (*a*) the relative rate-constants and (*b*) differences in accessibility of the hydroxyl groups within the sample.

Starch has also been found to react preferentially at the 2-hydroxyl group in some Williamson reactions[5,12]. However, the reactivity of the 2-hydroxyl group in starch has been reported to be somewhat lower, and more nearly equal to that of the 6-hydroxyl group, than for the corresponding reaction in cellulose. Croon[13] found that methylation of amylose and amylopectin with methyl sulfate and 18.9% sodium hydroxide solution yields a partially methylated starch in which reaction at the 6-hydroxyl group exceeds that at the 2-hydroxyl group. Some reaction occurs at the 3-hydroxyl group, but less than that obtained with cellulose under the same conditions.

Doane and co-workers[14] found that methylation of starch with methyl iodide in liquid ammonia results in 2-*O*-methyl-D-glucose as the preponderant monomethyl ether in the hydrolyzate of a preparation having a degree of substitution (D. S.) of 0.34. The 6- and 2-methyl ethers were found in about equal proportions in the hydrolyzate from a preparation having a D. S. of 0.90, and no 3-methyl ether was present in either case. However, significant proportions of 2,3- and 3,6-di-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose were present in the hydrolyzate of the *O*-methylstarch, suggesting that methylation at the 2- or 6-hydroxyl groups, or both, enhances the reactivity at the 3-hydroxyl group. A similar observation was made by Croon[13], who suggested that the reactivity at the 3-hydroxyl group is doubled when the 2-hydroxyl group is methylated.

Reaction at the 2-hydroxyl group in methyl 4,6-*O*-benzylidene-α-D-glucopyranoside exceeds that at the 3-hydroxyl group in most of the reactions thus far reported[15,16]. Willard and co-workers[15] found that one mole of (benzylthio)carbonyl chloride reacts with one mole of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside in dry pyridine to yield preponderantly the 2-*O* derivative, and a small proportion of disubstituted derivative; on the other hand, methyl 4,6-*O*-benzylidene-β-D-glucopyranoside[17], under the same conditions, yields an exceptionally large proportion of disubstituted derivative, together with a large proportion of starting material and a small proportion of monosubstituted product. Croon[13] found that methyl 4,6-*O*-benzylidene-β-D-glucopyranoside reacts with methyl sulfate in the presence of an excess of sodium hydroxide to yield equal amounts of the 2- and 3-methyl ethers, together with some of the 2,3-dimethyl ether.

Most of the studies thus far reported on the relative reactivities of the hydroxyl groups of carbohydrates have been based on reactions conducted in aqueous media, without particular attention having been paid to the effect of the reaction conditions

on the distribution of substitutents in a specific carbohydrate, or with no intent to compare the behavior of different carbohydrates under identical conditions of reaction. The present paper presents the results of a study of the reactivities of the hydroxyl groups in cellulose, starch, and the anomers of methyl 4,6-*O*-benzylidene-D-glucopyranoside with 2-(diethylamino)ethyl chloride under identical conditions, namely, in (*a*) aqueous and (*b*) nonaqueous medium.

EXPERIMENTAL

Gas–liquid chromatography. — The gas–liquid chromatograms were obtained with an F & M model 1609 instrument* equipped with a hydrogen-flame ionization detector. The column was based on a solid phase of acid-washed, dichlorodimethylsilane-treated Chromsorb W (80–100 mesh) from Johns–Manville Corporation, and a liquid phase consisting of XE-60 from Wilkens Instrument and Research, Incorporated. The column was prepared in a 2-ft tube, 1/4-in O. D.; it carried a liquid phase of 8.5%, and was operated isothermally at 140°. It had previously been shown that[6], for *O*-[2-(diethylamino)ethyl]-D-glucoses, separations were achieved on the XE-60 column, and the results were confirmed by those obtained on an SE-52 column. For this reason, only the XE-60 column was employed in the work reported here.

Determination of the relative distribution of 2-(diethylamino)ethyl groups in the monosubstituted D-*glucose fraction.* — The 2-*O*-, 3-*O*-, and 6-*O*-[2-(diethylamino)ethyl]-D-glucoses were identified by chromatographic comparison with authentic, known derivatives, and the distribution of 2-(diethylamino)ethyl groups among the 2-, 3-, and 6-hydroxyl groups was determined by gas–liquid chromatography as previously described by Roberts and Rowland[6]. Methyl α-D-glucopyranoside was added to each sample as an internal standard, and all samples were equilibrated overnight in dry pyridine before conversion into the trimethylsilyl ethers[18]. It was found necessary to remove most of the D-glucose from the hydrolyzates of *O*-[2-(diethylamino)ethyl]-starch by fermentation with yeast prior to gas–liquid chromatography of the D-glucose derivatives.

Quantitative determination of D-*glucose and mono-, di,- and tri-substituted* D-*glucoses.* — Paper electrophoresis in 0.75M formic acid at 75 volts per cm on Whatman 3MM paper proved to be effective for the separation of D-glucose and the mono-, di-, and tri-*O*-[2-(diethylamino)ethyl]-D-glucoses. The individual isomeric mono-*O*-[2-(diethylamino)ethyl]-D-glucoses are not separated by electrophoresis in this system; this fraction of the hydrolyzed 2-(diethylamino)ethyl carbohydrates was identified by electrophoretic comparison with authentic, known derivatives. The disubstituted fraction was identified by electrophoretic comparison with the fastest moving component of the hydrolyzate from the reaction product of 2-(diethylamino)ethyl chloride with

*Mention of a company and/or product by the U. S. Department of Agriculture does not imply approval or recommendation of the company or product to the exclusion of others which may also be suitable.

methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, as only the mono- and di-substituted products are possible in this case. The cotton of high D.S. was the only sample that contained a third component (other than D-glucose). This component had a mobility greater than that of the disubstituted derivatives, and was assumed to be the tri-substituted derivative because electrophoretic mobility depends largely upon ion formation in the derivatives. The mobilities of the mono-, di-, and tri-substituted derivatives, relative to D-glucose (1.0), were found to be 4.6, 7.0, and 9.3, respectively.

Each component separated by paper electrophoresis was located by means of guide strips cut from each edge of the paper, and each section of the paper containing a component was cut out and eluted with water. The D-glucose was determined colorimetrically by the phenol–sulfuric acid method of Smith and co-workers[19], and the D-glucose derivatives were determined by the hypoiodite oxidation method of Hirst and co-workers[20]. The 2-(diethylamino)ethyl derivatives were in the form of the formic acid salts; because formic acid is oxidized by hypoiodite, this fact was taken into consideration in calculating the results. The trisubstituted D-glucose from the *O*-[2-(diethylamino)ethyl]cellulose sample of D.S. 0.64 contained an interfering substance, and could not be determined directly; however, the proportion of this derivative was calculated from the nitrogen not accounted for by the mono- and di-substituted derivatives. The disubstituted derivatives from the other preparations also contained an interfering substance; the proportions of these derivatives could be calculated from the nitrogen not accounted for by the monosubstituted derivatives, as there were no trisubstituted derivatives present.

Preparation of O-[*2-(diethylamino)ethyl*]*cellulose* (**1**) *of high D.S. in aqueous system.* — Cotton linters were crosslinked with formaldehyde by the Form W process[21] to a formaldehyde content of 1.18%, in order to prevent dissolution of the cotton during the repeated treatments to be described[22]. The crosslinked linters (180 g, 1.11 mole of D-glucopyranose residues) were stirred into 600 ml of 20% sodium hydroxide solution, and the suspension was cooled in an ice bath for 30 min. A solution of 2-(diethylamino)ethyl chloride hydrochloride (105 g, 0.61 mole) in 135 ml of water was added with stirring, and the mixture was heated to 85° and stirred for 50 min at this temperature. The mixture was cooled, 1 liter of 2N sodium chloride was added, and the linters were collected on a filter and washed with N sodium hydroxide solution until the filtrate was colorless. The solid material was then alternately washed (twice) with N hydrochloric acid and 1N sodium hydroxide solution, and finally with water, and dried at 85°. The nitrogen content of the product was 1.53%, and the formaldehyde content was 0.95%. A second treatment of the linters in the same way yielded a product having a nitrogen content of 2.77% and a formaldehyde content of 0.80%. A third treatment afforded the final product (**1**), having a nitrogen content of 4.1%, a formaldehyde content of 0.70%, and D.S. 0.64.

Preparation of O-[*2-(diethylamino)ethyl*]*cellulose* (**2**) *of low D.S. in aqueous system.* — A swatch of de-sized, scoured, and bleached print-cloth (19×26 in) weighing 34 g (0.24 mole of D-glucopyranose residue) was immersed in, and squeezed through rollers (2 cycles) to 100% wet pickup (0.04 mole), in 100 g of a 20% aqueous

solution of 2-(diethylamino)ethyl chloride hydrochloride. The treated fabric was put onto a pin frame (16.5 × 22.5 in) and immersed for 3 min in 4 liters on a 20% solution of sodium hydroxide saturated with sodium sulfate. The wet sample on the frame was kept in a polyethylene bag for 19 h; it was subsequently rinsed with water, soaked for 15 min in N acetic acid, and then rinsed with water. The nitrogen content of the product (**2**) was 0.39%, corresponding to a D.S. of 0.048.

Preparation of O-[2-(*diethylamino*)*ethyl*]*cellulose* (**3**) *in* p-*dioxane system.* — Twenty grams (0.12 mole of D-glucopyranose residue) of de-sized, scoured, and bleached print-cloth was cut into 0.25-in squares, and swollen by boiling in water for 30 min. It was lyophilized and, immediately after being removed from the freeze-dryer, was covered with dry *p*-dioxane; 20 g of powdered potassium hydroxide and 22 g (0.13 mole) of 2-(diethylamino)ethyl chloride hydrochloride were added, and the mixture was refluxed with stirring for 3 h. The fabric was removed by filtration with suction, and the alkali and salts were washed out with water. The fabric was dried for 2 h at 110°, and equilibrated in the air for 16 h at room temperature. The product (**3**) weighed 19.1 g, and had a nitrogen content of 0.22%, indicating a D.S. of 0.026.

Hydrolysis of O-[2-(*diethylamino*)*ethyl*]*celluloses.* — A 5-g sample of each of the three *O*-[2-(diethylamino)ethyl]cellulose preparations (**1**, **2**, and **3**) was hydrolyzed with sulfuric acid by the procedure described by Rowland and co-workers[7], and the solutions were lyophilized. Each product was an amorphous solid; sample number, wt. (g), and nitrogen content: **1**, 4.55, 4.1%; **2**, 4.8, 0.38%; **3**, 4.8, 0.20%.

Preparation of O-[2-(*diethylamino*)*ethyl*]*starch* (**4**) *in aqueous system.* — To 50 g (0.3 mole of D-glucopyranosyl residue) of cornstarch was added 50 g of a 20% aqueous solution of 2-(diethylamino)ethyl chloride hydrochloride (0.06 mole). The mixture formed a paste that was slowly added, with rapid stirring, to 500 ml of an aqueous solution of sodium hydroxide (20%). After all of the lumps had been broken up, the mixture was kept for 19 h at room temperature. It was then poured into an equal volume of ethanol. The solid material was removed by filtration with suction, and washed on the filter with 500 ml of 50% ethanol. The product was suspended in 200 ml of water, and dialyzed for 48 h against a stream of water saturated with toluene (to remove salts); the resulting suspension was lyophilized, yielding 41 g of product (**4**) having a nitrogen content of 0.67%, corresponding to a D. S. of 0.083.

Preparation of O-[2-(*diethylamino*)*ethyl*]*starch* (**5**) *in* p-*dioxane system.* — Twenty grams (0.12 mole of D-glucopyranosyl residue) of cornstarch, predried for 16 h at 110°, was suspended in 250 ml of dry *p*-dioxane; 20 g of powdered potassium hydroxide and 22 g (0.13 mole) of 2-(diethylamino)ethyl chloride hydrochloride were added, and the mixture was stirred for 3 h under reflux. The mixture was cooled, and the solid material was removed by filtration with suction, and washed on the filter with 500 ml of 50% ethanol. The solid material was suspended in 200 ml of water, and dialyzed for 48 h against water saturated with toluene (to remove salts). Some of the water remaining was removed in a rotary evaporator, and the suspension was lyophilized, yielding 17.5 g of an amorphous solid (**5**) containing 0.20% of nitrogen, corresponding to a D.S. of 0.024.

Hydrolysis of O-[*2-(diethylamino)ethyl*]*starches.* — Five grams of each *O*-[2-(diethylamino)ethyl]starch preparation (**4** and **5**) was hydrolyzed as described for the hydrolysis of the similar cellulose derivatives. Each product was an amorphous solid; sample number, wt. (g), and nitrogen content: **4**, 4.2, 0.68%; **5**, 4.8, 0.20%.

*Reaction of methyl 4,6-*O*-benzylidene-α-*D*-glucopyranoside with 2-(diethylamino)-ethyl chloride in an aqueous system.* — To 14 g (0.049 mole) of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, prepared by the method of Freudenberg and co-workers[23], in 250 ml of 20% aqueous sodium hydroxide was added 9 g (0.052 mole) of 2-(diethylamino)ethyl chloride hydrochloride. The mixture was shaken for 16 h at room temperature, and the solid material was removed by filtration, and washed lightly with water. The filtrate and washings were combined, and extracted with two 100-ml portions of chloroform. The extracts were combined, dried (sodium sulfate), and evaporated to dryness, and the residue combined with the material collected on the filter; the composite was hydrolyzed by refluxing for 16 h in 100 ml of 2N sulfuric acid. The solution was cooled, and washed with two 100-ml portions of ether (to remove benzaldehyde and benzoic acid). The sulfuric acid was neutralized by adding barium hydroxide to slight alkalinity, the excess barium ions were removed with carbon dioxide, and the suspension was filtered; the filtrate was concentrated, and lyophilized, yielding 10.3 g of an amorphous, hygroscopic solid. This product (**6**) contained some sodium carbonate resulting from a small amount of sodium hydroxide that had remained in the reaction mixture prior to hydrolysis. The nitrogen content was 2.35% (corrected for the carbonate), corresponding to a D.S. of 0.36.

*Reaction of methyl 4,6-*O*-benzylidene-α-*D*-glucopyranoside with 2-(diethylamino)-ethyl chloride in a p-dioxane system.* To 14.0 g (0.049 mole) of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside in 250 ml of dry *p*-dioxane was added 14 g of powdered potassium hydroxide and 9.0 g (0.052 mole) of 2-(diethylamino)ethyl chloride hydrochloride. The mixture was stirred for 3 h under reflux, and the solid material was removed by filtration with suction, and washed on the filter with *p*-dioxane. The filtrate and washings were combined, and evaporated under diminished pressure to a syrup that was dissolved in 250 ml of ether. The solution was washed 3 times with water, evaporated under diminished pressure to a syrup, and the syrup hydrolyzed by refluxing it for 16 h in 100 ml of 2N sulfuric acid. The solution was cooled, washed with two 100-ml portions of ether (to remove benzaldehyde and benzoic acid), and the sulfuric acid was neutralized by adding barium hydroxide to slight alkalinity; the excess barium ions were removed with carbon dioxide, the suspension was filtered, and the filtrate was concentrated and lyophilized, yielding 10.0 g of amorphous, hygroscopic solid (**7**) containing 4.99% of nitrogen, corresponding to a D.S. of 0.88.

*Reaction of methyl 4,6-*O*-benzylidene-β-*D*-glucopyranoside with 2-(diethylamino)ethyl chloride in an aqueous system.* — Seven grams (0.025 mole) of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside, prepared by the method of Freudenberg and co-workers[23], in 175 ml of 20% sodium hydroxide solution was treated with 4.5 g (0.027 mole) of 2-(diethylamino)ethyl chloride hydrochloride in exactly the same way as described for the α-D anomer (**6**). On hydrolysis, there was obtained 4.2 g of an

amorphous, hygroscopic solid (**8**). The nitrogen content was 3.62% (corrected for the carbonate), corresponding to a D.S. of 0.62.

Reaction of methyl 4,6-O-benzylidene-β-D-glucopyranoside with 2-(diethylamino)-ethyl chloride in p-dioxane system. — Five grams (0.018 mole) of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside was treated with 3.1 g (0.019 mole) of 2-(diethylamino)ethyl chloride hydrochloride in exactly the same way as described for the α-D anomer (**5**). On hydrolysis, there was obtained 4.8 g of amorphous, hygroscopic solid (**9**) containing 4.44% of nitrogen, corresponding to a D.S. of 0.75.

RESULTS AND DISCUSSION

In a preceding paper[6], we have delineated the distribution of 2-(diethylamino)-ethyl substituents on the D-glucopyranosyl residues of 2-(diethylamino)ethylated cotton cellulose to be at the 2-, 3-, and 6-hydroxyl groups in the proportions of 1.27:0.35:1.00, and we noted the decrease in the ratio of substituents at the 2- and 3-hydroxyl groups, relative to that at the 6-hydroxyl group, with progressive increase in the molecular size of the reagent from methyl chloride (giving the proportions 2.5:0.5:1.0) to ethyl chloride (1.75:0.5:1.0) to 2-(diethylamino)ethyl chloride. This trend suggests that steric factors may exert a considerable influence on the distribution of substituents on the D-glucopyranosyl residues; we recognize, however, that subtle differences in (*a*) solubilities of reagents, (*b*) the processes of reaction, (*c*) the structures of the transition states, etc., may be responsible for changes in the distribution of the substituents. It is evident that information is needed regarding the effect, on the distribution of substituents, of reaction conditions and steric factors.

Process. — We have chosen to explore the effect of reaction conditions in terms of the effect of the medium in which the reaction is conducted: on the one hand, we have carried out the reaction in an aqueous medium in a manner generally conventional for etherification of cotton cellulose; and, on the other hand, we have employed *p*-dioxane as a nonaqueous medium having low dielectric properties. Examination of the effect of the process was considerably simplified by the initial observation that the relative distribution of substituents at the 2-, 3-, and 6-hydroxyl groups is independent of the D.S., as indicated by the fact that multiple treatments of cotton with 2-(diethylamino)ethyl chloride resulted in the same distribution in the mono-*O*-[2-diethylamino)-ethyl]-D-glucopyranose fraction as was noted for the once-treated cotton (see Table I). Thus, it is reasonable to compare the distributions of substituents in various etherified carbohydrates at significantly different levels of D.S.

Cotton cellulose treated with 2-(diethylamino)ethyl chloride in *p*-dioxane proved to have substantially higher ratios of substituents on the hydroxyl groups at C-2 and C-3, relative to that at C-6, than resulted when the reaction was conducted in an aqueous system (see Table I). An examination of the results of this reaction on the basis of the fraction of substituents in each of the three positions (see Table II) shows that the proportion of substituents increases at the 2-hydroxyl group, decreases at the 6-hydroxyl group, and remains essentially unchanged at the 3-hydroxyl group. Al-

TABLE I

DISTRIBUTION OF SUBSTITUENTS AS A FUNCTION OF THE REACTION CONDITIONS AND THE STRUCTURE OF D-GLUCOPYRANOSYL RESIDUES

	Cotton			*Starch*		*Methyl 4,6-O-benzylidene-D-glucopyranoside*			
	Linters	*Fabric*				*alpha-*		*beta-*	
	Aqueous	*Aqueous*	*p-Dioxane*	*Aqueous*	*p-Dioxane*	*Aqueous*	*p-Dioxane*	*Aqueous*	*p-Dioxane*
% N	4.10	0.39	0.22	0.67	0.20	2.35	4.99	3.62	4.44
D. S.	0.64	0.048	0.026	0.083	0.024	0.36	0.88	0.62	0.83
	Relative distribution of DEAE groups in monosubstituted D-glucose fraction[a]								
O-2	1.25	1.27	2.50	1.08	3.00	1.00	1.00	1.00	1.00
O-3	0.35	0.35	0.50	0.45	1.50	2.00	12.00	1.60	2.00
O-6	1.00	1.00	1.00	1.00	1.00	—	—	—	—
	Distribution of DEAE groups among the D-glucopyranosyl residues[b]								
C_0	0.320	0.910	0.944	0.856	0.932	0.595	0.071	0.468	0.215
C_1	0.296	0.073	0.033	0.136	0.035	0.185	0.715	0.195	0.428
C_2	0.173	0.007	0.007	trace	0.003	0.195	0.193	0.358	0.312
C_3	0.167	—	—	—	—	—	—	—	—
Σ[c]	0.956	0.990	0.984	0.992	0.970	0.975	0.984	1.021	0.955

[a] O-2, O-3, and O-6 denote the specific oxygen atoms to which the 2-(diethylamino)ethyl (DEAE) substituents are attached.
[b] C_0 denotes the weight fraction of D-glucose; C_1, C_2, and C_3 denote the weight fractions of mono-, di-, and tri-substituted D-glucoses, respectively.
[c] Σ denotes the sum of $C_0+C_1+C_2+C_3$, and the deviation from unity indicates the overall accuracy.

though the reaction of cotton in *p*-dioxane proceeded to a lower D.S., as might be expected from a lower rate of reaction (and, perhaps, a lower accessibility), the fraction of disubstituted D-glucopyranosyl residues was very substantially higher than that resulting from reaction in the aqueous system (see Table II).

Starch follows a similar pattern of changes on alteration of the conditions of reaction from aqueous to nonaqueous. The increases in substitution on the 2- and 3-hydroxyl groups, relative to the 6-hydroxyl group, are even more pronounced in this case (see Table I). The fraction of substituents on the 2-hydroxyl groups increases as a result of reaction in *p*-dioxane, the increase being remarkably similar, in terms of percentage, to that noted for cellulose (see Table II). The decrease in the fraction of substituents at the 6-hydroxyl group is substantially larger than that for cellulose; this decrease is in proportion to the significant increase in substitution at the 3-hydroxyl groups of starch occurring on reaction in *p*-dioxane. Again, reaction in the organic medium results in a product having a lower D.S. but a higher fraction of disubstituted D-glucopyranosyl residues.

Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside, which bears a resemblance to

cellulose as regards the D-glucosidic linkage, shows only a slight change in the distribution of the substituents at the 2- and 3-hydroxyl groups for reactions in aqueous as against nonaqueous media; in this respect, it behaves similarly to cotton, although the small changes that were observed were in opposite directions. Although the increase in D.S. effected by reaction in *p*-dioxane might be attributable to the higher solubility of the compound in this medium, the considerably lower proportion of disubstitution that accompanied the higher D.S. is seen to be opposite to the results already discussed for cellulose and starch.

TABLE II

DISTRIBUTION OF 2-(DIETHYLAMINO)ETHYL SUBSTITUENTS IN VARIOUS *O*-2-(DIETHYLAMINO)ETHYLATED CARBOHYDRATES

Reaction medium	*Fraction of isomeric components in monosubstituted* D-*glucopyranosyl residues*			*Fraction of disubstituted* D-*glucopyranosyl residues*
	O-2[a]	*O-3*[a]	*O-6*[a]	$C_2/(C_1+C_2)$[b]
		Cotton		
Aqueous	0.49	0.13	0.38	0.08
p-Dioxane	0.63	0.12	0.25	0.17
		Starch		
Aqueous	0.43	0.18	0.39	0.00
p-Dioxane	0.55	0.27	0.18	0.08
	Methyl 4,6-*O*-benzylidene-D-glucopyranoside			
		beta		
Aqueous	0.38	0.62	—	0.65
p-Dioxane	0.33	0.67	—	0.42
		alpha		
Aqueous	0.33	0.67	—	0.51
p-Dioxane	0.07	0.93	—	0.22

[a] O-2, O-3, and O-6 denote the specific oxygen atoms to which the 2-(diethylamino)ethyl groups are attached in the monosubstituted fraction.

[b] $C_2/(C_1+C_2)$ denotes the fraction of disubstituted D-glucopyranosyl residues in the total of mono-+di-+tri-substituted residues, but trisubstituted residues were zero in all of these cases.

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, which bears a resemblance to starch in respect to the D-glucosidic linkage, exhibits a large increase in the fraction of substituent at the 3-hydroxyl groups as a result of reaction in the nonaqueous medium. Even the extent of increase (42%) is similar to that observed for starch (50%) on proceeding from aqueous to nonaqueous medium. Unlike starch, but like the β-D anomer, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside reacts to a higher D.S. in *p*-dioxane, and affords a lower proportion of disubstituted product.

Steric factors. — From molecular models, it is apparent that a freely rotating substituent on the anomeric carbon atom may interfere considerably with the approach

of a reactant to the hydroxyl group on C-2 when the D-glucosidic linkage is α. The anomers of methyl 4,6-*O*-benzylidene-D-glucopyranoside provide a means for observing changes in reactivity caused by the difference in steric effects about C-2. For reactions conducted in aqueous medium, on proceeding from the α-D to the β-D anomer, there is a small but significant increase in the proportion of substitution at the 2-hydroxyl group, and a significant increase in the fraction of disubstituted product. Both of these changes, which confirm the existence of a steric effect at C-2 in the α-D anomer, are considerably more pronounced when the comparison is based on the reactions conducted in nonaqueous medium. It appears, therefore, that the steric effect exists, that it changes with the medium, and that it is minimized by solvation in the transition stage of the reaction.

In the aqueous medium of reaction, cotton and starch exhibit relatively similar distributions of 2-(diethylamino)ethyl substituents (see Table I), but it is evident (see Table II) that, for starch, there is a significant decrease in substitution at the 2-hydroxyl group, a corresponding increase at the 3-hydroxyl group, and a constancy in substitution at the 6-hydroxyl group. For starch, as compared with cotton, there is a decrease in the fraction of disubstitution. The decrease in the fraction of reaction at the 2-hydroxyl group in cotton, compared to starch, is very similar to that between the β- and α-D anomers of methyl 4,6-*O*-benzylidene-D-glucopyranoside. In *p*-dioxane medium, the decrease in the fraction of substitution at the 2-hydroxyl groups of cotton, compared to starch, is essentially identical to that noted for the aqueous medium; this is substantially smaller than the change noted for the β- and α-D anomers of methyl 4,6-*O*-benzylidene-D-glucopyranoside in *p*-dioxane. Again, in *p*-dioxane medium, the fraction of disubstitution is lower for starch than for cotton.

General. — Although it is evident from the foregoing results that (*a*) the distribution of substituents in the D-glucopyranosyl residues of these carbohydrates is a function of the process, (*b*) a steric effect is apparent for an α-D-glucosidic linkage, and (*c*) cotton cellulose, starch, and the methyl 4,6-*O*-benzylidene-D-glucopyranosides exhibit some pronounced similarities, there remain some differences that are highly intriguing. In reaction with 2-(diethylamino)ethyl chloride, the 3-hydroxyl groups in starch show a higher reactivity than those in cellulose; this conclusion is unexpected because of the results of Doane *et al.*[14], who found no mono-3-*O*-substituted derivative in the hydrolyzate of partially methylated starch.

It is most interesting that the main reaction of the methyl 4,6-*O*-benzylidene-D-glucopyranosides with 2-(diethylamino)ethyl chloride occurs at the 3-hydroxyl group, and that this behavior is not limited to the particular reagent employed in this study[24,25]. Because very little inductive effect, and no steric effect, on the 3-hydroxyl group would be expected from the benzylidene grouping of the methyl 4,6-*O*-benzylidene-D-glucopyranosides, the decreased reactivity of this hydroxyl group in cellulose and starch appears attributable to an inaccessibility in these polymers; *i.e.*, the 3-hydroxyl groups may be strongly bound by hydrogen bonds between neighboring D-glucopyranosyl residues, or this hydroxyl group may in some other manner be sterically hindered by adjacent D-glucopyranosyl residues.

SUMMARY

The reactivities of the hydroxyl groups in cellulose, starch, and the anomers of methyl 4,6-*O*-benzylidene-D-glucopyranoside with 2-(diethylamino)ethyl chloride have been investigated under identical conditions in (*a*) aqueous and (*b*) nonaqueous medium. For cotton cellulose, the relative distribution of 2-(diethylamino)ethyl groups on the 2-, 3-, and 6-hydroxyl groups in the mono-*O*-[2-(diethylamino)ethyl]-D-glucopyranose fraction of the hydrolyzate was found to be independent of the D.S. Cellulose treated with 2-(diethylamino)ethyl chloride in *p*-dioxane showed a higher ratio of substituents on the 2- and 3-hydroxyl groups, relative to the 6-hydroxyl group, than resulted when the reaction was conducted in an aqueous medium. Consideration of these results on the basis of the fraction of substituents in each of the three positions showed that the increase in substitution on the 2-hydroxyl group is accompanied by a decrease in substitution at the 6-hydroxyl group and little change in that at the 3-hydroxyl group. Starch follows a similar pattern, but the increase in substitution on the 2- and 3-hydroxyl groups, relative to the 6-hydroxyl group, is more pronounced than for cellulose under identical conditions. The fractions of substituents on the 2-hydroxyl groups increases as a result of reaction in *p*-dioxane. The increase in substitution at the 2-hydroxyl group is accompanied by a decrease in that at the 6-hydroxyl group, but here a substantial increase in substitution at the 3-hydroxyl group occurs. With regard to the change in fraction of substitution at the 3-hydroxyl group with change in the reaction medium, methyl 4,6-*O*-benzylidene-β-D-glucopyranoside behaves similarly to cellulose, and the α-D anomer behaves similarly to starch.

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STUDIES ON SOIL POLYSACCHARIDES I.

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INTRODUCTION

The slimy, bacterial, extracellular products, found by Waksman and Martin[1] to aggregate sand–clay mixtures, were later shown[2–6] to be polysaccharides. The subject of soil carbohydrates has been comprehensively reviewed[7]. Evidence from statistical correlations[8–12] indicates that polysaccharides may be important in the stabilization of soil aggregates. Chemical evidence[13,14], obtained by treating soil aggregates with sodium periodate and sodium tetraborate solutions (to break down the polysaccharides), has shown more convincingly that polysaccharides are involved in the stabilization of aggregates in cultivated and grassland soil.

The association (which might involve hydrogen bonds[15–17], or even weak, covalent bonds[18]) between polysaccharides and humic constituents in soil has made it difficult to extract uncontaminated polysaccharides.

Extractions carried out at elevated temperatures with water[17,19–22], anhydrous formic acid[15], *N,N*-dimethylformamide[23], and aqueous acid[22], could have aided the autohydrolysis of acidic polysaccharides[24], and extraction with strong base[25] could have caused degradation[26] of the polysaccharide. There are no such objections to the use of sodium pyrophosphate[16,17,27] or of cold, dilute acids[28] as solvents.

In this work, various mild methods for the extraction of polysaccharides from an organic soil have been evaluated, the fractionation and characterization of components extracted with dilute acid have been studied, and preliminary results of structural studies by the use of enzyme-induction procedures are reported.

EXPERIMENTAL

The soil. — The highly organic, Fenland soil used as the source of polysaccharides has[29] a pH value of 5.8; ash, 15.9 ±1.5%; and humic acid, 37% [measured by determining the amount of organic matter precipitated at pH 2 (by hydrochloric acid) from the extract obtained with 0.5N sodium hydroxide]. The cation-exchange capacity, expressed as mequiv. per 100 g of soil dried at 105°, was 305.0. Cations contributing to the base saturation (each is expressed as mequiv. per 100 g) were Ca^{2+} 230, Mg^{2+} 39.6, H^+ 30.9, K^+ 2.9, Fe^{2+} 1.2, and Fe^{3+} 0.4. The soil, when sampled, had a moisture content of 60% (drying at 105°). Soil in the H^+ form [soil (H^+)] was

prepared by washing with N hydrochloric acid, and then with water until the filtrate was chloride-free.

Extraction of the polysaccharides, and evaluation of solvent systems. — In order to find an efficient method for the extraction of polysaccharides, separate 100-g samples of the untreated soil were shaken for 24 h at room temperature with water (200 ml), *N,N*-dimethylformamide (200 ml), *N*-methylpyrrolidone (200 ml), Amberlite IR-120 (50 g; H^+ form) suspended in water (200 ml), 7% aqueous sodium ethylenediaminetetraacetate (200 ml), 0.6N sulphuric acid (300 ml), or 8M urea solution (300 ml). Soil (H^+) (100 g) was also extracted with *N,N*-dimethylformamide (200 ml). The filtrates from the *N,N*-dimethylformamide and *N*-methylpyrrolidone extracts were diluted with an equal volume of water. The extract with sulphuric acid was neutralised with solid sodium hydrogen carbonate. During the neutralisation process, a stable, brown precipitate was formed at pH 4.8. The precipitate was recovered by centrifugation (4500 r.p.m. for 1 h at 0°), dissolved in 0.3N hydrochloric acid (100 ml), dialysed for two days against this acid (2×2 l), and then further dialysed for 2 days against running water. Ethylenediaminetetraacetic acid was precipitated from the appropriate extract by adjusting the pH to 2 with 4N sulphuric acid. Humic acids were precipitated from the *N,N*-dimethylformamide extract of the soil (H^+) by adjusting the pH to 2 with 4N hydrochloric acid. The precipitated material was separated by centrifugation (4500 r.p.m. for 1 h at 0°), and the supernatant, as well as the various filtrates described, were dialysed for two days against running water; the non-dialysable materials were recovered by freeze-drying. Samples were dried (60°/15 mm) over

TABLE I

YIELDS AND SUGAR CONTENTS OF ORGANIC MATTER EXTRACTED FROM SOIL WITH DIFFERENT EXTRACTANTS

Soil extractant	*Organic matter extracted (% of dry soil)*	*Sugar content (%, as anhydroglucose)*
Water	0.02	
N,N-Dimethylformamide	0.08	10.8
N-Methylpyrrolidone	0.10	10.8
Amberlite IR-120 (H^+) + water	0.40	12.7
Sodium ethylenediaminetetraacetate	1.38	7.0
Sulphuric acid (0.6N) (fraction soluble at pH 7.0)	0.10	29.7
Sodium hydrogen carbonate (precipitate from sulphuric acid extract)	0.12	25.0
Soil (H^+) + *N,N*-dimethylformamide	7.46	3.4
8M Urea	1.42	10.4

phosphorus pentaoxide and analysed for incombustible ash and for sugar content (by comparison with data for an anhydroglucose standard[30]). The results are presented in Table I.

The freeze-dried urea extract (546 mg), which was dark coloured (whereas

extracts obtained by the other procedures were light brown or brown), was dissolved in 0.1N hydrochloric acid (20 ml) and dialysed against the same acid (500 ml) for 2 days at 0°. This resulted in loss of dark-coloured material; the residue was further dialysed for 2 days against distilled water, and the non-dialysable material was freeze-dried to yield a light-brown product (87 mg; water, 21%; ash, 7%).

Gel filtration of the soil extracts. — Samples of soil extracted with Amberlite IR-120–water (138 mg), sodium ethylenediaminetetraacetate (328 mg), 8M urea (85 mg; recovered, as described, from the acid treatment), and 0.6N sulphuric acid [two samples; one (41 mg), which did, and the other (39 mg) which did not precipitate when the acid extract was neutralised with sodium hydrogen carbonate] were dissolved in water (50 ml), and eluted with water from columns (4 × 100 cm) of Sephadex G-75 or G-100. Fractions (10 ml) were collected and monitored[30] for sugars and for absorption at 400 nm. The eluates that corresponded to the void volumes of the columns were combined and freeze-dried, and the polysaccharides of high molecular weight (HMW) were recovered. Yields (*ca.* 12 mg) of these polysaccharides were similar for the acid and urea extracts and for the IR-120 and sodium ethylenediaminetetraacetate fractions (21 and 27 mg, respectively). Only the fractions from the acid extract were colourless. The work done on the acid extract that was soluble at pH 7.0 is reported hereafter.

Large-scale extraction and fractionation of the polysaccharides. — Soil (5 kg, containing *ca.* 60% of water) was extracted by rolling for 24 h at 3° with 0.6N sulphuric acid (10 l). The filtrate was neutralised with solid sodium hydrogen carbonate, and centrifuged at 2000 r.p.m. for 30 min, and the supernatant solution was filtered through an EKS-2 bacterial filter pad (John C. Carlson Ltd., Ashton-under-Lyne, Lancashire) to remove all traces of suspended materials. The filtrate was exhaustively dialysed against running water, and the non-dialysable material was freeze-dried to yield a crude polysaccharide mixture (*ca.* 4 g) that was dissolved in distilled water (200 ml) and eluted from a Sephadex G-100 column (5.5 × 100 cm) with distilled water. Fractions (25 ml) were collected and monitored for sugars and colour as above (Fig. 1). Coloured components were completely separated from the HMW-polysaccharide materials eluted in the void volume (400–650 ml) of the column. The fractions eluted in the effluent volumes 400–650 (*A*, Fig. 1), 650–1400 (*B*), and 1400–2200 ml (*C*) were combined and freeze-dried.

Fractionation of the polysaccharide on DEAE Sephadex A-50. — A gradient elution (Fig. 2) with 0 to 2M sodium chloride in phosphate buffer showed that polysaccharide fractions were eluted at 0, (*F1*), 0.3M (*F2*), 0.6M (*F3*), and M (*F4*) concentrations of sodium chloride. HMW-polysaccharide (197 mg of fraction *A*, Fig. 1) was dissolved in phosphate buffer (50 ml, pH 6; KH_2PO_4 0.0371M, Na_2HPO_4 0.0043M), applied to a column (3.4 × 45 cm) of the ion exchanger (Cl^-) previously equilibrated with the buffer, and eluted successively with the buffer (600 ml) and 0.3M (800 ml), 0.6M (600 ml), and M (600 ml) solutions of sodium chloride in the buffer. Fractions (10 ml) were collected and monitored[30] for sugars. Polysaccharide-containing fractions for each concentration of salt were combined, dialysed against distilled water, and freeze-dried. The relative proportions of fractions *F1–F4*, expressed

as the average, percentage weight of freeze-dried material in each fraction from four such separations, were 26.7, 31.6, 30.2, and 11.5, respectively.

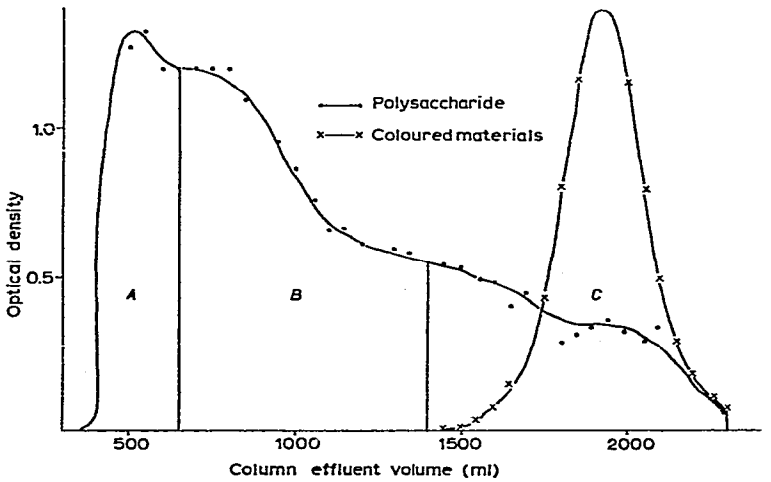

Fig. 1. Gel filtration (Sephadex G-100) of the large-scale extract of soil with sulphuric acid.

Titration of polysaccharides fractionated on DEAE Sephadex A-50. — Aqueous solutions of fractions *F1*, *F2*, *F3*, and *F4* were electrodialysed against running, distilled water for *ca.* 10 h, until the current had dropped to the predetermined minimum for distilled water (10 mamp at 200 volts). The solutions were then dialysed for 24 h against running, distilled water, concentrated to *ca.* 100 ml by freeze-drying, passed separately through columns (2 × 11 cm) of Amberlite IRC-50 (H^+), and then freeze-dried. Desalted samples of fractions *F1*, *F2*, *F3*, and *F4* were separately dissolved in demineralised water (30 ml), and dry, carbon dioxide-free nitrogen was bubbled through the solutions for 1 h before each was titrated with *ca.* 0.02N sodium hydroxide. The equivalent weights were 5470, 3560, 1200, and 740, respectively.

After titration, the polysaccharides were recovered by desalting with IRC-50 (H^+), dialysing against distilled water, and freeze-drying.

Molar ratios of sugars in the polysaccharide fractions. — Aqueous solutions (*ca.* 0.01%) from dried samples of fractions *F1*, *F2*, *F3*, and *F4* were assayed for pentose[31], hexose[32], 6-deoxyhexose[33], and hexuronic acid[34]; the results (Table II) are expressed as molar ratios relative to the anhydropentose content of each polysaccharide.

Identification of the sugars. — (*a*) *Hydrolysis.* Polysaccharide solutions (10 mg/ml) in N sulphuric acid were heated (100°) for 4 h in sealed tubes. The hydrolysates were centrifuged, and the supernatants were neutralized with barium carbonate and again centrifuged. The residues were washed with water, and the combined, supernatant solutions and washings were treated with Amberlite IR-120 (H^+) to remove barium ions, and then concentrated *in vacuo* at 30°.

(*b*) *Paper chromatography.* Paper chromatograms were irrigated with ethyl

acetate–pyridine–water (8:2:1), and detection was effected with alkaline silver nitrate[35] or with aniline hydrogen phthalate[36]. The following components were identified: arabinose, galactose, glucose, mannose, rhamnose, xylose, and glucuronic acid.

TABLE II

MOLAR RATIOS, EXPRESSED IN TERMS OF ANHYDROPENTOSE CONTENT, OF SUGARS IN POLYSACCHARIDE FRACTIONS OF HIGH MOLECULAR WEIGHT

Sugar	*Molar Ratio; Anhydropentose = 1*			
	F1	F2	F3	F4
Pentose (as anhydroxylose)	1	1	1	1
Hexose (as anhydroglucose)	14.6	7.7	3.4	5.2
6-Deoxyhexose (as anhydrorhamnose)	3.2	1.4	0.9	1.1
Hexuronic acid (as glucuronolactone)	0.7	1.3	2.1	1.4

(*c*) *Cellulose-column chromatography.* Dried HMW-polysaccharide (275 mg, fraction *A*, Fig. 1) was hydrolysed, as described above, and the hydrolysate was concentrated *in vacuo* to a light-brown syrup (188 mg), a part (142 mg) of which was chromatographed on a cellulose column (3.5 × 37 cm) packed in the above chromatography solvent. Fractions (25 ml) were collected, and monitored for sugars with alkaline silver nitrate[35]. The appropriate fractions were combined, and shaken with equal volumes of water, and the lower, aqueous layers were continuously extracted with ether until pyridine was removed, and then concentrated (rotary evaporator, 30°) until colourless syrups remained. The syrups were tentatively identified (in order of elution) by paper chromatography as glucurono-6,3-lactone (3 mg), an unidentified sugar (14 mg), rhamnose (20 mg), rhamnose plus a trace of ribose (8 mg), xylose (12 mg), arabinose (6 mg), mannose (7 mg), glucose (9 mg), and galactose (6 mg).

The sugars were characterised as follows: (*i*) The glucopyranurono-6,3-lactone (5 mg, obtained by combining the fractions from two column runs) was refluxed[37] for 6 h with 2% methanolic hydrogen chloride (1 ml). The ester glycoside was reduced with lithium aluminium hydride[38], and then hydrolysed for 3 h at 100° with 2N sulphuric acid. The hydrolysate was neutralised with barium carbonate, and concentrated to a syrup, and a solution of the residue in water (2 ml) was assayed (0.2 ml) for D-glucose by the glucose oxidase method[39] to give a value corresponding to 95 μg of D-glucose in the original solution. Authentic D-glucopyranurono-6,3-lactone (20 mg) yielded D-glucose (14%) when converted and assayed by the same procedures.

(*ii*) The fraction containing the unidentified sugar (15 mg, recovered from two column chromatograms) was purified by preparative chromatography on paper which resolved it into two components having R_G values of 5.6 and 6.2, respectively (ethyl acetate–pyridine–water, 8:2:1). Two components having M_G values of 0.34 and 0.75 were detected with aniline hydrogen phthalate[36] when the fraction was subjected to paper ionophoresis (Whatman No. 3 paper; borate buffer, pH 10.0). The syrup (12 mg) was dissolved in water (2 ml) and an aliquot (0.5 ml) was evaporated to dryness,

cooled to −80°, and dealkylated with boron trichloride[40]. Glucose and xylose were identified by paper chromatography as products of this reaction.

(*iii*) Rhamnose gave the benzoylhydrazone[41] (87.5%), m.p. 179°, mixed m.p. 180°.

(*iv*) Xylose gave the di-*O*-benzylidene dimethyl acetal[42] (46%), m.p. 212°, mixed m.p. 211°.

(*v*) Arabinose gave the benzoylhydrazone[41] (91%), m.p. 187°, mixed m.p. 188°.

(*vi*) Mannose gave the phenylhydrazone[41] (66%), m.p. 194°, mixed m.p. 199°.

(*vii*) Glucose gave the *p*-nitroaniline derivative[43] (65%), m.p. 184°, mixed m.p. 184°.

(*viii*) Galactose gave the 2-methyl-2-phenylhydrazone[41] (64%), m.p. 189°, mixed m.p. 190°.

Detection of fucose and ribose. — The fucose and ribose components of a hydrolysate of the HMW-polysaccharide (21 mg) were separated as above by means of preparative chromatography on paper. When subjected to paper ionophoresis (borate buffer, pH 10.0), spots corresponding to fucose (M_G 0.83) and xylose (M_G 0.99) were detected. By means of paper chromatography, a faint spot, with an R_G corresponding to ribose, was detected.

Incubation of HMW-polysaccharide with an induced α-L-rhamnosidase. — An extracellular α-L-rhamnosidase[44] in 0.1M phosphate buffer (100 ml, pH 7) was added to the HMW-polysaccharide (100 mg containing 16% of water) dissolved in the same buffer (25 ml). The combined solutions were sterilized by membrane filtration, and then incubated at 37°. Samples (1 ml) were aseptically withdrawn after incubation for 0, 3, 6, 10, and 24 h, and separately passed down a column (25 × 0.7 cm) of Sephadex G-25 packed in the phosphate buffer. The column was previously calibrated with a polysaccharide–rhamnose solution. The polysaccharide was eluted in Fractions 4 and 5 (1-ml fractions), and the rhamnose in Fractions 7 to 9. Fractions 7–9 were combined and concentrated to dryness, and the residue was redissolved in water (0.6 ml), and assayed for 6-deoxyhexose[33]. The percentage of anhydrorhamnose liberated after 6, 10, and 24 h was constant (*ca.* 1.7%). After incubation for 24 h, the bulk of the solution (120 ml) was applied to a column (3.5 × 57.5 cm) of Sephadex G-100 and eluted with water. Fractions (10 ml) were assayed for sugar[30], and monitored for protein by measuring the absorption at 260 nm (Fig. 3). Fractions 45–64, were combined and concentrated to 100 ml, and rhamnose could then be detected by chromatography. An aliquot (0.5 ml) contained 14.5 μg of 6-deoxyhexose[33], equivalent to 2.0 mg of rhamnose from the 100 mg of polysaccharide.

Fractions 15–36 were freeze-dried, and the polysaccharide "core" (96 mg) was recovered. Dried samples (10 mg) of the HMW-polysaccharide and of the "core" were dissolved in water (100 ml), and assayed for hexose[32] and for 6-deoxyhexose[33]. The hexose content of the HMW-polysaccharide, expressed as anhydroglucose, was 32.8%, that of the "core" was 39.0%, whereas the 6-deoxyhexose contents, expressed as anhydrorhamnose, were 7.5% and 4.0%, respectively.

DISCUSSION

The organic soil selected as the source of soil polysaccharides was highly humified, had a low ash content when in the H^+ form, and had a high microbial activity. The properties of the solutions used for the extractions met, at least in part, the requirements for an effective solvent for soil organic matter as stated by Whitehead and Tinsley[23].

The total yields of polysaccharide in fulvic acid (soil organic matter soluble in acid and in alkali solutions) fractions were in the relative order: soil (H^+) + *N*,*N*-dimethylformamide > 8M urea > sodium ethylenediaminetetraacetate > Amberlite IR-120 (H^+) + water > 0.6N sulphuric acid (extract soluble at pH 7.0) > material precipitated by sodium hydrogen carbonate from the sulphuric acid extract > *N*,*N*-dimethylformamide > *N*-methylpyrrolidone.

Urea (8M) and sulphuric acid (0.6N) were selected as extractants because of the ability of these reagents to break hydrogen bonds. Although the ratio of saccharide to organic matter in the fulvic acid materials was higher in the case of the acid extract, the total saccharide content of the organic matter in the urea extract was *ca.* 5 times greater than that in the acid extract. However, although the polysaccharides of high molecular weight were completely separated from the coloured components of the acid extract during gel filtration, it was not possible to resolve the brown materials from the polysaccharides by gel filtration of the urea extract, except after acid treatment.

The soil (H^+), apart from the cations and small proportions of soluble organic materials removed during its preparation, may be regarded as being chemically similar to the untreated soil. The conversion of untreated soil into soil (H^+) caused an approximately 37-fold increase in the amount of saccharide material extracted by *N*,*N*-dimethylformamide (Table I), although it decreased the ratio of saccharide to organic matter. The polysaccharides extracted could not, however, be separated by gel filtration from the co-extracted brown materials. These data indicate that the polysaccharides are present as insoluble salts in the untreated soil and/or bonded to the insoluble salts of humic acids in a soil predominantly in the Ca^{2+} and Mg^{2+} forms. Humic acids were precipitated at pH 2 from the extract from the soil (H^+). Subsequent work[45] has shown that appreciable proportions of humic acid-type materials can be precipitated from similar extracts in the pH range of 1–2.

The polysaccharide subfraction (soluble at pH 7) obtained from the sulphuric acid extract was chosen for study because it gave the highest yield of material of high molecular weight (32% of the total organic matter extracted; having ash, 2.2%; and N, 0.6%) which was separable by gel filtration from the brown, non-carbohydrate impurities (Fig. 1).

It had been shown[46] by means of free-boundary electrophoresis (in phosphate buffer pH 7.0) that a similar polysaccharide of high molecular weight had one essentially neutral and five negatively charged components. The pattern of gradient elution (Fig. 2) indicates that at least four components of different charge densities could be

separated on DEAE Sephadex A-50. For larger scale fractionations, it was more convenient to use stepwise elution.

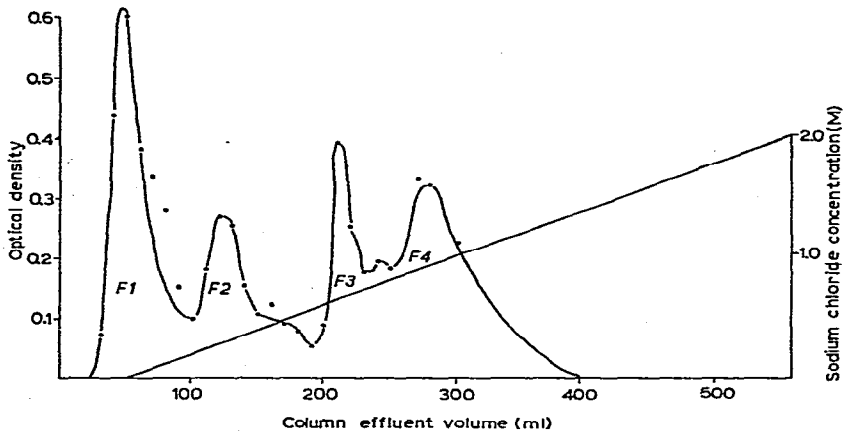

Fig. 2. Gradient elution (DEAE Sephadex A-50) of the polysaccharide of high molecular weight.

Exhaustive desalting was necessary in order to obtain a high degree of accuracy in the determination of equivalent weight. The ash content was reduced by electrodialysis from 2.2% in *A* (Fig. 1) to 0.5, 0.2, 0.1, and 0.3% in fractions *F1*, *F2*, *F3*, and *F4*, respectively. The data on equivalent weight, if it is assumed that uronic acids alone contributed to the polysaccharide acidity, indicate anhydrohexuronic acid contents of 3.2, 5.0, 14.7, and 23.8%, respectively, for fractions *F1*, *F2*, *F3*, and *F4*. The corresponding contents determined colorimetrically[34] were 2.3, 5.8, 13.1 and 5.1%. The value obtained for fraction *F4* indicates that acids other than uronic acids contributed towards the acidity of this fraction. Continuous extraction with ether of the hydrolysate of the HMW-polysaccharide yielded a brown extract, which, after chromatography, could be located by spraying with Bromocresol Green, but not with alkaline silver nitrate, aniline hydrogen phthalate, or 2.4-dinitrophenylhydrazine (for keto acids). Comparison of R_F values indicated that the compound was not succinic acid, which has been shown[47] to constitute 10% of a polysaccharide synthesised by a soil bacterium.

By means of paper chromatography, it was shown that fractions *F1–F4* contained the same, major monosaccharides, namely, arabinose, galactose, glucose, mannose, rhamnose, xylose, and hexuronic acid. A similar observation has been made by Mueller *et al.*[48] concerning the five soil-polysaccharide fractions isolated by use of DEAE Cellulose. However, it may be seen from Table II that the four fractions contained different proportions of the various sugars. The colorimetric procedures used in these analyses gave reproducible results for pentose, hexose, and 6-deoxyhexose, provided that a correction was made for the slight error introduced by 6-deoxyhexose in the analyses for pentose and hexose. The same was true in the case of analyses for hexuronic acid[34] where a correction had to be made for the presence of hexose.

The low yield of glucopyranurono-6,3-lactone precluded the formation of a derivative, and the possibility is recognised that other uronic acids might have been present in this fraction.

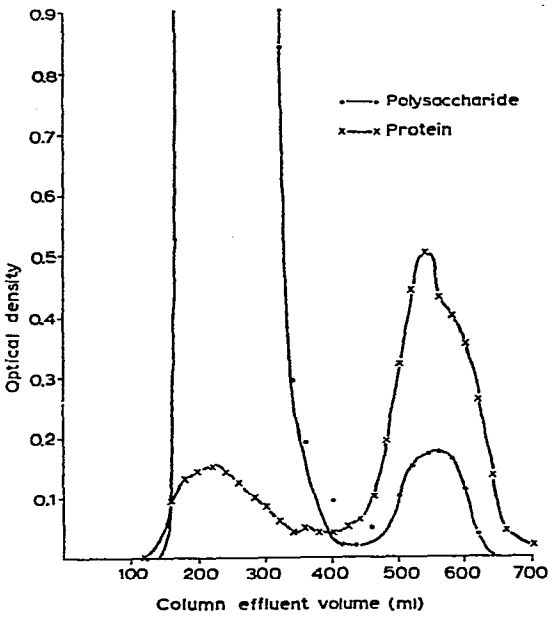

Fig. 3. Gel filtration (Sephadex G-100) of the digest of polysaccharide of high molecular weight.

No amino sugars were detected in the hydrolysate of the HMW-polysaccharide, although the conditions would not have hydrolysed chitin-like polysaccharides. The nitrogen content (0.6%, which might have included nitrogen-containing ash) of the materials of high molecular weight indicated that amino sugars could not be present in appreciable proportions. This is in agreement with other observations[19,20,24].

Ribose and fucose were barely detectable in the polysaccharide hydrolysate. It is possible that the trace amount of ribose was derived from nucleic acid residues, although no ultraviolet absorbing areas, indicative of purine or pyrimidine bases, could be detected on paper chromatograms. Fucose has been detected in soil polysaccharides[49,50].

It seems probable that the unidentified sugars were methyl ethers of glucose and xylose. Duff[51] has identified 2-*O*-methylxylose, 2-*O*-methylrhamnose, and 4-*O*-methylgalactose as components of soil polysaccharides. 2-*O*-Methylxylose[52], 3-*O*-methylgalactose[53], 3-*O*-methylrhamnose[54], and 4-*O*-methylglucuronic acid[55] have been found in hydrolysates of plant polysaccharides, but methyl ethers of glucose, 2-*O*-methylrhamnose, and 4-*O*-methylgalactose have not been similarly detected. It seems likely that the methylated sugars found in soil polysaccharides are of microbial origin.

Polysaccharides that contain six or more sugars are rare, and, at present, are known to occur only in the polysaccharides of pathogenic organisms[56]. For this

reason, it seemed unlikely that fractions *F1–F4* could be considered to be pure polysaccharides. This has been confirmed[57] for fraction *F1* by free-boundary electrophoresis.

The enzymic studies were performed in order to evaluate the potential of the technique for structural studies on soil polysaccharides. It was postulated that, should five or more sugars be present in purified soil polysaccharides, the classical methods for structural investigation would be of limited value because of the complexity of products that could be expected. Since rhamnose is often found in the terminal, non-reducing position of rhamnose-containing polysaccharides, it was decided to study the behaviour of polysaccharide *A* (Fig. 1) towards an α-L-rhamnosidase.

The difference of 3.5% (corresponding to 3.9% of rhamnose) between the anhydrorhamnose content of the polysaccharide and that of the "core" resulting from enzymic treatment agrees reasonably well with the observed loss (colorimetrically determined) of 2.4% from polysaccharide *A*. The discrepancy may be attributed to errors of the order of 10% in the analysis procedure. These errors can be doubled by taking a difference and are accentuated by the relatively low proportions of rhamnose in the polysaccharides. It may be concluded that from 25–50% of the L-rhamnose residues in the mixture of polysaccharides of high molecular weight are present as α-L-linked, end groups. The remainder of the rhamnose could be present as β-L-linked terminal units, as intercatenary units, or even as α-L-linked terminal units sterically shielded from attack by the α-L-rhamnosidase.

It is proposed to extend this technique by inducing other enzymes in order to study the structures of purified soil polysaccharides.

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The authors acknowledge the technical assistance of Mr. J. Harris and Mr. R. L. Jones, Dr. A. G. M. MacDonald for microanalytical data, and the Science Research Council for the award of a Research Studentship to one of us (R. G. S.).

SUMMARY

Polysaccharide material was extracted from an organic soil with *N*,*N*-dimethylformamide, *N*-methyl-2-pyrrolidone, sodium ethylenediaminetetraacetate, Amberlite IR-120 (H^+) resin plus water, 8M urea, and 0.6N sulphuric acid. By the use of a gel-filtration technique, it was possible, in the case of acid extracts, to separate the brown, acid-soluble components of soil organic matter from the polysaccharides of high molecular weight (HMW).

The HMW-polysaccharide contained arabinose, galactose, glucose, mannose, rhamnose, xylose and glucuronic acid as major constituents, and fucose, ribose, and, possibly, methylated xylose and glucose derivatives as minor constituents. Four fractions, obtained by anion-exchange chromatography of the HMW-polysaccharide, differed in their equivalent weights, but contained the same, major, sugar units,

although in different proportions. The most acidic fraction contained an unidentified acidic component, which was not a uronic acid.

Part (25–50%) of the rhamnose was present in the HMW-polysaccharide as α-L-linked terminal units.

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SYNTHESIS OF *O*-β-D-GALACTOPYRANOSYL-(1→3)-*O*-β-D-GALACTOPYRANOSYL-(1→4)-D-GLUCOSE*

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(Received February 20th, 1967)

INTRODUCTION

During the course of a study of the chemistry of complex glycosphingolipids containing the sequence 4-*O*-β-D-galactopyranosyl-D-glucose[1], we have investigated the possibility of synthesizing oligosaccharides involving the glycosidation of lactose at positions 3 or 4 of the galactose moiety. Very little work has been published on the selective substitution of lactose. An isopropylidene acetal has been described[2], but its suggested structure was later refuted[3]. An attempt, in our laboratory, to prepare trityl ethers of benzyl β-lactoside was unsuccessful. However, conditions were found for the preparation of an isopropylidene derivative of unambiguous structure which was readily converted into a lactoside with free hydroxyl groups at C-3 and C-4 of the galactopyranose ring. The synthesis of benzyl 2,6-di-*O*-acetyl-β-D-galactopyranoside has been recently described[4]. Treatment of this compound with acylglycosylhalides led to the formation of benzyl 3-*O*-glycosyl-β-D-galactopyranosides[5]. It was therefore expected that an analogous derivative of lactose would react similarly and provide a means of synthesizing *O*-glycosyl-(1→3)-*O*-galactopyranosyl-(1→4)-D-glucose derivatives. The synthesis of the trisaccharide *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose (**1**), described in this paper, illustrates the validity of this approach, and suggests a route to the preparation of other trisaccharides of biological interest having similar structures.

DISCUSSION

Catalytic deacetylation of benzyl hepta-*O*-acetyl-β-lactoside[6] (**2**) afforded benzyl β-lactoside (**3**) which was treated with acetone in the presence of acid catalysts to give crystalline benzyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). The use of anhydrous cupric sulfate as catalyst[7] failed to catalyze any reaction, whereas sulfuric acid[8] led to a moderate yield (about 40%). A much higher yield (76%) was obtained, however, with *p*-toluenesulfonic acid[8]. Excessive

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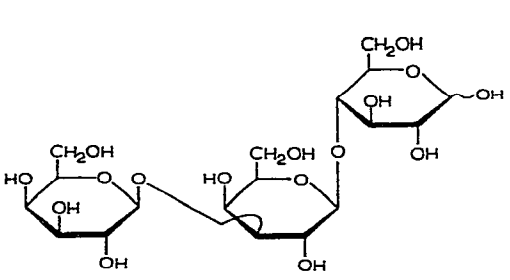
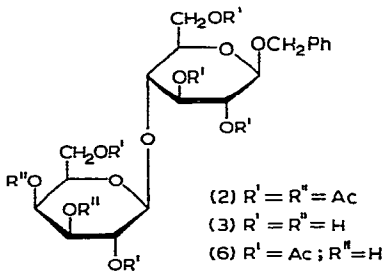
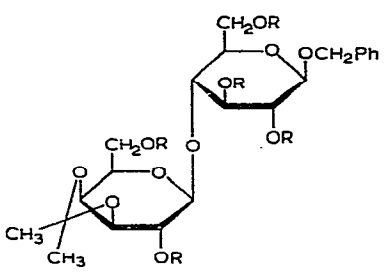
prolongation of the time of the reaction and a large excess of *p*-toluenesulfonic acid led to the cleavage of the disaccharide and formation of considerable amounts of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and benzyl β-D-glucopyranoside. The acetal (**4**) consumed 1 mole of periodate per mole at 20°, in agreement with the



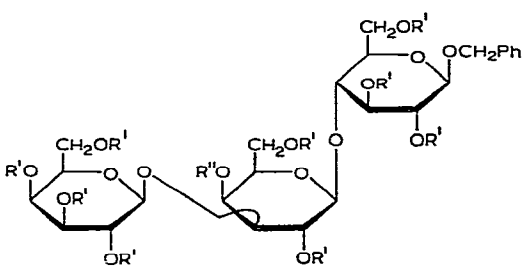
(1)



(2) R' = R'' = Ac
(3) R' = R'' = H
(6) R' = Ac ; R'' = H



(4) R = H
(5) R = Ac



(8) R' = Ac ; R'' = H
(9) R' = R'' = Ac
(10) R' = R'' = H

structure postulated. Acetylation of **4** gave the pentaacetate **5**, which was converted into benzyl 4-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6**) by treatment with hot aqueous acetic acid. Compounds **5** and **6** did not crystallize, but were purified by chromatography on silica gel, and gave satisfactory elementary analyses and the expected n.m.r. spectra. The periodate consumption of **6** (1 mole per mole of **6** at 20°) was in agreement with the structure assigned.

Treatment of **6** with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**) in the presence of mercuric cyanide in nitromethane–benzene solution[7], followed by chromatography on silica gel G, afforded an amorphous product which was formulated as benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**8**). This assignment was based on the expected greater reactivity at C-3, rather than at C-4 of the galactopyranoside ring, as shown previously in our laboratory[5]. Acetylation of **8** gave the decaacetate **9**, and catalytic deacetylation of **8** and **9** afforded benzyl *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**10**), which was purified by chromatography on Sephadex G-15. Periodate oxidation con-

firmed the structure of **10**, and hence, that of **8** and **9**. Catalytic hydrogenolysis of **10** gave the desired trisaccharide (**1**) as an amorphous, hygroscopic solid. The overall yield of **1** from benzyl β-lactoside (**3**) was 19%. Acetylation of **1** with hot acetic anhydride–pyridine gave a crystalline undecaacetate.

EXPERIMENTAL

Melting points were taken between glass slides on a Fisher–Johns apparatus and are corrected. Optical rotations were determined in semimicro tubes with a Perkin–Elmer No. 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, with tetramethylsilane as the internal standard and chloroform-*d* as solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used and elution was stepwise, in order of increasing polarity of the eluants. The proportion of weight of substance to weight of adsorbent added to the column was 1 to 50–100. The volume of the fractions eluted was 2 ml per g of silica gel. Thin-layer chromatograms (and column separations where specified in the text) were performed on silica gel G (Kieselgel G, E. Merck, Darmstadt). Analyses were made in the Institute's Microanalytical Laboratory under the direction of Mr. R. Heller. Periodate oxidations were performed with an adaptation of the spectrophotometric method[9]. A sample of the substance to be examined (about 5 mg) was dissolved in 0.01M sodium metaperiodate solution (10 ml). This solution and the standard solutions were kept in the dark at 20°. Aliquots from both solutions (0.5 ml) were withdrawn and diluted to 50 ml. The relative absorbances of the two solutions were determined at 223 mμ with a Perkin–Elmer 137 u.v. spectrophotometer. The absorbance was recorded as the depth of the peak below the reading at 300 mμ which was taken as base line. The difference in relative absorbances at 223 mμ of the two solutions gave a measure of the periodate consumption. A correction was applied for the absorbance of sodium iodate measured under these conditions (about 13% of that of an equimolar sodium metaperiodate solution).

Benzyl 4-O-β-D-galactopyranosyl-β-D-glucopyranoside (**3**). — A solution of benzyl hepta-*O*-acetyl-β-lactoside[6] (**2**, 9.0 g) in methanol (10 ml) containing a catalytic amount of sodium methoxide, was kept overnight at room temperature, and was then neutralized with a few drops of glacial acetic acid. The solution was evaporated *in vacuo* and the residue dissolved in water. The aqueous solution was passed through a column of Dowex-50 (H^+) and evaporated *in vacuo*. The dry residue crystallized from hot absolute ethanol as needles (4.5 g, 84%), m.p. 180°; it was homogeneous on t.l.c. in 4:5:1 butyl alcohol–acetone–water, $R_{lactose}$ 2.6; $[\alpha]_D^{25}$ −18.1° (*c* 1.32, water).

Anal. Calc. for $C_{19}H_{28}O_{11}\cdot H_2O$: C, 50.7; H, 6.67. Found: C, 50.6; H, 6.85. Loss in weight at 100° in high vacuum over P_2O_5, 4%, corresponding to 1 molecule of water.

Benzyl 4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**). — (*a*) A suspension of **3** (200 mg, dried over P_2O_5 in high vacuum overnight at 100°) in anhydrous acetone (60 ml) containing sulfuric acid (0.4 ml) was stirred for 5 h at room temperature. The resulting clear solution was neutralized with anhydrous sodium

carbonate and filtered. The filtrate was evaporated *in vacuo*, and the residue was crystallized from acetone–methanol; yield 82 mg (37%); m.p. 196–197°.

(*b*) A stirred mixture of **3** (3.0 g), anhydrous *p*-toluenesulfonic acid (0.5 g), and pure, anhydrous acetone (1500 ml) was boiled under reflux for 3 h. After being cooled, the colorless solution was neutralized with anhydrous sodium carbonate and filtered. The clear solution was evaporated *in vacuo*, and the product was crystallized from acetone–methanol; yield 2.5 g (76%), m.p. 196–197°, $[\alpha]_D^{25}$ −1.0° (*c* 0.73, pyridine). N.m.r.: τ 2.70 (5H, C_6H_5), τ 8.44 and 8.62 [6H, $C(Me)_2$]. T.l.c. in 65:25:4 chloroform–methanol–water: $R_{lactose}$ 2.75, identical with that of the product described under (*a*). Periodate oxidation showed the consumption of 0.98 mole of sodium metaperiodate per mole of compound **4** during 48 h at 20°; no further consumption during an additional 10 h was observed

Anal. Calc. for $C_{22}H_{32}O_{11}$: C, 55.9; H, 6.78. Found: C, 55.9; H, 6.70.

Benzyl 4-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**5**). — A solution of **4** (2.0 g) in pyridine (10 ml) and acetic anhydride (15 ml) was kept overnight at room temperature. The solution was concentrated *in vacuo* to a syrup from which traces of pyridine and acetic anhydride were removed by co-evaporation *in vacuo* with toluene. The syrupy residue was dissolved in benzene, and the solution was chromatographed on silica gel. Benzene–ether (1:1, v/v) eluted fractions (2.2 g, 84%) which were homogeneous on t.l.c. in 14:1 benzene–methanol, $R_{Compound\ 2}$ 0.94; $[\alpha]_D^{25}$ −10.7° (*c* 0.61, chloroform). N.m.r.: τ 2.70 (5 H, C_6H_5), τ 7.88–8.02 (15H, 5 OAc groups), and τ 8.46 and 8.66 [6H, C $(Me)_2$].

Anal. Calc. for $C_{32}H_{42}O_{16}$: C, 63.8; H, 6.99. Found: C, 63.8; H, 6.95.

Treatment of compound **3** *with acetone in the presence of a large excess of* p-*toluenesulfonic acid.* — A stirred mixture of **3** (5.0 g), *p*-toluenesulfonic acid (10 g), and acetone (1500 ml) was boiled for 3 h under reflux. The cooled solution was processed as previously described, and the residue was acetylated with acetic anhydride–pyridine. The product was chromatographed on silica gel. Benzene–ether (12:1) eluted benzyl tetra-*O*-acetyl-β-D-glucopyranoside (2.45 g) which crystallized from ether–hexane; m.p. 101–102° (reported[10] m.p. 101–104°).

Anal. Calc. for $C_{21}H_{26}O_{10}$: C, 57.5; H, 5.90. Found: C, 57.9; H, 5.90.

Benzene–ether (9:1) eluted 6-*O*-acetyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.27 g) identical with an authentic specimen[11]; no acetal (**4**) could be isolated from the reaction mixture.

Benzyl 4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**6**). — A solution of **5** (5.0 g) in 80% acetic acid (50 ml) was kept for 3 h at 100°. The acetic acid was removed by codistillation with water, and the residue was dried by addition of toluene and evaporation *in vacuo*. The dry residue was dissolved in dichloromethane and chromatographed on silica gel. Dichloromethane–ether (1:1) eluted fractions (4.0 g, 85%) which were homogeneous on t.l.c. in 4:1 benzene–methanol, $R_{Compound\ 2}$ 0.51. N.m.r.: τ 2.7 (5H, C_6H_5), τ 7.80–7.99 (15H, 5 OAc groups); the signals at τ 8.46 and 8.66 (CMe_2) present in **5** were absent in **6**; $[\alpha]_D^{25}$ −23.2° (*c* 0.60, chloroform). Periodate oxidation showed the consumption of 0.98 mole of

sodium metaperiodate per mole of **6** during 24 h at 20°; no further consumption was observed on prolongation of the reaction for an additional 30 h.

Anal. Calc. for $C_{29}H_{38}O_{16}$: C, 62.0; H, 6.76. Found: C, 61.8; H, 6.79.

*Benzyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-*O*-(2,6-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**8**). — A stirred solution of **6** (300 mg, 0.468 mmole) in 1:1 nitromethane–benzene (50 ml) was boiled until approx. 20 ml of the solvent mixture had distilled to ensure complete dehydration, and was then cooled to 60°. Mercuric cyanide (110 mg, 0.468 mmole) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**, 0.266 mg, 0.468 mmole) were added, and the reaction was continued for 24 h at 60–70°, additional portions of mercuric cyanide (0.234 mmole) and of the bromide (**7**, 0.234 mmole) being added after 18 h. The mixture was cooled, diluted with benzene (20 ml), washed successively with a cold solution of sodium hydrogen carbonate (5%) and water, dried with sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in dichloromethane and chromatographed on silica gel G. Dichloromethane–ether (1:1) eluted fractions which were homogeneous on t.l.c. in 4:1 benzene-methanol; $R_{Compound\ 6}$ 1.25; yield 260 mg (50%), $[\alpha]_D^{25}$ −5.2° (*c* 0.81, chloroform). N.m.r.: τ 2.70 (5H, C_6H_5), τ 7.82–8.01 (27H, 9 OAc groups).

*Benzyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**9**). — Syrup **8** (240 mg) was acetylated with pyridine–acetic anhydride at room temperature. The product (240 mg, 99%) did not crystallize, but was homogeneous on t.l.c. in 4:1 benzene–methanol, $R_{Compound\ 8}$ 1.15; $[\alpha]_D^{20}$ +3.6°(*c* 0.99, chloroform). N.m.r.: τ 2.70 (5H, C_6H_5), τ 7.85–8.02 (3 OH, 10 OAc groups).

Anal. Calc. for $C_{45}H_{58}O_{26}$: C, 53.2; H, 5.71. Found: C, 52.9; H, 6.05.

Benzyl O-β-D-*galactopyranosyl-(1→3)-*O*-β-D-*galactopyranosyl-(1→4)-β-D-glucopyranoside* (**10**). — A solution of **9** (200 mg) in methanol (10 ml) containing a catalytic amount of sodium methoxide was kept overnight at room temperature, and was then neutralized with a few drops of glacial acetic acid. The neutralized solution was concentrated *in vacuo*, and the residue dissolved in water. The aqueous solution was chromatographed on Sephadex G-15. Fractions (1 ml) were eluted with water. The fractions containing saccharides were identified by spot-tests on a microslide coated with silica gel G with anisaldehyde–sulfuric acid as detecting reagent[12]. The fractions which were homogeneous on t.l.c. in 4:5:1 butanol–acetone–water (R_F 0.69) were combined and conc. *in vacuo* to a syrup. Abs. ethanol precipitated a hygroscopic powder (90 mg, 77%); $[\alpha]_D^{20}$ +17.9° (*c* 0.95, water). On periodate oxidation, 3 moles of sodium metaperiodate were consumed per mole of **10** during 60 h at 20°.

Anal. Calc. for $C_{25}H_{38}O_{16} \cdot H_2O$: C, 49.0; H, 6.22. Found: C, 48.6; H, 6.04.

O-β-D-*Galactopyranosyl-(1→3)-*O*-β-D-*galactopyranosyl-(1→4)-D-glucose* (**1**). — A solution of **10** (120 mg) in 80% aqueous ethanol (50 ml) was hydrogenolyzed in presence of palladium–charcoal (10%) for 20 h at 50 lb.in^{-2}. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo*. The resulting syrup was dissolved in ethanol, and the solution was filtered to remove residual traces of charcoal,

and concentrated *in vacuo*. Addition of a few drops of ethanol gave 91 mg (92% yield) of a hygroscopic, white precipitate which was homogeneous on t.l.c. in 4:5:1 butanol–acetone–water, $R_{Compound\ 10}$ 0.54; $[\alpha]_D^{25}$ +26.1° (*c* 0.69, water).

Anal. Calc. for $C_{18}H_{32}O_{16} \cdot H_2O$: C, 41.4; H, 6.56. Found: C, 41.8; H, 6.50.

A solution of **1** (1.1 g) in pyridine (20 ml) and acetic anhydride (20 ml) was kept for 1 h at 100°. After removal of the acetic anhydride and pyridine *in vacuo*, the residue was crystallized from aqueous ethanol to yield 1.5 g (81%) of an undecaacetate, m.p. 108–110°, $[\alpha]_D^{15}$ +17.2° (*c* 0.61, chloroform). The product was homogeneous on t.l.c. in 4:1 benzene–methanol ($R_{Compound\ 2}$ 0.3).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.7; H, 5.63. Found: C, 49.3; H, 5.56.

SUMMARY

O-β-D-Galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose was obtained by selective glycosidation of benzyl 4-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside with tetra-*O*-acetyl-α-D-galactopyranosyl bromide.

The disaccharide was prepared from benzyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside, which was obtained in good yield by treatment of benzyl β-lactoside with acetone and *p*-toluenesulfonic acid.

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NUCLEOSIDES. XLI. THE CONFORMATION OF 1,3,4-TRI-*O*-BENZOYL-2-DEOXY-2-FLUORO-β-D-RIBOSE AND OF 1,3,4-TRI-*O*-BENZOYL-2-DEOXY-α-(AND β-)-D-*erythro*-PENTOSE*

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A previous paper[1] in this series reported the synthesis of 1,3,4-tri-*O*-benzoyl-2-deoxy-2-fluoro-D-ribose (**1**). A highly negative rotation value indicated that **1** had probably, by comparison with similar compounds[1-3], the β-configuration. The most promising method for determining the anomeric configuration of **1** appeared to be n.m.r. spectroscopy. Indeed, n.m.r. studies proved not only the configuration of this compound, but established its conformation as well. In the course of this work, comparison was made with n.m.r. spectra of both anomeric forms of 1,3,4-tri-*O*-benzoyl-2-deoxy-β-D-*erythro*-pentose (**2** and **3**).

The n.m.r. data for compound **1**, determined in three solvents, are given in Table I. The table shows that, at 100 MHz, $J_{1,2}$ is 2.9 Hz. Hence, the H-1–H-2 protons

TABLE I

N.M.R. PARAMETERS FOR 1,3,4-TRI-*O*-BENZOYL-2-DEOXY-2-FLUORO-D-RIBOSE (**1**)

Solvent[a]	*Chemical shifts* (δ)					
	H-1	*H-2*	*H-3*	*H-4*	*H-5e*	*H-5a*
Carbon tetrachloride (60 MHz)	6.57	4.88	5.74	~5.54	~4.23	~4.23
Acetone-d_6 (60 MHz)	6.66	5.23–5.27	5.97–6.02	~5.75	~4.26	~4.58
Methyl sulfoxide-d_6 (100 MHz)	6.50	5.13	5.82	~5.65 (5.65)[b]	4.12 (4.11)	4.42 (4.42)

Solvent	*Coupling constants* (*Hz*)								
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5,5}$	J_{H-1-F}	J_{H-2-F}	J_{H-3-F}
Carbon tetrachloride (60 MHz)	3	3	3	—	—	—	6	48	24–30
Acetone-d_6 (60 MHz)	3	3	3	~2	~3	~13	6.5	48–52	26–30
Methyl sulfoxide-d_6 (100 MHz)	2.9	3.0	3.0	1.6 (1.6)	3.2 (3.2)	13.0 (−13.0)	6.7	48.0	28

[a]With tetramethylsilane as internal standard. [b]Numbers in parentheses are values calculated by computer.

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are *gauche*, rather than *anti*. Also, *every other* vicinal H–H coupling constant is of the same magnitude. The chemical shift of H-5a was assigned to a lower field than H-5e by application of the rule[4] that an axial C-O bond deshields *syn*-axial protons. The chemical shift for H-4 is only approximate, since its signal is buried beneath part of the H-3 signal. The $J_{H\text{-}4-H\text{-}5a}$, $J_{H\text{-}4-H\text{-}5e}$, and $J_{H\text{-}5a-H\text{-}5e}$ values were obtained by a first-order analysis of the 100-MHz spectrum. They were found to be almost identical with values obtained from an ABX sub-spectrum computed on an IBM 1620 computer using the CHEM 3 program of Wilkins and Klopfenstein[5] (Table I). In these calculations, the geminal H-5a–H-5e coupling constant was assumed to be negative[6].

The proton–proton coupling constants given in Table I are consistent with expected values for either the α-D or β-D anomer in the *1C* conformation. The α-D anomer can be ruled out, however, on the basis of the vicinal proton–fluorine coupling constants (Table I). The *1C*(β-D) isomer would possess one *gauche* arrangement and one *anti* arrangement of proton and fluorine, whereas the *1C*(α-D) form would have two *anti* arrangements of the atoms. Therefore, the coupling constants ($J_{H\text{-}1-F}$ 6.7 Hz, $J_{H\text{-}3-F}$ 28 Hz)* are only compatible[7] with the *1C*(β-D) isomer. The magnitudes of the two vicinal H–F couplings strongly favor a pure *1C* conformer, and the conformational purity was established by temperature studies.

The n.m.r. spectrum of **1** was studied over the temperature range of 43–120° in deuterated methyl sulfoxide with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard, and of −40 to +60° in deutereated acetone with tetramethylsilane (TMS) as internal standard. The half-band widths of the H-1 triplet and AB sub-system due to the H-5 protons remained essentially unchanged over the entire temperature range when thermal broadening effects, as evidenced by the broadened TMS (or DSS) signal, were taken into consideration. Since the conformers almost certainly differ appreciably in energy, and since couplings and chemical shifts will be different from one conformation to the other, a change would be expected in the n.m.r. spectrum over a substantial temperature range if equilibrating species were present. The conformational purity of the sample was further supported by the nearly identical coupling constants (H–H and H–F) found in three different solvents, namely, deuterated methyl sulfoxide, carbon tetrachloride, and deuterated acetone.

Vicinal coupling constants depend on bond length and hybridization[8], electronegativity of substituents[9], orientation of electronegative substituents[10], as well as on bond angles[11]. Hence, as stated by Karplus[8], angles predicted from coupling constants can contain some uncertainty. Empirical approaches to a modified "Karplus curve" have led Abraham *et al.*[12] to modify the original Karplus equation for application to carbohydrates, and Lemieux *et al.*[13] to predict an upward displacement of about 2 Hz on the "Karplus curve" for carbohydrate derivatives. The predicted dihedral bond angles obtained from the coupling constants, as well as the actual values based on perfect geometrical models, for chair and flexible forms of **1** are given in Table II.

*These values have been substantiated by ^{19}F n.m.r. spectroscopy kindly carried out by Dr. L. D. Hall of the University of British Columbia.

Inspection of the table shows that a chair conformation fits the observed coupling constants best.

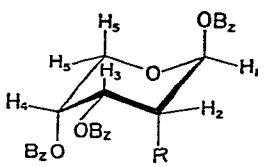
TABLE II

THEORETICAL AND CALCULATED DIHEDRAL ANGLES FOR 1,3,4-TRI-*O*-BENZOYL-2-DEOXY-2-FLUORO-β-D-RIBOSE

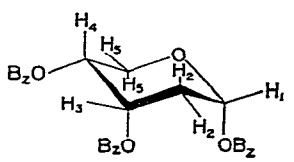
Conformation	*Theoretical dihedral angles (in degrees)*				
	H-1–H-2	*H-2–H-3*	*H-3–H-4*	*H-4–H-5a*	*H-4–H-5e*
1C(β-D)	60	60	60	60	60
1C(α-D)	60	60	60	60	60
S(β-D)[a]	87	71	33	153	33
B(β-D)[a]	120	60	60	120	0
	Dihedral angles calculated from 100-MHz spectrum (in degrees)				
	H-1–H-2	*H-2–H-3*	*H-3–H-4*	*H-4–H-5a*	*H-4–H-5e*
After Karplus[11]	52(126)	52(126)	52(126)	61(116)	50(127)
After Abraham[12]	54(124)	54(124)	54(124)	63(114)	52(126)
After Lemieux[13]	68(111)	67(112)	67(112)	83(96)	65(113)

[a]The skew (*S*) and boat (*B*) conformations are considered the most likely of the flexible forms.

Although the dipole–dipole interactions in the *1C*(β-D) form are likely to keep the values of the O–C-1–C-2 bond angles very close to those found in a full chair conformation, the *syn*-axial interactions between the substituents at C-2 and C-4 and between the 1-*O*-benzoyl group and the protons at C-3 and C-5 may cause some flattening of the pyranose ring, thus slightly varying the proton–proton dihedral angles.



1 R = F
2 R = H



3

This may account for the constancy in the magnitude of the coupling constants, despite the varying electronegativity of the substituents. Laszlo and Schleyer[14] have stated that electronegativity differences account for only a *maximum* deviation of 20% of the coupling constant as compared with that of the corresponding saturated compound.

Since the 2-deoxy-2-fluoro tribenzoate (**1**) was found to exist exclusively in the *1C* form, it was of interest to know whether the corresponding deoxy analog, namely 1,3,4-tri-*O*-benzoyl-2-deoxy-β-D-*erythro*-pentose[2] (**2**), showed the same conformation. N.m.r. data for both α-D and β-D anomers of 1,3,4-tri-*O*-benzoyl-2-deoxy-D-*erythro*-pentose are reported in Table III. The $J_{1,2}$ of 3.9 Hz for the H-1 triplet of the α-D

anomer (3), as well the $J_{4,5e}$ and $J_{4,5a}$ couplings of 4.0 and 7.9 Hz, respectively, establishes the *C1* conformation for the α-D anomer. The $J_{4,5}$ values for 3, in which H-5a is opposed by two *syn*-axial C-O bonds, show that H-5a occurs to lower field than H-5e. This result supports the assignment of the lower field signal to H-5a in the case of **1**, as described above.

In the case of the 2-deoxy-β-D compound (**2**), the values for $J_{1,2}$ and $J_{4,5}$ are almost identical with those found for the corresponding fluoro analog (**1**) given in Table I. Thus, the β-D anomer of the 2-deoxy tribenzoate (**2**) also exists in the *1C* conformation.

TABLE III

N.M.R. PARAMETERS FOR 1,3,4-TRI-*O*-BENZOYL-2-DEOXY-α- AND β-D-*erythro*-PENTOSE (3 AND 2) IN ACETONE-d_6[a]

Compound	*Chemical shifts (δ)*						
	H-1	*H-2a*	*H-2e*	*H-3*	*H-4*	*H-5a*	*H-5e*
2	6.72	2.68[b]		~6.0[b]	~5.79[b]	4.52	4.19
3	6.43	2.62[c,d]		5.82[d]	5.54[d]	4.52	4.08

Compound	*Coupling constants (Hz)*								
	$J_{1,2a}$	$J_{1,2e}$	$J_{2a,2e}$	$J_{2a,3}$	$J_{2e,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5a,5e}$
2	~2.9	~2.9	—	—	—	—	1.2	2.5	13.1
3	3.9	3.9	0[c,d]	4.8	4.8	~3[d]	7.9	4.0	11.4

[a]Tetramethylsilane as internal standard. [b]Unresolved multiplet. [c]Protons at C-2 appear to be magnetically equivalent. [d]Values obtained from spin-decoupling experiments.

Temperature studies were carried out with **2** and with **3**, as described above for **1**. The results for **2** over the temperature range of 43–140° show no change in the n.m.r spectrum of the H-5 protons. The spectrum remains sharp throughout the entire range. Attempts to obtain spectra below 43° were thwarted by insolubility. In the case of the α-D anomer (**3**), the H-1 and H-5 signals were studied in the temperature range of 0–120°. Again the half-band widths remained essentially unchanged over the entire range. Thus, the data are consistent with the existence of pure conformers for **2** and **3**.

The exclusive existence, in the compounds investigated, of the conformer having an axially-oriented aglycon may be attributed to the influence of the anomeric effect first described by Lemieux[15]. In recent papers[16,17], the anomeric effect has also been invoked to explain the *1C* conformation of several β-D-ribopyranosyl halides. Coxon[16] has also reported that 1,2,3,4-tetra-*O*-benzoyl-β-D-ribopyranose, the 2-*O*-benzoyl analog of compounds **2** and **1**, exists predominantly (67%) as the *1C* conformer. The tendency of this compound to exist in the *1C* conformation was attributed to the anomeric effect. The fact that the *1C* conformer of the tetrabenzoate possesses a *syn*-axial interaction between the benzoyl groups at C-2 and C-4 may explain why it does not exist exclusively in the *1C* conformation.

The H-5a–H-5e coupling constants of 13.0 Hz for **1** and 13.1 Hz for **2** are similar to the values reported by Coxon[16] who observed that sugars in the *1C* conformation possess H-5a–H-5e couplings of 13.4–14.0 Hz, whereas sugars in the *C1* conformation show values of 10.8–10.9 Hz. The value for $J_{5a,5e}$ of 11.4 Hz found for the *C1* conformer, **3**, is somewhat higher than the range (10.8–10.9) reported by Coxon[16].

The anomeric protons in **2** and **3** should be magnetically equivalent in terms of their immediate environment, since differences exist only in the orientation of substituents at C-3 and C-4. The absence of substituents other than H at C-2 enables one to detect of long-range effects on H-1 by the C-3 substituent[7]. The equatorial H-1 signal of **2** is found 0.29 p.p.m. lower than the equatorial H-1 signal of **3**. Evidently there is a long-range shielding effect due to the particular orientation of the benzoyl group at C-3, operating in the α-D anomer (**3**).

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SUMMARY

N.m.r. studies have been conducted on 1,3,4-tri-*O*-benzoyl-2-deoxy-2-fluoro-β-D-ribose (**1**) and on the β and α anomers of 1,3,4-tri-*O*-benzoyl-2-deoxy-D-*erythro*pentose (**2** and **3**, respectively). Temperature studies have shown that all exist as pure conformers having an axially oriented aglycon. The conformation of **1** and **2** was established as *1C*, that of **3** as *C1*.

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NUCLEOPHILIC DISPLACEMENT REACTIONS IN CARBOHYDRATES
THE FORMATION OF 1,4-ANHYDRO-6-DEOXY-2,3-*O*-ISOPROPYLIDENE-β-L-TALOPYRANOSE (1,5-ANHYDRO-6-DEOXY-2,3-*O*-ISOPROPYLIDENE-α-L-TALOFURANOSE)

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INTRODUCTION

In a recent report[1], we described a base-catalysed rearrangement of 2,3:6,7-di-*O*-isopropylidene-5-*O*-methanesulphonyl-D-*glycero*-D-*gulo*-heptofuranose, leading to the formation, amongst other products, of 1,4-anhydro-2,3:6,7-di-*O*-isopropylidene-α-D-*glycero*-D-*allo*-heptopyranose, which was considered to arise by an intramolecular, nucleophilic displacement. However, there is some confusion as to the stereochemistry of the products formed by such reactions, and some of the early reports on the formation of 1,4-anhydro sugars are not based on rigorous chemical characterisation and warrant closer scrutiny.

Treatment of 2,3,6-tri-*O*-methyl-α-D-glucosyl chloride with sodium in ether was reported by Freudenberg and Braun[2] to yield a 1,4-anhydro compound that was unreactive towards Fehling's solution, bromine water, and potassium permanganate solution. A later attempt[3] to reproduce this synthesis apparently yielded only unsaturated products. Micheel and Micheel[4] examined the action of trimethylamine in ethanol–benzene on 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide and obtained a crystalline product, which was weakly reducing towards Fehling's solution and was considered to be 2,3-di-*O*-acetyl-1,4-anhydro-α-L-rhamnopyranose. It was also claimed[5] that the reaction of 2,3,6-tri-*O*-methyl-4-*O*-toluene-*p*-sulphonyl-D-glucopyranose with sodium methoxide gave 1,4-anhydro-2,3,6-tri-*O*-methyl-β-L-idopyranose, with inversion occurring at C-5, rather than at the carbon atom bearing the sulphonate group.

The latter reaction was re-investigated by Kops and Schuerch[6], and it was demonstrated that the anhydro sugar has the D-*galacto* configuration, and so the displacement follows the expected stereochemical course. Similarly, an unresolved mixture of 2,3-di-*O*-methyl-4(5)-*O*-toluene-*p*-sulphonyl-D-xylopyranose(furanose) afforded 1,4-anhydro-2,3-di-*O*-methyl-α-L-arabinopyranose and 1,4-anhydro-2,3-di-*O*-methyl-α-D-xylopyranose on treatment with sodium isopropoxide[6].

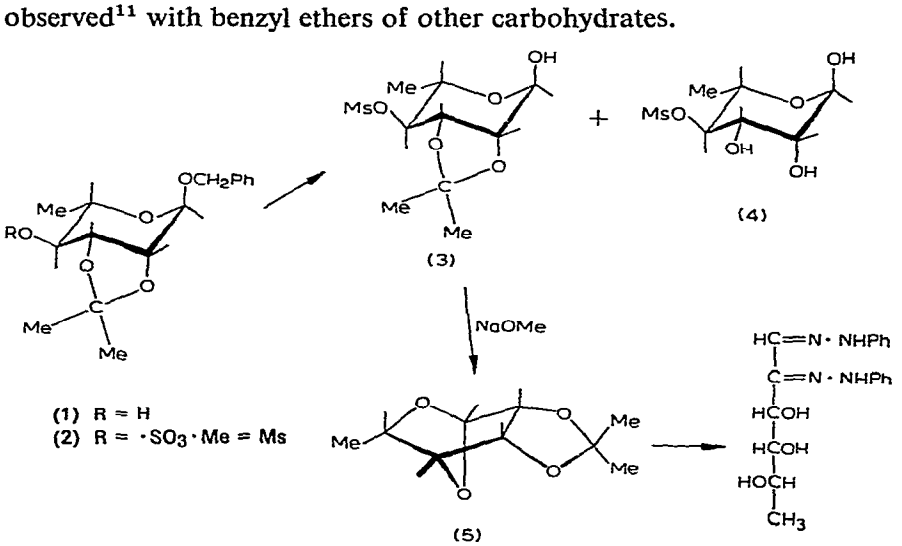
1,4-Anhydropyranoses may also be regarded[7] as 1,5-anhydrofuranoses, so that displacement reactions at C-5 of a furanose provide an alternative route to these compounds. Thus, 1-*O*-acetyl-2,3,6-tri-*O*-methyl-5-*O*-toluene-*p*-sulphonyl-D-glucofuranose is converted by the action of sodium methoxide into an anhydro sugar, which originally was assigned[8] the D-*gluco* configuration, but which, in the light of

present-day knowledge, can be assigned the structure 1,5-anhydro-2,3,6-tri-*O*-methyl-α-L-idofuranose (1,4-anhydro-2,3,6-tri-*O*-methyl-β-L-idopyranose). Vis and Fletcher[7] reported that both 2,3-*O*-benzylidene- and 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-D-ribofuranose are converted into the corresponding 1,5-anhydro-β-D-ribofuranoses with sodium isopropoxide.

In view of the controversy over the structures of some of the 1,4-anhydro sugars known at the start of our investigation, particularly those resulting from intramolecular displacements on a pyranoid ring, we have examined the formation of such compounds by using 2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-L-rhamnopyranose (**3**) as a representative molecule. Additionally, it was hoped to gain some information on the rate of formation of 1,4-anhydro sugars, under conditions comparable to those used in our previous investigation[1].

DISCUSSION

A convenient starting-point for the preparation of compound **3** is benzyl α-L-rhamnopyranoside, since subsequent removal of the glycosidic substituent can be accomplished under mild, catalytic conditions[9]. The rhamnopyranoside is isolated[10] only with difficulty from the acid-catalysed reaction of L-rhamnose with benzyl alcohol, but is smoothly converted into benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**) on treatment with acidified acetone. Later, it was found that compound **1** could be isolated, in crystalline form, by acetonation of the original rhamnoside mixture. Reaction of compound **1** with methanesulphonyl chloride in pyridine afforded benzyl 2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-rhamnopyranoside (**2**). In passing, it is interesting to note that the n.m.r. spectra of compounds **1** and **2** showed that the glycosidic benzyl group has a restricted rotation, since the signals of the methylene protons appear as an AB system (see Experimental); this effect has been observed[11] with benzyl ethers of other carbohydrates.



Catalytic debenzylation of glycoside **2** afforded crystalline 2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-L-rhamnopyranose (**3**), together with a small proportion of a product (**4**), arising from cleavage of the acetal group. The latter compound exhibited upwards mutarotation indicative of an α-L configuration. In agreement with this assignment, the n.m.r. spectrum (deuterium oxide) initially showed a narrow doublet at τ 4.91, ascribed to the anomeric proton of the α-L form, whilst that of the equilibrium solution exhibited two narrow doublets at τ 4.91 and 5.13. Compound **3** had a positive rotation (+36°) in methanol but did not mutarotate in this or several other solvents. Its n.m.r. spectrum (deuteriochloroform containing some deuterium oxide) revealed the presence of a single anomer that gave rise to a singlet for H-1 at τ 4.67; the spectrum was unchanged after two days, and, in the absence of deuterium oxide, the signal for H-1 appeared as a doublet due to coupling with the hydroxylic proton. The signal for the anomeric proton appeared as a singlet at τ 4.68 when the spectrum was measured in deuterium oxide, but since compound **3** was only sparingly soluble in this solvent, the presence of a small proportion of the other anomer cannot be excluded. Angyal *et al.*[12] have shown that for an aqueous, equilibrium solution of 2,3-*O*-isopropylidene-4-*O*-methyl-L-rhamnopyranose, the H-1 signal for the α-anomer appears as a singlet at τ 4.71 and for the β-anomer as a doublet at τ 4.90; in deuteriochloroform, these signals occur at τ 4.70 and 5.10, respectively. This evidence suggests that compound **3** crystallises as the α-L anomer.

On treatment with sodium methoxide in methanol at room temperature, compound **3** was rapidly (*ca.* 15 min) converted into a single product with the precipitation of sodium methanesulphonate. The product was obtained as a readily volatile solid, which was best purified by sublimation. It had a molecular weight (mass spectrometry) of 186 and exhibited no infrared absorption attributable to either C=C or OH groups. These data suggested that the product is 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-β-L-talopyranose (1,5-anhydro-6-deoxy-2,3-*O*-isopropylidene-α-L-talofuranose) (**5**), and this structure is supported unequivocally by n.m.r. spectroscopy and chemical evidence. The n.m.r. spectrum of **5** (Fig. 1) is extremely simple, since no coupling is observed between H-1–H-2, H-3–H-4, H-4–H-5, which subtend dihedral angles of *ca.* 80° on the bicyclic ring-system, and H-2 and H-3 appear to be equivalent. The quartet at τ 6.55, corresponding to one proton, can clearly be ascribed to H-5. Acid hydrolysis of anhydro sugar **5** proceeded as expected for a 1,4(1,5)-glycosan to yield 6-deoxy-L-talose (chromatographic identification) as the only reducing component. The sugar was characterised as the crystalline phenylosazone (**6**), which was identical with that prepared from L-fucose.

In view of our present experience and other evidence[6], it appears that intramolecular displacements of pyranose 4-sulphonates yield products having the expected stereochemistry. However, recent evidence[13,14] suggests that, in our case, the mechanism may not be as straightforward (see later).

As an alternative, we examined the preparation of anhydro sugar **5** by intramolecular displacement of the sulphonate group of 6-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-D-allofuranose (**10**). On treatment with sodium benzyl oxide in

benzyl alcohol, 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-L-rhamnofuranose (**7**) was converted into crystalline benzyl 6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranoside (**8**) by a rearrangement analogous to that observed with sodium methoxide[15]. Compound **8** gave 6-deoxy-D-allose on acid hydrolysis, and its infrared and n.m.r. spectra (see Experimental) were in complete agreement with the structure assigned;

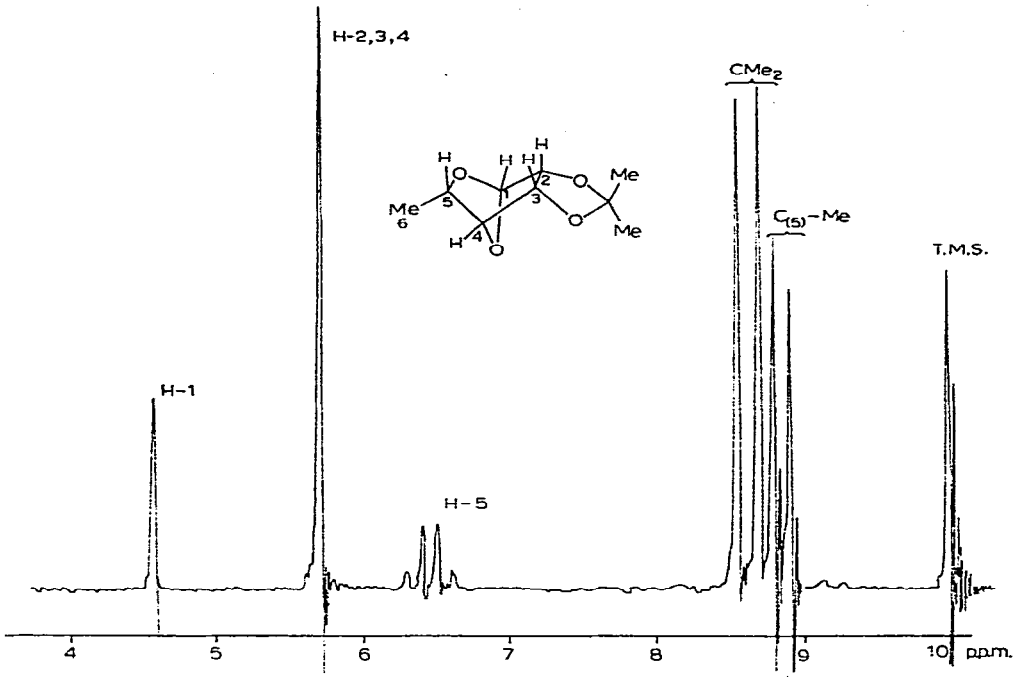
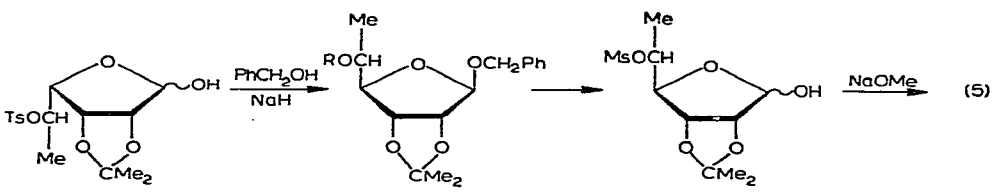


Fig. 1. The n.m.r. spectrum of 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-β-L-talopyranose (**5**) in deuteriochloroform at 60 MHz.

the methylene protons of the benzyl group again appeared as an AB system. The derived methanesulphonate (**9**) was obtained as a syrup that could not be freed entirely from impurities. It is likely that the impurities were responsible for deactivating the palladium–charcoal catalyst used in subsequent, unsuccessful attempts to remove the glycosidic benzyl group. Compound **9** was therefore hydrolysed under conditions to remove both the acetal and glycosidic substituents. The resulting syrup was re-acetonated to give a product that was presumed to be mainly 6-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-D-allofuranose (**10**), since treatment with sodium methoxide rapidly converted it into anhydro sugar **5**. The overall yield of anhydro sugar **5** obtained by this sequence was low, and the first procedure is clearly the method of choice.

If the conversion **3**→**5** proceeds by a normal, intramolecular displacement, it must involve the β-L anomer, since only in this configuration and by adopting a boat conformation of the pyranoid ring can the molecule attain the correct stereochemistry. There is evidence[13,14] to indicate that bimolecular, nucleophilic reactions of rhamno-

pyranoside 4-sulphonates occur *via* an unusual ring-contraction involving participation of the C-5–ring oxygen bond, which is *trans*-antiparallel to the bond undergoing cleavage. An analogous mechanism could presumably operate in the formation of anhydro sugar **5** by an intramolecular displacement and must necessarily occur through the α-L anomer. The rapid deposition of sodium methanesulphonate prevented any measurement of optical rotational changes when the base was added to a solution of compound **3** in methanol. A choice between these possibilities may be possible when the mechanism of displacement reactions of rhamnopyranoside 4-sulphonates is fully established.



(7) (8) R=H (9) R=Ms (10)

The dramatically different reactions of compounds **7** and **10** with base is also worthy of comment*. Two points can be made. With compound **7**, anhydro-ring formation would require that the nucleophile approach the sulphonate group from within the V-shape formed by the trioxabicyclo[3.3.0]octane ring-system, where it is likely to experience adverse steric and/or electronic interactions. Secondly, in the transition state for this reaction, a severe, steric interaction will be introduced between the C-5 hydrogen atom and the relatively inflexible 1,3-dioxolan ring. These interactions are presumably sufficient to divert the reaction to another course[15,21].

These considerations do not apply in the case of compound **10**, and it is significant that, in the transition state for the reaction leading to anhydro sugar **5**, the 1,3-dioxolan ring is approaching an *exo*-configuration with respect to the bioxabicyclo-[2.2.1]heptane ring-system being formed. The same consideration holds for other 2,3-*O*-isopropylidenefuranose sulphonates where anhydro-ring formation is observed[1,7]. The ease of intramolecular, nucleophilic displacement of the sulphonate group in this instance is also of interest in view of a report[14] that displacements with methyl 6-deoxy-2,3-*O*-isopropylidene-5-*O*-*p*-bromobenzenesulphonyl-β-L-allofuranoside involve participation and migration of the methoxyl group.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was performed on Kieselgel G and detection was effected with vanillin–sulphuric acid[16]. N.m.r. spectra were normally obtained

*We have included this discussion at the suggestion of a referee, to whom we are indebted for valuable comment. A detailed discussion of the effects governing anhydro-ring formation in other molecules is reserved for a later communication.

with a Varian A-60 spectrometer for *ca.* 10% solutions in deuteriochloroform with tetramethylsilane as internal reference; for solutions in deuterium oxide, sodium 4,4-dimethyl-4-silapentane-1-sulphonate was used as reference. Infrared spectra were recorded for Nujol mulls with a Perkin–Elmer Infracord spectrometer, or for comparative purposes, on *ca.* 3% solutions in carbon tetrachloride with a Perkin–Elmer 125 spectrometer. Molecular weights were measured on an A.E.I. MS9 mass spectrometer.

Benzyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (**1**). — L-Rhamnose monohydrate (25 g) was dissolved in redistilled benzyl alcohol (300 ml) containing conc. hydrochloric acid (3 ml), and the solution was stored for 3 days at room temperature and then neutralised with conc. ammonia (2.5 ml). The excess of benzyl alcohol was largely removed under diminished pressure to yield a syrup that contained a major component indistinguishable in its chromatographic behaviour (t.l.c., acetone–toluene, 1:1) from that of authentic benzyl α-L-rhamnopyranoside[10]. The syrup was taken up in dry acetone (1 litre) containing conc. sulphuric acid (3 ml) and kept for 1 h at room temperature. After the acid had been neutralised with conc. ammonia (10 ml), the solvents were removed, and the residue was extracted with chloroform (500 ml), which was washed with water (3 × 500 ml) and dried ($MgSO_4$). Removal of the chloroform afforded an oil, containing benzyl alcohol, which yielded the product, b.p. 155–165°/0.075 mm, on fractional distillation. The distillate crystallised on standing and gave compound **1** (11 g, 27%), m.p. 73–75°, $[\alpha]_D$ −55° (*c* 1, chloroform), on recrystallisation from light petroleum (b.p. 80–100°) (Found: C, 65.2; H, 7.2. $C_{16}H_{22}O_5$ calc.: C, 65.3; H, 7.5%). N.m.r. data: τ 2.70 (5-proton singlet, aromatic); 4.98 (singlet, H-1); 5.42 (AB quartet, *J* 12 Hz, benzyl methylene protons); 8.48, 8.66 (3-proton singlets, CMe_2); 8.70 (3-proton doublet, $J_{5,6}$ 6 Hz, $C_{(5)}$-Me).

The compound prepared by the above procedure was identical (t.l.c., infrared spectrum, m.p. and mixed m.p.) with that obtained by acetonation of crystalline benzyl α-L-rhamnopyranoside[10] (kindly donated by Dr. M. C. Cook).

Benzyl 2,3-O-isopropylidene-4-O-methanesulphonyl-α-L-rhamnopyranoside (**2**). — Methanesulphonyl chloride (5 ml) was added to a solution of compound **1** (3 g) in dry pyridine (75 ml), and the mixture was stored for 24 h at room temperature. Water (3 ml) was added and, after 15 min, the solution was added to ether (200 ml), which was washed with water (3 × 200 ml) and dried ($MgSO_4$). Removal of the solvent, with recrystallisation of the residue from aqueous methanol and finally from ether–light petroleum (b.p. 40–60°), gave compound **2** (3.1 g), m.p. 87–88°, $[\alpha]_D$ −28° (*c* 2, methanol). (Found: C, 55.05; H, 6.6; S, 8.4. $C_{17}H_{24}O_7S$ calc.: C, 54.8; H, 6.45; S, 8.6%). N.m.r. data: τ 2.68 (5-proton singlet, aromatic); 4.93 (singlet, H-1); 5.40 (AB quartet, *J* 12 Hz, benzyl methylene protons); 6.84 (3-proton singlet, $MeSO_2$); 8.43, 8.65 (3-proton singlets, CMe_2); 8.69 (3-proton doublet, $J_{5,6}$ 6 Hz, $C_{(5)}$-Me).

2,3-O-Isopropylidene-4-O-methanesulphonyl-α-L-rhamnopyranose (**3**) *and 4-O-methanesulphonyl-α-L-rhamnopyranose* (**4**). — A solution of glycoside **2** (3 g) in methanol (250 ml) containing a suspension of 5% palladium-on-charcoal (1 g) was shaken under a slight overpressure of hydrogen for 7 days, whereupon t.l.c. (acetone–toluene, 1:3) showed the complete disappearance of starting material (R_F 0.6) and the

formation of two products (R_F 0.1 and 0.4). The catalyst was filtered off, and the filtrate was concentrated, under reduced pressure, to a solid residue. Recrystallisation from methanol–ether gave compound **4** (0.36 g), m.p. 142–143° (decomp.), $[\alpha]_D$ −13° (2 min)→ *ca.* 0° (equil) (*c* 1, water) (Found: C, 34.9; H, 5.8; S, 13.3. $C_7H_{14}O_7S$ calc.: C, 34.7; H, 5.8; S, 13.25%). N.m.r. data for an equilibrated solution in deuterium oxide: τ 4.91, 5.13 (doublets, $J_{1,2}$ *ca.* 1.5 Hz, 1 proton, anomeric protons); 6.72 (3-proton singlet, $MeSO_2$); 8.70 (3-proton doublet, $J_{5,6}$ 6 Hz, $C_{(5)}$-Me). The mother liquors were concentrated, and the residue was recrystallised from ether–light petroleum (b.p. 40–60°) to yield compound **3** (1.4 g), m.p. 91–92°, $[\alpha]_D$ +36° (*c* 1, methanol); no mutarotation was observed in chloroform, methanol, or aqueous methanol (Found: C, 42.4; H, 6.2; S, 11.7. $C_{10}H_{18}O_7S$. calc.: C, 42.7; H, 6.4; S, 11.4%). N.m.r. data: τ 4.67 (doublet, *J* 3 Hz, H-1); 6.86 (3-proton singlet, $MeSO_2$); 8.43, 8.67 (3-proton singlets, CMe_2); 8.67 (3-proton doublet, $J_{5,6}$ 6 Hz, $C_{(5)}$-Me); on the addition of deuterium oxide, the signal at τ 4.67 collapsed to a singlet.

*Benzyl 6-deoxy-2,3-*O*-isopropylidene-β-*D*-allofuranoside* (**8**). — 2,3-*O*-Isopropylidene-5-*O*-toluene-*p*-sulphonyl-L-rhamnofuranose[15] (6 g) was added to a solution of sodium benzyl oxide [250 ml; prepared with sodium hydride (2 g)], and the mixture was stored for 7 days at room temperature. The excess of reagent was destroyed with carbon dioxide, and the mixture was dispersed in water (200 ml), which was extracted with ethyl acetate (2 × 200 ml). Concentration of the dried ($MgSO_4$) extracts yielded an oil that contained a substantial proportion of benzyl alcohol. This was removed by distillation under diminished pressure (*ca.* 63°/0.1 mm), and further distillation afforded compound **8** (4.5 g), b.p. 115–120°/0.05 mm, as a syrup that crystallised on standing. The alloside **8** was recrystallised, with difficulty, from methanol and had m.p. 66–67°, $[\alpha]_D$ −101° (*c* 1, chloroform) (Found: C, 65.2, H, 7.4. $C_{16}H_{22}O_5$ calc.: C, 65.3; H, 7.5%). N.m.r. data: τ 2.67 (5-proton singlet, aromatic); 4.82 (singlet, H-1); 5.32 (AB quartet, *J* 12 Hz, benzyl methylene protons); 8.52, 8.68 (3-proton singlets, CMe_2); 8.78 (3-proton doublet, $J_{5,6}$ 7 Hz, $C_{(5)}$-Me).

Hydrolysis of alloside **8** (40 mg) in boiling dioxane (1 ml) with 2N sulphuric acid (1 ml) for 4 h gave, after neutralisation and concentration, 6-deoxy-D-allose, m.p. and mixed m.p. 144–145° (lit.[15], m.p. 151–152°).

*Benzyl 6-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-β-*D*-allofuranoside* (**9**). — Compound **8** (0.1 g) in a mixture of chloroform (5 ml) and pyridine (5 ml) was treated at room temperature with methanesulphonyl chloride (0.2 ml) for 12 h, and the solution was then processed in the usual manner. The sulphonate (**9**) (0.14 g), $[\alpha]_D$ −80° (*c* 1, chloroform), was obtained as a chromatographically homogeneous syrup, which could not be induced to crystallise and was contaminated with solvent.

*6-Deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-D-allofuranose* (**10**). — Attempts to remove the glycosidic benzyl substituent by catalytic means were unsuccessful, and the following procedure was adopted.

The sulphonate **9** (0.14 g) in acetone (5 ml) was heated under reflux with N hydrochloric acid (5 ml) for 30 min, whereafter the hydrolysate was diluted with water (100 ml), extracted with ether (2 × 50 ml), and neutralised with Amberlite IRA-400

(CO_3^{2-}). The solvents were removed to give a syrup (68 mg), which appeared to be homogeneous by t.l.c. (toluene–acetone, 3:1). The syrup was acetonated with acidified acetone in the normal way to yield a slightly impure, syrupy product (73 mg), $[\alpha]_D$ +3° (*c* 2, ether). The syrup appeared to be essentially homogeneous by t.l.c., whilst n.m.r. spectroscopy demonstrated the presence of both sulphonate and acetal substituents, but also indicated the presence of contaminants, which were not readily removed.

1,4-Anhydro-6-deoxy-2,3-O-isopropylidene-β-L-talopyranose (**5**). — (*a*) 2,3-*O*-Isopropylidene-4-*O*-methanesulphonyl-L-rhamnopyranose (0.5 g) was treated with N sodium methoxide in methanol (5 ml) for 1 h at room temperature; t.l.c. showed that the reaction was essentially complete after 15 min. The reaction mixture was dispersed in ether (200 ml) and washed successively with dilute sulphuric acid (200 ml) and water (200 ml). Concentration of the dried ($MgSO_4$) organic layer yielded a crystalline residue (0.27 g), which was sublimed (*ca.* 70°/15 mm) to give anhydro sugar **5** (0.21 g, 60%), m.p. 71–73°, $[\alpha]_D$ −51° (*c* 1, methanol) (Found: C, 58.4; H, 7.65. $C_9H_{14}O_4$ calc.: C, 58.1; H, 7.5%). The mass spectrum of the product contained a top mass peak at 171 (M-15) corresponding[17] to a molecular weight of 186. Subsequent experience indicated that considerable loss of material occurred during evaporation of the ether solution.

Hydrolysis of the anhydro sugar (0.15 g) in dioxane (1 ml) was accomplished with 2N sulphuric acid (1 ml) in a sealed tube for 2 h at 90–100°. After neutralization with Amberlite IRA-400 (CO_3^{2-}), the solution was concentrated to yield a syrupy, reducing sugar (80 mg) with chromatographic properties indistinguishable from those of 6-deoxy-L-talose. The osazone (**6**), prepared in the usual manner[18], had m.p. 168–170° (from aqueous ethanol), which was not depressed on admixture with the osazone prepared from L-fucose. Reist *et al.*[19] repoited m.p. 168–171° for this derivative, whilst Votoček and Červaný[20] gave m.p. 177–178°. The infrared spectra of the osazones were identical.

(*b*) 6-Deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-D-allofuranose (73 mg) was treated with N sodium methoxide in methanol (0.5 ml), as described above, to give anhydro sugar **5** (14 mg), m.p. and mixed m.p. 71–73°, on sublimation; t.l.c. showed that only one product was formed. The infrared spectrum and chromatographic properties of the anhydro sugar were indistinguishable from those of previously prepared **5**.

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SUMMARY

The action of sodium methoxide in methanol on 2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-L-rhamnopyranose rapidly yields 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-β-L-talopyranose (1,5-anhydro-6-deoxy-2,3-*O*-isopropylidene-α-L-talofuranose) as the sole product. The structure is assigned on the basis of chemical and n.m.r. evidence. The anhydro sugar is also obtained by similar treatment of 6-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-D-allofuranose, prepared from benzyl 6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranoside.

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ASYMMETRIC SYNTHESIS

PART I. A STEREOSELECTIVE SYNTHESIS OF BENZYLIC CENTRES. DERIVATIVES OF 5-*C*-PHENYL-D-*gluco*-PENTOSE AND 5-*C*-PHENYL-L-*ido*-PENTOSE

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INTRODUCTION

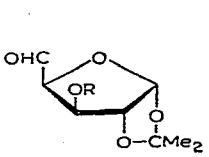
The biological importance of compounds that contain an asymmetic carbon atom attached directly to an aromatic ring (*i.e.*, the benzylic carbon atom), and the critical dependence of their biological activity on the absolute configuration of this atom[1] make it desirable that a stereospecific synthesis of such asymmetric centres should be readily available. One approach to asymmetric synthesis involves addition reactions to a carbonyl group where the direction of the addition is influenced by the proximity of a neighbouring asymmetric centre. This type of reaction has been widely investigated[2] and has led to the formulation of the empirical rules of Cram[3] and Prelog[4]. These rules make it possible to predict the configuration of the preferred isomer, but, generally, the stereoselectivity of most of the reported reactions has been low. In carbohydrate chemistry, however, it has long been recognised that additions to the aldehyde function are highly stereoselective[5], and, more recently, it has been shown that the addition of Grignard reagents to keto sugars is highly stereoselective and perhaps in some cases stereospecific[6].

In 1962, it was reported that the addition of methylmagnesium iodide to 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**2**) gave only 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose (**8**) and that no trace of the corresponding D-*gluco* isomer (**5**) could be detected[7]. This suggested that, if the addition of phenylmagnesium bromide to **2** followed the same path, it would constitute a stereospecific synthesis of a benzylic centre which was in a system that could be used to establish the absolute configuration of the new asymmetric centre. Moreover, by use of appropriate techniques, the asymmetric, benzylic carbon atoms of established configuration could then be detached from the carbohydrate substrate and incorporated into other molecules. This paper is concerned with the stereospecific synthesis and configurational assignment of benzylic carbon atoms.

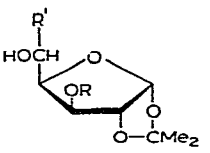
DISCUSSION

The reaction between 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**2**) and phenylmagnesium bromide in ether yielded two compounds

that were separated by chromatography over silica gel. The minor product (5%, $[\alpha]_D$ −76°, chloroform), which was eluted first, was subsequently shown to be 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (**3**). The major product (72%, $[\alpha]_D$ −33°, chloroform) was therefore formulated as 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose. The absolute configuration of the benzylic centre in **3**, and hence its structure, was established in the following fashion. Compound **3** was hydrogenolysed at atmospheric pressure over freshly prepared palladium to give **4**, which was converted into 5-*C*-phenyl-α-D-*gluco*-pentose (**9**) by acid hydrolysis. The α-D anomeric configuration is tentatively assigned to **9** on the basis of the observed mutarotation. When **9** was acetylated with acetic anhydride and sodium acetate at 100°, a crystalline tetraacetate (**10**, m.p. 184–186°, $[\alpha]_D$ +23°, chloroform) was obtained, whereas acetylation of **9** with acetic anhydride and pyridine at 0° gave a predominantly crystalline tetraacetate (**11**, m.p. 144–145°, $[\alpha]_D$ +104°, chloroform), which was purified by fractional crystallisation. Compounds **10** and **11** could not be separated by thin-layer chromatography in any of the solvent systems examined. Both **10** and **11** gave n.m.r. spectra that could be analysed by first-order methods. The parameters extracted from these spectra are listed in the Experimental section. The structures of **10** and **11** are immediately and unequivocally apparent if the Karplus[8] equation is valid and the assumption is made that large coupling constants (8–12 Hz) indicate a projected valency angle of *ca.* 180° (antiperiplanar), and that small coupling constants (2–4 Hz) indicate a projected valency angle of *ca.* 60° (gauche). Thus with **10**, where all of the coupling constants are large (8–10 Hz), each pair of vicinal protons must have an antiperiplanar relationship, and only where there is a *gluco* configuration can this result arise. Thus **10** must be 1,2,3,4-tetra-*O*-acetyl-5-*C*-phenyl-β-D-*gluco*-pentopyranose with the benzylic centre having the D configuration. It will be recognised that the acetylation conditions chosen for preparation of **10** generally give the β-anomer in the D-glucose series, whereas **11** was formed under conditions that favour formation of the α-anomer[9]. The n.m.r. data clearly indicate that **11** must be 1,2,3,4-tetra-*O*-acetyl-5-*C*-phenyl-α-D-*gluco*-pentopyranose, since the value of 3.7 Hz for $J_{1,2}$ clearly indicates a gauche relationship for the C-1 and C-2 protons. Again, the value of 10 Hz for the coupling constant between the C-4 and C-5 protons indicates that they must have an antiperiplanar relationship and confirms that the benzylic centre must have the D configuration, since these compounds are derivatives of 5-*C*-phenyl-D-*gluco*-pentose.

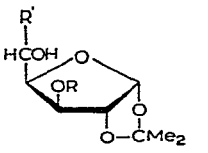


(1) R = H
(2) R = C_6H_5—CH_2



D-*gluco* series

(3) R' = C_6H_5 R = C_6H_5—CH_2
(4) R' = C_6H_5 R = H
(5) R' = CH_3, R = C_6H_5—CH_2



L-*ido* series

(6) R' = C_6H_5 R = C_6H_5—CH_2
(7) R' = C_6H_5 R = H
(8) R' = CH_3 R = C_6H_5—CH_2

These conclusions are further substantiated by a consideration of the chemical shifts. It has been shown that the chemical shifts of the C-3 and C-5 ring protons in the glucopyranose acetates depend predictably on the anomeric configuration[10]. Thus, where the C-1 acetoxy group is axial, the chemical shifts of the C-3 and C-5 protons tend to be at 0.25 p.p.m. to lower field than when the C-1 acetoxy group is equatorial. It will be seen that this generalisation also applies to the derivatives of 5-*C*-phenyl-D-*gluco*-pentopyranose.

Since **3** has the D-*gluco* configuration, it follows that **6** must have the L-*ido* configuration. Compound **6** was smoothly hydrogenolysed to 1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose (**7**), but subsequent hydrolysis with abid gave an amorphous product, acetylation of which failed to yield either of the pure anomeric tetraacetates. The n.m.r. spectrum of the anomeric mixture was too complex to be analysed by first-order methods.

It will be appreciated that, although the addition of phenylmagnesium bromide to **2** is not stereospecific it is highly stereoselective, and since the configurations of the products are readily determined, it provides a convenient route to a novel type of 5-*C*-substituted sugar of known configuration. Further, since such compounds as **3** and **6** contain a benzylic hydroxyl group at C-5, they can be converted into 4-*C*-benzyl-D-*xylo*-tetrose derivatives by prolonged hydrogenolysis. Thus, the hydrogenolysis of **3** to **4** and of **6** to **7** proceeded very rapidly, and either **4** or **7** was then slowly hydrogenolysed to 4-*C*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-tetrofuranose (**12**). Compound **12** was converted into 4-*C*-benzyl-D-*xylo*-tetrose by acid hydrolysis.

Since the addition of phenylmagnesium bromide to **2** resulted in the formation of two products, it was decided to repeat the experiment of adding methylmagnesium iodide to **2** by using essentially the conditions of Wolfrom and Hanessian[7]. The mother liquors, which remained after crystallisation of the 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose (**8**), were examined by thin-layer chromatography and found to contain a component having a higher mobility than that of **8**, in addition to a small proportion of **8**. The mother liquors were concentrated, and the residue was fractionated on silica gel. The faster-moving component was isolated and subsequently shown to be the expected 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**5**, $[\alpha]_D$ −64°, chloroform). That **5** was a derivative of 6-deoxy-D-glucose was confirmed by showing that the 5-benzoate was indistinguishable from the product obtained by treatment of 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-5-*O*-methanesulphonyl-β-L-idofuranose with sodium benzoate in *N*,*N*-dimethylformamide. This type of reaction is known to proceed with inversion of configuration[12]. The fact that some of the D-glucose derivative **5** was produced when methylmagnesium iodide was added to **2** is at variance with the results of Wolfrom and Hanessian[7], who claimed that they could find no trace of **5**. The proportion of isomers produced, however, corresponds to the proportion of **3** and **6** obtained when phenylmagnesium bromide was added to **2**.

The effect of changes in solvent and reagents on stereoselectivity have been severally reported[11]. It is not proposed now to further investigate the effect of such

changes, but the following points seem worthy of comment. In ether, the ratio of **3**:**6** produced when phenylmagnesium bromide is added to **2** is 1:14.4, whereas the addition of methylmagnesium iodide to **2**, under similar conditions, produces **5** and **8** in the ratio 1:7.7 However, since **3** and **5** were produced in low yield, a comparison of the relative ratios of the products from the two reactions may not be too meaningful. However, a more definite change in stereoselectivity is observed when the reaction medium is changed from ether to tetrahydrofuran. The product ratio **3**:**6** changes from 1:14.4, when the addition of phenylmagnesium bromide to **2** takes place in ether, to 1:3.3 when a similar reaction is performed in tetrahydrofuran.

The relative ratios of D-*gluco* and L-*ido* isomers can also be varied by slight modification of the carbohydrate substrate **2**. Wolfrom and Hanessian[7] suggested that 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**1***) failed to react with Grignard reagents because it exists in a dimeric form[13]. However, it has been subsequently shown that **1** reacts with ethynylmagnesium bromide in tetrahydrofuran[14]. When 1,2-*O*-isopropylidene-α-D-glucofuranose was oxidised with lead tetraacetate, and the crude product was allowed to react with phenylmagnesium bromide in ether, **4** and **7** were isolated in the ratio 1:2.3. Similarly, in tetrahydrofuran, **1** was treated with phenylmagnesium bromide to give **4** and **7** in the ratio 1:1. Thus, it appears that the addition of phenylmagnesium bromide to **1** and **2** is more stereoselective in ether than in tetrahydrofuran. Further, the addition reactions with **2** are more stereoselective than with **1**. Although a large number of investigations of the factors that govern the stereoselectivity of Grignard reactions have been carried out, the above considerations suggest that an investigation of these factors might be particularly fruitful with carbohydrate substrates of well-established and easily varied stereochemistry. The fact that the degree of stereoselectivity can be varied over a wide range, by suitable choice of solvent and substrate, increases the possibilities for the synthesis of asymmetric, benzylic carbon atoms by use of this approach.

EXPERIMENTAL

Melting points are uncorrected. Thin-layer chromatography was performed on microscope slides coated with Silica Gel G, and column chromatography was performed with Silica Gel of particle size 0.05–0.2 mm (both grades of Silica were obtained from E. Merck, Darmstadt, Germany). The chromatoplates were developed with 50% sulphuric acid and/or iodine vapour. For the Grignard reactions, ether was dried over sodium wire, and tetrahydrofuran was dried by cautious distillation from lithium aluminium hydride. Where stated, drying of organic solvents was effected with magnesium sulphate. N.m.r. spectra were measured with a JEOL JNM-4H-100 n.m.r. spectrometer at 100 MHz with deuteriochloroform as solvent and with tetramethyl

*Compound **1** is represented diagrammatically as the free aldehyde. The precise nature of this compound is discussed more fully in the following paper.

silane as an internal standard. Identification of compounds was based on mixed melting points and comparison of n.m.r. and infrared spectra.

Reaction of 3-O-benzyl-1,2-O-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**2**) *with phenylmagnesium bromide.* — (*a*) *In ether.* A solution of aldehyde **2** (15 g) in ether (100 ml) was added dropwise to a solution of the Grignard reagent [freshly prepared in the usual way by controlled addition of bromobenzene (18 ml) in ether (150 ml) to magnesium (4.5 g)], and the suspension was then boiled under reflux for 2 h. The solution was cooled, washed with dilute, aqueous ammonium chloride and water, dried, and concentrated. The residue was fractionated on silica gel with benzene–ether (4:1) as solvent. The first compound eluted was 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (**3**) (1 g, 5%), $[\alpha]_D^{23}$ −76° (*c* 1.0, chloroform). The major product was distilled at 280°(bath)/0.1 mm. to give 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose (**6**) (14 g, 72%), $[\alpha]_D^{23}$ −33.5° (*c* 2.0, chloroform).

(*b*) *In tetrahydrofuran.* Aldehyde **2** obtained from 5,6-di-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[15] (15 g) was dissolved in tetrahydrofuran (100 ml), and the solution was added dropwise to a solution of the Grignard reagent [from bromobenzene (15 ml) and magnesium (4 g) in tetrahydrofuran (100 ml)]. The solution was boiled under reflux for 1.5 h, cooled, and poured into dilute, aqueous ammonium chloride. Tetrahydrofuran was removed by spin evaporation, and the aqueous solution extracted with chloroform. The chloroform extracts were washed with water, dried, and concentrated. Chromatographic resolution of the residue, on a column of silica gel with benzene–ether (4:1), yielded **3** (1.8 g, 13%) and **6** (6 g, 43%), the percentage yields being calculated from the quantity of 5,6-di-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose used.

1,2-O-Isopropylidene-5-C-phenyl-α-D-gluco-pentofuranose (**4**). — A solution of **3** (1.5 g) in methanol was hydrogenolysed by using freshly prepared palladium as catalyst. When the theoretical quantity of hydrogen had been absorbed, the solution was filtered through a Celite pad and concentrated. The residue was crystallised from light petroleum (b.p. 60–80°)–diisopropyl ether to yield the product (0.4 g, 35%), m.p. 99–100°, $[\alpha]_D^{23}$ −26° (*c* 0.8, chloroform) (Found: C, 63.5; H, 6.9. $C_{14}H_{18}O_5$ calc.: C, 63.2; H, 6.8%).

1,2-O-Isopropylidene-5-C-phenyl-β-L-ido-pentofuranose (**7**). — A solution of **6** (3.6 g) in methanol was hydrogenolysed as described above for **3**. The resulting residue was crystallised from ethanol to yield the product (1.2 g, 44%), m.p. 156–157°, $[\alpha]_D^{23}$ +25° (*c* 1.1, chloroform) (Found: C, 63.2; H, 6.8. $C_{14}H_{18}O_5$ calc.: C, 63.2; H, 6.8%).

Reaction of 1,2-O-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**1**) *with phenylmagnesium bromide.* — The crude product (**1**) resulting from the oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose (15 g) with lead tetraacetate was divided into two equal portions that were allowed to react with phenylmagnesium bromide.

(*a*) *In tetrahydrofuran.* The crude aldehyde **1** in tetrahydrofuran was added dropwise to a solution of Grignard reagent in tetrahydrofuran, and the mixture was boiled under reflux for 1 h. The products were isolated as previously described for the

reaction performed in tetrahydrofuran. The products were separated by column chromatography with carbon tetrachloride–ethanol (11:1) as solvent. The first component isolated was 1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (**4**) (2.1 g, 23%), m.p. 102–103° (from light petroleum–diisopropyl ether). The second component isolated was 1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose (**7**) (2 g, 22%), m.p. 159–160° (from ethanol).

(*b*) *In ether.* Similar treatment of the crude aldehyde **1** in ether with the Grignard reagent in ether and similar column chromatography yielded **6** (1 g, 11%), m.p. 102–103°, and **7** (2.3 g, 25%), m.p. 158–159°.

5-C-Phenyl-α-D-gluco-pentose (**9**). — A solution of **4** (1.2 g) in 50% aqueous acetic acid (50 ml) was heated for 1 h at 100 ± 5°. The solution was concentrated, and the last traces of acetic acid were removed by spin evaporation with toluene. The residue was crystallised by trituration with ethanol and recrystallised from ethanol–light petroleum to give the product (0.58 g, 56%), m.p. 170°, $[\alpha]_D^{23}$ +53° (*c* 1.8, water) (5 min) → 20° (equil., 130 min) (Found: C, 58.2, H, 6.1. $C_{11}H_{14}O_5$ calc.: C, 58.4; H, 6.2%).

1,2,3,4-Tetra-O-acetyl-5-C-phenyl-β-D-gluco-pentopyranose (**10**). — A solution of 5-*C*-phenyl-α-D-*gluco*-pentose (0.15 g) and sodium acetate (0.5 g) in acetic anhydride (10 ml) was heated for 1 h at 100°. The solution was poured into water, neutralised with aqueous sodium hydrogen carbonate, and extracted with chloroform (3 × 50 ml). The chloroform extract was washed with water, dried, and concentrated, and the residue was crystallised from ethanol to give the product (0.14 g, 51%), m.p. 184–186°, $[\alpha]_D^{23}$ +23° (*c* 1.7, chloroform) (Found: C, 58.0; H, 5.8. $C_{19}H_{22}O_9$ calc.: C, 57.9; H, 5.6%). N.m.r. data. Chemical shifts (δ values, p.p.m.): H-1, 5.88; H-2, 5.39; H-3, 5.3; H-4, 5.15; H-5, 4.54; CH_3, 2.08, 2.05, 2.0, 1.79. Coupling constants (Hz): $J_{1,2}$, 7.8; $J_{2,3}$, 9.5; $J_{3,4}$, 9.5; $J_{4,5}$, 9.8.

1,2,3,4-Tetra-O-acetyl-5-C-phenyl-α-D-gluco-pentopyranose (**11**). — A solution of 5-*C*-phenyl-α-D-*gluco*-pentose (**9**) (0.15 g) in pyridine (3 ml) was cooled to 0°, acetic anhydride (0.4 ml) was added, and the solution was stored overnight at room temperature. The solution was concentrated, and the last traces of pyridine and acetic anhydride were removed by spin evaporation with toluene. The residue, on crystallisation from ethanol, had m.p. 150–155°. This solid was dissolved in ethanol, and the solution was seeded with compound **10**. After the first deposit of crystals (0.1 g, m.p. 152–155°) had been filtered off, the filtrate was concentrated to half volume. A second deposit of crystals was obtained (0.08 g) with m.p. 144–145°, $[\alpha]_D^{23}$ +104° (*c* 1.8, chloroform) (Found: C, 58.2; H, 5.7. $C_{19}H_{22}O_9$ calc.: C, 57.9; H, 5.6%).

N.m.r. data. Chemical shifts (δ values, p.p.m.): H-1, 6.47; H-2, 5.23; H-3, 5.60; H-4, 5.14; H-5, 4.85; CH_3, 2.18, 2.03, 2.0, 1.81. Coupling constants (Hz); $J_{1,2}$, 3.75; $J_{2,3}$, 9.4; $J_{3,4}$, 9.0; $J_{4,5}$, 10.0. The n.m.r. data indicated that this compound was essentially the pure α-D anomer (**11**).

4-C-Benzyl-1,2-O-isopropylidene-α-D-xylo-tetrofuranose (**12**). — A solution of 1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose (**7**) (2 g) in methanol (50 ml) was hydrogenolysed by using freshly prepared palladium as catalyst. The reaction was

slow, but, after 24 h, it was shown by t.l.c. with benzene–ether (7:3) that only a trace of **7** remained. The solution was filtered and concentrated, and the residue was chromatographed on silica gel with benzene–ether (7:3). The major component was crystallised from benzene–light petroleum or from diisopropyl ether to yield the product (1.2 g, 65%), m.p. 129–130° (Found: C, 67.4; H, 7.4. $C_{14}H_{18}O_4$ calc.: C, 67.2; H, 7.3%).

*4-C-Benzyl-*D-xylo-*tetrose* (**13**). — A solution of **12** (1 g) in 50% aqueous acetic acid (20 ml) was heated for 2 h at 100°. The solution was concentrated, and the residue crystallised from ethanol–light petroleum to yield the product (0.5 g, 60%), m.p. 122–123°, $[\alpha]_D$ +67° (*c* 0.9, water) (Found: C, 63.4; H, 6.7. $C_{11}H_{14}O_4$ calc.: C, 62.9; H, 6.7%).

*Reaction of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-*D*-*xylo*-pentodialdo-1,4-furanose* (**2**) *with methylmagnesium iodide.* — A solution of **2** (4 g) in ether (100 ml) was added dropwise to a solution of the Grignard reagent, freshly prepared by the controlled addition of methyl iodide (5 ml) in anhydrous ether (70 ml) to dry magnesium (2.5 g). After the addition of the aldehyde was completed, the solution was boiled under reflux for 1 h and cooled. The ether solution was decanted from residual magnesium, carefully washed with aqueous ammonium chloride and water, dried, and concentrated. The residue was crystalllised from ether–light petroleum (b.p. 60–80°) to give 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose (2 g), m.p. 92–93, $[\alpha]_D^{23}$ −63 (*c* 0.9, chloroform); Wolfrom and Hanessian recorded m.p. 93–94°, $[\alpha]_D^{25}$ −63.5° (*c* 1.3, chloroform). The mother liquors were concentrated, and examined by t.l.c. with benzene–ether (7:3). Two components were detected, and these were separated by column chromatography on silica gel with the same solvent system. The minor component had the highest mobility and was 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (0.4 g, 9%), $[\alpha]_D^{23}$ −64° (*c* 4.2, chloroform). The major component (1.0 g), crystallised from ether–light petroleum (b.p. 60–80°), had m.p. 97–98°, $[\alpha]_D^{23}$ −63° (*c* 1.3, chloroform), and was chromatographically pure 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose. (Total yield 3 g, 69%). The first crop of crystals, m.p. 92–93°, was contaminated by a trace of the D-glucose isomer.

*5-*O*-Benzoyl-3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-α-*D*-glucofuranose.* — (*a*). A solution of **5** (0.1 g) in pyridine was benzoylated with benzoyl chloride in the usual manner, and the product crystallised from ethanol (0.1 g, 75%), m.p. 101–102°, $[\alpha]_D^{22}$ −55° (*c* 0.9, chloroform) (Found: C, 69.4; H, 6.6. $C_{23}H_{26}O_6$ calc.: C, 69.4; H, 6.6%).

(*b*). 3-*O*-Benzyl-6-deoxy-1,2-*O*-isopropylidene-5-*O*-methanesulphonyl-β-L-idofuranose[7] (**14**) was prepared from **8** (1.8 g) and methanesulphonyl chloride (0.8 ml) in pyridine (3 ml) in the usual manner. The product (1.6 g, 70%) had m.p. 96–97° (from ether–light petroleum). Wolfrom and Hanessian[7] recorded m.p. 99–100°.

A mixture of the methanesulphonate (**14**) (1 g) and sodium benzoate (2.5 g) in *N,N*-dimethylformamide was boiled under reflux for 6 h. The solution was cooled, diluted with water (100 ml), and extracted with chloroform (2 × 50 ml). The chloroform extract was washed with water (4 × 100 ml), dried ($MgSO_4$), and concentrated. Two components were isolated from the residue by column chromatography on silica gel

with benzene–ether (9:1). The first component isolated was 5-*O*-benzoyl-3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (0.3 g, 29%), m.p. 102–103°, $[\alpha]_D^{23}$ −54° (*c* 0.7, chloroform). The second component isolated was unchanged **14** (0.2 g, 20%), m.p. 96–97°.

SUMMARY

Methods for the stereoselective synthesis and configurational assignment of asymmetric, benzylic carbon atoms have been investigated. The reaction between phenylmagnesium bromide and 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose in ether is highly stereoselective, giving a mixture of 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose and 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose in the ratio 1:14.4. The stereoselectivity of the reaction was appreciably different when ether was replaced by tetrahydrofuran as solvent. The novel, crystalline sugars, 5-*C*-phenyl-D-*gluco*-pentose and 4-*C*-benzyl-D-*xylo*-tetrose are described.

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GLYCOL-CLEAVAGE PRODUCTS FROM 1,2-*O*-ISOPROPYLIDENE-α-D-GLUCOFURANOSE

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INTRODUCTION

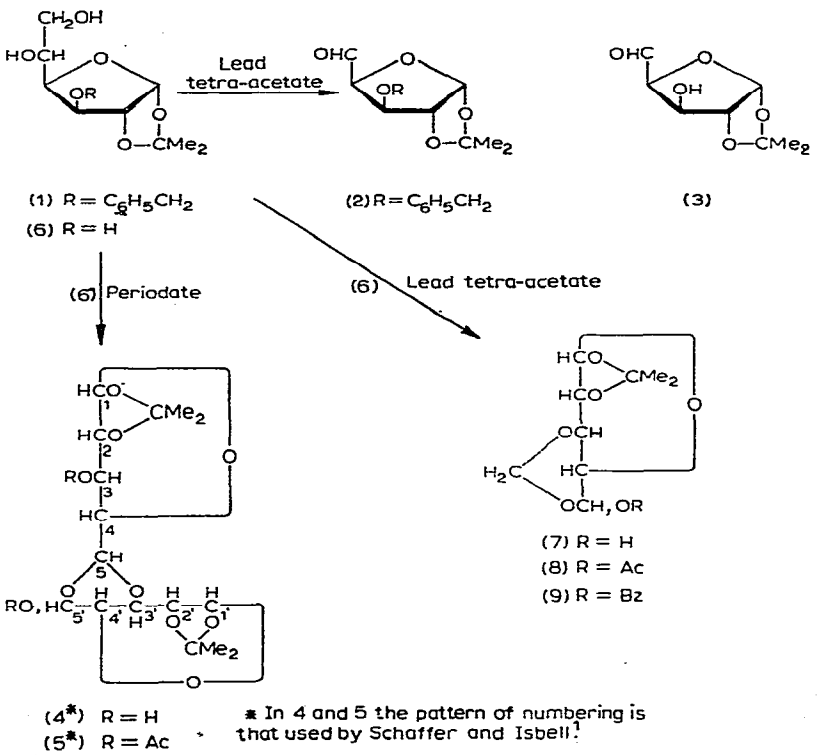
Schaffer and Isbell[1] isolated a dimeric product, and not the expected 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**3**), from the reaction between sodium periodate and 1,2-*O*-isopropylidene-α-D-glucofuranose (**6**). It was suggested[2] that Grignard reagents failed to condense with **3** probably because the aldehyde group is masked in the dimeric form of **3**. However, it has been subsequently shown[3,4] that Grignard reagents do react with the product that results from the oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose (**6**) with lead tetraacetate. Since the oxidation of **6** with both sodium periodate and lead tetraacetate would be expected to yield the same product, these reactions were investigated in detail to establish the true nature of the glycol-cleavage products and to determine which of these products reacts with Grignard reagents.

DISCUSSION

In an attempt to prepare an authentic sample of 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**3**), 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose[2] (**2**) was first prepared by oxidation of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[5] (**1**) with lead tetraacetate in benzene. A small sample, purified by chromatography on silica gel, had infrared and nuclear magnetic resonance spectra that were consistent with the expected structure. Catalytic hydrogenolysis of **2**, over freshly prepared palladium, gave a product whose infrared spectrum had only a weak absorption at 1725 cm^{-1} characteristic of the aldehyde group. This product was shown by thin-layer chromatography to contain a small proportion of **2** and a component of much lower mobility, which had chromatographic properties indistinguishable from those of an authentic sample of bis(5-aldo-1,2-*O*-isopropylidene-α-D-*xylo*-pentofuranose) 5,5′:3′,5-cyclic acetal (**4**)*. The slower-moving component was separated from unreacted **2** by column chromatography on silica gel, and its identity as the dimer **4** was confirmed by the usual methods.

*This name for the dimer **4** was proposed by Schaffer and Isbell[1]; **4** may also be named 1,2-*O*-isopropylidene-3,5-*O*-[4,5-*O*-isopropylidene-(L-*gluco*-tetrahydro-3,4,5-trihydroxyfurfurylidene]-(5-hydroxy-D-xylofuranose).

Since dimerisation of **3** apparently occurs so readily, it is not surprising that Schaffer and Isbell[1] obtained a good yield of **4** by oxidation of **6** with sodium periodate. This experiment was repeated by using essentially the conditions described[1], and the reaction product was examined by thin-layer chromatography. Of the two components detected, and subsequently separated by column chromatography on silica gel, the slower moving was chromatographically indistinguishable from the dimer **4**. The faster-moving component (15%, $[\alpha]_D$ +47°, chloroform) was identified as 1,2-*O*-isopropylidene-3,5-*O*-methylene-(5-hydroxy-α-D-xylofuranose) (**7**) and must result from condensation between **3** and the formaldehyde liberated during the glycol cleavage of **6**. The major component was the dimer **4** (47%). Use of chromatography gives the dimer in its anhydrous form, and not as the hydrate initially isolated by Schaffer and Isbell[1].



When 1,2-*O*-isopropylidene-α-D-glucofuranose (**6**) was oxidised with lead tetraacetate in benzene, and the reaction product chromatographed on silica gel, **7** (50%) and the dimer **4** (12%) were isolated. Again, no trace of the free aldehyde **3** was in evidence. Compound **7** was converted into an amorphous monoacetate (**8**) by acetylation with acetic anhydride in pyridine, and into a crystalline monobenzoate (**9**) by benzoylation under similar conditions. Conclusive proof of the structures of **7**, **8**, and **9**, and of the di-*O*-acetyl derivative (**5**) of **4** was obtained by analysis of their n.m.r. spectra. These results will be considered in detail later.

The supposed aldehyde **3** was characterised as its crystalline semicarbazone (m.p. 209°) by Iwadare[6] and by Wolfrom and Hanessian[2]. This semicarbazone can be obtained by treatment of either **4** or **7** with semicarbazide hydrochloride and potassium acetate. It seems probable therefore that the product previously obtained[6] from oxidation of **2** with lead tetraacetate was in fact **7** and not **3**, and also that the product obtained by Wolfrom and Hanessian[2] by hydrogenolysis of **2** was preponderantly the dimer **4**. It is interesting to contrast the reaction between formaldehyde and **3** to give **7**, which presumably occurs under weakly acidic conditions, with the reaction between formaldehyde and **4** under strongly basic conditions[7]. In sodium hydroxide solution, a mixed-aldol reaction apparently occurs, which is immediately followed by a crossed-Cannizzaro reaction, so that the product isolated is 4-*C*-(hydroxymethyl)-1,2-*O*-isopropylidene-L-*threo*-pentofuranose.

One of the main objects of this investigation was to establish which glycol-cleavage products of **6** react with Grignard reagents. From the results previously reported[4], it is obvious that **7** reacts with Grignard reagents, since **7** is the major product formed by oxidation of **6** with lead tetraacetate. It is not surprising therefore that the dimer **4** also reacts with Grignard reagents. When **4** was boiled under reflux with a freshly prepared solution of phenylmagnesium bromide in ether, two compounds were detected in the product. They were separated by column chromatography on silica gel and shown to be 1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose[4] (25%) and 1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose[4] (66%). Although the overall yield from this reaction was much higher than when the corresponding reaction was performed[4] on the crude oxidation product of **6**, the relative proportions of the isomers were little affected. Also, it was shown chromatographically that **4** reacts with methylmagnesium iodide to give products indistinguishable from 6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose and 6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose.

Proof of structure of oxidation products. — The n.m.r. parameters for **7**, **8**, **9**, and **5**, are listed in Table I. Compound **7** was identified as follows. The n.m.r. spectrum of **7** was integrated to reveal the presence of 6 methyl and 8 other protons. One of the latter protons was shown to be a hydroxyl proton, since it readily exchanged with deuterium oxide. It was immediately apparent that this proton count could be explained if condensation between formaldehyde and **3** had taken place. The ease of dimerisation of β-hydroxyaldehydes is well established[8], and Schaffer and Isbell[1] had already observed that the syrupy product from the oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose was difficult to free from formaldehyde, and that perhaps some unspecified reaction had occurred. The interpretation of the n.m.r. spectrum of **7** was facilitated by comparison with the spectra of authentic derivatives[9] of 1,2-*O*-isopropylidene-α-D-xylofuranose. Thus, for **7**, the doublets at 5.98 and 4.51 p.p.m., with a measured line-spacing of 3.6 Hz, may be assigned to the C-1 and C-2 ring protons, respectively. The doublets at 5.17 and 4.64 p.p.m., with a measured line-spacing of 6.5 Hz, may be assigned to the two methylene protons, since a coupling of this magnitude does not occur between ring protons in the 1,2-*O*-isopropylidene-α-D-xylofuranose system. Further, a value of 6.5 Hz for such a geminal coupling is consistent

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TABLE I

ASSIGNMENTS IN THE N.M.R. SPECTRA OF DERIVATIVES OF 1,2-*O*-ISOPROPYLIDENE-α-D-*xylo*-PENTODIALDO-1,4-FURANOSE

Compound	*Chemical shifts*[a]							*Coupling constants*[b]				
	C-1	C-2	C-3	C-4	C-5	CH_2	CH_3	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{C<^{H}_{H}}$
7	5.98d	4.51d	3.93d	4.33d	5.32s[d]	5.17d 4.64d	1.31s 1.47s	3.6	<0.5	2.2	<0.5	6.5
8	5.98d	4.54d	3.88	4.33d	6.20s	5.0d 4.75d	1.30s 1.46s 2.12s(OAc)	3.9	<0.5	2.3	<0.5	6.8
9	6.08d	4.59d	4.06d	4.45d	6.50s	5.12d 4.80d	1.30s 1.48s	3.4	<0.5	2.0	<0.5	6.4
5[c]	5.95d (6.01d)	4.46d (4.54d)	5.27d (3.86d)	4.23q (4.30d)	5.11d (6.36s)	— —	1.30(x2) 1.48(x2) 2.06(OAc) 2.15(OAc)	3.1 (3.7)	<0.5 (<0.5)	2.8 (2.7)	5.4 (<0.1)	

[a]In p.p.m. s, singlet; d, doublet; q, quartet. [b]Coupling constants in Hz. [c]For **5**, the figures in parentheses refer to the chemical shifts and coupling constants for the C′ protons. [d]The C-5 proton appeared as a doublet until **7** was equilibrated with deuterium oxide.

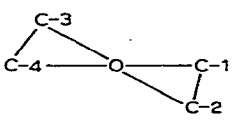
with the values listed by Cookson and co-workers[10]. The doublet at 5.32 p.p.m. collapsed to a single, sharp peak when a deuteriochloroform solution of **7** was shaken with deuterium oxide. Thus, the signal arises from the proton attached to the carbon atom also bearing the hydroxyl group. The doublets at 3.93 and 4.33 p.p.m. obviously result from the C-3 and C-4 protons, since they show the characteristic coupling of 2–3 Hz found for these protons in a 1,2-*O*-isopropylidene-α-D-xylofuranose system, in which it is unusual for there to be any measurable coupling between the protons at C-2 and C-3. From a consideration of the spectra of **7**, **8**, and **9**, it is not possible to make specific assignments for the doublets at 3.93 and 4.33 p.p.m., but, when their spectra are compared with the spectrum of **5**, it seems most likely that the doublets at 3.93 and 4.33 p.p.m. result from the protons at C-3 and C-4, respectively. Since the C-4 proton is only coupled to the C-3 proton, it is reasonable to assign the singlet at 5.32 p.p.m. to the C-5 proton. It will be noticed that acetylation of **7** causes the expected downfield-shift of the C-5 proton to 6.20 p.p.m., and that benzoylation of **7** similarly shifts the C-5 proton to 6.50 p.p.m. The chemical shifts of the other protons are little affected by esterification.

The structure assigned to the dimeric compound by Schaffer and Isbell[1] was confirmed by a consideration of the n.m.r. spectrum of the acetate **5**. The doublets at 5.95 and 6.01 p.p.m. may be assigned to the C-1 and C′-1 ring protons, and the doublets at 4.46 and 4.54 p.p.m. may be assigned to the C-2 and C′-2 ring protons. No specific assignment of these signals is possible. The doublet at 5.27 p.p.m. results from the C-3 proton, since this is the normal chemical shift for a proton attached to C-3 when C-3 also carries an acetoxy group and is in the 1,2-*O*-isopropylidene-α-D-xylofuranose system[9]. By comparison with the spectrum of **8**, it is possible to assign the single peak at 6.36 p.p.m. to the C′-5 proton. The low-field doublet at 5.11 p.p.m. must then result from the C-5 proton. The line spacing for the C-5 proton is 5.4 Hz, a spacing which is also found in the quartet at 4.23 p.p.m. The C-4 proton may therefore be placed at 4.23 p.p.m., and, in view of the close similarity between the two parts of the molecule, it seems reasonable to suggest that the doublet at 4.30 p.p.m. results from the C′-4 proton. The doublet at 3.86 must then result from the proton at C′-3. By analogy, the tentative assignments shown for the C-3 and C-4 protons in **7** are suggested. It will be recognised that the difference in chemical shift between the C-3 and C′-3 protons and between the C-5 and C′-5 protons clearly confirms their non-equivalence and is in agreement with the structure shown. A comparison of the spectra of the dimer acetate **5** and of the acetate (**8**) of the 3,5-*O*-methylene acetal shows the close relationship of the 1,3-dioxan ring formed in each case.

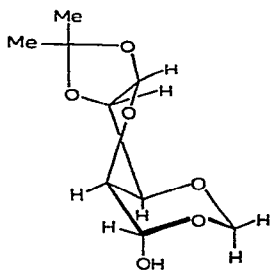
The formation of the 3,5-*O*-methylene acetal (**7**) involves the creation of one new asymmetric centre and thus could give two products, whereas formation of the dimer **4** involves the creation of two new asymmetric centres and could give four products. From the n.m.r. spectra, it is clear that one product has been isolated in each case, and, if the structures postulated are accepted, a further consideration of the n.m.r. parameters should enable a tentative assignment to be made for the configuration of the new asymmetric centres and for the conformation of the 1,3-dioxan

rings. Since the differences in chemical shift between adjacent protons in all of the reported spectra are much larger than the corresponding coupling constants, the measured line spacings can be considered to approximate closely to the true coupling constants. Various workers have attempted to substitute such constants into the Karplus equation[11] (or one of its subsequent modifications[12]), which relates coupling constants with dihedral angles, in order to determine precise molecular conformation. Although the general validity of this type of approach is well established, the dangers of attempting to make too exact a correlation between measured coupling constants and calculated dihedral angles have also been well documented[13]. Because of this, no attempt will be made here to calculate precise dihedral angles, but only the general, broad principles of the so-called Karplus curve will be invoked.

The conformation of the 1,2-*O*-isopropylidene-α-D-xylofuranose structure has been thoroughly investigated by Abraham and his co-workers[9], and the proposed skew-conformation for the puckered furanoid ring seems consistent with other evidence[14]. It was suggested that the coupling constants for the furanoid ring in this type of derivative could only arise if C-2 and C-3 are displaced below and above the other ring members (**10**). The observed coupling constants for compounds **7**, **8**, **9**, and **5** of $J_{1,2}$ 3–4 Hz, $J_{2,3}$ <0.5 Hz., and $J_{3,4}$ 2 Hz are in close agreement with the measured values from which the skew conformation for the 1,2-*O*-isopropylidene-α-D-xylofuranose ring was determined. It is therefore reasonable to assume that this conformation must have remained essentially unchanged when **3** dimerised or reacted with formaldehyde. It follows that the preferred conformation of the 1,3-dioxan ring in **7** and **4** will be that which most easily allows retention of the skew conformation postulated for the furanoid ring. A consideration of molecular models for **7** shows that one conformation for the 1,3-dioxan ring markedly favours the retention of this skew conformation. This is the conformation shown in **11**. Any other chair conformation for the dioxan ring is unfavourable for the retention of the skew conformation, and no flexible conformation for the dioxan ring appears to be sterically more favourable than the depicted chair conformation.



(10)



(11)

If this is the actual conformation, it might, at first sight, be expected that the hydroxyl substituent at C-5 would preferentially occupy the equatorial orientation. However, to explain the negligible coupling constant between the C-4 and C-5 protons,

the dihedral angle between them must be close to 90°. This only occurs when the C-5 proton is equatorially oriented. If the anomeric effect[15] operates, this will in fact be the preferred configuration, since it is well established that the anomeric effect creates preference for axial substitution at C-1 in most pyranose derivatives.

The n.m.r. parameters of **5** are consistent with a similar chair conformation of the intermediate 1,3-dioxan ring. The negligible coupling between the C′-4 and C′-5 protons again suggests that the C′-5 proton is equatorially situated. The coupling constant of 5.3 Hz between the C-5 and C-4 protons suggests that free rotation about the C-4 and C-5 bond occurs, resulting in the observed intermediate value for this vicinal coupling constant[16]. For free rotation of this type to occur, it is most likely that the 1,3-dioxan ring will be equatorially substituted at C-2. An essentially symmetrical molecule of this type is in agreement with the equivalent values observed for the methyl groups of both isopropylidene rings.

EXPERIMENTAL

Melting points are uncorrected. Thin-layer chromatography was performed on microscope slides coated with Silica Gel G, and column chromatography was performed with Silica Gel of particle size 0.05–0.22 mm (both grades of silica were manufactured by E. Merck, Darmstadt, Germany). The chromatoplates were developed with 50% sulphuric acid and/or iodine vapour. N.m.r. spectra were measured with a JEOL, JNM-4H-100 n.m.r. spectrometer at 100 MHz with deuteriochloroform as solvent and with tetramethylsilane as an internal standard. Identification of compounds was based on mixed melting points and comparison of n.m.r. and infrared spectra.

Reaction of 1,2-O-isopropylidene-α-D-glucofuranose (**6**) *with sodium metaperiodate.* — A solution of sodium metaperiodate (15 g) in water (75 ml) was added dropwise to a solution of 1,2-*O*-isopropylidene-α-D-glucofuranose (15 g) in water (100 ml) at 0°. The solution was then stored at room temperature for 30 min and concentrated by freeze drying. The residue was extracted with chloroform, and the chloroform extract was concentrated under diminished pressure at 40°. The product, shown to contain two components by t.l.c. in ether, was fractionated on silica gel with ether. The first component (2 g, 15%) had $[\alpha]_D$ +47° (*c* 10, chloroform), R_F 0.66, and was 1,2-*O*-isopropylidene-3,5-*O*-methylene-(5-hydroxy-α-D-xylofuranose) (**7**). The second component (6 g, 47%) had R_F 0.34 and $[\alpha]_D$ +21° (*c* 1.8, chloroform). This material was crystallised from ether or benzene and had m.p. 178–180° alone or 179–181° in admixture with an authentic sample of the 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose dimer (**4**); $[\alpha]_D$ 0° (10 min)→ −26° (equil.) (*c* 1.7, water).

Acetylation of dimer **4**. — A solution of **4** (0.4 g) and acetic anhydride (0.5 ml) in pyridine (3 ml) was stored at room temperature overnight. The solution was concentrated by spin evaporation with toluene, and the residue was purified by column chromatography on silica gel with ether as solvent. The diacetate **5** (0.4 g, 80%) had $[\alpha]_D$ +43° (*c* 2.0, ethanol),

Reaction of 1,2-O-isopropylidene-α-D-glucofuranose (**6**) *with lead tetraacetate.* —

Lead tetraacetate (50 g) in benzene was added to a suspension of 1,2-*O*-isopropylidene-α-D-glucofuranose (20 g) in benzene (1 litre), and the mixture was boiled under reflux for 30 min. The cooled suspension was filtered off, the filtrate neutralised with sodium hydrogen carbonate, filtered again, and concentrated. The residue was washed with ethanol, and the ethanol extract was concentrated. The residue was washed thoroughly with ether, and the washings were filtered, concentrated, and chromatographed on silica gel with ether as eluent. The first compound isolated was the 3,5-*O*-methylene acetal (**7**) (10 g, 50%), $[\alpha]_D$ +47° (*c* 2.4, chloroform). The second compound isolated was the dimer **4** (2 g, 12%), m.p. 182°.

Esterification of 1,2-O-isopropylidene-3,5-O-methylene-(5-hydroxy-α-D-xylofuranose). — A solution of **7** in pyridine at 0° was acetylated with acetic anhydride in the usual way to give the 5-acetate **8** as a chromatographically homogeneous syrup, $[\alpha]_D$ +100° (*c* 0.6, chloroform).

A solution of **7** in pyridine at 0° was benzoylated with benzoyl chloride in the usual way to give the 5-benzoate **9**, m.p. 148–149°, $[\alpha]_D$ +113° (*c*. 1.0, chloroform) (Found: C, 59.5; H, 5.9. $C_{16}H_{18}O_7$ calc.: C, 59.6; H, 5.6%).

1,2-O-Isopropylidene-α-D-xylo-pentodialdo-1,4-furanose semicarbazone. — (*a*) A solution of **7** (0.3 g), semicarbazide hydrochloride (0.3 g), and potassium acetate (0.45 g) in water (6 ml) was stirred for 3 h at room temperature. The precipitate was filtered off and recrystallised from water to give the product (0.2 g, 52%), m.p. 209–210°.

(*b*) A solution of **4** (0.3 g), semicarbazide hydrochloride (0.3 g), and potassium acetate (0.45 g) in aqueous ethanol (10 ml) was stirred for 3 h at room temperature. The precipitate was filtered off and recrystallised from water to yield the product (0.3 g, 76%), m.p. 210–211°. Wolfrom and Hanessian[2] recorded m.p. 208–209° and Iwadare[6] recorded m.p. 208–209.5° for this product.

Reaction of phenylmagnesium bromide with the dimer **4**. — A solution of **4** (0.5 g) in ether was added to excess of phenylmagnesium bromide in ether (100 ml), and the solution was boiled for 2 h under reflux. The solution was poured into dilute, aqueous ammonium chloride, and the aqueous solution was extracted with ether. The ether solution was concentrated, and the residue was chromatographed on silica gel with carbon tetrachloride–ethanol (11:1). The first compound isolated was 1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-*gluco*-pentofuranose (0.15 g, 25%), m.p. 94–95° (from di-isopropyl ether). The major product was 1,2-*O*-isopropylidene-5-*C*-phenyl-β-L-*ido*-pentofuranose (0.4 g, 66%), m.p. 159–160° [from ethanol–light petroleum (b.p. 60–80°)].

Hydrogenolysis of 3-O-benzyl-1,2-O-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**2**). — Compound **2** (prepared from 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**1**) by oxidation with lead tetraacetate in benzene and purified by chromatography on silica gel with benzene–ether) had $[\alpha]_D$ −75° (10 min) (*c* 3.7, chloroform). The n.m.r. spectrum of **2** had (δ values, p.p.m.) a doublet at 9.62 (J 2.1 Hz) (aldehyde proton), a doublet at 4.07 ($J_{1,2}$ 3.9 Hz) (C-1 proton), and peaks at 1.27 and 1.40 characteristic of the isopropylidene methyl groups.

A solution of **2** (1.0 g) in methanol (20 ml) was hydrogenolysed over freshly prepared palladium. The solution was filtered, concentrated, and chromatographed on silica gel with ether as solvent. The first compound isolated was unchanged **2** (0.1 g), and the second compound isolated was the dimer **4** (0.4 g, 60%), m.p. 180–181°.

ACKNOWLEDGMENTS

The author thanks Drs. R. Schaffer and H. S. Isbell for providing a sample of bis(5-aldo-1,2-*O*-isopropylidene-α-D-*xylo*-pentofuranose) 5,5′:3′,5-cyclic acetal. The valued counsel of Professor A. B. Foster is gratefully acknowledged.

SUMMARY

Oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose with sodium periodate has been shown to yield primarily bis(5-aldo-1,2-*O*-isopropylidene-α-D-*xylo*-pentofuranose) 5,5′:3′5-cyclic acetal (**4**) with 1,2-*O*-isopropylidene-3,5-*O*-methylene-(5-hydroxy-α-D-xylofuranose) (**7**) as the minor product. When 1,2-*O*-isopropylidene-α-D-glucofuranose was oxidised with lead tetraacetate the proportions of **4** and **7** produced were reversed, and **7** became the major product of the reaction. Neither method of oxidation gave any 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose.

A first-order analysis of the n.m.r. spectra of **7** and its derivatives and of the diacetate from **4** permits tentative assignments for their configurations and conformations.

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STUDIES ON CYCLIC POLYOLS

PART IX. DIRECTIVE EFFECTS IN THE REACTIONS OF POLYSUBSTITUTED CYCLOPENTANES*

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INTRODUCTION

In previous studies[1-3], directive influences in the synthesis and reactions of different classes of substituted cyclopentanes were investigated, and the preparation of several isomeric members of some of these series (*e.g.*, tetrols, aminotriols, aminotetrols, diaminotriols, and anhydrotetrols) was described. Conformational factors play a major role in determining the stereoselectivity of reactions of cyclohexane derivatives, but are of minor or negligible importance in reactions involving the corresponding cyclopentane derivatives. The observed selectivity could be rationalized on the basis of other directive influences, such as steric hindrance, inductive effects, and participation of neighboring groups.

In the present report, further examples are presented that appear to substantiate the general validity of the earlier conclusions about the directive influence, on epoxide opening, of an adjacent, electronegative substituent. *cis*-1,2:3,4-Diepoxycyclopentane has been prepared, and its behavior in nucleophilic, epoxide-opening reactions has been studied; when either water or Br^- is the nucleophile, no selectivity is seen in the direction of opening of the first of the two epoxide rings. A rational approach to stereoselective synthesis of the five known cyclopentanetetrols is indicated, and several new derivatives are described. In addition, the relative ease of hydrolysis of *O*-isopropylidene groups and epoxide rings present in the same molecule has been investigated; in the presence of dilute mineral acid, the *O*-isopropylidene residue is found to be hydrolyzed more easily than the epoxide ring. Many of the compounds synthesized are suitable for i.r. spectroscopy in nonaqueous solvents, permitting the detection of intramolecular hydrogen bonds (H-bonds). Usually, only one (or two)

*The nomenclature used for cyclitols has varied from author to author. Definitive proposals for official nomenclature for this field are at present being considered by the IUPAC/IUB Nomenclature Commission. Until a decision is reached by this Commission, no nomenclature may be considered official. The nomenclature used in this paper is the "Modified Maquenne" system proposed by Professors S. J. Angyal and L. Anderson. Although some of the compounds described in this paper are not strictly cyclitols (cycloalkanes containing one hydroxyl group on each of four or more ring atoms), the relationships among the compounds are such that it seems preferable to name all of them by one system. In the present paper, the same enantiomer is shown in each case, except where it is necessary to do otherwise to illustrate a sequence of reactions.

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of the possible conformations is consistent with the indicated presence of a strong, intramolecular H-bond, and, on this basis, tentative conformational assignments have been made.

RESULTS

Epoxide opening. — Earlier studies[1,2] showed that, in the presence of dilute acid, the epoxide ring of the *cis*-anhydrotetrol **1** is opened, by water or Br^-, stereo-

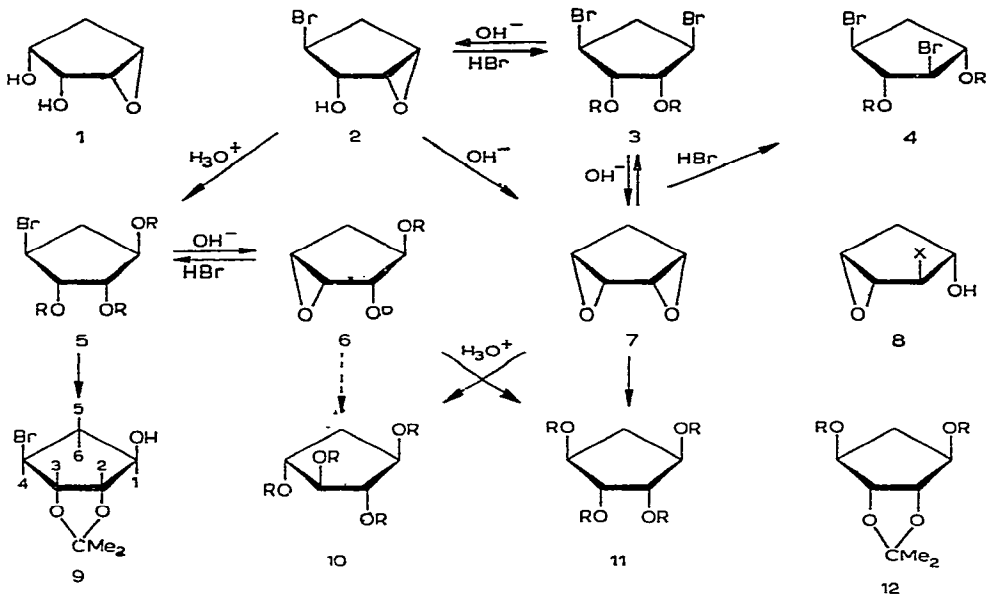

Fig. 1. Formation and opening of some epoxides of cyclopentane: R = H (a); Bz (b); X = OH (c); Br (d). The numbers in formula 9 show the numbering used in the discussion of the n.m.r. spectra.

selectively at the position farthest from an electronegative hydroxyl group. In the present work, similar results were obtained when the epoxybromohydrin[4] **2** and the anhydrotetrol **6a** were subjected to the same conditions. In ethanolic hydrogen bromide, **2** was converted into a product that crystallized during processing; it had m.p. 68–71° (compared with a m.p. for authentic **3a** of 75.5–76.5°). It was easily purified by a single recrystallization, and was identified as the dibromoglycol **3a**. Hydrolysis by dilute sulfuric acid converted **2** into a bromotriol identified, for several reasons, as **5a**. Thus, benzoylation of the product gave a tribenzoate **5b** that crystallized during processing, and that melted sharply at 136–7° after a single recrystallization. In our previous studies, such ease of purification has been characteristic only of mixtures consisting preponderantly of a single isomer. Acetonation of the presumed bromotriol **5a** gave a liquid *O*-isopropylidene derivative **9**. No conclusions concerning the stereoselectivity of opening of **2** in the hydrolytic reaction can be based on the yield of **9**, because yields of *O*-isopropylidene derivatives in our previous work have

been far from quantitative. However, the configurational assignment as **9** is unequivocal; the dioxolane ring can only be fused *cis* to the cyclopentane ring, and the n.m.r. spectrum[5] is consistent only with the 1,4/2,3 configuration. This proves that no rearrangement occurred during hydrolysis of the epoxide.

When the crude bromotriol **5** is treated with aqueous alkali, the *trans*-bromohydrin is converted into an epoxide, and the anhydrotetrol **6a** (m.p. 103.5–104.5°) is obtained in 49% yield; this constitutes further proof that the principal bromotriol present is **5a**. Previously[2], we were unable to distinguish between the two possible configurations **6** or **8c** for the anhydrotetrol, but since **5a** is the parent compound, structure **8** is untenable. Consistent with the proposed configuration is the observation[3c] that epoxides having a *trans*-hydroxyl group adjacent to one end of the oxirane ring, as in the epoxydiol **8c**, open stereoselectively at the less-hindered site. The product obtained by hydrolysis of **8c** should therefore be the 1,3/2,4 tetrol (**10a**), but the product actually obtained from the epoxydiol is preponderantly the 1,4/2,3 tetrol **11a** (see below). Unequivocal proof of its configuration was obtained by treatment of the anhydrotetrol **6a** with hydrogen bromide. That the resulting bromotriol is **5a** was shown by its conversion into an *O*-isopropylidene derivative whose i.r. spectrum was identical with that of an analytically pure sample of **9**.

In an earlier study[1], *trans*-1,2-cyclopentenediol (**13**) was epoxidized with peroxybenzoic acid (see Fig. 2) and the product was then hydrolyzed directly to a

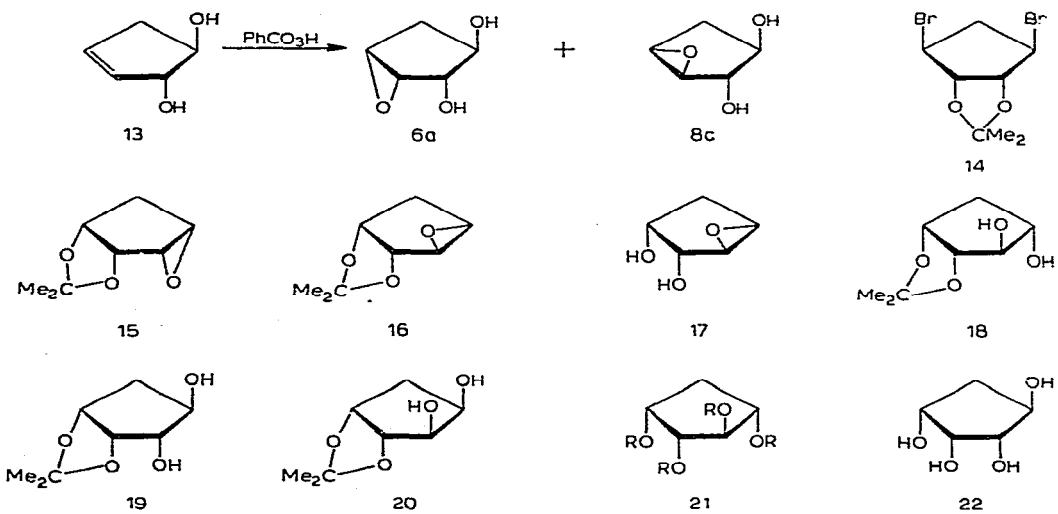

Fig. 2. Intermediates in synthetic reactions. **21a**, R = H; **21b**, R = Bz.

mixture of tetrols, composed of about 20–25% of **10a** and 75–80% of **11a**. The *cis*-directing influence of an allylic hydroxyl group in such epoxidation reactions is well known[6] and has been amply substantiated in our earlier studies[1–3a]. However, Darby *et al.*[7] have shown that a homoallylic hydroxyl group is also *cis*-directing, so that the

epoxydiol produced could have been a mixture of **6a** and **8c**, and no conclusions concerning stereoselectivity of epoxide opening could be drawn. In the present work, two different lines of evidence indicated the preferential opening of the oxirane ring of the epoxydiol **6a** at the position adjacent to the methylene group, *i.e.*, remote from an electronegative substituent. When the anhydrotetrol was hydrolyzed, the product crystallized during removal of the solvent. A single recrystallization gave the 1,4/2,3 tetrol **11a**, m.p. 132–4°, identical with authentic material previously prepared[1]. A more reliable indication of stereoselectivity is the quantitative analysis of the hydrolyzate by n.m.r. spectroscopy. As the methylene protons and the adjacent O–C–H protons of the tetrol **11a** form an AKX_2 system[8a,9], each of the methylene protons is represented in the spectrum by a six-line multiplet (doublet of triplets) (see Table III). The corresponding four protons of the tetrol **10a** form an A_2X_2 system, the methylene protons of which gave a triplet. Fortuitously, the two methylene signals of **11** are widely separated, and the methylene signal of **10** falls between them. Consequently, the composition of a mixture of these two isomers may be analyzed by comparing the integrated areas of the corresponding methylene signals, and such an analysis showed that the hydrolyzate consisted of 90% of **11** and 10% of **10**.

An entirely different and unexpected result was obtained when the opening of the diepoxide **7** was investigated (see below for the synthesis and proof of structure of **7**). When **7** was hydrolyzed and the hydrolyzate was analyzed by the n.m.r. method, 58% of the 1,3/2,4 tetrol **10a** and 42% of the 1,4/2,3 tetrol **11a** were found. For the following reasons, this result indicates that the first attack by water at an inner or outer end of an oxirane ring shows only slight stereoselectivity. If the first attack occurs at an outer position, the intermediate product would be the anhydrotetrol **6a**, and the final product would therefore contain 90% of the tetrol **11a**. On the other hand, if the first attack occurred at an inner position, the intermediate product would be the anhydrotetrol **8c**, and the final product would consist largely of the tetrol **10a**. We therefore conclude that both anhydrotetrols are intermediates in the hydrolysis, although no direct proof of the formation of either has been obtained. Partial hydrolysis was performed, and the hydrolyzate was examined by t.l.c. under conditions in which anhydrotetrols and tetrols are readily distinguished. At no time during the hydrolysis did demonstrable amounts of anhydrotetrols accumulate, presumably because the latter are hydrolyzed more rapidly than the parent diepoxide.

A similar lack of selectivity was observed when the diepoxide **7** was treated with aqueous hydrobromic acid. Quantitative analysis of the product was not accomplished, but evidence was obtained for the presence of both dibromoglycols, **3a** and **4a**. Treatment of the mixture with acetone and copper(II) sulfate produced a small amount of an *O*-isopropylidene derivative that could not be purified, but which is presumably **14**. The material that resisted acetonation was benzoylated and, after five recrystallizations of the product, a dibenzoate having m.p. 117.5–119° was isolated. A dibenzoate **3b**, prepared from the authentic dibromoglycol **3a**, melted at 123.5–124°, and a mixture of the two dibenzoates melted at 95°, proving that the two substances are not identical. The structure of the dibenzoate having m.p. 117.5–119° is probably **4b**, for the follow-

ing reasons. The first attack by Br^- can occur at an inner or outer end of either of the equivalent oxirane rings. Attack at an outer position would lead to the epoxybromohydrin **2**, which would then be opened stereoselectively by the second attack of Br^- to give **3a**, as was noted above. On the other hand, if the first attack occurs at an inner end of an oxirane ring, the epoxybromohydrin **8d** would be formed first. The bromo group of **8d** hinders the approach of the second Br^-, and the hindrance will be greater at the adjacent end than at the distant end of the oxirane ring; this would lead to the formation of the 1,3/2,4 dibromoglycol **4a**. The n.m.r. spectrum of the dibenzoate is consistent with the proposed structure **4b**, although application of computer techniques will be required for detailed analysis of the spectrum. Quantitative analysis of the crude mixture, by examination of the n.m.r. spectrum, could not be effected on the mixture of dibromoglycols, because of overlap of the methylene signals of the two compounds.

*Hydrolysis of anhydro-*O*-isopropylidene-tetrols.* — The anhydro-*O*-isopropylidene-tetrols **15** and **16** were previously found[1,2] to yield chiefly the 1,2,3/4 tetrol **22** and the 1,2,4/3 tetrol **21**, respectively, when subjected to acid-catalyzed hydrolysis, and preliminary evidence was obtained that the anhydrotetrols **1** and **17**, instead of the 1,2-mono-*O*-isopropylidenetetrols **18** and **19**, were intermediate products in the hydrolytic sequence. Further investigation has shown that the sequences are indeed **15**→**1**→**22**, and **16**→**17**→**21**. Authentic samples of each of these substances have been prepared, and used as reference materials in t.l.c. analysis. Table I shows the results of typical experiments in which partial hydrolyzates of the anhydro-*O*-isopropylidene-tetrols were analyzed by this technique (see Experimental for details). It is evident that, although compounds whose R_F values corresponded to those of the expected anhydrotetrols accumulated in partial hydrolyzates, the corresponding mono-*O*-isopropylidenetetrols could never be detected. In other experiments, not shown in Table I, known mixtures containing different proportions of the two tetrols **21** and **22** were used as reference materials for comparison with the tetrols contained in the complete hydrolyzate. By this semiquantitative method, we have estimated that, by acid hydrolyzis, the *cis*-anhydro-*O*-isopropylidene-tetrol **15** is converted into a mixture containing 90–98% of **22**, whereas the other isomer (**16**) yields 99% of **21**. The greater ease of acetal hydrolysis relative to the epoxide-opening reaction is in accord with a similar finding by Buchanan and Schwarz[11] concerning the *O*-benzylidene group in anhydro-*O*-benzylidene pyranoside derivatives of sugars.

cis-*1,2:3,4-Diepoxycyclopentane* (**7**). — The epoxybromohydrin **2** is an important intermediate for the synthesis of many of the compounds that we have studied, and it has been prepared many times by the procedure of Young *et al.*[4], *viz.*, by incubation of the dibromoglycol **3a** with aqueous alkali at room temperature. Usually, the observed yield of **2** has been lower than that reported by Young *et al.*, and the boiling point and viscosity of the product were often abnormal. A fraction having a boiling point lower than that of the epoxybromohydrin was obtained by fractional distillation. The i.r. spectrum of the low-boiling fraction showed no absorption in the O–H stretching region (3500–3600 cm^{-1}). Treatment of **2** with aqueous sodium

TABLE I

THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF HYDROLYZATES OF ANHYDRO-*O*-ISOPROPYLIDENETETROLS[a]

Substance hydrolyzed	*Hydrolyzate*[b]			*Reference compounds*[b]					
				Tetrol		*Anhydrotetrol*		*Mono-O-isopropylidenetetrol*	
				21	**22**	**1**	**17**	**18**	**19**
15	0.19 (major)	0.30 (minor)	0.52	0.30	(0.18)[c]	0.51		0.70	0.59
16	0.17 (minor)	0.27 (major)	0.63	0.27	(0.18)[c]	0.47	0.62	0.70	0.58

[a]Both experiments were performed with silica gel G and the developing solvent was butanone–acetic acid–2% boric acid (9:1:1 by volume). The detecting reagent was a periodate–permanganate spray[10]. [b]The numbers are R_F values and, unless otherwise stated, all the values in a row were determined on the same thin-layer plate. [c]These values were measured in a separate but comparable experiment.

TABLE II

INTRAMOLECULAR HYDROGEN BONDING

Compound		*Solvent*	*Δν in cm⁻¹ for H-bonded OH*[a]	*Bond length OH···O, Å*	*Compound*		*Solvent*	*Δν in cm⁻¹ for H-bonded OH*[a]
Dibromoglycol	**3a**	CS_2	40			**12a**	[e]	66
	28[b]	CCl_4	61	1.84		**18**	$CHCl_3$	51
	29[c]	CCl_4	63	1.82	Isopropylidenetetrols	**19**	CH_2Cl_2; $CHCl_3$	45
	32[b]	CCl_4	73	1.70		**20**	$CHCl_3$; CS_2	51
Diols	**30**[b]	CCl_4	39	2.22	Bromo-*O*-isopropyl-	**35**	CS_2	~50[f]
	31[b]	CCl_4	32	2.36	idenetriols	**9**	[e]	32
	33[d]	CCl_4	103	1.42				
	34[d]	CCl_4	102	1.41				

[a]The values of Δν are considered accurate to ±5 cm⁻¹. [b]Data of Kuhn[14]. [c]Data of Darby *et al.*[7]. [d]Data of Kwart and Vosburgh[15]. [e]The value is an average of measurements made on solutions of **12a** in CS_2, $CHCl_3$, or CH_2Cl_2, and **9** in CS_2 or $CHCl_3$. [f]The value is based on an average position of 3610–3615 cm⁻¹ for an unbonded OH.

hydroxide gave material whose i.r. spectrum was identical with that of the low-boiling fraction. The spectrum showed two strong bands at 3020 cm^{-1} and 3047 cm^{-1}, characteristic of oxirane or vinyl C–H stretching. The strongest band in the spectrum was at 845 cm^{-1}, coinciding exactly with the strong band that is found in the spectra of all of the epoxides we have studied. In our series, there was no absorption in the region (700–725 cm^{-1}) characteristic of out-of-plane C–H deformation[12] of vinylic compounds nor in the 1600–1680 cm^{-1} region characteristic[12] of C=C stretching. Consequently, the assignment as a diepoxide was considered certain. Since both oxirane rings originate from the *trans*-bromohydrin, functional groups of the dibromoglycol **3**, the *cis* configuration was anticipated[4], and this expectation is substantiated by the n.m.r. spectrum (see Fig. 3). Only two cyclopentane diepoxides are possible, the 1,2:3,4 (*i.e.*, *cis*, *syn cis*), and the 1,2/3,4 (*i.e.*, *cis*, *anti cis*). In the former compound

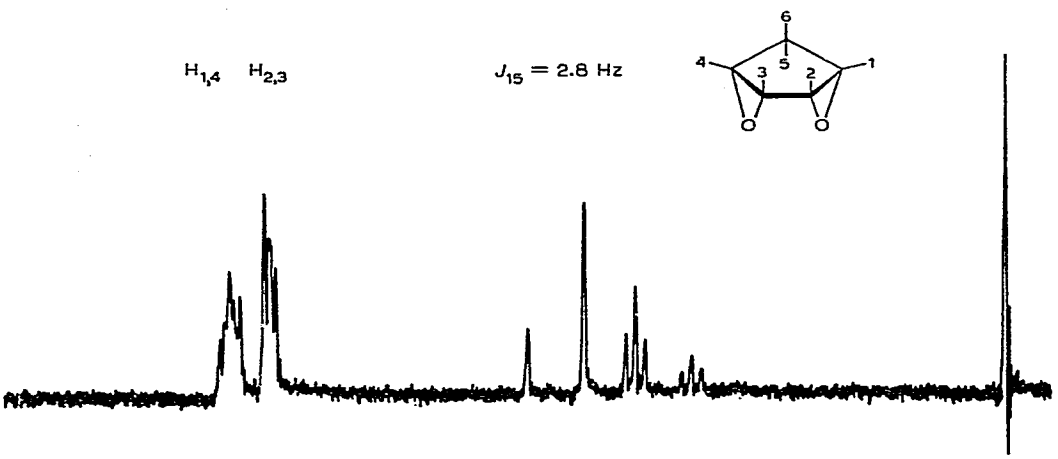

Fig. 3. 60-MHz n.m.r. spectrum of diepoxide **7**.

the protons of the methylene group are not equivalent, and are expected to be represented by an AB system in the spectrum[8,9], whereas for the (1,2/3,4)-diepoxide, the methylenic protons would be chemically equivalent and would be seen either as a single line, or as the A_2 portion of an A_2X_2 system; in the latter case, the signal would be a triplet. The spectrum shows an AB portion of an ABX_2 system in the methylene region, and the proposed *cis* configuration is, therefore, established. A more detailed discussion of the n.m.r. spectrum is given below.

Tetrols and O-isopropylidenetetrols. — Improved methods for synthesis of some of the tetrols have been developed. These methods, and the preparation of some *O*-isopropylidene derivatives useful in their purification, are reported.

The (1,2,4/3)-tetrol was originally prepared[1] by *trans*-hydroxylation of *cis*-1,3-cyclopentenediol (**23**). Although the product requires little purification, the starting material is difficult to prepare, and therefore, the tetrol is now prepared routinely by acid hydrolysis of the more readily accessible[1,2] anhydro-*O*-isopropylidenetetrol **16**. The tetrol has been obtained crystalline, m.p. 127.5–129°, and the small proportion of

contaminating (1,2,3/4)-tetrol is removed by recrystallization. The (1,2/3,4)-tetrol **24** was prepared[1] by *cis*-hydroxylation of the dibenzoate (**25b**) of *cis*-1,2-cyclopentenediol. A better preparative route involves *cis*-hydroxylation of the corresponding *O*-isopropylidene derivative **26**. The product is the corresponding mono-*O*-isopropyl-

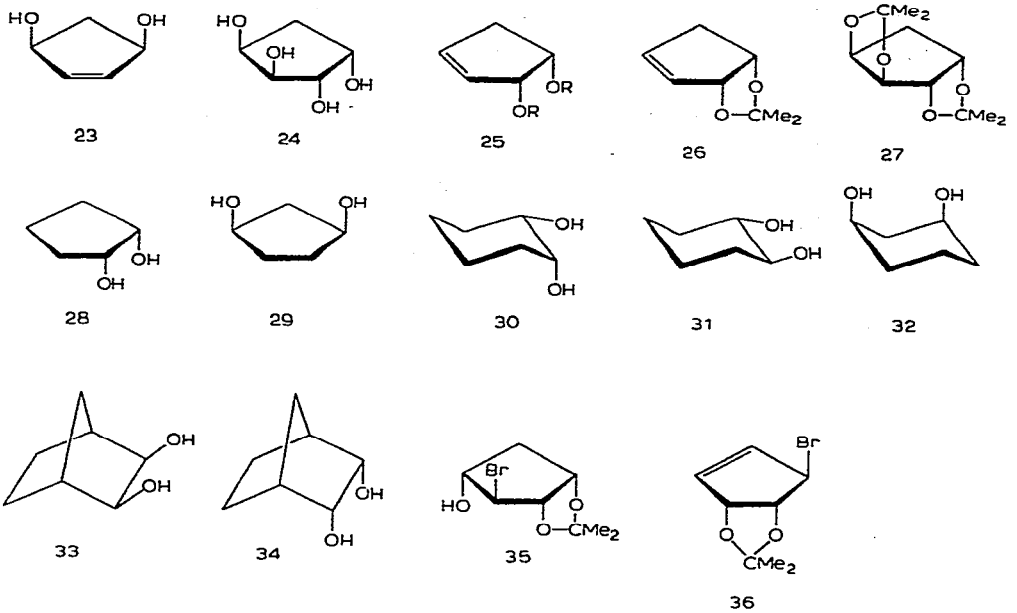

Fig. 4. Other compounds studied; **25a**, R = H; **25b**, R = Bz.

idenetetrol **20**, which is readily purified and then hydrolyzed to give the free tetrol. The preparation of the (1,4/2,3)-tetrol **11a** was formerly accomplished by *cis*-hydroxylation of *cis*-1,3-cyclopentenediol (**23**) with osmium tetraoxide, or by *trans*-hydroxylation of *trans*-1,2-cyclopentenediol (**13**). Both starting materials are difficult to obtain; and consequently, the tetrol **11a** is now prepared by the sequence **3a**→**2**→**5a**→**6a**→**11a**.

The mono-*O*-isopropylidene derivatives of the tetrols are useful intermediates in the synthesis and purification of the tetrols. They may be purified by sublimation, are soluble in a variety of solvents (water, ethanol, ethyl acetate, ether, hot benzene, and dichloromethane), can be selectively extracted from mixtures, and are readily recrystallized. Only the (1,2,3/4) isomer (**19**) has previously been described[1], although four halo-*O*-isopropylidenetriols were also described in the earlier work[2]. In the present study, the (1,4/2,3) and the (1,2,4/3) isomers, (**12a**) and (**18**), were prepared by the usual procedure of stirring the parent tetrol in an excess of dry acetone, with anhydrous copper(II) sulfate as the dehydrating agent and catalyst. When the (1,2/3,4)-tetrol **24** was treated in this way, the product did not contain a significant proportion of the mono-*O*-isopropylidene derivative **20**; but instead, a 94% yield of the di-*O*-isopropylidene derivative **27** was obtained. The i.r. and n.m.r.[9] spectra were consistent only with the proposed structure.

Production of the diol **23** by treatment of the corresponding dibromide with tetraethylammonium acetate and subsequent saponification[1b] suggested that tetrols might be produced directly from the *O*-isopropylidene derivative of the dibromoglycol **14**. The only product that could be identified when **14** was treated with tetraethylammonium acetate was, however, the corresponding, unsaturated, monobromo compound **36**, indicating that elimination of hydrogen bromide occurred, instead of nucleophilic displacement of bromide; this result is probably attributable to steric hindrance related to the *endo* methyl group of the *O*-isopropylidene residue.

Conformational analysis. — Substituted cyclopentanes are known to exist in nonplanar conformations[13a], either as an envelope form, in which one ring atom is out of the plane of the other four, or as a twist conformer in which one atom is above and an adjacent atom is below the plane of the other three. The relief of nonbonded repulsions thus obtained more than compensates for the increased angular strain caused by the "pseudorotation". We believe that the all-*cis* diepoxide **7** may be an exception to this rule. Examination of Dreiding models and of plane projections of

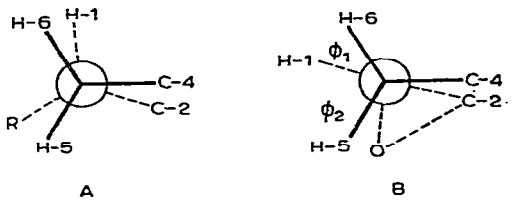

Fig. 5. Comparison of dihedral angles and internuclear distances. A, "planar" cyclopentane. Dihedral angle (H-1)–C–C–(H-6), 0°; H-1 to H-6 internuclear distance 2.2–2.3Å. B, Diepoxide **7**, planar conformation. Dihedral angles: $\varphi_1 = 35$–$38°$; $\varphi_2 = 82$–$85°$. Distances: H-1 to H-6 = 2.55 Å; H-1 to H-5 = 2.8 Å. In both cases, the observer is looking at the methylenic carbon atom (C-5), in the direction of the bond from C-5 to C-1. See reference 13b for an explanation of the Newman projections used.

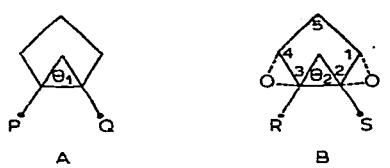

Fig. 6. Projected valence-angles and internuclear distances. A "planar" cyclopentane; $\theta_1 = 70°$; distance PQ = 2.2–2.3 Å. B, Diepoxide **7**; $\theta_2 = 78°$; distance RS = 2.6–2.7 Å.

these models shows the relations illustrated in Figs. 5 and 6. Atoms H-5 and H-6 are not eclipsed with the vicinal hydrogen atoms H-1 and H-4 of the oxirane ring, but the four oxirane hydrogen atoms are mutually eclipsed, *i.e.*, H-1 with H-2, H-2 with H-3, and H-3 with H-4. However, the internuclear distance is 2.6–2.7 Å, as against 2.2–2.3 Å in the eclipsed, ethane-like portion of cyclopentane. Furthermore, the projected, vicinal C–H bonds in cyclopentane intersect at an angle of 70°, whereas those of the diepoxide intersect at an angle of 78°. For all of these reasons, the non-

bonded repulsions in the diepoxide are expected to be significantly smaller than those in a saturated derivative of cyclopentane. In addition, because of the rigidity of the three-membered ring, H-1 (H-4) would tend to remain eclipsed with H-2 (H-3), and the only repulsion that might be relieved is that of H-2 with H-3. The energetic cost of puckering this fused-ring system would probably far exceed the small gain obtained from the relief of eclipsing. This explanation also agrees with the generalization[13a]* that "an sp^2-hybridized atom in planar surroundings is not seriously troubled by bond eclipsing". For all of these reasons, we propose that the cyclopentane ring of 7 exists in the planar conformation.

The conformations of the tetrols and related compounds are more difficult to assign. Many of the substances (but not the tetrols) are sufficiently soluble in non-hydroxylated solvents to permit detection and assessment of the strength of intramolecular H-bonds by i.r. spectroscopy. The presence of such bonds defines an additional ring, and thus diminishes the number of conformations possible. Some of the relevant data are shown in Table II. The difference, $\Delta\nu$, in O–H stretching frequency between free and H-bonded hydroxyl groups has been related[14] to the length of the H-bond. The value found for *cis*-1,2-cyclopentanediol (**28**) is compatible only with a conformation in which there is a torsional angle of 20–30°, and an O··· H bond-length of 1.84 Å. Darby *et al.*[7] reported that $\Delta\nu$ for *cis*-1,3-cyclopentanediol (**29**) is 63 cm^{-1}, corresponding to an O··· H distance of 1.82 Å. Examination of Dreiding models shows that, in order that this H-bond may exist, the cyclopentane ring must be puckered (see Fig. 7). The simplest puckering produces the V conformation (the terminology is that suggested by Hall[16] and used by Bishop and Cooper[17]). In order to accommodate the H-bond in the molecule, C-2 must be displaced by 0.4 to 0.5 Å out of the plane of the other ring atoms, in the direction away from the oxygen atoms. Although this conformation is logical in the sense that it explains the length of the H-bond, it still includes an eclipsed, ethane conformation involving C-4 and C-5, with a corresponding increase in energy of about 3 kcal[13b]. Consequently, the T_2^3 conformation, which also can accommodate the H-bond, must be considered. Similar arguments apply to the dibromoglycol 3a. In this case, the alternative conformations T_2^1 and T_1^5 differ with respect to the carbon atom (C-4 or C-3, respectively) that has its substituents in the bisectional orientation. Somewhat different considerations apply to **18**. Again, the simplest deformation would produce either the V^3 or V^5 conformation, depending on whether O-2 or O-1 is involved in the intramolecular H-bond. For the reasons outlined above, a twist conformer seems more likely; and, in the T_2^3 and T_1^5 conformations (see Fig. 7), the substituents are correctly disposed for H-bonding to O-2 and O-1, respectively. These conformations differ in the orientation of the C-3 hydroxyl group. In the T_2^3 conformation, the C-3 hydroxyl is quasi-axial; whereas, in the T_1^5 conformation, it is bisectional and one of the methylenic hydrogen atoms is quasi-axial (see Discussion).

*In this discussion, we have assumed that the diepoxide 7 resembles cyclopropane[13c] with respect to the approximate sp^2 hybridization of the orbitals involved in the C–H bonds.

Nuclear magnetic resonance studies. — Configurational assignments for many cyclopentane cyclitols and related compounds have previously been made on both chemical and spectroscopic grounds[1,2,9], and further details of the n.m.r. spectra of

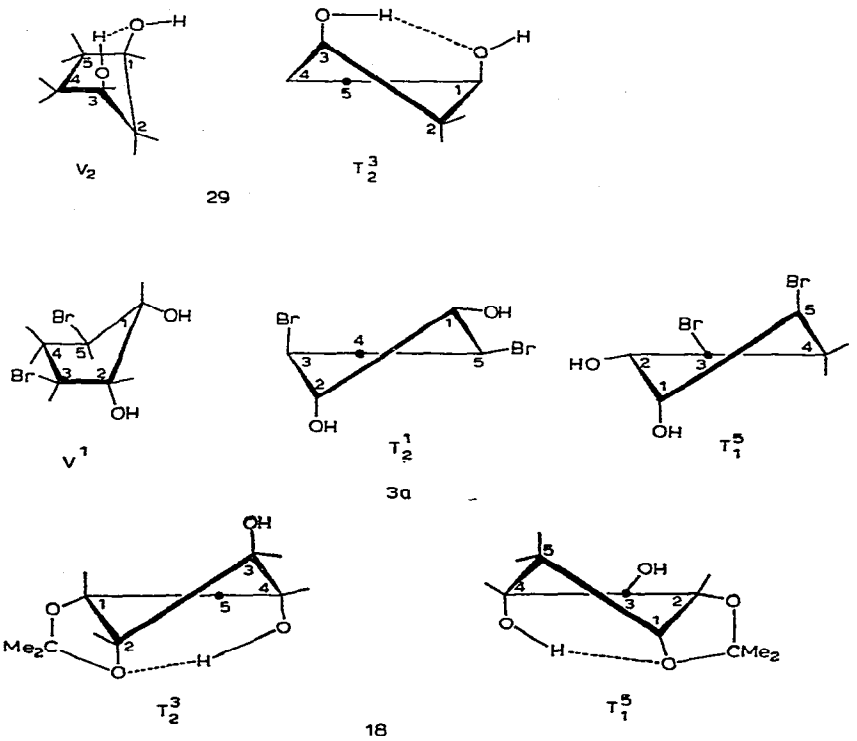

Fig. 7. Conformations considered reasonable for compounds **3a**, **18**, and **29**.

these compounds have been reported[5]. The spectral parameters of several of the compounds now reported are listed in Table III. In some cases, unequivocal assignments of some of the signals cannot yet be made; but the partial descriptions are included, because the spectra have nevertheless been useful in identifying the compounds. The numbering of the protons is as shown for **9** in Fig. 1, in which H-6 is *cis* and H-5 is *trans* with respect to H-1.

The spectrum of the dibenzoate **3b** resembles in general that of **3a**, reported earlier[9]. In the spectrum of that dibenzoate for which structure **4b** is proposed, the difference in chemical shift between H-5 and H-6 is much smaller than the corresponding difference in chemical shift in the spectrum of **3b**. The principal reason for this difference is the angular dependence of the deshielding effect of the vicinal, electronegative substituents[9,18]. In a compound having configuration **4**, the deshielding effects on the two methylenic protons should be approximately equal, whereas in compounds having configuration **3**, the methylenic proton that is *trans* to both vicinal substituents is subjected to a stronger deshielding influence

than is the *cis* proton. The spectrum of the unsymmetrical dibromoglycol supports, therefore, the proposed configurational assignment **4b**.

Most of the compounds under consideration having the same geometry as tetrol

TABLE III

N.M.R. SPECTROSCOPIC DATA[a]

Substance	*Protons*	*Appearance of signal*	δ	*Coupling constants*[a], *Hz*			
				$J_{1,2}$	$J_{1,5}$	$J_{1,6}$	$J_{5,6}$
3b	H-1,4	multiplet of at least 8 lines	~4.45[b]				
	H-2,3	multiplet of at least 4 lines	5.29	4.5	7.2	8.1	15.5
	H-5	doublet of triplets	2.60				
	H-6	5-line multiplet	3.36				
4b	H-5,6	irregular multiplet of at least 8 lines	~2.76[b]	width 27 Hz			
	All Br-C-H	irregular multiplet	~4.47[b]	width 24 Hz			
	O-C-H	quartet	5.67	"J" = 4.3 Hz			
	O-C-H	triplet	5.93	"J" = 4.3 Hz			
7	H-1,4	6-line multiplet[c]	3.75	width 5.6 Hz			
	H-2,3	4-line multiplet[c]	3.56	width 3.1 Hz			
	H-5	doublet of triplets	1.69[d]	?	2.8	0	16.5
	H-6	doublet	2.13[d]				
24	H-5,6	triplet	2.03	"J_{AX}" = 5.2 Hz			
	All O-C-H	irregular multiplet	4.06[b]	width 26 Hz			
10a	H-5,6	triplet	1.98	"J_{AX}" = 7.4 Hz			
	O-C-H	multiplet (probably H-2,3)	3.71[b]	width 13 Hz			
	O-C-H	multiplet (probably H-1,2)	4.03[b]	width 23 Hz			
11a	H-5	multiplet of at least 24 lines[e]	~1.4	width ~30 Hz			
	H-6	multiplet of at least 20 lines	~2.5	width ~32 Hz			
					6.5[e]	7.5[e]	17[e]
	All O-C-H	multiplet	~3.95	width ~30 Hz			
22	H-5,6	multiplet	2.00[b]	width 49 Hz			
	All O-C-H	irregular multiplet	4.12[b]	width 42 Hz			
21	H-5	doublet of multiplets	1.55	width 29 Hz			
	H-6	doublets of multiplets	2.48	width 38 Hz			
				$J_{5,6}$ ~16 Hz			
	All O-C-H	irregular multiplet	3.9[b]	width 37 Hz			

[a] **3b**, **4b**, and **7**: solutions in chloroform-*d*, with internal TMS; **10a**, **21**, **22**, and **24**: solutions in deuterium oxide, with internal DSS. The coupling constants shown are averages of all of the appropriate line separations in each of the signals in which the coupling may be assigned. Unless otherwise stated, the chemical shifts reported are the central point of the appropriate signals. [b] Value given is the midpoint of this multiplet, and is not necessarily the chemical shift of either of the nuclei represented. [c] The apparent multiplicity was determined from a spectrum recorded on an expanded scale at a lower sweep-rate. [d] Chemical shifts of H-5 and H-6 were in this case calculated by treating these two protons as an AB system[8b]. [e] See text for explanation.

11 (for example, **3a**, **3b**, **11b**, **12a**, **12b**, and **14**) have simple spectra that are amenable to first-order analysis[5,9]. In all of these instances, the bonds (H-5)–C–C–C–(H-2) and (H-5)–C–C–C–(H-3) assume the "W" conformation, which is known to favor long-range coupling[5]; and, in the examples cited, this long-range coupling is also amenable to first-order interpretation. The only compound in this series whose spectrum cannot be interpreted by the first-order rules is the free tetrol **11a**. The signals of H-5 and H-6 are not the simple doublets of triplets observed in the other cases, and the signals of H-1 (H-4) and H-2 (H-3) comprise an overlapping multiplet. On the basis of the total width of each of these signals, and the recurrence of certain separations between spectral lines, tentative values for $J_{1,5}$, $J_{1,6}$ and $J_{5,6}$, are reported in Table III, but these values will have to be confirmed by computation before they may be considered to have been established. The reason for the complexity of the spectrum is not easy to assess. One possible explanation is that the spectrum observed is the result of combined real and virtual[8c] coupling, which causes each proton in the molecule to be involved in non-zero coupling with all of the other protons.

For the diepoxide **7**, at least two different interpretations are possible. The assignment of the signals of H-5 and H-6 has been made by analogy with compounds of similar geometry in which the internal chemical shift[9,18] between H-1 (H-4) and H-5 is larger than that between H-1 (H-4) and H-6. We have assumed that this assignment is valid for substituted epoxides of cyclopentane. However, we have observed strong, long-range shielding and deshielding effects[19], ascribed to the diamagnetic anisotropy of the C–O bonds of the oxirane ring, and it is conceivable that the assignment of resonance frequencies to the two methylenic protons should be reversed (*cf.*, Tori *et al.*[20] and Jefferies *et al.*[21]). Consequently, until the assumption is unequivocally proved, *e.g.*, by specific replacement of H-5 or H-6 by deuterium, the assignment can be only tentative. In the epoxycyclopentanes that we have studied[9,19], and in anhydrofuranosides[16], vicinal spin–spin coupling of sp^3-hybridized protons with oxirane protons is associated with small or zero line-separation in the n.m.r. spectra. The relation between the dihedral angle and the magnitude of such vicinal coupling constants is not known. If the assignment of the resonance frequencies of the methylenic protons is correct, the tripling of the H-5 signal may be ascribed to $J_{1,5} = J_{4,5} =$ 2.8 Hz. If this conclusion is correct, it follows that $J(85°)$ is much larger than $J(35°)$, opposite to the situation described by Karplus' calculations[22]. This apparently anomalous coupling adds to the uncertainty of the assignments of the protons of the methylene group. A spin–spin decoupling experiment, by nuclear-magnetic, double resonance[19], showed that the nuclei corresponding to the signal at δ 3.75 are coupled to the nuclei that we have designated H-5. The signal at δ 3.75 is, therefore, assigned to H-1 (H-4). The remaining signal, at δ 3.56, must, therefore, be that of H-2 (H-3). If first-order rules for multiplicity apply, the signal at δ 3.75 should be a doublet of doublets due to $J_{1,2}$ ($J_{3,4}$) and $J_{1,5}$ ($J_{4,5}$); the signal at δ 3.56 should be a doublet due to $J_{1,2}$. The additional multiplicity might then be ascribed to long-range coupling ($J_{1,3}$ and $J_{2,4}$); indeed, the C–H bonds concerned appear to be well oriented for such long-range coupling.

An entirely different interpretation of the spectrum seems equally reasonable. The methylenic protons might be assumed not to be coupled with H-1 and H-4. Instead, the tripling in the signal at highest field is ascribed to long-range coupling equally with H-2 and H-3. The latter nuclei would, therefore, be assigned to the signal at δ 3.75. In addition, H-2 is coupled with H-1 and, by long range, with H-4. Since H-1 and H-4 have the same chemical shift, the line-splitting caused in H-2 is one half of $(J_{1,2} + J_{2,4})$, causing a tripling[23] of the signal; H-2 appears, therefore, as a six-line signal. H-1 (H-4) is a "triplet" due to $J_{1,2}$ and $J_{1,3}$; the coupling constants are unequal, but, as the chemical shifts of H-2 and H-3 are identical, the line-separation in the H-1 (H-4) signal is one half of $(J_{1,2} + J_{1,3})$.

DISCUSSION

Conformational analysis of cyclopentanoid systems is subject to more uncertainty than that of cyclohexanoid or pyranoid systems. In the examples cited above, the conformations proposed are not necessarily valid, except in the solutions in non-hydroxylic solvents in which the intramolecular hydrogen-bonds were observed. Even in these restricted situations, the choice between alternative possibilities (see Fig. 7) does not yet have an unequivocal basis. For example, in cyclohexanoid systems, the choice between conformers corresponding to T_2^3 and T_1^5 of the mono-*O*-isopropylidene tetrol **18** would be relatively easy; that conformer having fewer axial substituents would be favored, because of the decreased 1,3-diaxial repulsion. In the example shown in Fig. 7, the cost in energy of the quasi-axial hydroxyl group is probably much less than that of an axial group in a cyclohexanoid system, because the hydroxyl group of T_2^3 does not enter into serious 1,3-interaction with another group. Consequently, we favor conformation T_2^3 over T_1^5 because the mutual repulsion between the C-3 and C-4 hydroxyl groups in the former is minimized. Gagnaire and Vottero[24] have studied a related system (2,4/3,5)-3,4-dibromo-2,5-dimethoxytetrahydrofuran. On the basis of n.m.r. coupling-constants of the ring protons, these authors have concluded that a T_3^4 conformation, with all the bulky groups in quasi-axial orientation, is favored. In this example also, the mutual repulsions of the substituents are minimized.

Assignment of absorption bands in the 1300–800 cm^{-1} region of the i.r. spectra to particular modes of vibration of epoxides is still associated with some uncertainty[12b]. The band at 845 cm^{-1} that is characteristic of cyclopentane epoxides is probably the same as the band at 830 cm^{-1} that some authors have tentatively assigned to ring vibrations in *cis*-epoxides[12b]. In general, this region of the spectrum is related to alkyl C–O stretching and to C–H rocking vibrations. Cope *et al.*[25] prepared *cis*-cyclodecene oxide and the corresponding compound in which the oxirane hydrogen atoms are replaced by deuterium. In December, 1964, Professor Cope sent us tracings of the i.r. spectra of the normal and deuterated epoxides, determined with thin films of the pure liquids. Both spectra have strong bands in the 845–835 cm^{-1} region. The possibility that this band is due to a C–H rocking mode is, therefore, eliminated.

Biological studies of the compounds reported have not yet been conducted.

Diepoxides have been found to be good alkylating agents, and they are cytotoxic, mutagenic, and carcinogenic[26-31]. It will be interesting to test the diepoxide **7** and other compounds, such as the epoxybromohydrin and dibromoglycol **2** and **3a**, which may also be good bis-alkylating agents.

EXPERIMENTAL

I.r. spectra were measured with a Perkin–Elmer Model 237B spectrophotometer. n.m.r. spectra were measured with a Varian Associates A-60 n.m.r. spectrometer, with either tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal standard. Melting points were determined on a Kofler micro hot-stage (Athur H. Thomas and Co.) and are corrected. Boiling points are uncorrected. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

cis-*1,2:3,4-Diepoxycyclopentane* (**7**). — A. *From 3,5-dibromo-(1,2/3,5)-cyclopentane-1,2-diol* (**3a**). The dibromoglycol **3a** (12.8 g, 49 mmoles) was dissolved in 70 ml of 0.72N sodium hydroxide (50 mmoles) and the solution was extracted with ether continuously for several h. The syrupy residue obtained after evaporation of the ether was separated into two fractions by vacuum distillation; (a) b.p. 62° (0.7 torr), and (b) b.p. 87° (0.3 torr). Fraction (b) was identified as **2** by its i.r. spectrum. Fraction (a) was redistilled to give **7**, b.p. 35° (0.2 torr).

Anal. Calc. for $C_5H_6O_2$: C, 61.21; H, 6.17, Found: C, 61.15; H, 6.22.

B. *From* DL-*1,2-anhydro-4-bromo-(1,2,3/4)-cyclopentane-1,2,3-triol* (**2**). The epoxybromohydrin **2** (2.9 g, 16.2 mmoles) was treated with sodium hydroxide as above, the aqueous solution was extracted for 42 h with dichloromethane and the residue was distilled, giving 1.5 g of **7** (15.4 mmoles, 95%).

DL-*Tri*-O-*benzoyl-4-bromo-(1,4/2,3)-cyclopentane-1,2,3-triol* (**5b**) *and* DL-4-*bromo-2,3*-O-*isopropylidene-(1,4/2,3)-cyclopentane-1,2,3-triol* (**9**). — A solution of **2** in 0.25N sulfuric acid was heated for 1h at 100° to produce **5a**. Excess barium carbonate was added, barium sulfate was removed by filtration, and the solvent was evaporated under diminished pressure. An aliquot sample of the resulting syrup (126 mg) was dissolved in 0.6 ml of pyridine, the solution was chilled in ice, 0.4 ml of benzoyl chloride was added and the mixture was kept overnight at room temperature. The product was isolated in the usual way[2] and it crystallized after brief contact with ethanol. Recrystallization gave **5b**, m.p. 136–7°.

Anal. Calc. for $C_{26}H_{21}BrO_6$: C, 61.31; H, 4.15; Br, 15.69. Found: C, 61.02; H, 4.13; Br, 15.80.

The remainder of the syrup (1.2 g) was stirred for 40 h with 100 ml of anhydrous acetone to which was added 2 g of anhydrous copper(II) sulfate and 3 drops of conc. hydrochloric acid. Conc. aqueous ammonia was added, the mixture was filtered, and the solvent was evaporated in a current of warm air. The residue was extracted with dichloromethane and the solvent was evaporated to give a semi-solid product. The product was extracted with carbon disulfide, and after removal of this solvent the oily residue was distilled to give **9**, yield 282 mg, b.p. 100–110° (0.3 torr).

Anal. Calc. for $C_8H_{13}BrO_3$: C, 40.52; H, 5.52; Br, 33.70. Found: C, 40.45; H, 5.65; Br, 33.66.

DL-*1,2-Anhydro-(1,2,3/4)-cyclopentane-1,2,3,4-tetrol* (**6a**). — The crude bromotriol **5a** (13.5 g, 68 mmoles) was added to a solution of 2.7 g (68 mmoles) of sodium hydroxide in 70 ml of water, and the solution was extracted with ether for 24 h. The ether was evaporated and the syrupy product was crystallized from hot ethyl acetate, yield 3.95 g (35 mmoles, 50%), m.p. 103.5–104.5°.

Anal. Calc. for $C_5H_8O_3$: C, 51.72; H, 6.94. Found: C, 51.76; H, 7.07.

The dibenzoate **6b** was prepared from the anhydrotetrol **6a** (70 mg) in 0.19 ml of pyridine, with 0.14 ml of benzoyl chloride; recrystallized from ethanol it had m.p. 119–120°.

Anal. Calc. for $C_{19}H_{16}O_5$: C, 70.86; H, 4.97. Found: C, 70.43; H, 5.08.

2,3-O-Isopropylidene-(1,4/2,3)-cyclopentane-1,2,3,4-tetrol (**12a**) *and its dibenzoate* (**12b**). — The anhydrotetrol **6a** was hydrolyzed by heating for 1 h at 100° in 0.25N sulfuric acid, and the solution was neutralized (barium carbonate) and evaporated. The syrupy product crystallized spontaneously. Analysis by n.m.r. spectroscopy showed that 90% of tetrol **11a** and 10% of tetrol **10a** had been produced. The hydrolyzate was stirred with acetone and copper(II) sulfate for two days and worked up in the usual way. The product (**12a**) crystallized spontaneously and was recrystallized from benzene; it had m.p. 136.5–138.5°.

Anal. Calc. for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.34; H, 8.25.

Benzoylation of **12a** (71 mg) with benzoyl chloride (0.09 ml) and pyridine (0.4 ml) gave the product **12b**, which crystallized rapidly from ethanol, and was recrystallized from the same solvent; it had m.p. 139–141°.

Anal. Calc. for $C_{26}H_{22}O_6$: C, 69.10; H, 5.80. Found: C, 68.91; H, 5.76.

DL-*(1,2,4/3)-Cyclopentane-1,2,3,4-tetrol* (**21a**). — The *O*-isopropylideneanhydrotetrol **16** was prepared as described previously[1a], and hydrolyzed (N sulfuric acid, 1 h, 100°) and worked up as usual. The syrup obtained after removal of solvent was desiccated for several weeks, during which time crystals formed slowly. After recrystallization from absolute ethanol, the product **21a** melted at 127.5–129°.

Anal. Calc. for $C_5H_{10}O_4$: C, 44.77; H, 7.52. Found: C, 44.64; H, 7.38.

Benzoylation of **21a** gave a product identical[1a] with authentic **21b**.

DL-*1,2-O-Isopropylidene-(1,2,4/3)-cyclopentane-1,2,3,4-tetrol* (**18**). — The cyclopentanetetrol **21** was prepared in the usual way, and the syrupy product was stirred overnight with anhydrous acetone and copper(II) sulfate. After the mixture had been filtered, the acetone was evaporated in a stream of warm air, and the residue was extracted with dichloromethane. The solvent was evaporated as before, and the liquid obtained was subjected to a high vacuum for 1h. After a few h at room temperature, the oil solidified to a mass of crystals, m.p. 60–65°. The product was purified by sublimation at 65° (0.3 torr) with the condenser at 0°; the product had m.p. 65–66°.

Anal. Calc. for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.20; H, 8.13.

DL-*1,2-O-Isopropylidene-(1,2/3,4)-cyclopentane-1,2,3,4-tetrol* (**20**). — A solution of *cis*-1,2-*O*-isopropylidene-3-cyclopentene-1,2-diol[4] (**26**) (3 g, 21.4 mmoles) in 300 ml

of acetone was cooled to −10° and 60 ml of 5% (w/v) aqueous potassium permanganate added dropwise, with good stirring, during 2.5 h. Stirring was continued and the temperature was maintained for 0.5 h at 0° after the addition had been completed. When the solution was allowed to warm slightly, the precipitate became flocculent and was removed by filtration. The filtrate was concentrated, and the resultant syrup was desiccated under vacuum for 2 days, during which time **20** crystallized, yield 2.0 g (11.3 mmoles, 53%), m.p. 87–92°. The product was dissolved in hot ether, and the solution was filtered and evaporated to give crystals, m.p. 91–94°, and the product was finally recrystallized from benzene; m.p. 95–96°.

Anal. Calc. for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.32; H, 8.00.

Hydrolysis of **20** under the usual conditions gave the free tetrol **24**, m.p. 124.5–127°, and benzoylation of the latter under the usual conditions gave the tetrabenzoate, m.p. 144–6°. The tetrol and the tetrabenzoate were identical to the authentic materials previously described[1a].

DL-*1,2:3,4-Di*-O-*isopropylidene-(1,2/3,4)-cyclopentane-1,2,3,4-tetrol* (**27**). — The tetrol **24** (0.50 g, 3.73 mmoles) was dissolved in 100 ml of anhydrous acetone, 3 drops of conc. hydrochloric acid and 2 g of anhydrous copper(II) sulfate were added, and the mixture was stirred overnight. After having been adjusted to pH 7 with conc. ammonium hydroxide, the mixture was filtered and the solvent was evaporated in a stream of cool air to give a white, crystalline product. Sublimation at 55° (0.3 torr), with the condenser at 0°, gave **27** (0.75 g, 3.50 mmoles, 94%) m.p. 49–50°.

Anal. Calc. for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47. Found: C, 61.39%; H, 8.50.

DL-*5-Bromo-1,2*-O-*isopropylidene-(1,2/5)-3-cyclopentene-1,2-diol* (**36**). — A solution of 3,5-dibromo-1,2-*O*-isopropylidene-(1,2/3,5)-cyclopentane-1,2-diol[4] **14** (2.5 g, 8.3 mmoles) in 15 ml of anhydrous acetone was added over a period of 20 min to a solution of tetraethylammonium acetate (3.4 g, 16.5 mmoles) in 25 ml of anhydrous acetone, and the temperature was maintained at 0°. A white precipitate formed slowly during the addition. The mixture was stirred for 24 h at room temperature, filtered and the solvent was evaporated. The syrupy residue was distilled, giving 0.2 ml of **36**, b.p. 68° (120 torr). The i.r. spectrum showed bands characteristic of vinyl C-H vibrations at 690, 755, and 3065 cm^{-1}, and of *O*-isopropylidene groups at 1370–1380, 1253, and 1154 cm^{-1}.

Anal. Calc. for $C_8H_{11}BrO_2$: C, 43.86; H, 5.06; Br, 36.47. Found: C, 44.01; H, 5.12; Br, 36.23.

Epoxide opening of epoxybromohydrin **2**. — Hydrolysis of **2** is described above. For opening by hydrogen bromide, 0.28 g of **2** was dissolved in 10 ml of abs. ethanol, and hydrogen bromide gas was bubbled vigorously through the solution for 1 min. The solution became hot. It was kept for 0.5 h and then concentrated. The syrup was diluted with 15 ml of water, the pH was adjusted to 6 by addition of solid sodium hydrogen carbonate, and the solution was extracted overnight with dichloromethane. Evaporation of the extract gave a crystalline product, m.p. 68–71°. The i.r. spectrum showed that the substance was the dibromoglycol **3a**. After recrystallization from

dichloromethane–low-boiling petroleum[4], it had m.p. 75.5–76.5°, mixture m.p. with authentic **3a** 75–77°.

Epoxide opening of anhydrotetrol **6a**. — A. *Hydrolysis.* Substance **6a** (300 mg) was dissolved in 0.02N sulfuric acid (50 ml) and the solution was heated for 1h at 100°. Excess barium carbonate was added, the mixture was filtered, and solvent was removed under vacuum. The product was dissolved in deuterium oxide (0.60 ml) and the n.m.r. spectrum of the solution was recorded.

B. *Opening with hydrogen bromide.* Substance **6a** (0.253 g, 2.2 mmoles) was dissolved in 25 ml of ethanol, and hydrogen bromide gas was bubbled vigorously through the solution for 2 min. The solution was kept overnight at room temperature and then concentrated *in vacuo* to an orange syrup. Anhydrous acetone (50 ml) and copper(II) sulfate (3 g) were added and the mixture was stirred for 22 h at room temperature. The pH was adjuted to 7 with conc. ammonium hydroxide, the mixture was filtered, and the solvent was removed by a current of cool air. The syrupy product was extracted with dichloromethane, the extract was evaporated, and the residue was distilled; b.p. 120° (0.75 torr), yield 0.234 g (0.1 mmole, 45%). The i.r. and n.m.r. spectra of the *O*-isopropylidene derivative were identical to those of **9** prepared from epoxybromohydrin **2**.

Epoxide opening of the diepoxide **7**. — A. *Hydrolysis.* Substance **7** (290 mg) was dissolved in 0.02N sulfuric acid (50 ml), the solution was heated for 1 h at 100°, and the product was worked up as described above. The syrupy product (118 mg) was dissolved in deuterium oxide (0.50 ml) and its n.m.r. spectrum was recorded.

B. *Opening by hydrogen bromide.* Substance **7** (1.67 g, 17 mmoles) was added to 15 ml of N aqueous hydrobromic acid and kept overnight at room temperature. The solution was neutralized with N sodium hydroxide and was then extracted for 3 days with dichloromethane. Evaporation of the solvent gave 1.2 g of an oil, which was treated overnight with acetone (70 ml) and copper(II) sulfate (2 g), and the product was worked up as usual. A yellow oil was obtained, which was extracted with low-boiling petroleum (35–60°); most of the material was insoluble. Evaporation of the petroleum left a liquid whose i.r. spectrum was identical to that of authentic[4] **14**. The petroleum-insoluble material was benzoylated in 8 ml of pyridine with 5.5 ml of benzoyl chloride. The product, DL-1,3-di-*O*-benzoyl-2,4-dibromo-(1,3/2,4)-1,3-cyclopentane-diol (**4b**), recrystallized 5 times from abs. ethanol, had m.p. 117.5–119°.

Anal. Calc. for $C_{19}H_{16}Br_2O_4$: C, 48.75; H, 3.44; Br, 34.12. Found: C, 48.69; H, 3.25; Br, 34.14.

The dibenzoate **3b**, prepared from the authentic dibromoglycol **3a**, had m.p. 123.5–124°. *Anal.* Found: C, 48.63; H, 3.51; Br, 34.24. The mixture m.p. of **3b** and **4b** was 97–107°.

Products of hydrolysis of the O-*isopropylideneanhydrotetrols* **15** *and* **16**. — A. *Partial hydrolysis.* Aqueous solutions were prepared, containing 7 mg/ml of the respective compounds. Aliquots (0.4 ml) were added to 1.2 ml of 1.8×10^{-3}N sulfuric acid, and the solutions were heated for 5 min at 100°. The reaction was stopped by chilling in ice and adding an excess of barium carbonate. The mixtures were filtered,

the filtrates were evaporated to dryness, and the residues were dissolved in water (0.2 ml). These solutions were used for t.l.c. analysis.

B. *Semi-quantitative analysis of products of complete hydrolysis.* Mixtures, prepared as above, were heated for 30 min and analyzed as described.

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SUMMARY

Earlier conclusions that an electronegative substituent exerts a strong influence on the direction of opening of an unhindered, adjacent epoxide by a nucleophilic reagent have been substantiated; the nucleophile attacks stereoselectively at the end of the oxirane ring farthest from the vicinal, electronegative substituent (OH or Br). *cis*-1,2:3,4-Diepoxycyclopentane **7** has been prepared, and, unlike the substituted monoepoxides of cyclopentane, this compound shows no stereoselectivity for the attack of a nucleophile (Br^- or H_2O). When the anhydro-*O*-isopropylidene cyclopentanetetrols are subjected to acid-catalyzed hydrolysis, the first products are anhydrotetrols, because of the greater lability of the *O*-isopropylidene residue. The corresponding *O*-isopropylidenetetrols are not detected in partial hydrolyzates, which contain tetrols, anhydrotetrols, and starting material. When a hindered halocyclopentane, 3,5-dibromo-1,2-*O*-isopropylidene-(1,2/3,5)-1,2-cyclopentane-diol (**14**), reacts with tetraethylammonium acetate, the expected nucleophilic displacement of halogen by acetate is not observed; instead a 1,2-elimination of hydrogen bromide occurs. Favored conformations of several derivatives of cyclopentane cyclitols are assigned, on the basis of the presence of strong, intramolecular, hydrogen bonds and the need to minimize nonbonded repulsion. Improved methods of synthesis, and several new derivatives of cyclopentane cyclitols, are described. The strong band near 840 cm^{-1} in the infrared spectra of epoxides is assigned to a C–O stretching, rather than to a C–H rocking mode.

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STUDIES ON 2-AMINO-2-DEOXY-D-GLUCOSE DERIVATIVES.
PART XV. SYNTHESIS OF 1-*N*-ACYL-2-ACYLAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLAMINES*

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The carbohydrate–peptide bond in glycopeptides isolated from hen-egg albumin[1–4] and from other sources[5–7] was shown, by comparison of the physical properties of synthetic and natural products[8–13], to be an amido linkage between the β-carboxyl group of L-aspartic acid and the amino group of 2-acetamido-2-deoxy-β-D-glucopyranosylamine. Synthesis of 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine was performed[8] by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine with α-benzyl *N*-(benzyloxycarbonyl)-L-aspartate, followed by removal of the protecting groups from the product.

In an earlier paper from our laboratory[14], a new synthesis of 2-acetamido-2-deoxy-1-*N*-glycyl-β-D-glucopyranosylamine, *via* the key intermediate 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamine[15] (**1**), was presented. In the present paper, this method is used for the synthesis of 1-*N*-acyl-2-acylamino-2-deoxy-β-D-glucopyranosylamines, wherein one of the two acylamino groups is an acetamido group and the other is an (aminoacyl)amino group. Compound **1** was prepared by heating 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucose in methanolic ammonia in a sealed tube. Treatment of **1** with 60% acetic acid for 1 h at 100° gave only 2-acetamido-2-deoxy-D-glucose (papergram[16]) and none of its 2-epimer.

Condensation of **1** with suitably protected amino acid derivatives (*N*-benzyloxycarbonyl-L-alanine, *N*,*N*′-dibenzyloxycarbonyl-L-lysine, α-benzyl *N*-benzyloxycarbonyl-L-aspartate, and β-benzyl *N*-benzyloxycarbonyl-L-aspartate) in pyridine, at room temperature, by the carbodiimide method afforded 1-*N*-(protected aminoacyl)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamines (**2**, **3**, **4**, and **5**) in 30–60% yield. Their assigned structures were supported by their characteristic i.r. absorptions (urethan, amido, or ester carbonyl groups) and by their positive Fehling's reaction. The benzylidene group of these compounds was resistant to hydrogenolysis, but was removed by hydrolysis with 60% acetic acid for 1 or 2 h at 100°. 1-*N*-(Protected aminoacyl)-2-acetamido-2-deoxy-β-D-glucopyranosylamines (**6**, **7**, **8**, and **9**) thus obtained in about 80% yield were catalytically hydrogenolyzed to give the 2-acetamido-1-*N*-aminoacyl-2-deoxy-β-D-glucopyranosylamines (**10**, **11**, **12**, and **13**) in 60–80% yield.

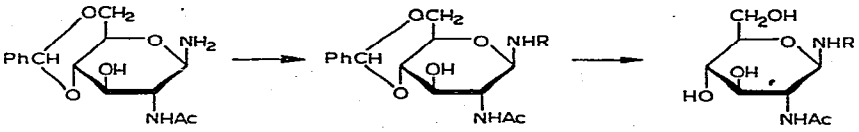
*Part of this work was presented at the International Symposium on the Chemistry of Natural Products held in April 1964 at Kyoto, Japan.

TABLE I

1-*N*-AMINOACYL-2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLAMINES AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*		c	*Carbon*		*Hydrogen*		*Nitrogen*		*Yield* %
			$[\alpha]_D^{23}$ *degrees*	*Solvent*		*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	
2	$C_{26}H_{31}N_3O_8$	—	—	—	—	60.81	60.53	6.08	5.92	8.18	8.07	50
3	$C_{37}H_{44}N_4O_{10}$	228–229	0.0	C_5H_5N	1.0	63.05	62.69	6.29	6.27	7.95	7.73	30
4	$C_{34}H_{37}N_3O_{10}$	214–216	−0.6	$HCONMe_2$	1.0	63.05	63.07	5.76	5.91	6.49	6.67	55
5	$C_{34}H_{37}N_3O_{10}$	224	−26.9	$HCONMe_2$	1.0	63.05	63.25	5.76	5.97	6.49	6.73	45
6	$C_{19}H_{27}N_3O_8$	198–199	14.4	$HCONMe_2$	1.0	53.64	53.77	6.40	6.63	9.88	9.90	75
7	$C_{30}H_{40}N_4O_{10}$	219–220	0.0	$HCONMe_2$	1.0	58.43	58.02	6.54	6.28	9.09	9.36	79
8	$C_{27}H_{33}N_3O_{10}$	188–189	13.6	$HCONMe_2$	1.0	57.95	57.84	5.94	6.02	7.51	7.62	80
9	$C_{27}H_{33}N_3O_{10}$	218–220	−15.1	$HCONMe_2$	1.0	57.95	57.74	5.94	5.98	7.51	7.90	80
10	$C_{11}H_{21}N_3O_6 \cdot H_2O$	200	43.8	H_2O	1.5	42.71	42.74	7.49	7.45	13.59	13.33	80
11	$C_{14}H_{28}N_4O_6 \cdot 2H_2O$	syrup	17.8	H_2O	1.0	43.73	43.84	8.39	8.37	14.57	14.51	90
12	$C_{12}H_{21}N_3O_8 \cdot 0.5H_2O$	232–235	25.0	H_2O	1.5	41.26	41.90	6.44	6.38	12.21	11.93	70
13	$C_{12}H_{21}N_3O_8 \cdot 1.5H_2O$	230–240	20.6	H_2O	1.5	39.77	39.96	6.68	7.06	11.59	11.18	70

The above-mentioned reactions were also applied to the preparation of 2-[(aminoacyl)amino]-2-deoxy-β-D-glucopyranosylacetamides in which the (aminoacyl)amino group is a glycylamino and a L-lysylamino group (**30** and **31**).

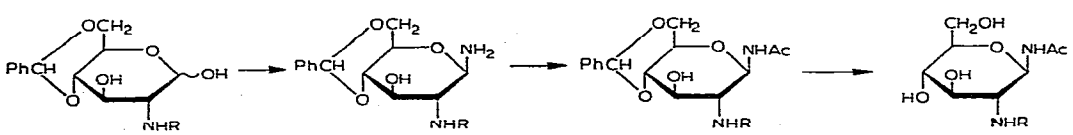


1 **2–5** **6–13**

2 and **6**, R = *N*-(benzyloxycarbonyl)-L-alanyl
3 and **7**, R = *N*,*N*′-di(benzyloxycarbonyl)-L-lysyl
4 and **8**, R = α-benzyl *N*-(benzyloxycarbonyl)-β-L-aspartyl
5 and **9**, R = β-benzyl *N*-(benzyloxycarbonyl)-α-L-aspartyl
10, R = L-alanyl
11, R = L-lysyl
12, R = β-L-aspartyl
13, R = α-L-aspartyl

Benzylidenation of 2-[(*N*-benzyloxycarbonylglycyl)amino]- and 2-{[*N*,*N*′-(dibenzyloxycarbonyl)-L-lysyl]amino}-2-deoxy-D-glucose, which were synthesized by the method of Kochetkov *et al.*[17], afforded, in 60% yield, the corresponding 4,6-*O*-benzylidene derivatives (**14** and **18**), which gave in 35–50% yield 4,6-*O*-benzylidene-2-[(*N*-benzyloxycarbonylglycyl)amino]- and 4,6-*O*-benzylidene-2-{[*N*,*N*-di(benzyloxycarbonyl)-L-lysyl]amino}-2-deoxy-β-D-glucopyranosylamine (**15** and **19**) by treatment with methanolic ammonia in a sealed tube. The anomeric configurations of **15** and **19** were assumed to be β-D from their specific rotations.

The procedure outlined above was unsuccessful, however, in the case of the aspartic acid derivative. The crude syrupy 2-{[α-benzyl *N*-(benzyloxycarbonyl)-L-aspartyl]amino}-2-deoxy-D-glucose, prepared by condensation of α-benzyl *N*-(benzyloxy-



14,18,22,26 **15,19,23,27** **16,20,24,28** **17,21,25,29–35**

14, **15**, **16**, and **17**, R = *N*-(benzyloxycarbonyl)glycyl
18, **19**, **20**, and **21**, R = *N*,*N*′-di(benzyloxycarbonyl)-L-lysyl
22 and **34**, R = α-benzyl *N*-(benzyloxycarbonyl)-β-L-aspartyl
23, **24**, and **25**, R = *N*-(benzyloxycarbonyl)-β-L-asparaginyl
26, **27**, **28**, and **29**, R = benzyloxycarbonyl
30, R = glycyl
31, R = L-lysyl
32, R = β-L-asparaginyl
33, R = H·HCl
35, R = β-L-aspartyl

TABLE II

2-[(AMINOACYL)AMINO]-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDES AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*		c	*Carbon*		*Hydrogen*		*Nitrogen*		*Yield* %
			$[\alpha]_D^{23}$ *degrees*	*Solvent*		*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	
14	$C_{23}H_{26}N_2O_8$	217–219	15.0	$HCONMe_2$	1.0	60.25	60.25	5.72	5.62	6.11	6.18	52
15	$C_{23}H_{27}N_3O_7$	187–189	−26.5	$HCONMe_2$	1.0	60.38	59.53	5.95	5.88	9.19	9.07	42
16	$C_{25}H_{29}N_3O_8$	254–255	6.6	$HCONMe_2$	1.0	60.11	60.02	5.85	6.09	8.41	8.68	55
17	$C_{18}H_{25}N_3O_8$	214–215	18.2	AcOH	1.0	52.55	52.14	6.13	6.58	10.21	10.42	89
18	$C_{35}H_{41}N_3O_{10}$	185–187	15.8	$HCONMe_2$	1.0	63.33	63.08	6.23	6.31	6.33	6.33	60
19	$C_{35}H_{42}N_4O_9$	201–202	−27.4	$HCONMe_2$	1.0	63.43	63.27	6.39	6.50	8.45	8.32	50
20	$C_{37}H_{44}N_4O_{10}$	234–237[a]	10.5	$HCONMe_2$	1.0	63.05	63.10	6.29	6.19	7.95	7.92	80
21	$C_{30}H_{40}N_4O_{10}\cdot H_2O$	164–166[a]	11.0	AcOH	1.0	56.85	57.23	6.67	6.98	8.83	8.54	80
30	$C_{10}H_{19}N_3O\cdot 0.5(COOH)_2\cdot H_2O$	205	44.7	H_2O	1.5	38.82	39.09	6.47	6.79	12.34	12.16	70
31	$C_{14}H_{28}N_4O_6\cdot 2HCl$	184–185	29.4	H_2O	1.5	39.92	40.22	7.16	7.39	13.33	13.25	60

[a]No dec.

carbonyl)-L-aspartate and 2-amino-2-deoxy-D-glucose in presence of *N,N'*-dicyclohexylcarbodiimide in aqueous acetone, was directly benzylidenated to give 4,6-*O*-benzylidene-2-{[*N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-D-glucopyranose (**22**) in crystalline form. When the condensation was performed in aqueous pyridine, the analytical results for the benzylidene derivative were consistent with those of a lactone derivative, in which the benzyl ester of the aspartyl moiety had been hydrolyzed and the resulting carboxyl group had formed a lactone with a hydroxyl group of the sugar moiety; the i.r. spectrum showed a characteristic absorption at 1725 cm^{-1} instead of the ester group absorption of **22** at 1745 cm^{-1}. After treatment with methanolic ammonia, both benzylidene derivatives gave the same glucosylamine, namely, 4,6-*O*-benzylidene-2-{[*N*-(benzyloxycarbonyl)-β-L-asparaginyl]amino}-2-deoxy-β-D-glucopyranosylamine (**23**), in which the α-carboxyl group converted spontaneously into a carbamoyl group. Although **23** was transformed into 2-[(β-L-asparaginyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (**32**) through the series of reactions mentioned previously, the carbamoyl group could not be successfully hydrolyzed into a carboxyl group without cleavage of the glycosidic bond.

In order to link the β-L-aspartyl group to the 2-amino group of 2-amino-2-deoxy-D-glucosylamine after *N*-acetylation at C-1, 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose (**26**), obtained by benzylidenation of 2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose[18] was transformed into 2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (**29**) *via* 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylamine (**27**) and its *N*-acetyl derivative **28**. The benzyloxycarbonyl group of **29** was hydrogenolyzed, and the resulting 2-amino-2-deoxy-β-D-glucopyranosyl hydrochloride (**33**) was then condensed with α-benzyl *N*-benzyloxycarbonyl-L-aspartate to give 2-{[*N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-β-D-glucopyranosylacetamide (**34**) which was again hydrogenolyzed to give the desired product, 2-[(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (**35**).

4,6-*O*-Benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucopyranose (**26**), **27**, and **29** were fully acetylated to give the corresponding 1,3-di-*O*-acetyl, *N*acetyl-*O*-acetyl, and 3,4,6-tri-*O*-acetyl derivatives, respectively, and the last-named compound was tranformed into 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosylacetamide hydrochloride.

The behavior of **12** during chromatography and electrophoresis was compared with that of the 2-acetamido-2-deoxy-D-glucose–L-aspartic acid compound isolated from hen-egg albumin. In these tests, the two compounds were found to be identical. On the other hand, no difference between **12** and **35** could be found in these tests. Recently, Micheel *et al.*[19] reported that the hydrolysis of **12** in N hydrochloric acid for 1 h at 100° gives the α-D anomer of **12** and 2-[(β-L-aspartyl)amino]-2-deoxy-D-glucose. Such an anomerization and $N \rightarrow N$ acyl migration complicates the reaction. However, the hydrolysis rate of each *N*-acetyl and *N*-aspartyl group in compounds **12** and **35**, in which the two groups are interchanged, should be different. The results of this study will be reported later.

TABLE III

2-(β-L-ASPARTYL)AMINO-4,6-*O*-BENZYLIDENE-2-DEOXY-D-GLUCOSE, 2-(β-L-ASPARAGINYL)AMINO-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDE AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*		c	*Carbon*		*Hydrogen*		*Nitrogen*		*Yield*
			$[\alpha]_D^{23}$ *degrees*	*Solvent*		*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	%
22	$C_{32}H_{34}N_2O_{10}$	166–168	−26.0	$HCONMe_2$	1.0	63.36	63.03	5.65	5.77	4.62	4.79	50
23	$C_{25}H_{30}N_4O_8$	208–209	−15.4	$HCONMe_2$	1.0	58.36	58.70	5.88	6.10	10.89	10.80	28
24	$C_{27}H_{32}N_4O_9$	252–254	0.0	$HCONMe_2$	1.0	58.26	57.92	5.80	6.09	10.07	10.28	50
25	$C_{20}H_{28}N_4O_9$	237–238	53.0	AcOH	1.5	51.27	50.82	6.02	6.30	11.96	11.81	80
32	$C_{12}H_{22}N_4O_7 \cdot H_2O$	190–192	—	—	—	40.91	40.65	6.87	6.98	15.92	15.72	70

TABLE IV

4,6-*O*-BENZYLIDENE-2-[(BENZYLOXYCARBONYL)AMINO]-2-DEOXY-D-GLUCOSE, 2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDE, 2-(β-L-ASPARTYL)AMINO-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDE AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*		c	*Carbon*		*Hydrogen*		*Nitrogen*		*Yield*
			$[\alpha]_D^{23}$ *degrees*	*Solvent*		*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	%
26	$C_{21}H_{23}NO_7$	240	−18.0	$HCONMe_2$	1.0	62.83	62.68	5.78	5.99	3.49	3.60	72
27	$C_{21}H_{24}N_2O_6$	195–196	−39.0	$HCONMe_2$	1.0	62.99	63.00	6.04	6.26	7.00	7.27	50
28	$C_{23}H_{26}N_2O_7$	278–279	−13.0	$HCONMe_2$	1.0	62.43	62.13	5.92	6.64	6.33	6.73	90
29	$C_{16}H_{22}N_2O_7$	206	28.0	MeOH	1.5	54.23	54.46	6.26	6.45	7.91	7.96	85
33	$C_8H_{16}N_2O_5 \cdot HCl \cdot H_2O$	176	0.0	H_2O	1.5	36.16	36.35	6.78	7.16	10.55	10.66	95
34	$C_{27}H_{33}N_3O_{10}$	222–223	26.0	$(Me_2N)_3PO$	1.0	57.95	58.18	5.95	6.28	7.51	7.77	40
35	$C_{12}H_{21}N_3O_8 \cdot 1.5H_2O$	270	23.6	H_2O	1.5	41.90	41.67	6.44	6.91	12.20	12.54	60

EXPERIMENTAL

Melting points are uncorrected. The solutions were evaporated under diminished pressure at a bath temperature not exceeding 45°. Optical rotations were measured in a 0.5-dm tube. The content of water of crystallization was calculated from the analytical values; some values were ascertained by titration with Fischer's reagent[20] of the water liberated in 0.5 h into a nitrogen atmosphere at 150°. For example, the water content of compounds **12** and **35** was estimated as 2.92% (theoretical value, 2.60%) and 6.99% (theoretical value, 7.64%), respectively.

1-N-(*Protected aminoacyl*)-*2-acetamido-4,6-O-benzylidene-2-deoxy-β-*D-*glucopyranosylamines* (**2**, **3**, **4**, *and* **5**). — To a stirred mixture of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranosylamine (1 mole)[14] and protected amino acid (1 mole) in pyridine was added a conc. solution of *N,N'*-dicyclohexylcarbodiimide (1.5 mole) in pyridine. The solution was stirred for several h at room temperature. *N,N'*-Dicyclohexylurea was filtered off, and the filtrate was evaporated. The residue was washed with hot ethanol and precipitated from *N,N*-dimethylformamide by addition of ether to give an amorphous solid.

1-N-(*Protected aminoacyl*)-*2-acetamido-2-deoxy-β-*D-*glucopyranosylamines* (**6**, **7**, **8**, and **9**), *2-deoxy-2-*[(*protected aminoacyl*)*amino*]-*β-*D-*glucopyranosylacetamides* (**17**, **21** *and* **25**), *and 2-*[(*benzyloxycarbonyl*)*amino*]-*2-deoxy-β-*D-*glucopyranosylacetamide* (**29**). — A suspension of **2**, **3**, **4**, **5**, **16**, **20**, **24**, or **28** (1 part) in 60% acetic acid (about 10 parts) was heated at 100° for 1 or 2 h until the compound was completely dissolved. In the case of glycine, L-alanine, and L-aspartic acid derivatives, the solutions were directly evaporated, and the residues were crystallized from 70% aq. ethanol. The L-lysine derivative was easily obtained as crystals by addition of water to the hydrolyzate, and was recrystallized from ethanol–ether.

Compound **29** was acetylated with acetic anhydride in pyridine to give 3,4,6-tri-*O*-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-*β*-D-glucopyranosylacetamide, m.p. 233° (dec.), $[\alpha]_D^{23}$ −18° (*c* 1.0, chloroform), yield 87%.

Anal. Calc. for $C_{22}H_{28}N_2O_{10}$: C, 54.99; H, 5.87; N, 5.83. Found: C, 54.64; H, 6.10; N, 6.10%.

1-N-(*Aminoacyl*)-*2-acetamido-2-deoxy-β-*D-*glucopyranosylamines* (**10**, **11**, **12** and **13**) and 2-[(*aminoacyl*)*amino*]-*2-deoxy-β-*D-*glucopyranosylacetamides* (**30**, **31**, **32**, *and* **35**). — A suspension of **6**, **7**, **8**, **9**, **17**, **21**, **25**, or **34** (1 part) in 50% aq. ethanol (about 10 parts), occasionally containing acetic acid (a few parts), was hydrogenolyzed in the presence of pailadium–charcoal. As the reaction proceeded, the compounds dissolved completely. The catalyst was filtered off, and the filtrate was evaporated. The residue was crystallized from water–ethanol, usually as a hydrate or a salt with hydrochloric acid or oxalic acid.

4,6-O-Benzylidene-2-{[N-(*benzyloxycarbonyl*)*aminoacyl*]*amino*}-*2-deoxy-*D-*glucoses* (**14** *and* **18**) *and 4,6-O-benzylidene-2-*[(*benzyloxycarbonyl*)*amino*]-*2-deoxy-*D-*glucose* (**26**).— The preparation of **26** is described to illustrate the method.

A mixture of 2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose (50 g)[18], benzal-

dehyde (300 ml), and anhydrous zinc chloride (70 g) was shaken for 24 h at room temperature. The mixture was extracted twice with petroleum ether (300 ml). The residue was stirred with ice and water (1000 ml), filtered, and washed with petroleum ether and then with water. The ppt. was triturated with a small amount of methanol, filtered and then recrystallized from pyridine–methanol (yield 45 g).

The corresponding *N*-(benzyloxycarbonyl)glycyl and *N,N'*-di(benzyloxycarbonyl)-L-lysyl derivatives were recrystallized from ethanol and methanol, respectively, sometimes resulting in a gelatinous mass.

Compound **26** was acetylated with acetic anhydride in pyridine to give 1,3-di-*O*-acetyl-4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucopyranose, m.p. 246–247° (dec.), $[\alpha]_D^{23}$ 0° (*c* 0.8, *N,N*-dimethylformamide), yield 96%.

Anal. Calc. for $C_{25}H_{27}NO_9$: C, 61.85; H, 5.60; N, 2.89. Found: C, 61.66; H, 5.42; N, 2.84%.

2-{[α-Benzyl N-(benzyloxycarbonyl)-β-L-aspartyl]amino}-4,6-O-benzylidene-2-deoxy-D-glucose (**22**). — To an ice-cold mixture of 2-amino-2-deoxy-D-glucose hydrochloride (10 g, 46.5 mmoles) in N sodium hydroxide (46 ml) and α-benzyl *N*-benzyloxycarbonyl-L-aspartate (16.5 g, 45.3 mmoles) in acetone (140 ml) was added dropwise *N,N'*-dicyclohexylcarbodiimide (28.6 g, 139 mmoles) in acetone (40 ml). After the reaction mixture had been stirred for several h at room temperature, the ppt. (*N,N'*-dicyclohexylurea and sodium chloride, 28.7 g) was filtered off, and a few drops of acetic acid were added to the filtrate. The solution was stirred again for a few h, and the urea was filtered off (8.1 g). The filtrate was evaporated and the residue was dissolved in hot ethanol (50 ml). The solution was kept overnight in a refrigerator and the crystalline product was filtered off. One half of it (3.5 g) was washed with acetone and ethyl acetate, and recrystallized from ethanol–acetone. After filtration of a small amount of ppt., evaporation of the filtrate to one half gave *O*-(α-benzyl *N*-benzyloxycarbonyl-L-aspartyl)-*N,N'*-dicyclohexylpseudourea as needles, m.p. 97–98°, yield 1.5 g.

Anal. Calc. for $C_{32}H_{41}N_3O_6$: C, 68.18; H, 7.33; N, 7.46. Found: C, 68.13; H, 7.34; N, 7.53%.

The ethanolic mother liquor was evaporated, and the resulting syrup (10 g), which could not be crystallized, was condensed with benzaldehyde to give the crystalline product (**22**), which was recrystallized from ethanol (yield 9 g).

When the condensation of 2-amino-2-deoxy-D-glucose with the aspartate derivative was performed in pyridine–water, however, the resulting benzylidene compound showed m.p. 179–181°, $[\alpha]_D^{23}$ −26° (*c* 1.1, *N,N*-dimethylformamide). Analytical values were in agreement with those calculated for a lactone of a benzylidene-benzyloxycarbonyl-aspartylaminodeoxy-hexose.

Anal. Calc. for $C_{25}H_{26}N_2O_9$: C, 60.23; H, 5.65; N, 5.62. Found: C, 59.66; H, 5.28; N, 5.75%.

4,6-O-Benzylidene-2-[(N-benzyloxycarbonylaminoacyl)amino]-2-deoxy-β-D-glucopyranosylamines (**15** *and* **19**) *and 4,6-O-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylamine* (**27**). — Compounds **14**, **18**, or **22** (1 part) were heated with saturated methanolic ammonia (15–20 parts) for 2 h to 2 days at 70–90°

in a sealed tube, until the starting material was completely dissolved. The presence of a small amount of ammonium chloride accelerated the reaction. The solution was gradually cooled to room temperature, and the crystals precipitated were filtered. In all cases, the product was analyzed and used for the next reaction without recrystallization. The glycosidic configuration of these compounds was determined to be β-D from their rotational values.

Compound **27** was acetylated with acetic anhydride in pyridine to give 3-*O*-acetyl-4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide, m.p. 298° (dec), $[\alpha]_D^{23}$ −8.2° (*c* 1.0, *N*,*N*-dimethylformamide), yield 96%.

Anal. Calc. for $C_{25}H_{28}N_2O_8$: C, 61.97; H, 5.83; N, 5.78. Found: C, 62.07; H, 5.88; N, 6.20%.

4,6-O-Benzylidene-2-{[N-(benzyloxycarbonyl)-β-L-asparaginyl]amino}-2-deoxy-β-D-glucopyranosylamine (**23**). — A suspension of **22** (4 g) in saturated methanolic ammonia (130 ml) was heated for 2 days in a sealed tube at 60–70°, and the gelatinous ppt. was completely dissolved. After cooling, the ppt. was filtered and dried. It darkened gradually from about 190° on, and decomposed at 209°. The same compound was obtained in a lower yield (20%) from the lactone of 4,6-*O*-benzylidene-2-{[*N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-D-glucose by the same procedure.

4,6-O-Benzylidene-2-deoxy-2-[(protected aminoacyl)amino]-β-D-gluco-pyranosylacetamides (**16**, **20**, *and* **24**) *and 4,6-O-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide* (**28**). — To a solution or suspension of **15**, **19**, **23**, or **27** in *N*,*N*-dimethylformamide–methanol was added under refrigeration 2 equimolar amounts of acetic anhydride, and the solution was kept for several h at room temperature. The acetylation was usually exothermic, the suspended mass dissolved at once, and then the corresponding *N*-acetyl derivatives were precipitated. Dilution of the reaction mixture with water increased the yield of the product, which was usually recrystallized from *N*,*N*-dimethylformamide–ether.

2-Amino-2-deoxy-β-D-glucopyranosylacetamide hydrochloride (**33**). — A suspension of **29** (5 g) in 50% aq. methanol (80 ml) was hydrogenated in the presence of 10% palladium–charcoal (1 g) and an equimolar amount of hydrochloric acid until the starting material was completely dissolved. After removal of the catalyst, the filtrate was evaporated to give quantitatively the hydrochloride. The pure crystals, which were recrystallized from ethanol–water, decomposed at 176° after gradual browning from 135° on.

2-{[α-Benzyl N-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-β-D-glucopyranosylacetamide (**34**). — A solution of **33** (3.6 g, 14 mmoles) in water (30 ml) was neutralized with triethylamine (1.52 g, 15 mmoles) in acetone (30 ml) and α-benzyl *N*-benzyloxycarbonyl-L-aspartate (7.3 g, 13 mmoles) was added. To the resulting solution was added dropwise, with stirring, *N*,*N'*-dicyclohexylcarbodiimide (5.35 g, 26 mmoles) in acetone (40 ml), and the mixture was kept one day at room temperature. The resulting *N*,*N'*-dicyclohexylurea was filtered off, and the filtrate was evaporated. The residual syrup was triturated with ether (150 ml), and then with water. The residue (4.8 g) was extracted with ethyl acetate (150 ml), and the extract was evaporated to

give an amorphous mass (3.5 g). Crystallization from ethanol (450 ml) afforded the pure product (2.3 g), m.p. 222–223° (dec). The mother liquor gave an additional crop (1.0 g), m.p. 209–211° (dec.). From the ether extract and water washings, α-benzyl *N*-benzyloxycarbonyl-L-aspartate (4.3 g) and the starting material (0.7 g) were recovered.

3,4,6-Tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranosylacetamide hydrochloride. — A solution of 3,4,6-tri-*O*-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (2.4 g) in chloroform (40 ml) and methanol (10 ml) was treated with an equimolar amount of conc. hydrochloric acid (0.8 ml); 5% palladium–charcoal (1 g) was added, and the mixture was hydrogenated. After 10 min, the catalyst was filtered off, and concentration of the filtrate gave fine needles (1.8 g) which gave a positive Beilstein test, m.p. 242–243° (dec.), $[\alpha]_D^{23}$ 40° (*c* 0.5, methanol).

Anal. Calc. for $C_{14}H_{23}N_2O_8Cl$: C, 43.92; H, 6.05; N, 7.51. Found: C, 44.07; H, 6.32; N, 7.29%.

Comparison of the synthetic products **12**, **13**, *and* **35** *with the product isolated from hen-egg albumin.* — Compounds **12**, **13**, and **35** were compared by chromatographic and electrophoretic procedures with the 2-acetamido-2-deoxy-D-glucose–L-aspartic acid compound isolated from hen-egg albumin (Table V). The values observed for **13** were slightly different from those of the other compounds. After treatment with a 1% solution of ninhydrin in acetone–pyridine (49:1)[21] only **13** showed a purple spot, whereas the others gave brown spots.

TABLE V

RATE OF MIGRATION OF COMPOUNDS **12**, **13**, AND **35**, AND OF THE NATURAL PRODUCT ISOLATED FROM HEN-EGG ALBUMIN ON PAPER CHROMATOGRAMS AND ELECTROPHORETOGRAM

Compound	*Paper chromatography*[a]		*Paper electrophoresis*[b]	
	Conditions[c]			
	A	*B*	*C*	*D*
12	0.47	0.40	3.9	5.2
13	0.52	0.41	4.1	5.6
35	0.48	0.39	3.9	5.0
Natural product	0.47	0.40	3.9	5.1

[a] R_F value. [b] Distance of migration in cm. [c] A, 2-methylpropionic acid–0.5N aq. ammonia (5:3). B, phenol–water (10:1). C, 5N acetic acid, 20 volts cm^{-1}, 3.5 h. D, borate buffer (pH 8.0), 20 volts cm^{-1}, 2 h.

SUMMARY

2-Acetamido-1-*N*-[(aminoacyl)amino]-2-deoxy-β-D-glucopyranosylamines, whose aminoacyl groups L-alanyl, L-lysyl, β-L-aspartyl, and α-L-aspartyl, were synthesized by condensation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamine with suitably protected amino acids in presence of *N*,*N*′-dicyclohexylcarbodiimide, followed by removal of the protecting groups.

This method was applied to the synthesis of 2-[(aminoacyl)amino]-2-deoxy-β-D-

glucopyranosylacetamides using 2-[(protected aminoacyl)amino]-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamines as intermediates these were obtained, in the case of neutral and basic amino acid derivatives, by amination of the corresponding starting materials with methanolic ammonia.

For the acidic amino acid derivative, however, the protected carboxyl group was converted into the amido group by amination. 2-Amino-2-deoxy-D-glucopyranosylacetamide hydrochloride was synthesized from 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose by similar reactions, and then it was condensed with a protected amino acid. 2-[(β-L-Aspartyl)amino]-2-deoxy-β-D-glucopyranosylacetamide, thus obtained, was compared with 2-acetamido-1-[*N*-(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylamine, obtained from ovalbumin, and no significant difference could be found.

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Notes

Reactions at position 1 of carbohydrates
Part VIII. Further studies of the polarographic reduction of carbohydrates

An investigation of the polarographic reduction of carbohydrates[1] has been extended by examination of the compounds listed in Table I. The methods adopted were those described[1] in Part I of this series. The results are summarised in Table I, the symbols having the same significance as in the earlier publication[1].

TABLE I

GENERAL PROPERTIES OF THE POLAROGRAPHIC WAVES OF SUGARS AT 25.0° AND pH 9.73 IN 0.002M TETRAMETHYLAMMONIUM PHOSPHATE BUFFER

Compound	*Mercury head dependence*	$E_{\frac{1}{4}}$ (*S.C.E.*) (*v*)	A (*microamp. l. mole*$^{-1}$)	$10^5 k_s \alpha\beta$ (*cm. sec*$^{-1}$)	$k_f \alpha\beta$ (*sec*$^{-1}$)	$10^8 K$	$10^3 B$ (*sec*$^{-\frac{1}{2}}$)
5-Deoxy-L-ribose	$\propto H^\circ$	−2.18	1.26	32.7	286	5600	193
4-Deoxy-L-*erythro*-pentose ("4-deoxy-L-ribose")	$\propto H^\circ$	−2.05	0.14	3.73	56	370	22.0
D-Ribose (values taken from ref. 1, at pH 8.0)	$\propto H^c$	−2.03	1.2	36	600	3200	220
6-*O*-Methyl-D-glucose	$\propto H^\circ$	−2.27	0.05	1.3	21	120	7.7
D-Fucose	$\propto H^\circ$	−1.97	0.031	0.56	22	21	3.3
L-Rhamnose	$\propto H^\circ$	−1.98	0.019	0.29	11	1.1	1.7
4-Deoxy-D-*xylo*-hexose ("4-deoxy-D-glucose")	$\propto H^\circ$	−1.80	0.010	0.22	8.8	7.8	1.3
Methyl 2-*C*-formyl-β-L-arabinopyranoside	$\propto H^c$	−2.08	3.10	70.5	747	9940	416

It was shown by Overend, Peacocke, and Smith[1] that the polarographic reduction of the five-membered, cyclic sugar, 2,3,5-tri-*O*-methyl-D-ribofuranose was diffusion-controlled, and this was attributed to a very high rate of conversion of ring form into acyclic aldehydo-form. Methylation of the hydroxyl groups led to steric interactions that resulted in destabilisation of the furanoid ring. The observation now reported that 5-deoxy-L-ribose (which may be considered as a non-methylated analogue of 2,3,5-tri-*O*-methyl-D-ribofuranose) gives kinetically controlled, limiting currents supports this view, since methylation otherwise results in diminished B-values.

It is interesting to note that the polarographic reduction characteristics of D-ribose correspond more closely to those of 5-deoxy-L-ribose than to those of

4-deoxy-L-*erythro*-pentose ("4-deoxy-L-ribose"). Since replacement of a hydroxyl group at C-4 or C-5 by hydrogen would be expected to have little effect on the polarographic reduction (*cf.* "4-deoxy-D-glucose" and D-glucose), other than that caused by a change in ring size, this result may have interesting implications about the structure of ribose in solution. There have been several n.m.r. spectral investigations of aqueous solutions of D-ribose and other pentoses[2-4], but the exact nature of all the species of D-ribose in water is still not completely resolved. It does appear, however, that in equilibrated solutions a significant proportion of the furanoid forms is present.

The half-wave potential of −2.08 volts found for methyl 2-*C*-formyl-β-L-arabinopyranoside is similar to that observed for other aldoses that exist in cyclic hemi-acetal forms, but quite different from that noted for 2,4:3,5-di-*O*-ethylidene-*aldehydo*-L-xylose for which a value of −1.42 volts was found. This result lends support for the structure proposed by Burton *et al.*[5] for the branched-chain sugar derivative. The high B-value indicates a high rate of ring-opening of the hemi-acetal ring at the mercury surface, which suggests that the five-membered ring in the bicyclic system is more highly strained than in monocyclic 5-deoxyribose.

There was nothing unusual about the results obtained for 6-*O*-methyl-D-glucose, D-fucose, L-rhamnose, and 4-deoxy-D-*xylo*-hexose ("4-deoxy-D-glucose").

EXPERIMENTAL

Materials. — The following sugars were recrystallised twice from 80% ethanol, washed with 96% ethanol, and dried first in air and then *in vacuo* over phosphoric oxide: D-fucose, m.p. 120°, $[\alpha]_D^{20}$ +76° (*c* 1, water); α-L-rhamnose monohydrate, m.p. 93–94°, $[\alpha]_D^{22}$ +8.2° (equil.) (*c* 3.5, water). 4-Deoxy-D-*xylo*-hexose, m.p. 131–132°, $[\alpha]_D^{20}$ +60.3° (equil.) (*c* 1, water) was recrystallised twice from ethyl acetate, and dried in air and then *in vacuo* over phosphoric oxide. Methyl 2-*C*-formyl-β-L-arabinopyranoside, m.p. 153–153.5°, $[\alpha]_D^{20}$ +124° (*c* 0.58, methanol) was recrystallised from a mixture of ethyl acetate (95 vol.) and ethanol (5 vol.), and dried in air and then *in vacuo* over phosphoric oxide.

6-*O*-Methyl-D-glucose, m.p. 144–145°, $[\alpha]_D^{20}$ +91.4 (3 min) → + 55° (equil.) (*c* 1.37, water) was kindly provided by Dr. O. Mérész. 4-Deoxy-L-*erythro*-pentose {$[\alpha]_D$ +22.9° (equil.) (*c* 0.72, water)} and 5-deoxy-L-ribose {$[\alpha]_D$ +12.9° (equil.) (*c* 1.0, water)} were both syrups, the former being provided by Dr. N. R. Williams, and the latter by Dr. J. Sibley.

Buffer solution. — A 0.002 M solution of tetramethylammonium phosphate, prepared as described in Part I, was used. It had a pH of 9.73, and, as addition of acid to produce buffers of lower pH always led to the introduction of significant quantities of alkali-metal ions, all experiments were performed at a pH of 9.73.

Method. — The method described in Part I of this series was used.

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Synthesis of β-D-xylopyranosides of substituted and branched-chain aliphatic alcohols

In Table I, we describe a series of tri-*O*-acetyl-β-D-xylopyranosides of substituted and branched-chain aliphatic alcohols, obtained by the reaction of tri-*O*-acetyl-α-D-xylopyranosyl bromide[1] with the appropriate alcohol in the presence of silver oxide[2]. Deacetylation with sodium methoxide[3] yielded the corresponding β-D-xylopyranosides (Table II).

EXPERIMENTAL

Absolute benzene (200 ml), silver oxide (0.1 mole), tri-*O*-acetyl-α-D-xylopyranosyl bromide (0.1 mole), Sikkon (calcium sulphate, Fluka; 30 g), and the appropriate alcohol (0.1 to 0.5 mole) were stirred until the supernatant liquid was free of bromide ions. The salts were filtered off, and the clear solution was concentrated *in vacuo* to yield the tri-*O*-acetyl-β-D-xylopyranosides (Table I).

The purity of the products was tested by t.l.c on Silica Gel G (Merck) in acetic acid–water–ethyl acetate (1:1:3) for the D-xylosides, and ethyl acetate–benzene (3:7) for the acetates; detection reagent, 5% sulphuric acid in ethanol (10 min at 120°). The optical rotations were measured in chloroform (acetates, *c* 2) or methanol (D-xylosides, *c* 2).

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Carbohyd. Res., 5 (1967) 93–95

Synthesis of β-D-xylopyranosides of substituted and branched-chain aliphatic alcohols

In Table I, we describe a series of tri-*O*-acetyl-β-D-xylopyranosides of substituted and branched-chain aliphatic alcohols, obtained by the reaction of tri-*O*-acetyl-α-D-xylopyranosyl bromide[1] with the appropriate alcohol in the presence of silver oxide[2]. Deacetylation with sodium methoxide[3] yielded the corresponding β-D-xylopyranosides (Table II).

EXPERIMENTAL

Absolute benzene (200 ml), silver oxide (0.1 mole), tri-*O*-acetyl-α-D-xylopyranosyl bromide (0.1 mole), Sikkon (calcium sulphate, Fluka; 30 g), and the appropriate alcohol (0.1 to 0.5 mole) were stirred until the supernatant liquid was free of bromide ions. The salts were filtered off, and the clear solution was concentrated *in vacuo* to yield the tri-*O*-acetyl-β-D-xylopyranosides (Table I).

The purity of the products was tested by t.l.c on Silica Gel G (Merck) in acetic acid–water–ethyl acetate (1:1:3) for the D-xylosides, and ethyl acetate–benzene (3:7) for the acetates; detection reagent, 5% sulphuric acid in ethanol (10 min at 120°). The optical rotations were measured in chloroform (acetates, *c* 2) or methanol (D-xylosides, *c* 2).

TABLE I

ACETYLATED β-D-XYLOPYRANOSIDES

Aglycon group	*M.p., degrees*	*Solvent*[a]	$[\alpha]_D^{22}$, *degrees*	*Yield, %*	*Found, %* C	H	*Formula*	*Calc., %* C	H
2-Chloroethanol	135	b	−56.3	57	46.3	5.6	$C_{13}H_{19}ClO_8$	46.1	5.6
Ethylene glycol	76	a	−54.2	55	48.7	6.4	$C_{13}H_{20}O_9$	48.8	6.3
2-Methoxyethanol	92	d	−59.8	51	50.2	6.7	$C_{14}H_{22}O_9$	50.3	6.6
3-Bromo-1-propanol	88	b	−35.0	23	42.8	5.5	$C_{14}H_{21}BrO_8$	42.3	5.3
4-Chloro-1-butanol	82	c	−58.2	36	49.2	6.6	$C_{15}H_{23}ClO_8$	49.1	6.3
2-Methyl-1-propanol	108	b	−58.6	53	54.2	7.3	$C_{15}H_{24}O_8$	54.2	7.2
2,2-Dimethyl-1-propanol	128	c	−56.0	30	55.7	7.7	$C_{16}H_{26}O_8$	55.5	7.5
3-Methyl-1-butanol	65	a	−52.7	40	55.5	7.5			
3-Pentanol	95	e	−66.0	65	55.5	7.5			
2-Methyl-2-butanol	94	c	−44.2	41	55.4	7.7			
2,2-Dimethyl-1-butanol	101	e	−54.4	20	56.7	8.0	$C_{17}H_{28}O_8$	56.6	7.8

[a]Crystallisations were effected from the following solvents: a, ether–light petroleum; b, ethanol; c, ethanol–water; d, methanol; e, methanol–water.

TABLE II

β-D-XYLOPYRANOSIDES

Aglycon group	*M.p., degrees*	*Solvent*[a]	$[\alpha]_D^{22}$, *degrees*	*Yield, %*	*Found, %* C	H	*Formula*	*Calc., %* C	H
2-Chloroethanol	87	b	−44.0	80	39.8	6.5	$C_7H_{13}ClO_5$	39.5	6.1
Ethylene glycol	64	a	−42.5	85	39.8	7.0	$C_7H_{14}O_6$	43.3	7.2
2-Methoxyethanol	39	b	−50.4	52	46.1	7.7	$C_8H_{16}O_6$	46.2	7.7
3-Bromo-1-propanol	82	c	−35.2	60	34.8	5.7	$C_8H_{15}BrO_5$	35.4	5.5
4-Chloro-1-butanol	75	c	−50.8	50	44.7	7.0	$C_9H_{17}ClO_5$	44.9	7.1
2-Methyl-1-propanol	103	d	−58.4	87	52.3	8.8	$C_9H_{18}O_5$	52.5	8.7
2,2-Dimethyl-1-propanol	131	c	−58.0	70	54.5	9.1	$C_{10}H_{20}O_5$	54.5	9.1
3-Methyl-1-butanol	106	d	−53.5	85	54.4	9.2			
3-Pentanol	108	a	−61.0	80	54.6	9.1			
2-Methyl-2-butanol	87	e	−35.7	80	54.3	9.0			
2,2-Dimethyl-1-butanol	100	f	−50.9	80	56.5	9.4	$C_{11}H_{22}O_5$	56.4	9.4

[a]Crystallisations were effected from the following solvents: a, acetone; b, ethyl acetate; c, ethyl acetate–light petroleum; d, butanone; e, hexane; f, ether–light petroleum.

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Synthesis of 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-DL-threonine methyl ester and study of its stability toward basic reagents*

A previous paper from this laboratory[1] describes the synthesis and properties of *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-serine methyl ester. The stability of this compound in the presence of a base was found to be different from that of other *N*-acylserine glycosides of 2-amino-2-deoxy-D-glucose synthesized earlier[2,3]. Recent work of Tanaka *et al.*[4], Anderson *et al.*[5–7], and others[8–11] prompted a similar study of the threonine glycoside (**1**). Its synthesis was accomplished by Helferich's method[12,13], modified for base-labile glycosides[1]. This procedure is described in the experimental section, because the *N*-(2,4-dinitrophenyl)-threonine methyl ester required more rigorous glycosidation conditions than did the serine analog[1]. A crystalline glycoside was isolated by thin-layer chromatography (t.l.c.). Structure **1** of the glycoside was confirmed by elementary analysis, optical rotation, i.r., and t.l.c. analysis of the acid hydrolyzate. Glycosidations under similar conditions with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[14] gave predominantly β-D-linked glycosides[2,3,15]. In agreement with these observations, application of Hudson's rules of isorotation[16] to the glycoside **1** ($[\alpha]_D^{25}$ +4°, $[M]_D$ +2500°) suggested a β-D anomeric configuration, since the DL-aglycon is optically inactive; in comparison, 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α- and β-D-glucopyranose show $[M]_D$ +35,991° and +581° respectively[14].

The glycosidic linkage of **1** was split rapidly, even in the presence of small concentrations of base to give the unsaturated product, methyl α-(2,4-dinitrophenyl)-aminocrotonate[16]. This elimination reaction was followed easily by t.l.c., and the reaction products were removed from the plate and determined spectrophotometrically (λ_{max}^{MeOH} 343 mμ). About 85% of the glycosidic linkage of *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-L-serine methyl ester[1] (**2**)

*This study was supported by the Agricultural Research Service, U. S. Department of Agriculture, Grant 12-14-100-7666(71), administered by the Northern Utilization Research and Development Division, Peoria, Illinois 61604.

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Synthesis of 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-DL-threonine methyl ester and study of its stability toward basic reagents*

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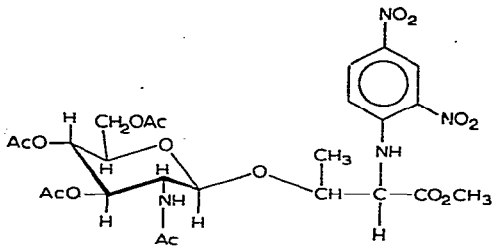
was split in the presence of 2 equivalents of sodium carbonate within 10 seconds at room temperature. In the presence of 1 equivalent of the same base, 65% of the glycos-



(1)

idic linkage was split in 10 seconds*. The glycoside **1** is split completely (100%) under identical conditions of temperature, time, and concentration. Dilution did not appreciably retard this reaction of **1**. Details of these experiments are found under Experimental and in Table I. In the presence of a mild base, methyl 2-(2,4-dinitrophenyl-

TABLE I

RELATIVE ELIMINATION RATES OF TWO β-HYDROXY AMINO ACID GLYCOSIDES[a]

Compound	*Glycoside μmoles*	*Na_2CO_3 μequiv.*	*Reaction vol. (ml)*	*Glycoside remaining μmoles*	*% degraded*
Glycoside of *N*-2,4-(dinitro-	2.00	2.00	0.8	0.36	82
phenyl)serine methyl ester (2)	2.00	2.00	0.8	0.76	68
Glycoside of *N*-2,4-(dinitro-					
phenyl)threonine methyl ester	0.21	0.21	2.1	0.00	100
(1)[b]	0.21	0.105	2.1	0.00	100

[a]Reaction time, 10 seconds. Neutralization by addition of an equivalent quantity of hydrochloric acid. [b]Concentrations of **1** identical to those used for **2** result in complete elimination of the glycoside. Diluted samples are reported, to illustrate an attempt to retard the reaction by concentration change.

aminocrotonate, like methyl 2-(2,4-dinitrophenyl)aminoacrylate[17], undergoes an intramolecular nitro oxidation at position *beta* to the carbonyl group, retroaldol scission of formaldehyde and acetaldehyde, respectively, and ring closure to 2-(methoxycarbonyl)-5-nitrobenzimidazole *N*-oxide[17,18]. This reaction is somewhat slower than β-elimination of the glycosyl residue, however. The kinetics of the elimination of the sugar residue were followed, therefore, by the rate of disappearance of the glycoside instead of by the rate of formation of the unsaturated ester, which is rapidly oxidized. Acid hydrolysis (N H_2SO_4, water bath, 100°) of **1** was completed in 4 h. Acetic anhydride–pyridine acetolysis of **1**, as well as the serine glycoside previously reported[1], resulted also in rapid cleavage of the glycosidic linkage. Whether elimination or

* This experiment was performed by Sr. St. Therese Lang.

acetolysis predominates during acetic anhydride–pyridine treatment is being studied at the present time; chromatographic evidence indicates that both processes occur during acetolysis.

EXPERIMENTAL

3-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-N-(2,4-dinitrophenyl)-DL-threonine methyl ester (**1**). — *N*-2,4-(Dinitrophenyl)-DL-threonine methyl ester was prepared by the procedure reported for the serine analog[1,17]. An optically active product first crystallized, $[\alpha]_D^{25}$ −1.32° (*c* 1.15, acetone). After 3 recrystallizations from benzene, the optically inactive DL-threonine derivative was obtained, m.p. 128–129°, identical with the compound previously described[17].

The procedure of Helferich[12,13], as modified by Vercellotti and Luetzow[1], was followed. Precautions against HCN fumes were taken by performing all operations in a well ventilated hood. *N*-(2,4-Dinitrophenyl)-DL-threonine methyl ester (1.49 g, 5 mmoles) and mercuric cyanide (3.85 g, 7.5 mmoles) were dissolved in anhydrous benzene (50 ml). A Newman adapter having a stopcock was attached below the condenser, which was fitted with a calcium chloride drying-tube. The mixture was heated to reflux (oil bath), and, after 20 min, benzene (20 ml) was distilled through the adapter sidearm. 3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-D-glucopyranosyl chloride[14] (4.55 g, 12.5 mmoles) in anhydrous benzene (25 ml) was added in three portions (8.3 ml) at 0.5 h intervals. The reaction mixture was then concentrated to a final vol. of 30 ml, and reflux was continued for 15 h. Periodic examination of the reaction mixture on silica gel G in ethyl ether–ethyl acetate (1:1) (solvent A) showed that the maximum formation of **1** occurred at 15 h. The reaction mixture was diluted with chloroform (50 ml) and filtered through a sintered glass Büchner funnel (medium porosity) covered with a pad of Celite. The filtrate was washed successively with water (3 times, 25 ml portions), a saturated sodium chloride solution (once, 25 ml), and again water (2 times, 25 ml portions). After drying with anhydrous sodium sulfate, the mixture was concentrated to a syrup, which was purified by t.l.c. in ether–ethyl acetate (6:1) on 33 silica gel G plates (200 × 200 mm, 0.65 mm thick activated for 3 h at 100°) with an elution time of 24 h[1]. Compound **1** was eluted from silica gel with acetone. After concentration of the solution, the residue was dissolved in isopropyl alcohol, and the solution was filtered and evaporated. The resulting syrup was dissolved in butyl alcohol (8 ml), and petroleum ether (b.p. 30–60°, about 12 ml) was added to turbidity. The solution was kept in the refrigerator overnight. The crystals (170 mg, 5.4%) were centrifuged, carefully washed 3 times with petroleum ether (10-ml portions), and dried at room temperature under vacuum; m.p. 107–109° (soft. 104°) after recrystallization from the same solvent system; $[\alpha]_D^{24}$ +4±2° (*c* 0.26, acetone); R_F 0.18 (solvent A, yellow zone, chromatographically homogeneous; no additional zones on charring with sulfuric acid spray); λ_{max}^{KBr} 2.80 (NH), 3.20, 5.60 (OAc), 5.78 (CO_2Me), 6.01 (amide I), 6.19 (Ar), 6.36 (NO_2), 6.47 (amide II), 6.85 (NO_2), 7.20, 7.40, 8.00, 8.48, 9.50, 10.75, 11.85, 13.35, 13.72 μm.

*Anal.** Calc. for $C_{25}H_{32}O_{15}N_4$: C, 47.76; H, 5.13; N, 8.91. Found: C, 48.03; H, 5.31; N, 8.75.

Acid hydrolysis of compound **1** in N H_2SO_4 for 4 h at 100° showed complete disappearance of the glycoside on silica gel t.l.c. (solvent A). After neutralization ($BaCO_3$), examination of the hydrolyzate on cellulose plates[19] (butyl alcohol–acetic acid–water, 3:1:1) revealed only 2 zones having mobilities corresponding to 2-amino-2-deoxy-D-glucose (ninhydrin and $AgNO_3$–NaOH spray reagents) and *N*-2,4-(dinitrophenyl)-DL-threonine.

Compound **1** (1 mg., 1.6 μmole, in 0.5 ml acetone) was treated with N sodium carbonate (2μl; about 1 equiv.). Immediate examination of the solution by t.l.c. with silica gel G (solvent A) without neutralization (about 3 sec of reaction before spotting) revealed three yellow zones: a small amount of compound **1** remaining, a zone having a mobility (R_F 0.87) identical to that of authentic methyl 2-(2,4-dinitrophenyl)-aminocrotonate[17] as well as a strong u.v.-absorbing zone containing 2-(methoxycarbonyl)-5-nitrobenzimidazole *N*-oxide and located at the baseline, as reported earlier[18]. No *N*-(2,4-dinitrophenyl)-DL-threonine methyl ester (R_F 0.72, solvent A) was observed as a base-catalyzed hydrolytic product from **1**.

Alkaline cleavage of **1** *and* O-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-N-(*2,4-dinitrophenyl*)*serine methyl ester* (**2**).—Typical β-elimination experiments of these glycosides are listed in Table I. Solutions of the glycosides in methanol were treated with sodium carbonate in water according to the conditions described in Table I. The solutions were neutralized with equivalent amounts of hydrochloric acid, and the components of each mixture were separated on silica gel G plates (50 × 200 mm, solvent A). The zone containing residual unreacted glycoside was eluted with absolute methanol, and the volume of the solution was adjusted with methanol for reading at 343 mμ on a Bausch and Lomb "Spectronic 600" spectrophotometer. Glycoside concentrations were calculated from standard absorbance curves. Duplicates of samples differed by only ±2%. Controls of glycoside in aqueous methanol did not react.

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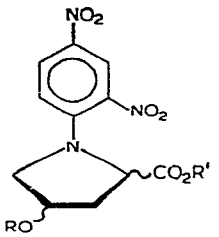
A 4-Hydroxy-L-proline glycoside of 2-amino-2-deoxy-D-glucose*

Previous papers from this laboratory described the synthesis and stability of *N*-2,4-dinitrophenylserine[1] and *N*-2,4-dinitrophenylthreonine[2] glycosides of 2-amino-2-deoxy-D-glucose. The present paper extends this series to 4-hydroxy-L-proline[3–6]. Glycosides of hydroxyproline are postulated as connective linkages in fibril proteins[7,8] and in the junction of glycoproteins to polysaccharide ground-substance[9–12].

A Koenigs–Knorr condensation[2,13,14] was used to couple 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[15] to the syrupy *N*-2,4-dinitrophenyl-4-hydroxy-L-proline methyl ester (**2**). A crystalline glycoside (**4**) was isolated by preparative thin-layer chromatography (t.l.c.). The structure of the glycoside was confirmed by elementary analysis, optical rotation, i.r. and n.m.r. spectra, and by acid hydrolysis and identification of the products by t.l.c. The H-1 signal of the hexosamine moiety (τ 5.20) showed a splitting ($J_{1,2}$ 9 Hz) indicative of the β-D anomeric configuration[16–18]. An orthoacetate structure for the glycoside[19,20] was considered improbable because no signal at *ca.* τ 8.3–8.5 (orthoacetate methyl group[21]) was observed. The acid stability of the glycoside, as well as steric considerations, also ruled out an orthoacetate structure.

Pure *trans*-4-hydroxy-L-proline was the starting material for synthesis of the *N*-dinitrophenyl derivative (**1**), the *N*-dinitrophenyl methyl ester (**2**), and the 4-*O*-*p*-tolylsulfonyl-*N*-dinitrophenyl methyl ester (**3**). Substances **1–3** gave n.m.r. spectra very similar to the hydroxyproline signals in the spectrum of the glycoside **4**. Nevertheless,



1 R=H, R'=H
2 R=H, R'=Me
3 R=Ts, R'=Me
4 R=2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl R'=Me

the geometric configuration of the pyrrolidine ring in **4** cannot be stated definitively, because the effects of esterification or glycosidation on the ring configuration are not known. The structure of the glycoside is simply assigned as 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-2,4-dinitrophenyl-4-hydroxy-L-proline methyl ester (**4**).

Glycosides of *N*-2,4-dinitrophenylserine[1] and *N*-2,4-dinitrophenyl-threonine[2],

*This study was supported by the Agricultural Research Service, U. S. Department of Agriculture, Grant 12-14-100-7666(71), administered by the Northern Utilization Research and Development Division, Peoria, Illinois 61604.

and also substance 3, are unstable to base, but the glycoside 4 is quite stable to base. Glycosides of 4-hydroxy-L-proline in glycoprotein substances should likewise possess alkaline stability.

EXPERIMENTAL

Ascending t.l.c. was performed on silica gel G (E. Merck Co., Darmstadt, Germany; activated for 1 h at 110°), with 3:1:1 *n*-butyl alcohol–acetic acid–water (solvent A,) 1:1 ethyl acetate–ether (solvent B), 1:6 ethyl acetate–ether (solvent C), and 1:3 ethyl acetate–petroleum ether (b.p. 30–60°) (solvent D). M.ps. were determined in capillaries with a National Instruments Co. "Melt Meter" and are uncorrected. I.r. spectra were recorded with a Perkin–Elmer Model 137 "Infracord" spectrometer with potassium bromide pellets. N.m.r. spectra were recorded at 60 MHz with a Varian A-60 spectrometer with saturated solutions in chloroform-*d* or acetone-d_6, with tetramethylsilane as internal reference. Deuteration was effected by shaking the sample solutions with deuterium oxide. All n.m.r. spectra gave satisfactory integral ratios for protons. Microanalyses were performed by Weiler and Strauss Microanalytical Laboratories, Oxford, England.

N-*2,4-Dinitrophenyl*-trans-*4-hydroxy*-L-*proline* (**1**). — *trans*-4-Hydroxy-L-proline, $[\alpha]_D^{25}$ −76° (*c* 1, water), was 2,4-dinitrophenylated by the method of Sober and Rao[22]. The product, recrystallized from anhydrous ethyl ether–petroleum ether, had m.p. 174–176° (lit.[22] m.p. 175°); R_F 0.85 (solvent A); $[\alpha]_D^{25}$ −1142±10° (*c* 0.14, acetone). I.r. spectral data were in accord with those reported for **1** by Friedberg[23]. N.m.r. data: τ 1.37 (1-proton doublet, $J_{3',5'}$ and $J_{3',6'}$ 3 Hz, H-3′), τ 1.76 (1-proton quartet, $J_{5',3'}$ and $J_{5',6'}$ 9 Hz, H-5′), τ 2.94 (1-proton doublet, $J_{6',5'}$ and $J_{6',3'}$ 8 Hz, H-6′), τ 5.15 (1-proton triplet, $J_{2,3}$ 8 Hz, H-2), τ 5.35 (1-proton broad singlet, H-4), τ 6.16 (2-proton multiplet, H-5), τ 7.35 (2-proton multiplet, H-3), τ 3.11 (1-proton broad singlet, disappeared on deuteration, CO_2H), τ 7.40 (1-proton broad singlet, disappeared on deuteration, OH).

N-*2,4-Dinitrophenyl*-trans-*4-hydroxy*-L-*proline methyl ester* (**2**). — Substance **1** (5.4 g, 0.0181 mole) was dissolved in abs. methanol (100 ml). Following the esterification procedure of Fischer (*cf.* ref. 24) the solution was saturated with anhydrous hydrogen chloride and refluxed for 20 min (reaction completion discerned by t.l.c., product R_F 0.66, solvent B). The syrup was codistilled with isopropyl alcohol several times to remove hydrogen chloride. The syrupy product was dried *in vacuo* to a yellow, hromatographically homogeneous powder; $[\alpha]_D^{25}$ −884±10° (*c* 0.32, acetone); λ_{max}^{KBr} 2.77 (OH), 5.70 (CO_2Me), 6.21, 6.32 (aryl), 6.56, 6.65 (NO_2), 6.96, 7.53, 8.27, 8.54, 8.70, 8.86, 9.30, 10.04, 10.50, 10.92, 11.36, 12.01, 12.32, 13.16, 13.44, 13.71, 14.70 μm; n.m.r. data: τ 6.24 (3-proton singlet, OMe). Other chemical shifts were nearly identical with those of **1**.

Anal. Calc. for $C_{12}H_{13}N_3O_7$: C, 46.31; H, 4.21; N, 13.50. Found: C, 46.41; H, 4.44; N, 13.65.

N-*2,4-Dinitrophenyl-4-hydroxy-4*-O-p-*tolylsulfonyl*-L-*proline methyl ester* (**3**). —

Substance **2** (0.5 g, 1.6 mmole) was dissolved in pyridine (25 ml) containing *p*-toluenesulfonyl chloride (2 g, 15.7 mmoles) and the solution was kept in the dark at room temperature. Complete conversion into **3**, R_F 0.95 (solvent B), was observed after 7 days. After workup[25], the resulting syrup was dissolved in benzene (10 ml), petroleum ether (b.p. 30–60°) added to turbidity, and the solution was refrigerated for 2 days to give dense, yellow crystals, yield, 0.23 g (31%). Although t.l.c. showed more product to be present in solution, no further crop of crystals was isolable. The product, recrystallized from benzene–petroleum ether (b.p. 30–60°), had m.p. 112–113°; $[\alpha]_D^{25}$ −640 ±10° (*c* 0.34 acetone); λ_{max}^{KBr} 5.73 (CO_2Me), 6.23, 6.34 (aryl), 6.55, 6.67 (NO_2), 6.92, 7.35, 7.54, 8.20, 8.50, 8.88, 9.14, 9.49, 10.05, 10.26, 10.47, 11.12, 11.84, 12.35, 13.43, 13.75, 14.40 μm; n.m.r. data: τ 4.80 (1-proton broad singlet, H-4), τ 2.51 (4-proton quartet, aromatic A_2B_2 system, *p*-tolyl group), τ 7.51 (3-proton singlet, *p*-tolyl Me). With the exception of the OH and H-4 signals, the other n.m.r. signals were identical with those of **2**.

Anal. Calc. for $C_{19}H_{19}N_3O_9S$: C, 49.03; H, 4.11; N, 9.03; S, 6.89. Found: C, 49.19; H, 4.27; N, 9.20; S, 7.18.

In the presence of base, or on prolonged exposure to light, this compound underwent decomposition (t.l.c., solvent D).

4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-N-2,4-dinitrophenyl-4-hydroxy-L-proline methyl ester (**4**). — A Koenigs–Knorr coupling reaction was carried out according to Helferich's[13,14] procedure, as modified by Hardy[26], and utilized in a previously reported synthesis of a threonine glycoside[2]. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride was prepared according to Horton[15]. Reaction progress was monitored by t.l.c. (solvent C, sulfuric acid detection). Maximum formation of product (at 2.5 h) coincided with the appearance of a decomposition product, R_F 0.00. After workup the mixture was resolved by preparative t.l.c.[2]. Six isolable zones were separated in solvent C; R_F 0.78 (yellow), 0.56 (yellow), 0.45 (yellow) mixed with 0.43 (colorless, H_2SO_4 detection), 0.31 (yellow), and 0.00 (brown). Upon concentration of the eluates (ethyl acetate) from these zones, each was preparatively rechromatographed. The purified eluates were evaporated, dissolved in benzene, and treated with decolorizing carbon. The filtrates were concentrated to syrups which were again dissolved in benzene (5 ml). The benzene solutions were diluted with petroleum ether (b.p. 30–60°) until turbid and refrigerated overnight. The zone having R_F 0.31 crystallized, but the product was not characterized. The zone having R_F 0.45 did not crystallize. The zone having R_F 0.56 crystallized giving **4**, yield 0.22 g (7%), m.p. 119–120°; $[\alpha]_D^{25}$ −400 ± 10° (*c* 0.12, chloroform); λ_{max}^{KBr} 2.90 (NH), 3.32 (CH), 5.70 (CO_2Me, OAc), 5.94 ($CONH_2$), 6.20 (aryl), 6.30 (NO_2), 6.65, 6.94, 7.28, 7.49, 8.09, 8.49, 8.69, 8,96, 9.55, 10.94, 11.99, 13.12, 13.39, 13.70, 14.61 μm; n.m.r. data: τ 3.41 (1-proton doublet, $J_{NH,2}$ 10 Hz, disappears on deuteration, NH of hexosamine), τ 5.20 (1-proton doublet, $J_{1,2}$ 9 Hz, H-1), τ 4.01-4.97 (6-proton multiplet, H-2, H-3, H-4, H-5, H-6), τ 7.83, 7.91, 7.97 (9 protons, OCOMe), τ 8.15 (3-proton singlet, NAc). Other chemical shifts and coupling constants were similar to those for **2** (OH of **2** absent).

Anal. Calc. for $C_{26}H_{32}N_4O_{15}$: C, 48.75; H, 5.04; N, 8.75. Found: C, 49.09; H, 5.21; N, 8.43.

To a solution of **4** in methanol (5 ml) was added 1 drop of 10% hydrochloric acid and 1 drop of water. The solution was refluxed for 6 h, at the end of which time, t.l.c. (solvent A, sulfuric acid as well as ninhydrin locating reagent) indicated complete disappearance of starting material. Only two zones formed, R_F 0.14 and R_F 0.74 (mobilities identical to those of 2-amino-2-deoxy-D-glucose and *N*-2,4-dinitrophenyl-hydroxyproline, respectively).

The glycoside **4** was treated with base[2] (up to 10 equivalents of sodium carbonate or sodium methoxide in methanol). T.l.c. indicated no scission to hexosamine and an elimination product of the aglycon. This stability of **4** in the presence of base was contrasted (t.l.c.) to that of **3** above and other glycosides of *N*-2,4-dinitrophenyl-hydroxyamino acid esters reported[1,2].

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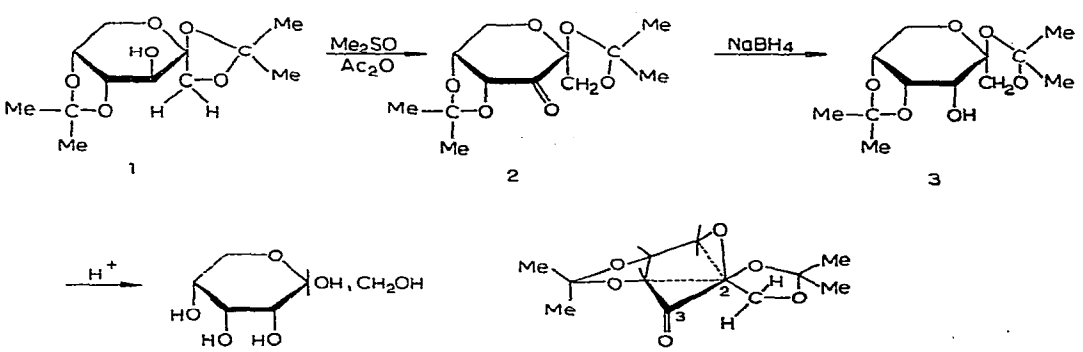
Carbohyd. Res., 5 (1967) 102–106

A new synthesis of D-psicose (D-*ribo*-hexulose)

D-Psicose has previously been prepared by the epimerization of D-altrose[1,2], and by the action of diazomethane on tetra-*O*-acetyl-D-ribonyl chloride with subsequent deacetylation of the resulting *keto*-D-psicose pentaacetate[3]. Recent work[4,5] in which methyl sulfoxide–acetic anhydride was used to oxidize secondary alcoholic groups to ketonic groups suggested that 1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-2,3-hexodiulose (**2**) might be preparable by similar oxidation of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**1**).

Reduction of this diulose acetal (**2**) should then afford a mixture of 1,2:4,5-di-*O*-isopropylidene-β-D-*ribo*-hexulopyranose (**3**) and the corresponding D-fructose acetal (**1**). Study of a molecular model of **2** suggested that the favored conformer is that skew form (S^{O}_{3}) in which both dioxolane rings are *exo* (**2a**), and indicated that borohydride reduction would favor formation of **3**. From seven of the eleven conformations of **2** considered, the D-*ribo* isomer (**3**) alone would be expected, whereas, for the four others,



1 2 3 4 2a

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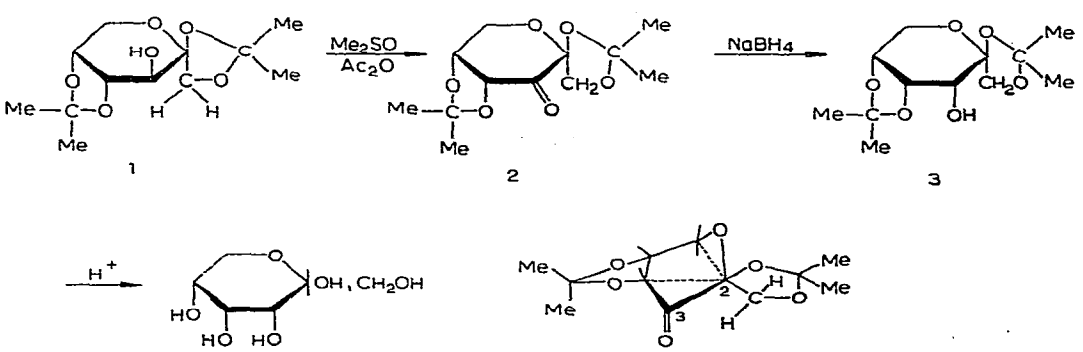

1 2 3 4 2a

there seemed to be little difference in the freedom of approach of the borohydride to the carbonyl group, and hence both the *ribo* and *arabino* isomers would be expected to be formed. Experimentally, it was found that **3** is formed almost exclusively; acid hydrolysis then gives **4**. With this relatively simple procedure, D-psicose (**4**) becomes readily available.

EXPERIMENTAL

General. — The oxidation, reduction, and hydrolysis reactions were followed by means of t.l.c. on Silica Gel G, with 3:1 (v/v) ethyl acetate–petroleum ether as the developer, and a spray of concentrated sulfuric acid, followed by charring, as the indicator: R_F of **1**, unity; of 3-*O*-acetyl-1,2:4,5-di-*O*-isopropylidene-β-D-fructose (**5**), 1.26; of **2**, 1.18; of **3**, 0.86; and of **4**, 0.00.

1,2:4,5-Di-O-isopropylidene-β-D-erythro-2,3-hexodiulo-2,6-pyranose (**2**). — Acetic anhydride (2 ml) was added to a solution of compound **1** (5.2 g; 0.02 mole) in 100 ml of methyl sulfoxide. After 48 h, t.l.c. indicated that compound **1** had been converted into **2** and **5**. The reaction mixture was evaporated under diminished pressure to a syrup, from which **2** crystallized; yield 3.6 g (70%). Compound **2** was recrystallized from methanol, m.p. 101–102°; $[\alpha]_D^{25}$ −100.4° (*c* 2.41, acetone).

Anal. Calc. for $C_{12}H_{18}O_6$: C, 55.81; H, 7.03. Found: C, 56.15; H, 7.08.

The mother liquor contained both **2** and **5**.

1,2:4,5-Di-O-isopropylidene-β-D-psicopyranose (**3**). — To a solution of **2** (2.8 g; 0.011 mole) in 1:3 (v/v) water–methanol (80 ml), cooled in an ice bath, was added alkaline sodium borohydride (100% excess). After 30 min, the conversion of **2** into **3** was complete, and only a trace of **1** was detected in the product. After addition of 50 ml of water, the solution was evaporated to dryness under diminished pressure, and the residue was dissolved in water. The solution was repeatedly extracted with chloroform, and the extracts were combined, dried (sodium sulfate), and evaporated to dryness; the residue was dissolved in petroleum ether (b.p. 30–60°), and the solution cooled to give **3**, yield 1.89 g (65%), m.p. 60–61°, lit. m.p.[2] 57–58.5°.

D-*Psicose* (**4**). — A solution of **3** (1 g) in water (5 ml) was mixed with 0.5 g of Dowex-50 W x8 (H^+) cation-exchange resin, and the suspension was stirred for 75 min at 95°; t.l.c. then showed that hydrolysis was complete. The mixture was filtered through activated carbon, and the filtrated was evaporated to dryness under diminished pressure to a syrup that was identified as D-psicose by conversion into its phenylosotriazole, m.p. 135–136°, lit. m.p.[6] 135–136°.

The product was also identified as D-psicose by high-voltage electrophoresis in sodium borate buffer. An authentic sample of **4** was prepared by an earlier procedure[2]. Both samples had R_{Fru} 0.82. The electrophoresis was performed in an apparatus built according to the specifications of Gross[7], and the separations were made under the following conditions: Whatman No. 3 MM filter paper; voltage gradient, 86 volts/cm; temp., 13 ± 1°; time, 40 min; and buffer, 0.05M $Na_2B_4O_7$ (pH 9.2). Chromatograms were sprayed with an aqueous solution of urea (0.5M) and tartaric acid (M), and then

heated[8] at 105° for 10 min. In the presence of borate ions, D-psicose (**4**) and D-fructose give a blue color with this reagent.

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STUDIES ON CYCLIC POLYOLS

PART X. SYNTHESIS AND NUCLEAR MAGNETIC RESONANCE STUDIES OF CYCLITOLS DERIVED FROM BICYCLO[2.2.1]HEPTANE*

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INTRODUCTION

In connection with the program of synthesis and other investigations of cyclitols derived from cyclopentane that are in progress in this laboratory[1-5], model compounds have been sought. The well known cyclitols derived from cyclohexane[6-8] are not entirely adequate models, because many of these compounds are in a chair conformation, and the substituents are in either the axial or equatorial orientation, whereas, in cyclopentane derivatives, truly axial or equatorial orientation is not achieved[2,5,9]. The bicyclo[2.2.1]heptane (norbornane) system **1**, consisting of two five-membered rings, appeared to offer many advantages as a model for cyclopentane, and was therefore investigated. Several norbornanediols have been reported[10-13], but of these, only the symmetrical glycols **2a** and **3a**, reported by Kwart and Vosburgh[10], seemed applicable to our studies. The related bornanediols have also been described[14] and studied by n.m.r. spectroscopy[15], but these appeared to be less suitable than norbornane compounds. Attempts to make *trans*-2,3-norbornanediol by solvolysis of the epoxide[13] obtained from norbornene (bicyclo[2.2.1]hept-2-ene) have led to rearrangement products, and the attempted epoxidation of norbornadiene (bicyclo[2.2.1]hepta-2,5-diene, **7**) has resulted[16] in deep-seated rearrangement of the ring system, with the formation of bicyclo[3.1.0]hex-2-ene-6-*endo*-carboxaldehyde. The present report describes the preparation of several derivatives (**2** and **4**) of *exo*-2,3-norbornanediol, the synthesis of *exo-exo*-2,3,5,6-norbornanetetrol and some of its derivatives (**5** and **6**), and n.m.r. studies of these compounds.

RESULTS AND DISCUSSION

Synthetic procedures. — *exo-cis*-Norbornane-2,3-diol (**2a**) was prepared by treatment of norbornene (**8**) with buffered permanganate as indicated by previous authors[10,11]. Purification was unexpectedly difficult, and a pure product was obtained only by sublimation of the crude oxidation mixture, followed by recrystallization from toluene. The usual procedures for acetonation[1,2,11] and acylation[1,4a] gave the iso-

*For Part IX see reference 5.

propylidene acetal (**4**), acetate (**2b**), and benzoate (**2c**). Spectral studies of these compounds are discussed below, together with those on the tetrol **5a** and its derivatives.

On treatment with permanganate, the diene **7** was converted into a tetrol. The yields in the hydroxylation reaction were uniformly low, the highest being just under 15% (see Experimental). Buffered permanganate, either zinc permanganate or a potassium permanganate–magnesium sulfate mixture, gave poorer yields than the alkaline permanganate procedure described. Treatment of alkenes with buffered permanganate may lead to products more highly oxidized than glycols[17]; for example, norbornene has been found to undergo fission of the double bond under these conditions[18], and oxidation of tertiary C–H to C–OH has been reported[19]. Consequently, the low yield of product in the present study is not surprising.

When the tetrol was treated under acetonating conditions, a di-*O*-isopropylidene derivative was obtained, showing that two vicinal, *cis*-glycol functions are present in the tetrol. Examination of Dreiding models shows that the di-*O*-isopropylidene derivative of *endo-endo*-2,3,5,6-norbornanetetrol could not exist, because the two "inner" methyl groups would have to occupy the same space. Consequently, only the *exo-exo* configuration (**6**) and the 2,3-*exo*-5,6-*endo* configuration (**9**) are possible. The n.m.r. spectra of the tetrol and of three of its derivatives are not compatible with the latter configuration, and these compounds are therefore represented by structures **5a**, **5b**, **5c**, and **6**. The formation of the *exo-exo*-tetrol is analogous to the formation of the *exo*-diol **2** from norbornene.

N.m.r. spectra of norbornane cyclitols. — (*a*) *Chemical shifts.* The chemical shifts of the identifiable proton-signals of the diol, tetrol, and three derivatives of each are summarized in Table I. The values are expressed on the δ-scale in p.p.m. downfield from the internal reference, [tetramethylsilane (TMS) in chloroform solutions and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in aqueous solutions]; the value of δ is positive in this direction. The difference in chemical shifts, $\Delta\delta$, between signals of the bridgehead protons and O–C–H protons of the diol **2a** is almost exactly the same when the spectrum is measured with a solution in chloroform-*d* or in deuterium oxide. This result shows that, although different solvents must be used for the free cyclitols and the derivatives, valid conclusions may be based on comparison of the spectral data.

The chemical shifts of the O–C–H protons of the glycols **2a** and **5a** are identical, as are those of the acetals **4** and **6**. The chemical shifts of the acetyl-methyl groups of **2b** and **5b** are also identical. The methyl groups of the *O*-isopropylidene groups of **6** give two narrow singlets (W_h* = 2.0 Hz, W_h of 1% TMS = 1.5 Hz) of intensity equivalent to 6 protons, proving the presence of two pairs of identical methyl groups. In pyranoid and cyclohexanoid chair conformers, axial ring-protons resonate 0.3–0.5 p.p.m. to higher field than corresponding equatorial protons[20,21], and the signals of the methyl groups of axially oriented acetoxyl groups are found about 0.2 p.p.m. to lower field than those of equatorial acetoxyl groups[20,22]. The geometry of the norbornane ring differs

*W_h denotes width of signal at half-height.

from that of a cyclohexanoid chair conformation, so that *endo* and *exo* protons cannot be equated with either axial or equatorial protons. Nevertheless, the environment of *exo* and *endo* protons and other substituents is so different that significant differences in chemical shifts would be expected. Indeed, Musher[23] has reported that *exo* protons

TABLE I

N.M.R. SPECTRA OF NORBORNANE CYCLITOLS[a]

Compound	*Solvent*	*Chemical shift of protons, δ*				
		Bridge CH_2	*Bridge-head*	*O–C–H*	*Acetyl $-CH_3$*	*Isopropylidene $-CH_3$*
Diol **2a**	D_2O		2.08	3.68		
	$CDCl_3$		2.14	3.69		
Diacetate **2b**	$CDCl_3$		2.27	4.74	2.02	
Dibenzoate **2c**	$CDCl_3$		2.47	5.09		
Acetal **4**	$CDCl_3$		2.26	3.98		1.28 1.43
Tetrol **5a**	D_2O	1.76	2.10	3.68		
Tetraacetate **5b**	$CDCl_3$	1.94	2.41	4.84	2.02	
Tetrabenzoate **5c**	$CDCl_3$	2.34	2.88	5.38		
Diacetal **6**	$CDCl_3$	1.60	2.38	3.98		1.28 1.46

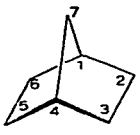
[a]TMS was used as the internal reference when chloroform-*d* was the solvent; DSS was the internal reference for solutions in deuterium oxide.

in hydroxybornane derivatives are deshielded by 0.35–0.5 p.p.m., relative to corresponding *endo* protons. Laszlo and Schleyer[24] reported that the O–C–H-*exo* proton of DL-5-*endo*-acetoxynorbornene (**10**) resonates at δ 5.16, and is 0.3–0.4 p.p.m. deshielded relative to the O–C–H protons of the di- and tetra-acetates **2b** and **5b**. However, the long-range, anisotropic effect of the double bond of **10** may be a complicating factor. The identity of chemical shifts seen in the present study is strong evidence in favor of the symmetrical configuration proposed (**5** and **6**).

Additional evidence in favor of the proposed configuration is obtained from the signals representing the protons of the bridge methylene group. In this configuration, the methylenic protons are sterically equivalent, and they therefore have the same chemical shift. Consequently, even though the geminal coupling constant[25] is nearly 10 Hz, this coupling will not be seen in the spectrum. On the other hand, in a compound having the *exo-endo* configuration, such as the diacetal **9**, the methylenic protons are not sterically equivalent and should give rise to an AB system[26a]. In actuality, the signals of the methylenic protons of the tetrol and its three derivatives show only the small, vicinal coupling with the bridgehead protons (H_b and $H_{b'}$ of **11**), that is required by the proposed configuration.

(*b*) *Spin–spin coupling constants.* Supporting evidence for the assigned configuration is obtained by examination of the spectra for proton spin–spin coupling effects involving the O–C–H protons. Musher[23] and Laszlo and Schleyer[25] have made detailed analyses of the spectra of bornane and norbornane derivatives. In the com-

pounds that they studied, the coupling of *endo* protons with vicinal, bridgehead protons, (H_n and H_b in structure **11**) was always zero, whereas, for *exo* protons, $^3J_{bx}$* was always measurable and varied in magnitude between 3.2 and 6.0 Hz. They also observed long-range coupling, involving H_a and H_n, of 1.4–1.9 Hz. In the present



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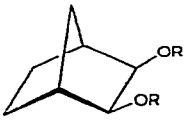

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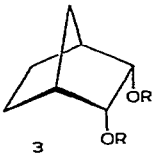

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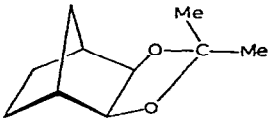

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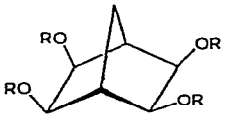

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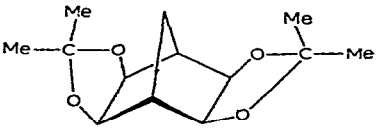

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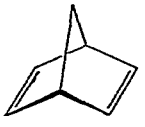

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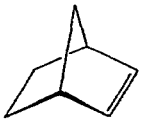

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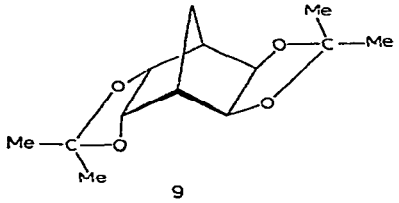

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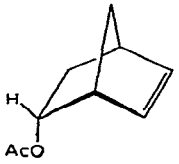

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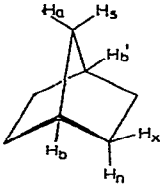

11

R =
a H
b Ac
c Bz

study, there is no evidence of coupling between the O–C–H protons and the bridgehead protons in the spectra of the tetrol and its derivatives, showing that the O–C–H protons are *endo*, in agreement with the structures proposed. All of the disubstituted compounds (see Table II) show small coupling (1.3–1.7 Hz.), involving the O–C–H protons**, that we attribute to long-range coupling with the *anti* proton of the bridge-methylene groups ($^4J_{an}$). Similar ^4J effects might, therefore, be expected in the spectra of the tetrol and its derivatives, as the geometry is the same. However, Lazslo and

*The following abbreviations are used: ^3J, vicinal coupling, through 3 bonds; ^4J, long-range coupling through 4 bonds.

**This long-range coupling is in conformity with the generalization that a "W" conformation of four bonds is favorable for such coupling (see references 25 and 3b for discussion of this point). A referee has suggested that our failure to observe long-range coupling in the spectra of the tetrasubstituted compounds may be due to the fact that the O–C–H protons and the equivalent methylenic protons constitute an $A_2'X_2'$ case. A related system, 7-oxanorbornane has been studied[26c] recently. Comparison of these two systems will be of interest.

Schleyer[25] have pointed out that "although definite geometrical arrangements appear to be required for such coupling to take place, little more is known about this phenomenon and precise prediction is not possible". Consequently, our failure to observe long-range coupling in the signals of the O–C–H protons of the tetrasubstituted compounds does not invalidate our conclusions concerning the configuration.

TABLE II

SPIN–SPIN COUPLING OF O–C–H PROTONS

Compound	*O–C–H Signal appearance*[a]	*J, Hz*	*Interpretation*[b]	*Compound*	*O–C–H Signal appearance*[a]	W_h, *Hz*	*TMS or DSS* W_h, *Hz*	*Interpretation*[b]
2a	d	1.75	$^4J_{na}$	**5a**	s	1.6	1.4	no coupling
2b	d	1.7	$^4J_{na}$	**5b**	s	1.8	1.0	no coupling
2c	d	~1.5	$^4J_{na}$	**5c**	s	2.5	1.5	no coupling
4	d	~1.3	$^4J_{na}$	**6**	s	1.8	1.5	no coupling

[a]s=singlet, d=doublet.

[b]The interpretation $^4J_{na}$ refers to coupling of *endo*-protons with the *anti*-proton of the bridge methylene group. See structure **11**.

(*c*) *Long-range effects of anisotropic groups.* The data in Table I suggest that, in the compounds under investigation, shielding of various groups of nuclei includes a long-range component[26b], due to diamagnetic anisotropy of some of the substituents. The deshielding of the bridgehead protons of dibenzoate **2c** relative to diacetate **2b**, and of tetrabenzoate **5c** relative to tetraacetate **5b**, is ascribed to the anisotropy of the phenyl ring. Part of the deshielding of the bridgehead protons of these four compounds, relative to the parent cyclitols, may be due to the anisotropy of the carbonyl group of the esters. Long-range deshielding is the most obvious explanation for the deshielding of the methylenic protons of **5b** and **5c** relative to **5a**. In this case also, there is a large effect (0.40 p.p.m.) that must be due to the phenyl ring. Similarly, the shielding of the methylenic protons of the diacetal **6** may be ascribed to the influence of the nearby *O*-isopropylidene groups, although it is difficult to specify which bonds are concerned in the overall effect. Deshielding of the bridgehead protons of **5b** and **5c** is larger than the corresponding effect in **2b** and **2c**, and this is probably due to the larger number of anisotropic groups in the two former compounds. Long-range deshielding by the additional phenyl rings may also account for the difference of 0.3 p.p.m. between the O–C–H protons of the dibenzoate and those of the tetrabenzoate.

As already noted, axial protons in cyclohexanoid chair conformers are shielded relative to equatorial protons[20,21], whereas axial acetoxyl groups are deshielded relative to equatorial acetoxyl groups[20,22]. If the analogy may be extended to the norbornane system, then, because *exo* protons are deshielded relative to *endo* protons[23], *exo* acetoxyl groups might be expected to be shielded relative to *endo* acetoxyl groups. Indeed, the chemical shift of the acetoxyl groups of **2b** and **5b** corresponds closely to

that of equatorial acetoxyl groups in cyclohexanoid systems[22,27]. It would be interesting to compare these values with those of the corresponding *endo* compounds.

Biological activity of norbornanetetrol. — Dr. Douglas Davidson of the Biology Department of this University has observed an influence of cyclitols on mitotic cells on man and plants. Tetrol **5a** closely resembles *myo*-inositol and DL-(1,2,3/4)-cyclohexanetetrol in its effects on these cells. "All these compounds induce an increase in chromosome contraction during prophase, and their effect is still visible in anaphase chromatids. They also prolong the stage of lateral adhesion of sister-chromatids during pre-metaphase, but this effect does not prevent subsequent separation of sister-chromatids at anaphase"[28].

EXPERIMENTAL

General. — N.m.r. spectra were recorded with a Varian Associates A-60 n.m.r. spectrometer, with TMS or DSS as the internal standard. TMS was present in 1% concentration. The concentration of DSS was not measured. Line positions are considered accurate to ±0.02 p.p.m., and coupling constants are considered accurate to ± 0.2 Hz. Melting points were determined on a Kofler Micro Hot-Stage (A. H. Thomas and Co.) and are corrected. Boiling points are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

exo-cis-*2,3-Bicyclo[2.2.1]heptanediol* (**2a**) *and derivatives.* — *A. Diol* **2a**. Bicyclo-[2.2.1]hept-2-ene (14.1 g, 0.15 mole) was dissolved in 1450 ml of 95% ethanol, and chilled in a dry ice–acetone bath. A solution of $Zn(MnO_4)_2 \cdot 6H_2O$ (40 g in 750 ml of water) was added during 1 h, with constant mechanical stirring. The mixture was warmed to −10°, and kept for 1 h at that temperature. A stream of sulfur dioxide was bubbled through the solution to dissolve the precipitate. The solution was concentrated, and extracted with dichloromethane overnight in a continuous extractor. Evaporation of the solvent gave crystals, m.p. 59–68°. Sublimation of this product at 80° (bath temp.)/0.1–0.3 torr gave colorless crystals, m.p. 125–137°. After recrystallization from toluene, the product had m.p. 138–9° (lit.[11] 139–140°).

B. Diacetate **2b**. Diol **2a** (524 mg, 4.1 mmoles) was dissolved in 5 ml of dry pyridine, acetic anhydride (4.5 ml) was added at 0°, and the vessel was stoppered. After 1 h at 0°, the mixture was kept overnight at room temperature. The pale-yellow solution was diluted with water (300 ml), and the mixture was extracted twice with 200-ml portions of ether. The ether extract was washed successively with 2N hydrochloric acid, 2N sodium hydroxide, and water, and was then dried (sodium sulfate) for 1 h, and filtered. The ether was evaporated, to leave a pale-yellow syrup that crystallized when chilled in ice, but which melted below room temperature. Purification was achieved by sublimation, but the product still melted below room temperature.

Anal. Calc. for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60. Found: C, 62.33; H, 7.65.

C. Dibenzoate **2c**. Diol **2a** (1.12 g, 8.7 mmoles) was dissolved in 6.4 ml of pyridine, and 4.6 ml of benzoyl chloride was added at 0°. The vessel was stoppered, kept for

1 h at 0°, and then kept overnight at room temperature. The mixture was cooled to 0°, water (1.0 ml) was added, and after 1 h at 0°, the mixture was kept for 1 h at room temperature. The product was dissolved in dichloromethane (75 ml), and the solution was washed twice with N sulfuric acid, twice with 5% sodium carbonate, and twice with water. The solvent was evaporated, the oily residue was dissolved in 5 ml of hot ethanol, and 5 ml of water was added. Crystals formed overnight (1.42 g, 4.2 mmoles), m.p. 75–83°. Recrystallized twice from ethanol, the product had m.p. 86–87°. An analytical sample had m.p. 88.5–89°.

Anal. Calc. for $C_{21}H_{20}O_4$: C, 74.98; H, 5.99. Found: C, 74.72; H, 5.99.

D. Acetal **4**. Diol **2a** (1.0 g, 7.8 mmole) was dissolved in 100 ml of anhydrous acetone, 2 g of anhydrous copper(II) sulfate and 0.01 ml of conc. sulfuric acid were added, and the mixture was stirred overnight in a stoppered flask. More copper(II) sulfate (1 g) was then added, and the stirring was continued for 4 h. The mixture was filtered, the filtrate was evaporated to dryness, and crude acetal (1.4 g) was distilled in a micro-distillation apparatus; yield 0.9 g (5.4 mmole, 69%), b.p. 102°/120 torr.

Anal. Calc. for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59. Found: C, 71.33; H, 9.80.

exo-exo-*2,3,5,6-Bicyclo[2.2.1]heptanetetrol* **5a** *and derivatives.* — *A. Tetrol* **5a**. Freshly redistilled bicyclo[2.2.1]hepta-2,5-diene, b.p. 88° (26 g, 206 mmoles) was dissolved in 1 liter of acetone. The solution was cooled in a Dry Ice–acetone bath, the temperature being kept between −20 and −30° during the addition of a solution of potassium permanganate (60 g, 380 mmole) and potassium carbonate (4.8 g, 35 mmole) in 1.2 liter of water; addition of the oxidant was complete in 2 h. The mixture was kept overnight at −10°, and was then centrifuged in an International PR-2 centrifuge. The precipitate was discarded, and to the dark-brown, supernatant solution was added 3N perchloric acid to pH 4. The solution was then concentrated under diminished pressure to about half the original volume, and the crystalline precipitate of potassium perchlorate was removed by filtration. The filtrate was concentrated to 750 ml, chilled in ice, and filtered. An equal volume of 95% ethanol was added, and the solution was chilled for one h, and filtered. The filtrate was concentrated to 400 ml, and was found to have pH 6. The solution was adjusted to pH 3.5 with 2 ml of 5N sulfuric acid, and was then extracted overnight, in a continuous extractor, with dichloromethane. The aqueous phase was treated with 0.3N barium hydroxide to bring the pH to 7, and the precipitate was removed by filtration. Concentration, chilling, and filtration were repeated until the volume of the solution was 100 ml, and residual, ionic material was removed by passing the solution through columns of Duolite-A4 (OH^-) and Dowex-50 (H^+). The eluate was evaporated to dryness, giving a mass of crystals. The product **5a** was recrystallized from abs. ethanol; yield 4.8 g (31 mmoles, 15%), m.p. 189–193°. Two further recrystallizations raised the m.p. to 196.5–197°.

Anal. Calc. for $C_7H_{12}O_4$: C, 52.49; H, 7.55. Found: C, 52.10; H, 7.53.

B. Tetraacetate **5b**. Tetrol **5a** was acetylated, and the product was purified as described above. A crystalline product **5b** was obtained; this was obtained pure by one recrystallization from abs. ethanol; it had m.p. 134–136°.

Anal. Calc. for $C_{15}H_{20}O_2$: C, 54.87; H, 6.14. Found: C, 54.82; H, 6.00.

C. Tetrabenzoate **5c**. Tetrol **5a** was benzoylated, and the product was purified as described above for the diol. The product had m.p. 259–263°. After several recrystallizations from glacial acetic acid, an analytically pure material was obtained, m.p. 263.5–264.5°.

Anal. Calc. for $C_{35}H_{28}O_8$: C, 72.91; H, 4.89. Found: C, 73.11; H, 4.98.

D. Diacetal **6**. Tetrol **5a** was acetonated as described above. When the acetone was evaporated from the reaction mixture, the residue crystallized. Sublimation at 110°/0.05 torr gave a product having m.p. 191–193°.

Anal. Calc. for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39. Found: C, 65.18; H, 8.27.

ACKNOWLEDGMENTS

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SUMMARY

exo-exo-2,3,5,6-Bicyclo[2.2.1]heptanetetrol (**5a**) has been synthesized by treatment of bicyclo[2.2.1]hepta-2,5-diene with alkaline permanganate. The configurational assignment is based on (*a*) analogy with similar reactions involving bicyclo[2.2.1]-heptene, (*b*) reactivity of the tetrol, and (*c*) the n.m.r. spectra of the tetrol, three of its derivatives, and the corresponding 2,3-diol **2a** and its derivatives. Long-range shielding and deshielding effects in these spectra are discussed. The influence of the tetrol on mitotic human and plant cells is noted.

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REACTIONS OF SUGAR CHLOROSULFATES

PART VIII. D-RIBOSE AND ITS DERIVATIVES

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INTRODUCTION

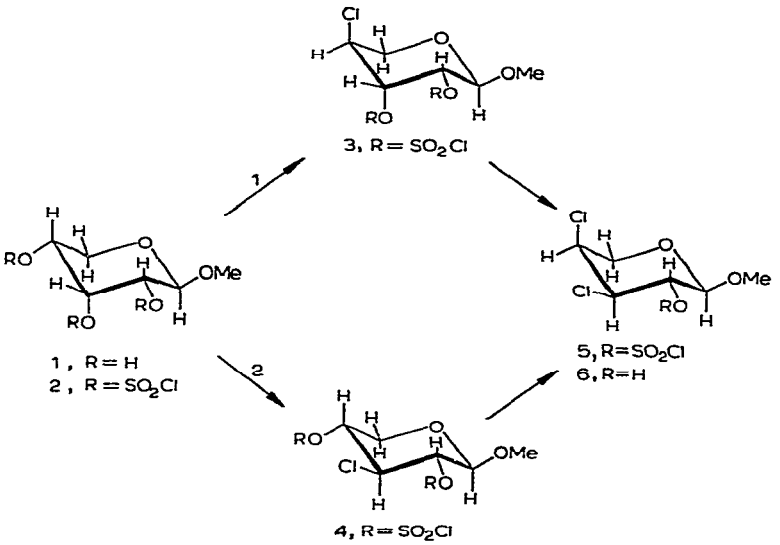
The displacement of the chlorosulfate (OSO_2Cl) group by chloride ion has been shown to occur at C-4 and at C-3 of chlorosulfate derivatives in hexopyranosides with inversion of configuration[1,2], which is in accord with a bimolecular nucleophilic displacement (S_N2) mechanism. Chlorosulfates of the pentopyranose series have been investigated to a lesser extent, but the available evidence shows that displacement of OSO_2Cl at C-4 proceeds with inversion here also. On the other hand, no example has yet been recorded of chlorosulfate displacement at C-3 of a pentopyranose derivative (see, however, ref. 3). The present work reports on such a displacement, and provides evidence of the stereochemistry of the process.

RESULTS AND DISCUSSION

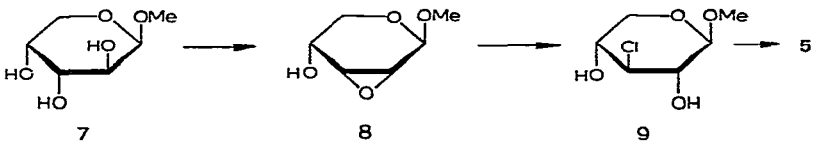
Methyl β-D-ribopyranoside (**1**) on treatment with sulfuryl chloride and pyridine in chloroform solution yields the tri(chlorosulfate) (**2**). Treatment of the latter with chloride ion results in replacement of two of the chlorosulfate groups and formation of a product that may be formulated as methyl 3,4-dichloro-3,4-dideoxy-α-L-arabinopyranoside 2-chlorosulfate (**5**), on the assumption that inversion of configuration has taken place at C-3 and C-4. In addition to providing structural proof of the stereochemistry of the process, it would be of interest to know whether the intermediate, monosubstituted product is **3** or **4**, formed by substitution at C-4 or C-3 (reactions 1 and 2), respectively. No monochloro-deoxy compound could, in fact, be isolated.

The stereochemistry of the substitution process was elucidated as follows. Methyl β-D-arabinopyranoside (**7**) was converted into methyl 2,3-anhydro-β-D-ribopyranoside (**8**) *via* the 2-*p*-toluenesulfonic ester of **7**. The anhydro ring of **8** is known[4] to be opened, by hydrogen bromide, with preponderant formation of a β-D-xyloside derivative. In the present work, this reaction (with hydrogen chloride) gave methyl 3-chloro-3-deoxy-β-D-xyloside (**9**) which, as expected[5], was unaffected by sodium periodate solution. Treatment of **9** with sulfuryl chloride and pyridine hydrochloride yielded the same dichlorodideoxy mono(chlorosulfate) (**5**) as had been obtained from **2**, even when the reaction was carried out under very mild conditions. Dechlorosulfation of the product **5** yielded a methyl dichlorodideoxypentoside (**6**), which may be formu-

lated as methyl 3,4-dichloro-3,4-dideoxy-α-L-arabinopyranoside on the expectation[1,2] that substitution at C-4 had occurred with inversion of configuration. The methyl

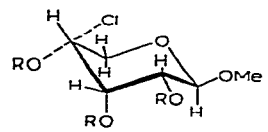


glycoside **6** could be hydrolyzed with difficulty to yield the crystalline reducing sugar which consumed 1 mole of periodate/mole rapidly and yielded formic acid slowly, presumably because of slow hydrolysis of the formic ester.



The mild conditions under which **9** is converted, *via* the 2,4-di(chlorosulfate), into **5** suggest that formation of **5** from **2** proceeds *via* intermediate **4**, rather than **3**. Indeed, it could be argued that, had substitution first occurred at C-4, the axially oriented chlorine of **3** would deactivate the neighbouring chlorosulfate group at C-3 towards further substitution, as had been observed previously[2,6] in related cases. However, the following discussion, based on conformational considerations, differentiates satisfactorily between the two alternatives (reactions 1 and 2). The transition state of the S_N2 displacement process in the pyranose series may be represented by the chairlike structures[7,8] **10** and **11**. The transition state for substitution at C-4, [**10**, and the end-on representation (**10a**) from a viewpoint along the C-4–C-3 bond] possesses considerable strain as a result of steric interaction between the near-eclipsed, adjacent, chlorosulfate groups. On the other hand, the transition state (**11**, **11a**) (or the equivalent of **11a** from a viewpoint along the C-3–C-2 bond) for substitution at C-3 is free from such unfavorable interactions and will, therefore, be of lower potential energy, thus

favoring this reaction path. A similar argument shows that further substitution at C-4 of **4** can take place without introduction of such unfavorable interactions in the transition state of the process; hence, this reaction will also occur readily.



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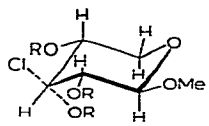
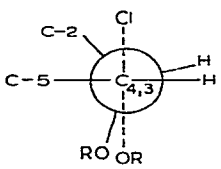
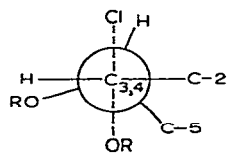


11

$R = SO_2Cl$



10a



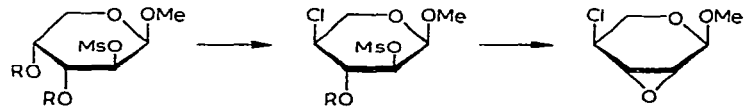
11a

D-Ribose, with sulfuryl chloride and pyridine, yielded a crystalline D-ribopyranosyl chloride 2,3,4-tri(chlorosulfate). The n.m.r. spectrum of this compound in acetonitrile with external tetramethylsilane as standard, shows the following features. The resonance farthest downfield is attributed to H-1 and appears as an ill-defined doublet at τ 3.71 with a width at half-height of 3 Hz. The narrow splitting shows that H-1 and H-2 are not *trans*-diaxial, and the rather complex appearance of the H-1 signal can be explained on the basis of long-range coupling with H-5e. Hall* has recently observed this effect in similar ribopyranosyl compounds. The H-3 signal, at τ 4.36, is a very sharp triplet with coupling constants of 3.6 Hz. The H-2 and H-4 signals appear together as a highly complex multiplet, upfield from the H-3 signal, at τ 4.76. The two H-5 signals, centered at τ 5.75, appear as the AB part of an ABX pattern. These spectral characteristics strongly indicate that the compound is the β-D anomer in the *1C*(D) conformation. A closely similar n.m.r. spectrum was obtained by Horton and Turner[9] for tri-*O*-acetyl-β-D-ribopyranosyl bromide; these authors also concluded that the compound under study exists in the *1C*(D) conformation.

It seemed of interest also to study the effect of substitution at C-2 of methyl β-D-arabinopyranoside on the process of chlorosulfate displacement. The 2-methanesulfonic ester **12** was prepared for this purpose and converted into the 3,4-di(chlorosulfate) derivative **13** with sulfuryl chloride and pyridine. The latter, on treatment with pyridinium chloride, yielded methyl 4-chloro-4-deoxy-2-*O*-(methylsulfonyl)-α-L-xyloside 3-chlorosulfate (**14**) which, on dechlorosulfation, produced methyl 4-chloro-4-deoxy-2-*O*-(methylsulfonyl)-α-L-xyloside (**15**). Base converted this substance mainly into methyl 2,3-anhydro-4-chloro-4-deoxy-α-L-lyxoside (**16**), which also can be obtained by the reaction of sulfuryl chloride and pyridinium chloride with methyl 2,3-

*Personal communication from Professor L. D. Hall.

anhydro-β-D-ribopyranoside (**8**), thus proving that, in **13**, it was the chlorosulfate group at C-4, and not the one at C-3, that had been displaced by chloride. Presumably, the chlorosulfate residue at C-3 of **13** is not displaced, because of an unfavorable diaxial interaction between an incoming chloride ion and the axial methoxyl group at C-1.



12, R = H
13, R = SO_2Cl

14, R = SO_2Cl
15, R = H

16

EXPERIMENTAL

General. — Melting points were determined on a Kofler Hot Stage and are uncorrected. Solutions were concentrated under diminished pressure at 50°, or below, with a rotary evaporator.

Paper chromatograms were made by the descending method with Whatman No. 1 filter paper and the following solvent systems (v/v): (a) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water, (b) 3:1:1 butyl alcohol–ethanol–water, and (c) 5:3:3:1 butyl alcohol–pyridine–benzene–water. The sugars were detected on the chromatograms by using alkaline silver nitrate spray[10] or *p*-anisidine hydrochloride spray followed by application of heat[11]. Chlorosulfate derivatives of sugars were detected by the specific butyl alcohol–aniline–pyridine spray reagent followed by heating[12]. Sugar epoxides were detected[13] by spraying with a solution of sodium iodide and Methyl Red in butyl alcohol and heating the paper chromatogram for a few minutes at 140°.

Thin-layer chromatograms were made by the ascending technique on glass plates that had been coated with silica gel G and dried at 150–160° for 2 h. The following solvent systems were used: (d) 10:1 benzene–methanol, (e) 20:1 benzene–methanol, and (f) 2:1 benzene–isopropyl alcohol. The sugars were detected by spraying the plates with 5% sulfuric acid in ethanol followed by heating them in an oven at 110°.

Deionization of solutions was effected by passing them through Amberlite IR-120 (H^+) cation-exchange resin and Duolite A-4 anion-exchange resin.

I.r. spectra were measured with a Perkin–Elmer Model 21 spectrophotometer by using either a 5% (w/v) chloroform solution of the compound or a potassium bromide pellet containing 0.8% of the substance.

Methyl β-D-ribopyranoside tri(chlorosulfate) (**2**). — Methyl β-D-ribopyranoside (**7**, 21.4 g) was converted into the tri(chlorosulfate) (**2**, 13.2 g) in the usual way[2]. The product had m.p. 151–152°, $[\alpha]_D^{20}$ −63° (*c* 1.1, methanol).

Anal. Calc. for $C_6H_9Cl_3O_4S_3$: C, 15.7; H, 1.98; Cl, 23.1; S, 21.0. Found: C, 15.7; H, 1.87; Cl, 23.1; S, 20.6.

Reaction of methyl β-D-ribopyranoside tri(chlorosulfate) (**2**) *with an excess of pyridine hydrochloride.* — Compound **2** (13.2 g) in chloroform (250 ml) containing pyridine hydrochloride (13.2 g, 4 moles) was heated for 2 days under reflux. The

crystalline product **5**, isolated in the usual way[2], was recrystallized from aqueous acetone; yield 4.5 g (52%), m.p. 130°, $[\alpha]_D^{20}$ +49° (*c* 3.0, methanol), R_F 0.76 [t.l.c., solvent (f)].

Anal. Calc. for $C_6H_9Cl_3O_5S$: C, 24.05; H, 3.01; Cl, 35.6; S, 10.7. Found: C, 24.2; H, 2.83; Cl, 36.2; S, 10.9. The compound gave a positive test for the $-OSO_2Cl$ group.

*Methyl 3,4-dichloro-3,4-dideoxy-α-*L*-arabinoside* (**6**). — Compound **5** (4.1 g) was dechlorosulfated by treating it in methanol (100 ml) in the presence of calcium carbonate (12 g) with one drop of N sodium iodide. After 6 h, the reaction was complete (t.l.c.). The solution was filtered and deionized, and the ash-free solution evaporated to yield crystals that were recrystallized from acetone–petroleum ether (b.p. 35–60°). The product (1.4 g, 51%) had m.p. 108°, $[\alpha]_D^{27}$ +88° (*c* 1.0, methanol), R_F 0.63.

Anal. Calc. for $C_6H_{10}Cl_2O_3$: C, 35.9; H, 4.98, Cl, 35.3. Found: C, 36.0; H, 4.78; Cl, 35.5.

*3,4-Dichloro-3,4-dideoxy-*L*-arabinose.* — The methyl L-arabinoside derivative **6** (680 mg) was heated in 4N sulfuric acid (60 ml) for 36 h, after which time hydrolysis was complete (t.l.c.). The solution was neutralized (barium carbonate), and the product (300 mg, 47%) was isolated in the usual way. It had m.p. 123°, $[\alpha]_D^{22}$ +167–174° (*c* 0.17, water, 25 h), R_F 0.48.

Anal. Calc. for $C_5H_8Cl_2O_3$: C, 32.1; H, 4.28; Cl, 38.0. Found: C, 32.2; H, 4.22; Cl. 38.0. Attempts to make a phenylosazone failed.

*Periodate oxidation of 3,4-dichloro-3,4-dideoxy-*L*-arabinose.* — A sample of the sugar (9 mg) was oxidized with aqueous sodium metaperiodate in the usual manner. Found (moles of periodate reduced/mole of formic acid produced in given time): 6 min, 0.477/0.04; 35 min, 0.996/0.00; 220 min, 0.954/0.5.

*β-*D*-Ribopyranosyl chloride 2,3,4-tri(chlorosulfate).* — D-Ribose (25 g) in dry pyridine (40 ml) and dry chloroform (100 ml), cooled to −70°, was treated with sulfuryl chloride (26 ml) to give a syrupy product that crystallized on standing. The compound was recrystallized from aqueous ethanol, yield 5 g (11%), m.p. 132°, $[\alpha]_D$ −108° (*c* 0.8, chloroform).

Anal. Calc. for $C_5H_6Cl_4O_{10}S_3$; C, 12.95; H, 1.29; Cl, 30.6; S, 20.8. Found: C, 13.0; H, 1.26; Cl, 30.8; S, 20.6.

The product gave a positive test for $-OSO_2Cl$ groups, and its infrared spectrum (in $CHCl_3$) exhibited strong absorption bands at 1435 and 1200 cm^{-1}.

When a portion of the compound was treated in methanol with aqueous sodium iodide in the presence of calcium carbonate at 20°, dechlorosulfation occurred. The only sugar detectable in solution after this procedure was D-ribose.

*Methyl 3-chloro-3-deoxy-β-*D*-xylopyranoside* (**9**). — A solution of **8** (4 g) in acetone (600 ml) containing 5N hydrochloric acid (16 ml) was refluxed for 4 h. The reaction was then complete (t.l.c.). The solution was neutralized (lead carbonate), and the product, crude **9**, was isolated in the usual way. Analysis (t.l.c.) indicated the presence of a major component (**9**) and a minor component, presumably the methyl 2-chloro-2-deoxy-D-arabinoside.

Methyl 2,4-di-O-acetyl-3-chloro-3-deoxy-β-D-xylopyranoside. — Crude **9** (0.5 g) was acetylated with a mixture of pyridine (6 ml) and acetic anhydride (3 ml). After 21 h, the crystalline product was isolated in 62% yield. It was recrystallized from ethanol–water, and had m.p. 116–117°, $[\alpha]_D$ −50° (*c* 1.2, chloroform).

Anal. Calc. for $C_{10}H_{15}ClO_6$: C, 45.0; H, 5.7; Cl, 13.3. Found: C, 45.3; H, 5.6; Cl, 13.0.

The i.r. spectrum was in agreement with this structure.

Conversion of **9** *into* **5.** — Substance **9** (2 g) was dissolved in a mixture of pyridine (4.5 ml) and chloroform (16 ml), and treated with sulfuryl chloride (2.5 ml) for 2 h at −70°. The reaction mixture was then warmed to 25° and, after 4 h at this temperature, was diluted with chloroform; the product was isolated in the usual manner. The recrystallized compound (m.p. 130–131°, 54% yield) was identical in all respects with **5** obtained from methyl β-D-ribopyranoside.

Attempted conversion of **9** *into* **4** ($R = SO_2Cl$). — Compound **9** in pyridine–chloroform solution was treated with sulfuryl chloride, as in the above experiment, but, following the 2-h period at −70°, the temperature was raised only to −20°; monitoring by t.l.c. over 6 h showed that reaction proceeded to only a very small extent and that mostly starting material was present. The temperature was then raised to 0° and, after 8 h, the product was isolated as previously described; it was identified as **5**.

Methyl 2-O-(methylsulfonyl)-β-D-arabinoside 3,4-di(chlorosulfate) (**13**). — Compound **12** (10 g) was dissolved in dry pyridine (15 ml) and chloroform (50 ml), and the mixture was treated with an excess of sulfuryl chloride (10 ml) at −70°. The product was isolated after 6 h, yield 81%, m.p. 110–111°, $[\alpha]_D^{20}$ −161° (*c* 3.3, chloroform). The i.r. spectrum showed the presence of the OSO_2Cl group[14].

Anal. Calc. for $C_7H_{12}Cl_2O_{11}S_3$: C, 19.1; H, 2.7; Cl, 16.2; S, 21.9. Found: C, 19.2; H, 3.0; Cl, 16.0; S, 21.8.

Methyl 4-chloro-4-deoxy-2-O-(methylsulfonyl)-α-L-xyloside 3-chlorosulfate (**14**). — The above di(chlorosulfate) (14 g) was dissolved in chloroform (250 ml), and an excess of pyridine hydrochloride (11 g) was added to the solution, which was then boiled for 5 h. The product, isolated in the usual way, was recrystallized from methanol; yield 76%, m.p. 99–100°, $[\alpha]_D^{20}$ −38° (*c* 5.4, chloroform).

Anal. Calc. for $C_7H_{12}Cl_2O_8S_2$: C, 23.4; H, 3.3; Cl, 19.8; S, 17.8. Found: C, 23.7; H, 3.7; Cl, 19.5; S, 17.7.

Prolonged reaction with pyridine hydrochloride failed to cause any further substitution of chlorosulfate by chlorine in **14**.

Methyl 4-chloro-4-deoxy-2-O-(methylsulfonyl)-α-L-xyloside (**15**). — The above chlorosulfate **14** (4 g) was dechlorosulfated in the usual way, and the product (2.5 g, 76%) was recrystallized from methanol. It was homogeneous by t.l.c. and had m.p. 119–120°, $[\alpha]_D$ −74° (*c* 3.25, chloroform). The i.r. spectrum showed the absence of $-OSO_2Cl$, and the presence of -OH.

Anal. Calc. for $C_7H_{13}ClO_6S$: C, 32.2; H, 5.0; Cl, 13.6; S, 12.3. Found: C, 32.0; H, 5.0; Cl, 13.6; S, 12.6.

Methyl 2,3-anhydro-4-chloro-4-deoxy-α-L-lyxoside (**16**). — Compound **15** (2 g) was dissolved in methanol (60 ml), and sodium methoxide (2 g) was added to the solution. After 24 h at 20°, the reaction was complete (t.l.c.), and the product was isolated in the usual way. The product was a mixture, both components giving a positive test for an anhydro ring (Methyl Red–sodium iodide spray). The major component was isolated by t.l.c., and was found to be identical with methyl 2,3-anhydro-4-chloro-4-deoxy-α-L-lyxoside (see below).

Methyl 2,3-anhydro-β-D-ribopyranoside (**8**, 2g) was dissolved in a mixture of dry pyridine (3.5 ml) and chloroform (9 ml) cooled to −70°, and sulfuryl chloride (2.1 ml) was added. After 4 h, the reaction was complete (t.l.c.), and the product, a syrup (1.4 g), was isolated. It was purified by distillation, b.p. 40°/15 mm; it had $[\alpha]_D$ −84° (*c* 4.2, in chloroform). The compound **16** was pure by t.l.c. and was free from -OSO_2Cl and -OH groups (i.r.).

Anal. Calc. for $C_6H_9ClO_3$: C, 43.8; H, 5.5; Cl, 21.6. Found: C, 43.9; H, 5.4; Cl, 21.7.

Attempts to prepare the intermediate methyl 2,3-anhydro-β-D-ribopyranoside 4-chlorosulfate were unsuccessful. Cleavage of the ring of **8** by hydrogen chloride yielded a complex mixture of products.

ACKNOWLEDGMENTS

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SUMMARY

The course of the reaction of methyl β-D-ribopyranoside (**1**) with sulfuryl chloride and pyridinium chloride is described. Compound **1** may readily be converted into the 2,3,4-tri(chlorosulfate) (**2**) which, with chloride ion, yields methyl 3,4-dichloro-3,4-dideoxy-α-L-arabinopyranoside 2-chlorosulfate (**5**). A consideration of conformational aspects of the substitution process suggests that the 3-chlorosulfate group of **1** is first displaced by chloride and that substitution then occurs at C-4. This conclusion is supported by a reaction series in which 3-chloro-3-deoxy-β-D-xylopyranoside was synthesized and converted into **5**. The stereochemical outcome at C-3 of **2** during the substitution process is also proved thereby.

The reaction of D-ribose with sulfuryl chloride yielded a D-ribopyranosyl chloride tri(chlorosulfate) which, on the basis of n.m.r. evidence, is described as the β-D anomer in the *1C*(D) conformation.

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SUBSTITUTED CEREBROSIDES.

PART IV. 1-*O*-(3-*O*-β-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSYL)DIHYDROCERAMIDE

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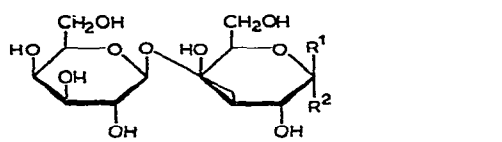
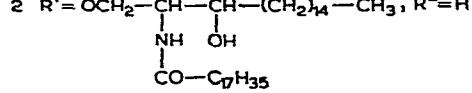
INTRODUCTION

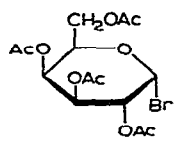
Various 3-*O*-β-linked derivatives of D-galactopyranose occur naturally, and several disaccharides containing a 3-*O*-β-substituted D-galactose residue have been isolated from their degradation products[1]. They include 3-*O*-β-D-galactopyranosyl-α-D-galactose (**1**) which had been synthesized earlier in low yields[2]. We have recently described the synthesis of two other 3-*O*-β-D-glycosyl-D-galactose disaccharides[3]. As part of a program of preparation of oligosaccharide derivatives of sphingosine, it was considered of interest to investigate the synthesis of the disaccharide-lipid **2** (a dihydroceramide glycoside of **1**) and to compare its properties with those of other known disaccharide-lipids[4].



1 $R^1 = H$, $R^2 = OH$



2 $R^1 = OCH_2-CH(NH-CO-C_{17}H_{35})-CH(OH)-(CH_2)_{14}-CH_3$, $R^2 = H$



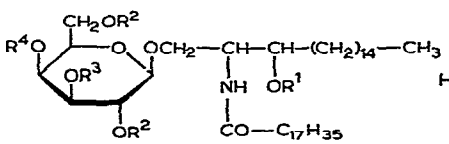
4

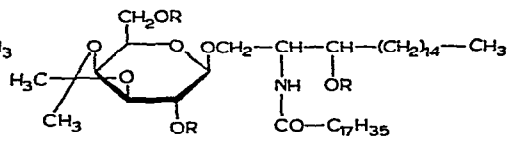
We have already discussed in some detail[4,5] arguments for a stepwise approach to the synthesis of disaccharide-lipids. Apart from its theoretical interest, such a pathway may well offer the only practical access to the disaccharide-lipid, when the disaccharide itself is not readily available. The yield reported of the disaccharide **1** was very low[2]; further conversion of **1** to a suitable *O*-acylglycosyl halide for reaction with the requisite lipid aglycon may also involve considerable losses. The present work provides an example of the practical value of the synthesis of a disaccharide-lipid, in reasonable yield, by attachment of a monosaccharide residue to a readily available protected cerebroside.

DISCUSSION

The substituted dihydrocerebroside **3**, the preparation of which was described

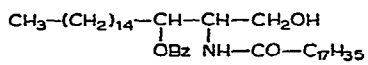
recently[6], was purified by chromatography on silica gel. Condensation of the product with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**), followed by catalytic deacylation, afforded a mixture from which the disaccharide-lipid (**2**) could be isolated in about 20% yield. The product consumed 2 moles of periodate per mole of **2**, thus confirming the structure assigned.





3 R^1=Bz, R^2=R^4=Ac, R^3=H
5 R^1=R^2=Ac, R^3=R^4=H
6 R^1=R^2=R^3=R^4=H
9 R^1=R^2=R^3=R^4=Ac

7 R=H
8 R=Ac



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An alternative intermediate for reaction with the bromide **4** was 3-*O*-acetyl-1-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-*N*-octadecanoyl-DL-dihydrosphingosine {*N*-[D,L-*erythro*-3-acetyloxy-1-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)octadecan-2-yl]octadecanamide} (**5**) which was prepared from the galactocerebroside **6** as follows: Treatment of **6** with acetone–sulfuric acid gave the acetal **7** in good yield. The location of the isopropylidene group was based on the observation that galactopyranosides easily give 3,4-*O*-isopropylidene derivatives, the reaction involving the two vicinal *cis* hydroxyl groups[7], and also on the stability of this compound towards periodate at 20°. Compound **7** was acetylated to give the triacetate (**8**), and deacetonation under mild acid conditions led to formation of **5**. This product consumed, at 25°, 1 mole of periodate per mole. Acetylation of **6** afforded the pentaacetate **9**. Comparison of the n.m.r. spectra of compounds **5**, **8**, and **9** gave additional evidence for their structure. Reaction of **5** with the bromide **4**, followed by catalytic deacetylation and chromatography on silicic acid, afforded the desired disaccharide-lipid **2**. Under similar conditions, compounds **3** and **5** reacted with the bromide **4** and gave the disaccharide **2** in almost 20% yield. The product consumed 2 moles of periodate per mole, in agreement with the value calculated for the glycoside of a disaccharide having an interglycosidic linkage located at C-3, which confirms the structure of **2**. The i.r. spectrum of **2** showed the presence of a β-D-galactopyranoside; no evidence for an α-D-linkage was obtained. The compound was homogeneous on t.l.c. in two-solvent systems.

EXPERIMENTAL

Melting points were determined between glass slides on a Fisher–Johns apparatus and are corrected. Optical rotations were determined in semimicro tubes, with a Perkin–Elmer No. 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, with tetramethylsilane as the internal standard and chloroform-*d*

as the solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used and elution was stepwise, in order of increasing polarity of the eluants. The proportion of weight of substance to weight of adsorbent added to the column was 1 to 50–100. The fractions eluted were 2 ml/g of adsorbent. "Silicic acid" refers to Bio-Rad silicic acid, used after a preliminary heating for 60 min at 120°. Thin-layer chromatograms (and column separations where specified in the text) were performed on Kieselgel G (E. Merck, Darmstadt). Analyses were performed in the Institute's Microanalytical Laboratory under the direction of Mr. Heller. Periodate oxidations were performed by an adaptation of the spectrophotometric method[8]. A solution of sodium metaperiodate (about 0.008M) was prepared by dissolving the salt in water (3 ml) and diluting to 100 ml with absolute alcohol in a volumetric flask. A sample of the substance to be examined (10–15 mg) was dissolved in the standard metaperiodate solution (10 ml) in a volumetric flask. The two solutions were kept in the dark. Aliquots from both solutions (0.5 ml) were withdrawn and diluted to 50 ml. The relative absorbances of the two solutions were read at 223 mμ on a Perkin-Elmer 137 u.v. spectrophotometer. (The absorbance was recorded as the depth of the peak below the reading at 300 mμ which was taken as base line). The difference in relative absorbances at 223 mμ of the two solutions gave a measure of the periodate consumption. A correction was applied for the absorbance of sodium iodate measured under the same conditions (about 13% of that of an equimolar sodium metaperiodate solution).

3-O-Benzoyl-N-octadecanoyl-1-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-DL-dihydrosphingosine — N-[DL-erythro-*3-benzoyloxy-1-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)oxyoctadecan-2-yl]octadecanamide* (**3**). — Crude compound **3** was prepared from 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine [*N*-(DL-*erythro*-3-benzoyloxyoctadecan-2-yl)octadecanamide] (**10**) as described in a previous publication[4]. The product (1.0 g from 0.8 g of **10**, 44% yield) was dissolved in 3:1 benzene–ether and chromatographed on a column of silica gel G (50 g, washed with 3:1 benzene–ether). The early fractions (0.25 g total weight) eluted with benzene–ether (1:1) were homogeneous on t.l.c. in 9:1 benzene–methanol (R_F 0.8); the spot on the plate was located with iodine vapor, but no reaction was shown by the anisaldehyde–sulfuric acid reagent[9] for sugars. Crystallization from methanol gave a compound (m.p. 73–75°) identical with **10**. Later fractions, eluted from the column with 1:1 benzene–ether, migrated more slowly on t.l.c. (R_F 0.5). They were combined and dissolved in hot methanol. On cooling, a white solid precipitated (0.60 g, 26% based on **10**), m.p. 50–52°, $[\alpha]_D^{26}$ −8.3° (*c* 0.84, chloroform).

Anal. Calc. for $C_{55}H_{93}NO_{12}$: C, 68.79; H, 9.76. Found: C, 68.63; H, 9.82.

1-O-(3,4-O-Isopropylidene-β-D-galactopyranosyl)-N-octadecanoyl-DL-dihydrosphingosine — N-[DL-erythro-*1-(3,4-O-isopropylidene-β-D-galactopyranosyl)oxyoctadecan-2-yl]octadecanamide* (**7**). — A suspension of 1-*O*-(β-D-galactopyranosyl)-*N*-octadecanoyl-DL-dihydrosphingosine[4] {*N*-[DL-*erythro*-1-(β-D-galactopyranosyl)oxyoctadecan-2-yl]octadecanamide} (**6**, 1.1 g) in anhydrous acetone (200 ml) containing sulfuric acid (1 ml) was stirred mechanically for 18 h; after a few hours, complete dissolution

was obtained. Excess solid sodium carbonate was added, and the solution was filtered. The clear filtrate was concentrated *in vacuo* to a waxy solid, which was dissolved in hot methanol. On cooling, a white precipitate (0.90 g, 77%) was obtained, m.p. 67–69°; $[\alpha]_D^{35}$ +3.0° (*c* 2.65, chloroform). The product was homogeneous on t.l.c. in 65:25:4 chloroform–methanol–water, $R_{compound\ 6}$ 1.55. No consumption of periodate was observed during 48 h at 20°.

Anal. Calc. for $C_{45}H_{87}NO_8$: C, 70.17; H, 11.39. Found: C, 70.40; H, 11.37.

3-O-Acetyl-1-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-N-octadecanoyl-DL-dihydrosphingosine — N-[DL-erythro-*1-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)oxyoctadecan-2-yl]octadecanamide* (**8**). — Compound 7 (0.85 g) was acetylated with acetic anhydride–pyridine overnight at room temperature. The solution was poured onto crushed ice, and the amorphous precipitate was separated by filtration, and dried. The product (0.9 g) was crystallized from methanol to give 0.70 g (71%), m.p. 61–63°; $[\alpha]_D^{22}$ +11.4° (*c* 1.28, chloroform); n.m.r. data: τ 7.85 (3H), τ 7.92, and τ 7.96 (3 OAc).

Anal. Calc. for $C_{51}H_{93}NO_{11}$: C, 68.34; H, 10.46. Found: C, 68.75; H, 10.85.

3-O-Acetyl-1-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-N-octadecanoyl-DL-dihydrosphingosine — N-[DL-erythro-*3-acetoxy-1-(2,6-di-O-acetyl-β-D-galactopyranosyl)-oxyoctadecan-2-yl]octadecanamide* (**5**). — A solution of **8** (0.51 g), in methanol (10 ml) and 60% acetic acid (14 ml) was heated for 1 h under reflux on a steam-bath. Addition of water precipitated an amorphous solid which was shown to be homogeneous on t.l.c. in 4:1 benzene–methanol (R_F 0.5). Crystallization from methanol gave 0.45 g (92%), m.p. 53–55°; $[\alpha]_D^{24}$ +2.3° (*c* 1.67, chloroform): n.m.r. data: τ 7.94 (OAc). The product consumed 0.91 mole of periodate per mole during 24 h at 20° and 1.13 during 48 h; no further consumption was observed during the next 24 h.

Anal. Calc. for $C_{48}N_{89}NO_{11}$: C, 67.33; H, 10.48. Found: C, 67.14, H, 10.53.

3-O-Acetyl-N-octadecanoyl-1-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-DL-dihydrosphingosine — N-[DL-erythro-*3-acetoxy-1-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)oxyoctadecan-2-yl]octadecanamide* (**9**). — Compound **6** (0.10 g) was acetylated with acetic anhydride–pyridine overnight at room temperature, followed by 30 min at 50°. A few drops of methanol were added to the cooled solution, which was poured onto crushed ice. The voluminous white precipitate was separated by filtration, washed thoroughly with water, and dried to give 0.09 g (78%), m.p. 42–44°. Recrystallization from cold methanol gave a product having m.p. 44–46°; $[\alpha]_D^{20}$ −5.8° (*c* 1.32, chloroform); n.m.r. data: peaks at τ 7.83, 7.85, 7.90, 7.96, and 8.04 (OAc).

Anal. Calc. for $C_{52}H_{93}NO_{13}$: C, 66.42; H, 9.97. Found: C, 66.85; H, 10.07.

1-O-(3-O-β-D-Galactopyranosyl-β-D-galactopyranosyl)-N-octadecanoyl-DL-dihydrosphingosine — N-[DL-erythro-*1-(3-O-β-D-galactopyranosyl-β-D-galactopyranosyl)-oxyoctadecan-2-yl]octadecanamide* (**2**), *from* (**3**). — To a stirred solution of **3** (0.64 g, 0.71 mmole) in 1:1 nitromethane–benzene (50 ml)[4] at 50° were added mercuric cyanide (0.2 g) and tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**, 0.30 g, 0.72 mmole). After 18 h at 60°, an additional portion of mercuric cyanide (0.1 g), and bromide **4** (0.10 g, 0.24 mmole) were added, and the mixture was stirred for 24 h at 50°. After

being processed[4], the crude product was dissolved in anhydrous methanol and deacylated by treatment with a solution of sodium methoxide in methanol. Addition of ice precipitated an amorphous powder, which was separated by filtration, washed thoroughly with water, and dried. The product was dissolved in 19:1 chloroform–methanol and chromatographed on silicic acid. Chloroform–methanol (19:1) eluted fractions (0.24 g, 49%) which crystallized from methanol to give a compound identical with **6** on t.l.c.; m.p. 192–193°, $[\alpha]_D^{26}$ −0.4° (*c* 1.0, pyridine)[4]. Chloroform–methanol (9:1) eluted fractions (0.11 g, 19.5%) which crystallized from methanol; m.p. 207–209°, $[\alpha]_D^{20}$ +4.3° (*c* 1.16, pyridine); λ_{max}^{KBr} 890 cm^{-1} (β-D-galactopyranoside); no band at 850 cm^{-1} (α-D). The compound consumed 2.03 moles of periodate per mole during 48 h at 25°; prolongation of the oxidation for 24 h (at 35°) gave no further periodate consumption.

Anal. Calc. for $C_{48}H_{93}NO_{13}$: C, 64.61; H, 10.01. Found: C, 64.41; H, 10.36.

From **5**. — To a stirred solution of **5** (0.35 g, 0.41 mmole) in 1:1 nitromethane–benzene (30 ml) at 50° was added mercuric cyanide (0.10 g, 0.39 mmole), and bromide **4** (0.10 g, 0.24 mmole). The mixture was kept for 3 days at 50°. Additional portions of both **4** and mercuric cyanide (0.12 mmole of each) were added after 18 h and 42 h. T.l.c. in 9:1 ether–ethyl acetate showed the presence of increasing proportions of a product having $R_{compound\ 5}$ 1.1. After 3 days, the reaction mixture was processed[4], and the resulting product was deacetylated catalytically. On chromatography on silicic acid, chloroform–methanol (19:1) eluted 0.17 g (57%), which was identical with **6**. Chloroform–methanol (9:1) eluted 0.067 g (19%) which crystallized from methanol; m.p. 206–208°, $[\alpha]_D^{22}$ +4.5° (*c* 0.88, pyridine). This product was identical, on t.l.c., with the product described above; t.l.c. in chloroform–methanol–water (80:20:1) ($R_{compound\ 6}$ 0.60, $R_{compound\ X}$ 1.68) and in chloroform–methanol–aq. ammonia (80:20:0.4) ($R_{compound\ 6}$ 0.47, $R_{compound\ X}$ 2.3), **X** being 1-*O*-(6-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl)-*N*-octadecanoyl-DL-dihydrosphingosine {*N*-[DL-*erythro*-1-(6-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl)oxyoctadecan-2-yl]octadecanamide}[4].

ACKNOWLEDGMENT

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SUMMARY

The synthesis of the disaccharide-lipid 1-*O*-(3-*O*-β-D-galactopyranosyl)dihydroceramide (**2**) was achieved from two key intermediates **3** and **5**. The purification of 3-*O*-benzoyl-*N*-octadecanoyl-1-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-DL-dihydrosphingosine (**3**) is described. Reaction of **3** with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**4**), followed by catalytic deacylation and chromatographic separation afforded **2**. 1-*O*-(β-D-Galactopyranosyl)-*N*-octadecanoyl-DL-dihydrosphingosine (**6**) was converted into the acetal 1-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-*N*-

octadecanoyl-DL-dihydrosphingosine (**7**) which was acetylated and deacetonated to give 3-*O*-acetyl-1-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-*N*-octadecanoyl-DL-dihydrosphingosine (**5**). Reaction of **5** with bromide **4** afforded the disaccharide **2**. Structures **5**, **7**, and **2** were confirmed by periodate oxidation.

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THE ACID-CATALYSED CONDENSATION OF D-XYLOSE WITH BENZALDEHYDE IN THE PRESENCE OF ALCOHOLS. TWO DIASTEREOISOMERIC 1,2:3,5-DI-*O*-BENZYLIDENE-α-D-XYLOFURANOSES

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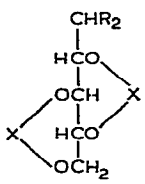
INTRODUCTION

Reaction of D-xylose with benzaldehyde–methanol mixtures in the presence of an acid catalyst affords 2,4:3,5-di-*O*-benzylidene-D-xylose dimethyl acetal[1] (**1**; R = OMe, X = CHPh), which can also be prepared in high yield by mercury-catalysed solvolysis of 2,4:3,5-di-*O*-benzylidene-D-xylose diethyl dithioacetal (**1**; R = SEt, X = CHPh) in methanol[2,3]. Although it has been used on many occasions to characterise D-xylose, and also in the quantitative determination of this sugar[1,4], the dimethyl acetal has apparently not found other use; the related 2,4-*O*-benzylidene-D-xylose dimethyl acetal, prepared after partial hydrolysis of the thioacetal (**1**; R = SEt, X = CHPh), has, however, been employed in the synthesis of 3-*O*-β-D-xylopyranosyl-D-xylose[3].



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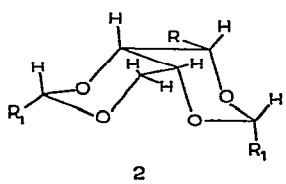

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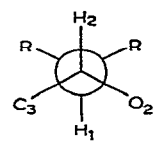

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We now report on experiments designed to provide a route to a series of di-*O*-benzylidene-D-xylose dialkyl acetals and, from them, to D-xylose dialkyl acetals, which were required for comparison with compounds detected amongst the products of alcoholysis of this pentose[5].

RESULTS AND DISCUSSION

The use of ethanol, propyl alcohol, butyl alcohol, and isobutyl alcohol in place of methanol in the initial condensation afforded the corresponding 2,4:3,5-di-*O*-benzylidene-D-xylose dialkyl acetals (**1**; R = OEt, OPr, OBu, OBu^i X = CHPh) in good yield, each of which, together with the dimethyl compound (**1**; R = OMe, X = CHPh), gave syrupy D-xylose dialkyl acetals on hydrogenolysis in 2-methoxyethanol; these were

characterised by conversion into crystalline bis(benzeneboronic) esters (**1**; R=O-alkyl, X=BPh). Previously[6], the methyl and ethyl acetals had been prepared by lengthier procedures from D-xylose diethyl dithioacetal. With ethylene glycol, under the same conditions, a cyclic ethylene acetal derivative was obtained from which the benzylidene groups were removed to give crystalline D-xylose ethylene acetal.

The 60-MHz nuclear magnetic resonance (n.m.r.) spectra of the dibenzylidene dialkyl acetals (**1**; R=O-alkyl, X=CHPh) were closely similar, except for the normal variations in the alkyl-group resonances, and were consistent with "*O*-inside", *cis*-decalin conformations [**2**; R=CH(O-alkyl)$_2$, R$_1$=Ph; $J_{4,5}$ and $J_{4,5'}$, *ca.* 2 Hz] as was expected, since (*a*) in this conformation, the group at C-2 is equatorial, and (*b*) this is the ring shape adopted by the parent bicyclic compound[7] (**2**; R=R^1=H)* and by 1,3:2,4-di-*O*-benzylidene-D-threitol[8] (**2**; R=H, R$_1$=Ph)*. Each spectrum showed two singlets for benzylic protons, with separations that increased with the size of the alkyl groups [dimethyl acetal, τ4.37, 4.37 (unresolved); diethyl acetal, 4.38, 4.41; dipropyl acetal, 4.38, 4.43; dibutyl acetal, 4.36, 4.42; di-isobutyl acetal, 4.36, 4.43], which suggests that large alkyl groups cause small, specific shieldings of the nearer benzylic protons. Alternatively, both alkyl groups on each compound showed identical resonance patterns (with tetra-*O*-acetyl-D-xylose diethyl dithioacetal, the signals from each ethyl group can be recognised[5]), suggesting that free rotation occurs about the C-1–C-2 bonds. This conclusion is supported by the finding that the H-1 splitting of 7.5 Hz observed near τ 5.15 for each dibenzylidene dialkyl acetal is unaffected by raising the temperature to 60°, and so it most probably is a weighted mean value of the sort that is observed for the ethyl group (6.7–7.2 Hz)[9], rather than a coupling constant derived from the most stable rotamer (**3**) with H-1 and H-2 opposed.

In the case of the ethylene compound (**1**; R=OCH$_2^-$, X=CHPh), the benzylic protons resonate unexceptionally at τ 4.34 and 4.37, and the other spectral features are consistent with those of the derivatives of the alkyl acetals, apart from the H-1 signal which appears as a doublet (splitting, 7.0 Hz) at τ 4.70. The deshielding of H-1 is attributed to the influence of the unshared electrons on the adjacent oxygen atoms held in the dioxolane ring in orbitals eclipsed with the C-1–H bond; this may also explain, to some extent, why benzylidene acetal protons on dioxolane rings resonate at lower fields than those on 1,3-dioxane rings[10].

Attempts to repeat the condensation reactions by using benzaldehyde together with secondary or tertiary alcohols (isopropyl alcohol, *sec*-butyl alcohol, *tert*-butyl alcohol) resulted in the isolation of crystalline, non-reducing products devoid of alkyl groups, and identified by n.m.r. spectroscopy as mixtures of di-*O*-benzylidene-D-xyloses. Thin-layer chromatography revealed the presence of two components (*A* and *B*) which were isolated after separation on a column of alumina, and had m.p 132–133°, $[\alpha]_D$+25° (chloroform), and m.p. 155–156°, $[\alpha]_D$+27° (chloroform), respectively. The only products of direct condensation of benzaldehyde and a xylose previously reported

*In these papers, 1,3:2,4-di-*O*-methylene-and 1,3:2,4-di-*O*-benzylidene-L-threitol were examined, and the conformations of the enantiomers are inferred.

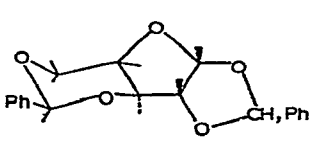
have m.p. 132°, $[\alpha]_D$ +26° (chloroform)*[11], and m.p. 130°, $[\alpha]_D$ +37.5° (methanol)**[12], respectively.

Partial hydrolysis of a mixture of isomers *A* and *B* afforded a mixture (n.m.r. analysis; see below) of monobenzylidene-D-xyloses, which did not reduce Fehlings' solution or sodium metaperiodate, and from which, on methylation and removal of the second benzylidene group, 3,5-di-*O*-methyl-D-xylose alone was obtained. The monobenzylidene compounds were consequently diastereoisomeric 1,2-*O*-benzylidene-D-xylofuranoses, and compounds *A* and *B* were 1,2:3,5-di-*O*-benzylidene-D-xylofuranoses.

The n.m.r. spectrum of compound *B* comprised a phenyl signal (10 protons), a doublet at τ 3.80 (H-1, $J_{1,2}$ 4.0 Hz), two benzylidene-acetal singlets (τ 4.10 and 4.54), a doublet at τ 5.31 (H-2, $J_{2,3}$ 0.5 Hz), and a doublet at τ 5.49 (H-3, $J_{3,4}$ 2.2 Hz) which overlapped the low-field part of the H-4, H-5e, H-5a ABC system. This was analysed by reference to computer-calculated, theoretical spectra[13], and $J_{4,5e} \simeq J_{4,5a} \simeq 2$ Hz and $J_{5a,5,e} \simeq 12$ Hz were thus obtained. The assignments were uncomplicated, except for H-3, and H-4 (τ 5.84), and were confirmed by a spin-decoupling experiment in which secondary irradiation at τ 5.8 caused decoupling of the assigned H-3 and H-5e signals. Compound *A* gave a spectrum differing in only two respects from that of *B*: (*a*) the low-field benzylidene resonance occurred at τ 3.83, instead of 4.10, and (*b*) H-3 gave a signal at τ 5.37. All other spectral features were alike; chemical shifts were within 3 Hz of the corresponding signals in the spectrum of isomer *B*, and the splittings were identical. Partial hydrolysis of both isomers exclusively removed the high-field benzylidene acetal signals (τ 4.51 and 4.54), indicating that they were derived from a common six-membered 3,5-ring, so that the two di-*O*-benzylidene-D-xyloses differ only in stereochemistry at the acetal centre of the 1,2-ring.

Since benzylic protons on dioxolane rings fused directly to five-membered cyclic systems are deshielded when *endo*[14], compounds *A* and *B* are assigned the H-*endo* and H-*exo* configurations, respectively. Consistent with this are the observations that, under kinetic control, preponderant proportions of isomer *B* are formed, whereas, at equilibrium, the compound with the phenyl groups *exo* is preponderant[14], and that H-3 is specifically shielded when the phenyl group on the dioxolane ring is *endo* (*B*).

It may be assumed, on the basis of extensive studies of benzylidene acetals[15], that the phenyl group on the 1,3-dioxane ring is equatorial in both isomers, but, since the compounds can exist with this ring in either of two slightly distorted chair conformations (**4**, "*O*-inside"; **5**, "H-inside"), this assumption does not permit a configu-



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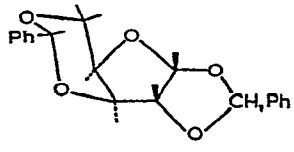

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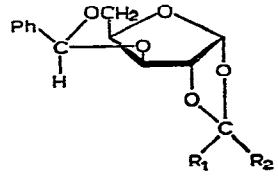

6 R_1 = H, R_2 = Ph
7 R_1 = Ph, R_2 = H

*This compound was described as the L enantiomer.

**The enantiomeric form was not indicated, but it has been assumed[11] to be L.

rational assignment at the second benzylidene centre. The H-4 and H-5 signals in the n.m.r. spectra of compounds *A* and *B*, however, allow a clear choice to be made in favour of structure **4**, since a large $J_{4,5a}$ value would be observed for conformation **5**, in which the C-4–H/C-5a–H angle is near 150°. A 4,5a splitting of 2 Hz precludes this possibility. In keeping with the "O-inside" assignment, $J_{2,3}$ is found to be < 1 Hz, and the measured dihedral angle is *ca.* 90°, whereas the coupling constants would be about 7 Hz for the alternative conformation (measured angle, *ca.* 150°).

Compounds *A* and *B* are assigned structures **6** and **7**. D-Xylose reacts with benzaldehyde, therefore, as with ketones to give 1,2:3,5-diacetals, which, on mild hydrolysis with acid, preferentially lose the six-membered ring, and, as has been found elsewhere with benzylidene derivatives[14,16], diastereoisomerism occurs only on the dioxolane system. The "O-inside" conformation adopted by those carbohydrate diacetals having a *cis*-decalin type of ring system[7,8,14] is also favoured by the oxygenated *cis*-hydrindanes.

EXPERIMENTAL

Analytical n.m.r. spectra were measured at 60 MHz and the double-resonance and elevated-temperature experiments at 100 MHz, with deuterated chloroform as solvent and tetramethylsilane as internal standard.

2,4:3,5-Di-O-benzylidene-D-xylose dialkyl acetals. — Freshly distilled benzaldehyde (20 ml) was added at 0° to a mixture of D-xylose (5 g) and dry alcohol (methanol, ethanol, propyl alcohol, butyl alcohol, isobutyl alcohol, or ethylene glycol; 100 ml) containing hydrochloric acid (4 ± 1%), and kept for 0.5 h at 0°. The heterogeneous mixtures were then allowed to warm to room temperature and kept for 2 days, after which they were again cooled to 0° and kept for a further 2 days. The crude acetals were removed by filtration, washed with water and methanol, and recrystallised from dichloromethane–methanol. The physical constants and analytical data are given in the Table, and the n.m.r. spectra were consistent with the assigned structures.

Parent acetal	*Yield* (%)	*M.p.* (*degrees*)	$[\alpha]_D$ (*degrees*) *in chloroform*	*Found, %* C	H	*Calc., %* C	H
Dimethyl[a]	70	209–210	−8	—	—	—	—
Diethyl[b]	60	185–186	−3	68.6	7.0	69.0	7.0
Dipropyl	60	168–170	−4	70.0	7.6	70.1	7.5
Dibutyl	50	160–161	−3	71.3	8.0	71.0	8.0
Di-isobutyl	30	158–159	+7	70.9	7.9	71.0	8.0
Ethylene	60	219–221	−22	67.9	6.0	68.1	6.0

[a]Lit.[1], m.p. 211–212°, $[\alpha]_D$ −9° (chloroform). [b]Lit.[6], m.p. 179°, $[\alpha]_D$ −2.8° (chloroform).

D-Xylose dialkyl acetals. — As a safeguard against acid-catalysed ring closure of the acetals, all processes, except the final evaporation, were carried out in the

presence of small amounts of basic resin (Deacidite FF, OH^-). The benzylidene acetals (1–2 g) in 2-methoxyethanol (300–500 ml) were hydrogenated at atmospheric temperature and pressure in the presence of a palladium catalyst (0.2–0.5 g, 10% on charcoal). After completion of the reaction (4 mol. of gas consumed; reaction times 0.5–3 h), the catalyst and solvent were removed to leave syrups that were taken up in water and extracted with carbon tetrachloride. The aqueous phases were taken to dryness, to give chromatographically pure products in yields of 80–95%. The $[\alpha]_D$ values (water) were: dimethyl acetal, +22° (lit.[6], +19.3°); diethyl acetal, +18° (lit.[6], +20.7°); dipropyl acetal, +18°; dibutyl acetal, +20°; di-isobutyl acetal, +18°; ethylene acetal, +1°. The last compound had m.p. 86.5–87.5° (Found: C, 43.3; H, 7.2. $C_7H_{14}O_6$ calc.: C, 43.3; H, 7.3%). The R_F values on chromatograms eluted with ethyl methyl ketone saturated with water were 0.13, 0.44, 0.78, 0.89, 0.87, and 0.10, respectively.

D-*Xylose dialkyl acetal bis(benzeneboronates)*. — The acetals (*ca.* 1 g) in *p*-dioxane (1%) were heated under reflux for 0.5 h with triphenylboroxole (0.66 mol.), and the majority of the solvent was then removed slowly by distillation. Final traces of *p*-dioxane were removed under vacuum, and the crystalline residues were recrystallized (light petroleum, b.p. 60–80°, for the dimethyl and diethyl compounds, b.p. 80–100°, for the dipropyl and ethylene compounds; aqueous methanol for the dibutyl compound) or sublimed (di-isobutyl compound).

Parent acetal	*Yield* (%)	*M.p.* (*degrees*)	$[\alpha]_D$ (*degrees*) *in* p-*dioxane*	*Found*, % *C*	*H*	*B*	*Calc.*, % *C*	*H*	*B*
Dimethyl	74	168–169	+30	61.7	6.0	6.0	62.0	6.0	5.9
Diethyl	58	150–151	+28	64.1	6.7	5.6	63.7	6.6	5.4
Dipropyl	67	135–136	+22	64.8	6.9	5.2	65.1	7.1	5.1
Dibutyl	25	114–115	+25	66.4	7.3	5.0	66.4	7.6	4.8
Di-isobutyl	75	130–132	+23	66.2	7.6	5.0	66.4	7.6	4.8
Ethylene	70	180–181	+1	62.8	5.7	6.0	62.4	5.5	5.9

1,2:3,5-Di-O-*benzylidene*-D-*xylofuranoses*. — Reactions with isopropyl alcohol, *sec*-butyl alcohol, and *tert*-butyl alcohol were carried out in the same way as with the primary alcohols, and mixed products were obtained as follows:

Alcohol used	*Yield* (%)	*M.p.* (*degrees*)	$[\alpha]_D$ (*degrees*) *in chloroform*	% endo-*phenyl isomer*	*Found* % *C*	*H*	*Calc.*, % *C*	*H*
Isopropyl	32	136–137	+26 ±1	50	69.9	5.6	69.9	5.6
sec-Butyl	32	138–143	+26 ±1	78	—	—	—	—
tert-Butyl	55	140–142	+26 ±1	75	69.9	5.5	69.9	5.6

The yield of products obtained from *tert*-butyl alcohol was increased to 70% when anhydrous sodium sulphate was incorporated in the reaction mixture as a desiccant.

The mixture (2.0 g) obtained from isopropyl alcohol, and containing equal proportions of isomers (n.m.r. analysis), was resolved on a column of alumina (Camag M.F.C., neutral) by graded elution with light petroleum (b.p. 60–80°) and ether. The resolution was followed by t.l.c. on alumina, and two chromatographically pure fractions were obtained. Eluted first was (*1,2-endo-phenyl*)-1,2:3,5-di-*O*-benzylidene-α-D-xylofuranose* (0.24 g), m.p. 155–156°, $[\alpha]_{5890}$ +27° (chloroform), $[\alpha]_{4000}$ −138°, $[\alpha]_{2990}$ +95°, $[\alpha]_{2620}$ +285°, $[\alpha]_{2380}$ +605° (Found: C, 69.7; H, 5.6. $C_{19}H_{18}O_5$ calc.: C, 69.9; H, 5.6%). This was followed by (*1,2-exo-phenyl*)-1,2:3,5-di-*O*-benzylidene-α-D-xylofuranose* (0.13 g.), m.p. 132–133°, $[\alpha]_{5890}$ +25° (chloroform). $[\alpha]_{4000}$ +75°, $[\alpha]_{3080}$ +196°, $[\alpha]_{2380}$ +422° (Found: C, 69.9; H, 5.6%).

A mixture of di-*O*-benzylidene-D-xyloses (3 g, containing 78% of the higher-melting form) was kept in benzaldehyde (40 ml) containing hydrochloric acid (2.5%). Equilibration occurred within a few hours, and, after 48 h, the mixture was neutralised, and the isomers were analysed by n.m.r. spectroscopy, and found to be present in the ratio *endo*-phenyl–*exo*-phenyl, 1:2. Heating for 30 min at 100° prior to neutralisation caused no change.

Partial hydrolysis of the di-*O*-benzylidene-D-xyloses (30 g, mixture containing 78% of 1,2-*endo*-phenyl isomer) was carried out in chloroform (150 ml) by adding methanolic hydrogen chloride (200 ml, 0.5%) and water (10 ml). After 2 days at 0°, the solution was neutralised with lead carbonate, filtered, and deionised with cationic and anionic resins. Water (100 ml) was added, and the bulk of the organic solvents removed under diminished pressure. The unhydrolysed acetals crystallised spontaneously and were removed by filtration (20 g), and the filtrate was taken to dryness. The syrupy residue was dissolved in chloroform (100 ml) and extracted with water (100 ml) to remove free D-xylose. The chloroform phase was taken to dryness to give a thick syrup (4.0 g), $[\alpha]_D$ +14° (ethanol), which solidified on trituration with light petroleum (b.p. 40–60°). T.l.c. and n.m.r. analysis showed it to contain mono-*O*-benzylidenexyloses exclusively. Similar treatment of the recovered diacetals afforded a further 4.3 g (total, 37%) of the partially hydrolysed products. They did not reduce Fehlings' solution, were not oxidised by sodium metaperiodate, and were shown by n.m.r. spectroscopy to contain two isomeric components in the ratio 78:22. Treatment with benzeneboronic acid in the usual way, with recrystallisation from light petroleum (b.p. 60–80°), afforded (*1,2-endo-phenyl*)-1,2-*O*-benzylidene-α-D-xylofuranose 3,5-benzeneboronate, m.p. 116–7°, $[\alpha]_D$ −6° (*p*-dioxane) (Found: C, 66.8; H, 5.5; B, 3.3. $C_{18}H_{17}BO_5$ calc.: C, 67.0; H, 5.25; B, 3.3%).

The mixed monoacetals (2.9 g) were treated with sodium hydride (1.5 g) in tetrahydrofuran (100 ml) for 3 h at room temperature, methyl iodide (30 ml) was added, and the mixture was kept for 18 h. After removal of the solids and the solvent, the residue was extracted with dichloromethane. Removal of this solvent gave a

*In the absence of an accepted rule, it is convenient to indicate the stereochemistry at the acetal carbon atom in these compounds by reference to the position of the phenyl group with respect to the fused-ring system.

syrup (2.7 g, 84%), $[\alpha]_D$ −8° (chloroform), which was homogeneous on thin-layer chromatograms and gave an n.m.r. spectrum consistent with that to be expected from 1,2-*O*-benzylidene-3,5-di-*O*-methyl-α-D-xylofuranoses. The dimethyl compound (2.5 g) was hydrogenolysed in 2-methoxyethanol (50 ml) in the presence of palladium-on-charcoal (1.7 g, 10%); 2 mol. of gas were consumed, and removal of the catalyst and solvent afforded 3,5-di-*O*-methyl-D-xylose (1.5 g, 90%, $[\alpha]_D$ +23° (water), +15° (chloroform) [lit.[17], +23° (water); +11° (chloroform)]. It was indistinguishable from 3,5-di-*O*-methylxylose on paper chromatograms, and reduced Fehlings' solution and sodium metaperiodate (1.0 mol., spectrophotometric determination[18]). Treatment of the free sugar (0.52 g) with triphenylboroxole (0.31 g, 0.33 mol.) in *p*-dioxane (30 ml), with slow removal of the solvent, gave a syrupy residue that, on distillation, afforded 3,5-di-*O*-methyl-α-D-xylofuranose 1,2-benzeneboronate (0.46 g, 60%), m.p. 53–54° [from light petroleum (b.p. 60–80°)], $[\alpha]_D$ −8° (*c* 1, *p*-dioxane) [lit.[17], m.p. 54–55°, $[\alpha]_D$ −9° (*p*-dioxane)]. The infrared spectrum was identical to that of an authentic sample and a mixed melting point was undepressed.

SUMMARY

D-Xylose condenses with benzaldehyde–primary alcohol mixtures to give 2,4:3,5-di-*O*-benzylidene-D-xylose dialkyl acetals from which the dialkyl acetals can be obtained by hydrogenolysis. Ethylene glycol affords the analogous ethylene acetal derivatives. With benzaldehyde–secondary or tertiary alcohol mixtures, two 1,2:3,5-di-*O*-benzylidene-D-xylofuranoses, which differ only in their stereochemistry at the new acetal centre on the 1,2-ring, are formed. The absolute configurations at the benzylidene positions are assigned, and the conformations of the six-membered 3,5-rings are found to be "*O*-inside".

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NITROGEN-CONTAINING CARBOHYDRATE DERIVATIVES.

PART IV*. STUDIES ON THE OPTICAL ROTATORY DISPERSION AND CIRCULAR DICHROISM OF CARBOHYDRATE *C*-NITRO ALCOHOLS. III

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INTRODUCTION

The nitromethane synthesis of Sowden and Fischer[1,2], one of the methods for lengthening the carbon chain of monosaccharides, is composed of two reactions. The first is the condensation of an aldose with nitromethane to produce a carbohydrate *C*-nitro alcohol, and the second is decomposition (according to Nef) of the nitro alcohol to give an aldose having an additional carbon atom. Generally, the first reaction gives an epimeric pair of carbohydrate *C*-nitro alcohols, whose configuration at C-2 has been established by the structure of the aldoses derived from them. This assignment has usually been made by comparing with each other the values of the molecular optical rotation of each of the aldoses[1-5], and also by comparing with each other the values of the aldonic acids[6] that are obtained by oxidation.

The nitromethane method has been applied to the synthesis of 2-amino-2-deoxy sugars[7-9]. When the acetylated nitro alcohols in methanol were treated with anhydrous ammonia[7], epimeric mixtures of 2-acetamido-2-deoxy-1-nitro alcohols were obtained. The same result was obtained, regardless of whether the starting material was a mixture of acetylated epimeric nitro alcohols or one of the epimers. The 2-acetamido-2-deoxy-1-nitro alcohols were converted into 2-acetamido-2-deoxy sugars by removal of the nitro group. In this case, determination of the configuration at C-2 was also an important problem. The sequence for the synthesis of aldoses and 2-acetamido-2-deoxy sugars is illustrated in the scheme on the next page.

In a preliminary communication[10], we reported the optical rotatory dispersion of carbohydrate *C*-nitro alcohols, and discussed the relation between the absolute configuration at C-2 of carbohydrate *C*-nitro alcohols and the sign of the Cotton effect. We have recently examined the circular dichroism (c.d.) curves of the carbohydrate *C*-nitro alcohols, such as 1-nitro-1-deoxy alcohols and 2-acetamido-1,2-dideoxy-1-nitro alcohols, in parallel with the o.r.d. curves, and have again found that the signs, not only of the Cotton effect, but also of the c.d. maximum (or shoulder) at about 310 mμ, are closely related to the absolute configuration at C-2 of the carbohydrate *C*-nitro alcohols.

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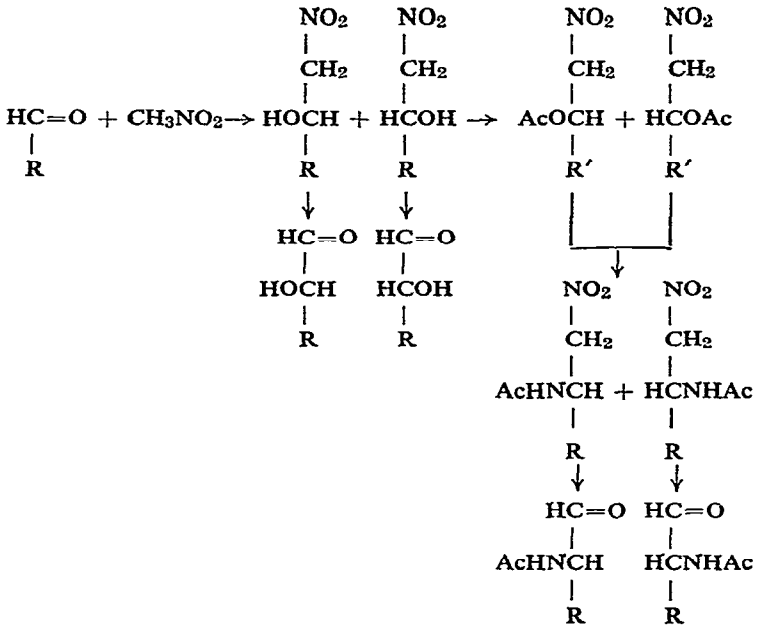
*Part III; CHIYOKO SATOH AND AKIO KIYOMOTO, *Carbohyd. Res.* 3 (1966) 248.



Several reports on the c.d. and o.r.d. curves of nitro steroids[11] have been published, and the relation between the conformation and the signs of the ellipticity and/or the Cotton effect has been discussed[12]. No report has, however, been published describing the usefulness of the o.r.d. and c.d. data for the determination of the stereochemistry of acyclic *C*-nitro compounds.

DISCUSSION

The o.r.d. and c.d. curves, and the ultraviolet spectra, of four epimeric pairs of carbohydrate *C*-nitro alcohols are given in Figs. 1 to 8. All of the compounds examined exhibited anomalous o.r.d. curves having the first extremum at about 340 mμ. In the u.v. absorption spectra, these compounds showed a peak (or a shoulder) in the 280–290 mμ region, but this band was not associated with the first extremum of the Cotton effect. C.d. curves of carbohydrate *C*-nitro alcohols indicate the presence of two optically active absorptions, shown by maxima at 310 mμ (or, frequently, a shoulder) and at 280 mμ*. It is evident that a maximum (or a shoulder) at 310 mμ in the c.d. curves is associated with the Cotton effect. The Cotton effect due to the absorption around 280 mμ is almost hidden, overlapping the Cotton effect due to the absorption in the longer wavelength region.

As shown in Figs. 1 to 8, 1-deoxy-1-nitro-D-glucitol (**1**)[1,2], 1-deoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (**3**)[4], 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol (**5**)[5], and 2-acetamido-1,2-dideoxy-1-nitro-D-glucitol (**7**)[7–9] showed positive Cotton effects

*Nitro steroids[11,12] have been shown to give two c.d. maxima, at 270–290 and 330 mμ. The former maximum is usually associated with the Cotton effect of nitro steroids.

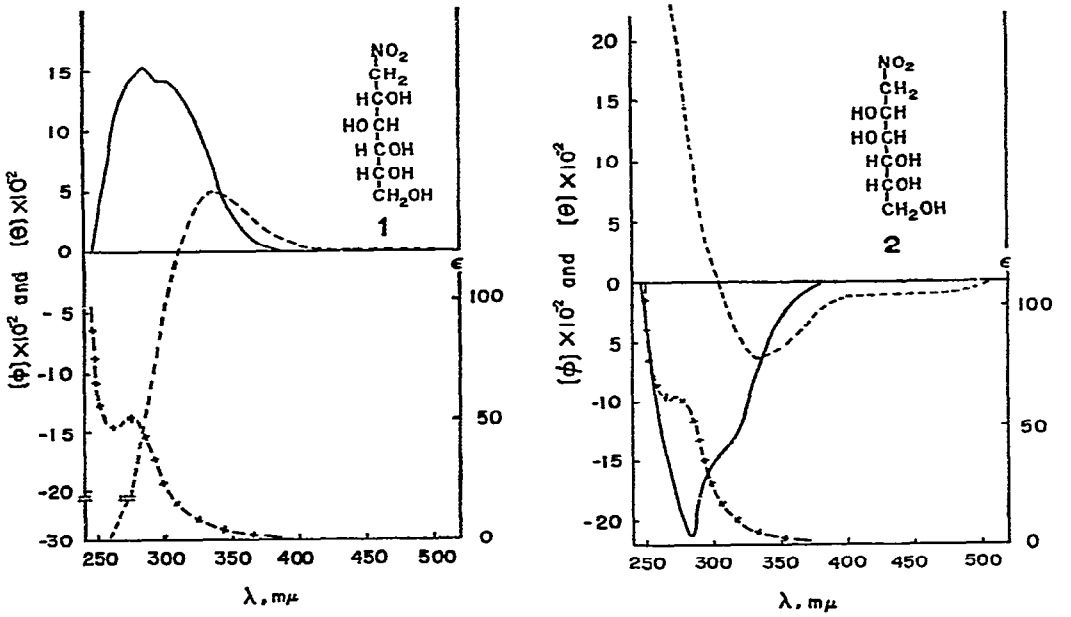


Fig. 1. Circular dichroism (————), optical rotatory dispersion (— — — —), and ultraviolet absorption (+ — + — +) curves of 1-deoxy-1-nitro-D-glucitol (1) in water.
Fig. 2 Circular dichroism (————), optical rotatory dispersion (— — — —), and ultraviolet absorption (+ — + — +) curves of 1-deoxy-1-nitro-D-mannitol (2) in water.

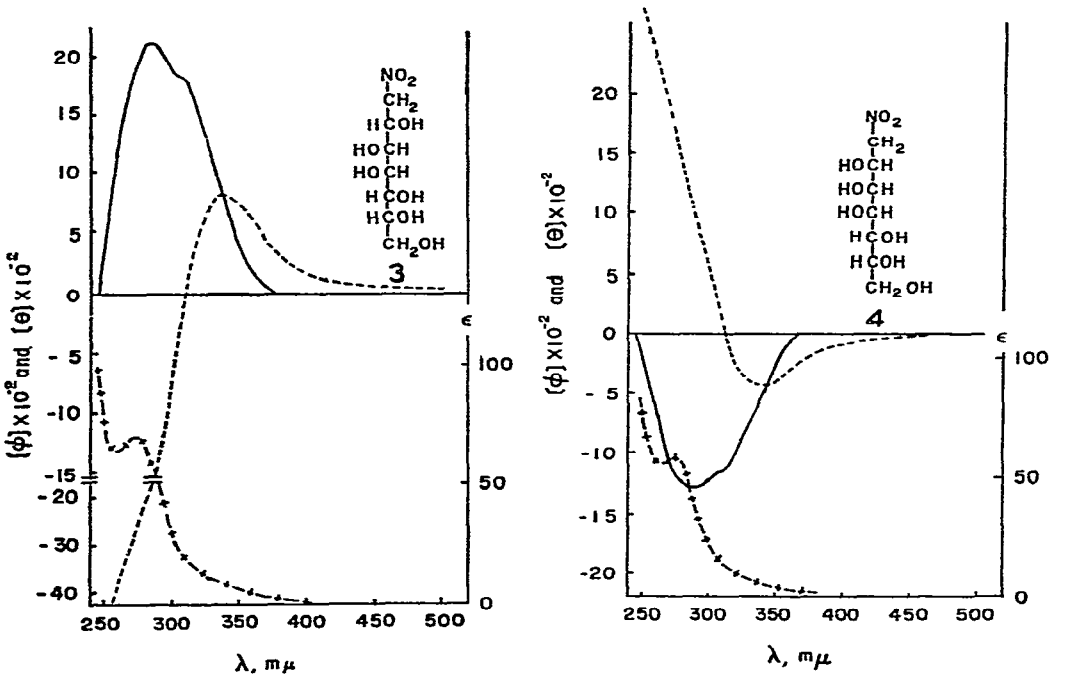


Fig. 3. Circular dichroism (————), optical rotatory dispersion (— — — —), and ultraviolet absorption (+ — + — +) curves of 1-deoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (3) in water.
Fig. 4. Circular dichroism (————), optical rotatory dispersion (— — — —), and ultraviolet absorption (+ — + — +) curves of 1-deoxy-1-nitro-D-*glycero*-D-*talo*-heptitol (4) in water.

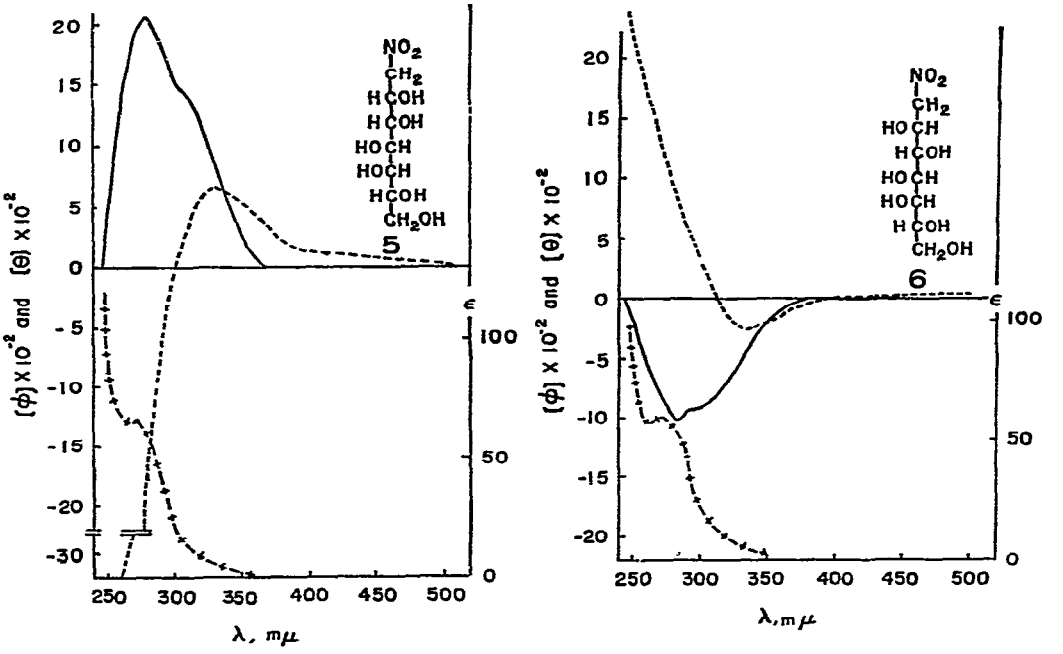


Fig. 5. Circular dichroism (————), optical rotatory dispersion (— — — — —), and ultraviolet absorption (+ − + − +) curves of 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol (5) in water.
Fig. 6. Circular dichroism (————), optical rotatory dispersion (— — — — —), and ultraviolet absorption (+ − + − +) curves of 1-deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol (6) in water.

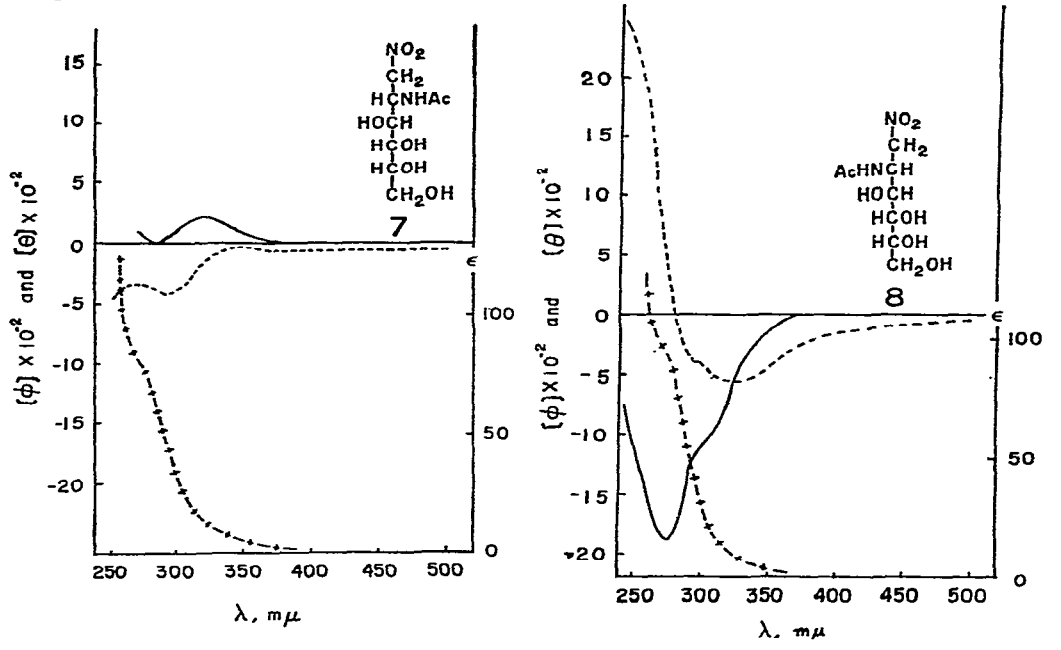


Fig. 7. Circular dichroism (————), optical rotatory dispersion (— — — — —), and ultraviolet absorption (+ − + − +) curves of 2-acetamido-1,2-dideoxy-1-nitro-D-glucitol (7) in water.
Fig. 8. Circular dichroism (— — — — —), optical rotatory dispersion (————), and ultraviolet absorption (+ − + − +) curves of 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol (8) in water.

centered at 310 mμ, whereas their epimers, 1-deoxy-1-nitro-D-mannitol (**2**)[1,2], 1-deoxy-1-nitro-D-*glycero*-D-*talo*-heptitol (**4**)[4], 1-deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol (**6**)[5], and 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol (**8**)[7-9] exhibited negative Cotton-effect curves. The c.d. curves of the first group of compounds showed a positive maximum (or a shoulder) near 310 mμ and a positive maximum at about 280 mμ. The second group of compounds exhibited c.d. curves having similar maxima or shoulders, but of the opposite sign. This marked difference obviously reflects the opposite absolute configuration at C-2 adjacent to the nitromethyl group. The absolute configuration of compounds **1**, **3**, **5**, and **7** at C-2 is S, whereas that of **2**, **4**, **6**, and **8** at C-2 is R (by the Cahn, Ingold, and Prelog convention[13]). Therefore, the following rules are now proposed relating the absolute configuration at C-2 of carbohydrate *C*-nitro alcohols and the signs of the Cotton effect at about 310 mμ and of the ellipticity of the band at 310 mμ.

1. Carbohydrate *C*-nitro alcohols in which the asymmetric carbon atom adjacent to the nitromethyl group belongs to the S-series exhibit positive Cotton effects and positive c.d. maxima (or shoulders), whereas the *C*-nitro alcohols in which the adjacent carbon atom has the R-configuration show negative Cotton effects and negative c.d. maxima (or shoulders).

2. The sign of the Cotton effect or the ellipticity is not affected by substitution at C-2, whether by a hydroxyl or an acetamido group.

3. The absolute configurations of other asymmetric centers do not contribute to the sign of the Cotton effect.

For all carbohydrate *C*-nitro alcohols thus far examined, the sign of the ellipticity of the 310-mμ band is the same as that of the 280 mμ band.

The usefulness, as well as the validity, of these rules has been further demonstrated by examination of the two epimeric pairs of 2-acetamido-1,2-dideoxy-1-nitro alcohols, (**9**) and (**10**), and (**11**) and (**12**).

When the epimeric heptitols, **9** and **10**, were synthesized[14] by the nitromethane method, the absolute configuration at C-2 of the resulting products remained to be determined. On the basis of the o.r.d. and c.d. curves (Figs. 9 and 10) and of the above-mentioned rules, the structure of 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-D-*talo*-heptitol (R-configuration at C-2) (**10**) was predicted for the one that showed a negative Cotton effect at about 310 mμ and negative ellipticity at about 310 mμ. This structure was proved to be correct by chemical determination[15] of the absolute configuration at C-2 of **10**. Accordingly, the compound that showed a positive Cotton effect and positive ellipticity is 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (S-configuration at C-2) (**9**).

The absolute configuration at C-2 of another epimeric pair, namely **11** and **12**, (Figs. 11 and 12) was determined in the same way[14]. In this case also, the structure based on the above-mentioned rules was shown to be correct by chemical determination: the compound that exhibits a positive Cotton effect centered at 310 mμ and positive ellipticity at 310 mμ is 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-L-*manno*-heptitol (S-configuration at C-2) (**11**), and the compound that shows a negative

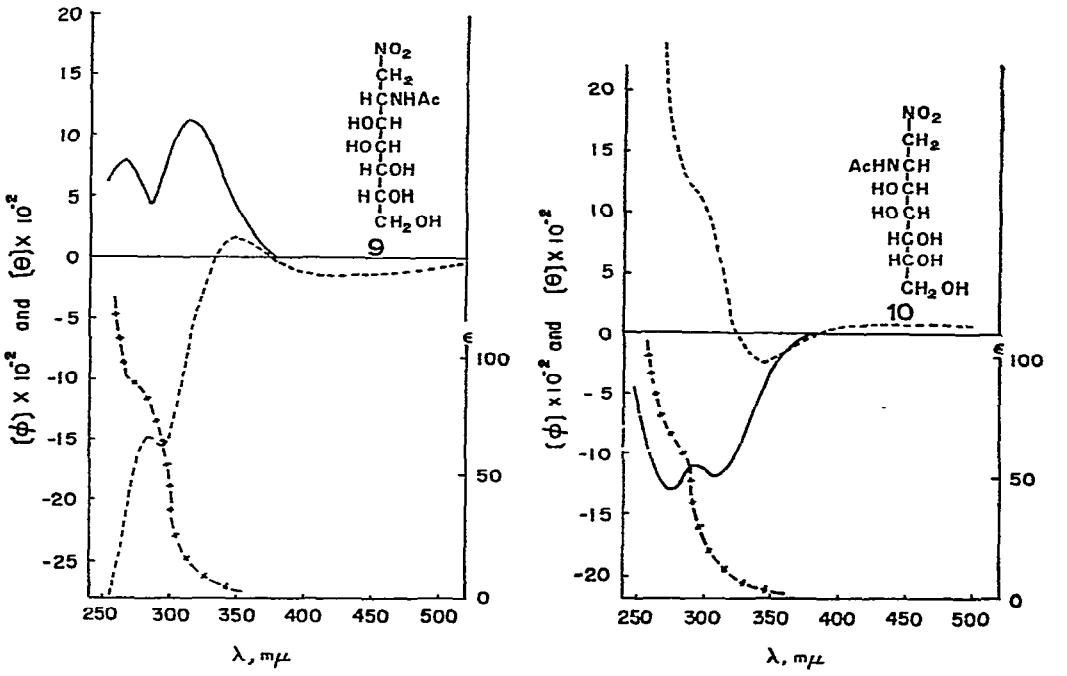


Fig. 9. Circular dichroism (————), optical rotatory dispertion (— — — —), and ultraviolet absorption (+ — + — + curves of 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (9) in water.

Fig. 10. Circular dichroism (————), optical rotatory dispersion (— — — —), and ultraviolet absorption (+ — + — +) curves of 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-D-*talo*-heptitol (10) in water.

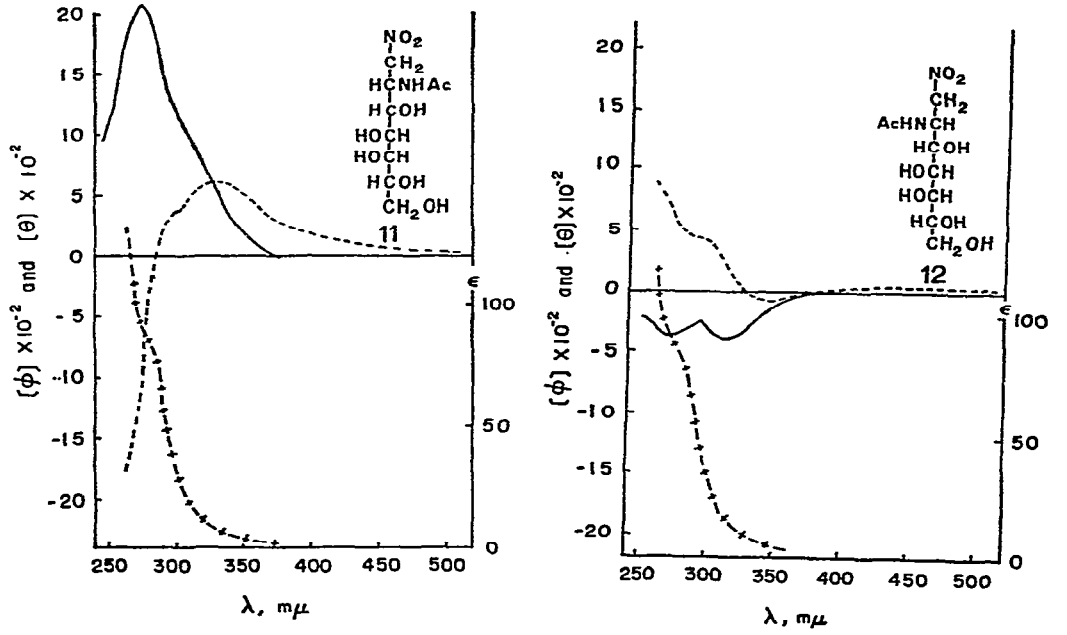


Fig. 11. Circular dichroism (— — — —), optical rotatory dispersion (————), and ultraviolet absorption (+ — + — +) curves of 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-L-*manno*-heptitol (11) in water.

Fig. 12. Circular dichroism (————), optical rotatory dispersion (— — — —), and ultraviolet absorption (+ — + — +) curves of 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol (12) in water.

Cotton effect and negative ellipticity is 2-acetamido-1,2-dideoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol (R-configuration at C-2) (**12**).

Thus, the absolute configuration at C-2 of epimeric pairs of carbohydrate *C*-nitro alcohols may be readily determined by use of the proposed rules.

EXPERIMENTAL

General. — The optical rotatory dispersion and circular dichroism of carbohydrate *C*-nitro alcohols were measured in water with a JASCO Model ORD/UV-5 automatically recording spectropolarimeter, Japan Spectroscopic Manufacturing Co. (Nippon Bunko).

The molecular rotation $[\phi]$ is defined by

$$[\phi] = \frac{[\alpha]M}{100} = \frac{M\alpha_r\, Sc}{c\,l}$$

where α_r is the reading of the rotation in degrees, M is the molecular weight, c is the concentration in g per 100 ml of solution, l is the path length in dm, and Sc is the scale setting.

The molecular ellipticity $[\theta]$ is defined by

$$[\theta] = \frac{3300\theta_r Sc}{c\,l}$$

where θ_r is the reading of ellipticity, c is the concentration in moles per liter, l is the path length in cm, and Sc is the scale setting.

The ultraviolet absorption spectra were measured on water solutions with a Hitachi recording spectrophotometer (Type EPS-2U).

1-Deoxy-1-nitro-D-*glucitol* (**1**) (*Fig. 1*). — O.r.d. (*c* 0.287): $[\phi]_{338-334}$ 490°. C.d. (*c* 0.0136): $[\theta]_{304-296}$ 1409, $[\theta]_{284}$ 1531. λ^{H_2O} 300 mμ (ε 21), $\lambda^{H_2O}_{max}$ 276–273 mμ (ε 51).

1-Deoxy-1-nitro-D-*mannitol* (**2**) (*Fig. 2*). — O.r.d. (*c* 0.30): $[\phi]_{334-332}$ −633°. C.d. (*c* 0.0141): $[\theta]_{310}$ −1369, $[\theta]_{280-276}$ −2135. λ^{H_2O} 310 mμ (ε 12), $\lambda^{H_2O}_{max}$ 273 mμ (ε 62).

1-Deoxy-1-nitro-D-glycero-D-galacto-*heptitol* (**3**) (*Fig. 3*). — O.r.d. (*c* 0.234): $[\phi]_{340-336}$ 824°. C.d. (*c* 0.0097): $[\theta]_{310}$ 1802, $[\theta]_{288-280}$ 2108. λ^{H_2O} 310 mμ (ε 18.5), $\lambda^{H_2O}_{max}$ 276–274 mμ (ε 71).

1-Deoxy-1-nitro-D-glycero-D-talo-*heptitol* (**4**) (*Fig. 4*). — O.r.d. (*c* 0.207): $[\phi]_{340-336}$ −445°. C.d. (*c* 0.0086): $[\theta]_{310}$ −1158, $[\theta]_{290-286}$ −1293. λ^{H_2O} 310 mμ (ε 13), $\lambda^{H_2O}_{max}$ 276–274 mμ (ε 58).

1-Deoxy-1-nitro-D-glycero-L-manno-*heptitol* (**5**) (*Fig. 5*). — O.r.d. (*c* 0.21): $[\phi]_{332-330}$ 667°. C.d. (*c* 0.0087): $[\theta]_{310}$1444, $[\theta]_{280}$ 2071. λ^{H_2O} 310 mμ (ε 12.5), $\lambda^{H_2O}_{max}$ 272 mμ (ε 66).

1-Deoxy-1-nitro-D-glycero-L-gluco-*heptitol* (**6**) (*Fig. 6*). — O.r.d. (*c* 0.226): $[\phi]_{337-333}$ −256°. C.d. (*c* 0.0094): $[\theta]_{300}$ −915, $[\theta]_{283}$ −1021. λ^{H_2O} 300 mμ (ε 23), $\lambda^{H_2O}_{max}$ 274 mμ (ε 60).

2-Acetamido-1,2-dideoxy-1-nitro-D-*glucitol* (**7**) (*Fig. 7*). — O.r.d. (*c* 0.862): $[\phi]_{350}$ −35°, $[\phi]_{292}$ −421°, $[\phi]_{270}$ −345°. C.d. (*c* 0.0342): $[\theta]_{320}$ 222. λ^{H_2O} 320 mμ (ε 14.5), $\lambda^{H_2O}_{shoulder}$ 290–280 mμ (ε 55–72).

2-Acetamido-1,2-dideoxy-1-nitro-D-*mannitol* (**8**) (*Fig. 8*). — O.r.d. (*c* 0.224): $[\phi]_{325}$ −563°, $[\phi]_{294}$ −394°. C.d. (*c* 0.0089): $[\theta]_{300}$ −1106, $[\theta]_{276}$ −1894. λ^{H_2O} 300 mμ (ε 35), $\lambda^{H_2O}_{shoulder}$ 282–270 mμ (ε 80–98).

2-Acetamido-1,2-dideoxy-1-nitro-D-glycero-D-galacto-*heptitol* (**9**) (*Fig. 9*). — Recrystallized from ethanol, m.p. 159–160.5° (dec.). O.r.d. (*c* 0.297): $[\phi]_{347}$ 171°, $[\phi]_{294}$ −1558°, $[\phi]_{283}$ −1482°. C.d. (*c* 0.0105): $[\theta]_{313}$ 1130, $[\theta]_{266}$ 801. λ^{H_2O} 313 mμ (ε 16), $\lambda^{H_2O}_{shoulder}$ 280–270 mμ (ε 84–90).

Anal. Calc. for $C_9H_{18}N_2O_8$: C, 38.29; H, 6.38; N, 9.92. Found: C, 38.62; H, 6.17; N, 9.94.

2-Acetamido-1,2-dideoxy-1-nitro-D-glycero-D-talo-*heptitol* (**10**) (*Fig. 10*). — Recrystallized from methanol, m.p. 180–181° (dec.). O.r.d. (*c* 0.293): $[\phi]_{346-343}$ −236°, $[\phi]_{297-292}$ 1158°. C.d. (*c* 0.0104): $[\theta]_{309-306}$ −1185, $[\theta]_{276}$ −1304. λ^{H_2O} 310 mμ (ε 15), $\lambda^{H_2O}_{shoulder}$ 280–270 mμ (ε 64–73).

Anal. Calc. for $C_9H_{18}N_2O_8$: C, 38.29; H, 6.38; N, 9.92. Found: C, 38.87; H, 6.13; N, 10.02.

2-Acetamido-1,2-dideoxy-1-nitro-D-glycero-L-manno-*heptitol* (**11**) (*Fig. 11*). — Recrystallized from methanol, m.p. 189–190° (dec.). O.r.d. (*c* 0.307): $[\phi]_{332-328}$ 607°, $[\phi]_{305}$ 405°. C.d. (*c* 0.0109): $[\theta]_{310}$ 942, $[\theta]_{275}$ 2067. λ^{H_2O} 310 mμ (ε 17), $\lambda^{H_2O}_{shoulder}$ 290–280 mμ (ε 60–84).

Anal. Calc. for $C_9H_{18}N_2O_8$: C, 38.29; H, 6.38; N, 9.92. Found: C, 38.57; H, 6.33; N, 9.63.

2-Acetamido-1,2-dideoxy-1-nitro-D-glycero-L-gluco-*heptitol* (**12**) (*Fig. 12*). — Recrystallized from methanol, m.p. 144.5–145.5° (dec.). O.r.d. (*c* 0.319): $[\phi]_{344}$ −80°, $[\phi]_{294}$ 425°. C.d. (*c* 0.0113): $[\theta]_{316-310}$ −409, $[\theta]_{270}$ −394. λ^{H_2O} 310 mμ (ε 19), $\lambda^{H_2O}_{shoulder}$ 290–280 mμ (ε 63–83).

Anal. Calc. for $C_9H_{18}N_2O_8$: C, 38.29; H, 6.38; N, 9.92. Found: C, 37.85; H, 6.16; N, 9.77.

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SUMMARY

The optical rotatory dispersion and circular dichroism of carbohydrate *C*-nitro alcohols were measured. Evidence is presented that the signs of the Cotton effect about 310 mμ and of the ellipticity of the 310 mμ band are closely related to the absolute configuration at C-2 of carbohydrate *C*-nitro alcohols. Carbohydrate *C*-nitro

alcohols in which the asymmetric carbon atom adjacent to the nitro methyl group belongs to the s-series exhibit positive Cotton effects and positive c.d. maxima (or shoulders), whereas *C*-nitro alcohols in which the adjacent carbon atom is of the R-series show negative Cotton effects and negative c.d. maxima (or shoulders). These deductions permit the ready determination of the absolute configuration of epimeric nitro alcohols obtained by the nitromethane synthesis for elongation of carbohydrate chains.

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SYNTHESIS OF 1,6-ANHYDRO-β-D-TALOPYRANOSE FROM DERIVATIVES OF D-GALACTOSE AND D-MANNOSE*†

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(Received May 13th, 1967)

Seven of the eight possible 1,6-anhydro-β-D-aldohexopyranoses have been described[2]. This paper reports the synthesis of the remaining isomer, 1,6-anhydro-β-D-talopyranose[1] (**6**), by two independent, high-yielding routes. One route involves net inversion at C-2 in 1,6-anhydro-β-D-galactopyranose, and the other entails net inversion at C-4 in 1,6-anhydro-β-D-mannopyranose. In each case, the *cis*-diol group in the precursor is protected by acetonation, the remaining secondary alcohol group is oxidized to the corresponding ketone, and the ketones are reduced stereospecifically to products having the D-*talo* configuration.

The 3,4-isopropylidene acetal (**1**) of 1,6-anhydro-β-D-galactopyranose, obtained[3] from lactose by pyrolysis followed by acetonation, was oxidized[4] with methyl sulfoxide–acetic anhydride or with ruthenium tetraoxide to give 1,6-anhydro-3,4-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-2-ulose (**2**), obtained crystalline in 85–90% yield. Reduction of the ketone **2** with lithium aluminum hydride in ether gave quantitative conversion into 1,6-anhydro-3,4-*O*-isopropylidene-β-D-talopyranose (**3**), which was also obtained crystalline. Mild, acid hydrolysis of **3** cleaved the *O*-isopropylidene group to give 1,6-anhydro-β-D-talopyranose (**6**) in high yield. Hydrolysis of **6** under more vigorous conditions gave a mixture of products from which crystalline D-talose was isolated; it gave an X-ray powder diffraction pattern identical with that of an authentic sample[5] of α-D-talose. The isolation of D-talose provides proof that the products **3** and **6** have the D-*talo* configuration, and supports the structural assignment made for the ketone **2**.

Reduction of the ketone **2** with sodium borohydride gave, again stereospecifically, the D-*talo* derivative **3**. The yield (84%) was somewhat lower than when lithium aluminum hydride was used, probably because of partial hydrolysis of **3** during the isolation procedure. The stereospecificity of the reduction of **2** to **3** was verified by g.l.c. of **1** and **3** as their 2-trimethylsilyl ethers. The latter were readily separated, and it was possible to detect $<1\%$ of **1** in admixture with **3**. None of the D-galactose derivative (**1**) was detected in the product when the ketone **2** was reduced with lithium aluminum hydride or with sodium borohydride. When the ketone **2** was reduced with

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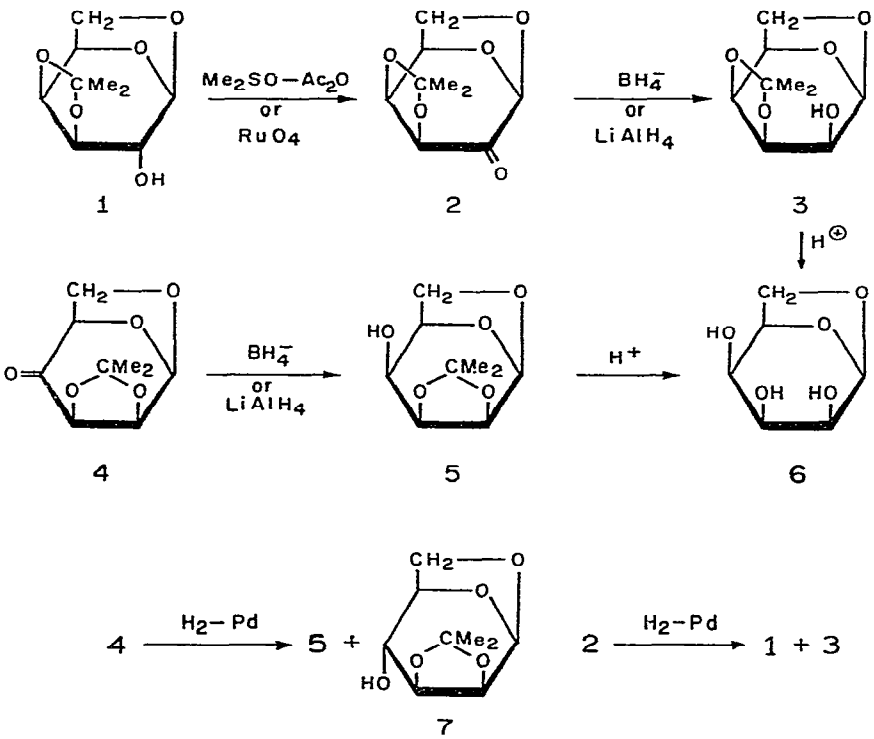
†For a preliminary communication, see ref. 1.

hydrogen over palladium, there resulted a 1:1 mixture of the D-*galacto* (**1**) and D-*talo* (**3**) derivatives, as revealed by t.l.c. and by g.l.c. of the 2-*O*-trimethylsilyl derivatives.

The conversion of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (**7**), obtained[6] from ivory-nut D-mannan by pyrolysis followed by acetonation, into 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (**4**) by oxidation with methyl sulfoxide–acetic anhydride, or with ruthenium tetraoxide, has already been described[4]. Reduction of the 4-ketone (**4**) with lithium aluminum hydride in ether gave



1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (**5**), obtained crystalline in quantitative yield. Similar results were obtained when sodium borohydride was used, although the yield (77%) was somewhat lower, probably because of partial hydrolysis during isolation. Again, g.l.c. (of the 4-trimethylsilyl ethers) indicated that the reduction with each reagent was stereospecific. When the reduction of **4** was effected with hydrogen over palladium, the reaction gave a 3:1 mixture of **5** and the parent D-*manno* derivative **7**, as verified by t.l.c., by direct isolation, and by g.l.c. of the trimethylsilyl ethers.

Mild hydrolysis of **5** to remove the *O*-isopropylidene group gave 1,6-anhydro-β-D-talopyranose (**6**), identical in all respects with **6** obtained by way of a D-galactose precursor.

Acetylation of **6** gave crystalline 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-talopyranose (**8**). The n.m.r. spectrum of this product in benzene-d_6 (see Fig. 1) was interpreted in terms of first-order vicinal and geminal couplings, together with extensive

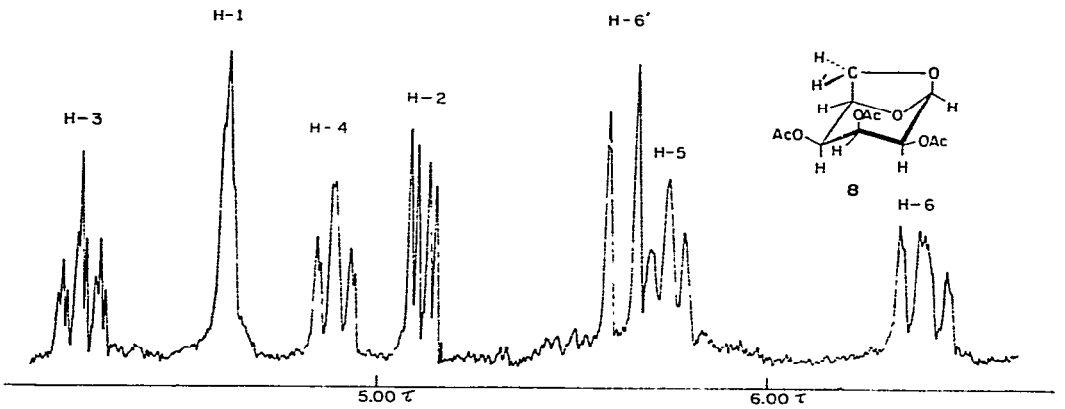


Fig. 1. The n.m.r. spectrum of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-talopyranose (**8**) in benzene-d_6 at 100 MHz.

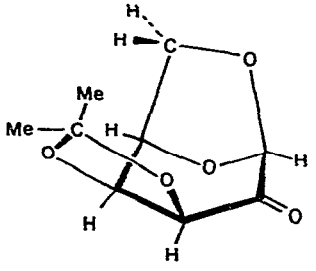
long-range couplings. The signal at lowest field, a triplet of narrow triplets, was assigned to the equatorial hydrogen atom at C-3; equal coupling (4.7 Hz) of this proton with the axial protons at C-2 and C-4 caused splitting into the principal triplet. Long-range coupling of 1,3-diequatorial protons ("W" conformation)[7-9] is possible with H-1 and with H-5, to give the observed splitting of each principal line in the H-3 signal into a narrow triplet ($J_{1,3} \approx J_{3,5} = 1.1$ Hz). The C-2 proton is axial and cannot exhibit this type of long-range coupling, and its signal is readily recognized as a sharp quartet at τ 5.12, from which the $J_{1,2}$ coupling (1.7 Hz) can be determined. The H-1 signal is not observed as a simple quartet through coupling with H-2 and long-range coupling with H-3, but exhibits additional multiplicity indicative of long-range coupling with H-5 ("W" conformation across the C-5 ring-oxygen atom) and possibly with H-6 (*exo*) ("W" conformation across the oxygen atom of the anhydro bridge). The H-4 signal is observed as a triplet of narrow doublets; the principal splitting indicates equal coupling of the axial H-4 with the equatorial protons at C-3 and C-5, and the narrow splitting of each line indicates long-range coupling of H-4 with H-6 (*exo*) through the "W"arrangement of bonds.

The signal at highest field was assigned to H-6 (*exo*). In addition to geminal coupling and coupling to H-5, additional multiplicity is caused by long-range coupling to H-4, and probably also to H-1. A sharp doublet observed at τ 5.63 was assigned to H-6(*endo*); its appearance indicated that geminal coupling, only, is involved, and that the coupling of this proton with H-5 is approximately zero. This is as would be anticipated for a projected-bond angle between H-5 and H-6(*endo*) of approximately 90°. In consequence, second-order effects between the H-6(*endo*) and the H-5 signals are not apparent, and the latter signal is observed as a triplet of narrow multiplets in

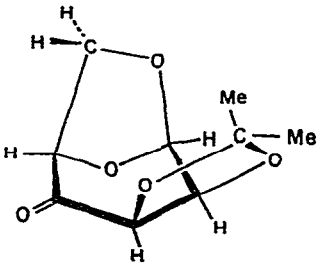
which principal couplings of H-5 with H-4 and H-6(*exo*) give rise to the large spacings, and long-range couplings with H-3 and H-1 cause additional multiplicity.

The n.m.r. data for the triacetate (**8**) are fully consistent with the pyranoid structure, and cannot be rationalized in terms of a furanoid form. This provides independent verification that the precursors **6**, **5**, **3**, and **2** have the pyranoid structure, also.

The hydride reducing agents appear to attack the ketones **2** and **4** (depicted as perspective representations in **2a** and **4a**) exclusively from the non-hindered side, below the ring; the two axial substituents that flank the carbonyl group presumably inhibit approach of the nucleophilic hydride species to the "top" face of the carbonyl group. Reduction with hydrogen over palladium proceeds from either side with equal facility in the case of **2**; but, with **4**, the favored mode of reaction is still by attack from he lower side of the molecule.



2a



4a

Either route to **6** provides an excellent preparative method from accessible starting materials, and the yields can be improved further if the intermediate ketones are not isolated (see Experimental section). The ketones **2** and **4** are of considerable interest in synthesis, as they offer a route[1] to derivatives of D-galactose, D-mannose, and D-talose that are specifically deuterated or tritiated at various positions. They can be ethynylated stereospecifically in high yield as a route to branched-chain sugars, reduction of their oximes provides a high-yielding route to amino sugars having the D-*talo* configuration, and they can be alkylated at C-3. Details of these investigations will be reported separately.

The ketones **2** and **4** both exhibit optically active absorption that corresponds to the n→π^* transition of a carbonyl group in an asymmetric environment[10,11]. The 4-ketone **4** shows an absorption maximum at 300 nm (ε 50), a negative Cotton effect in its o.r.d. spectrum (see Fig. 2), and a negative c.d. curve (see Fig. 2) having greatest magnitude at 304 nm. The 2-ketone **2** shows the n→π^* absorption in the u.v. at 330 nm, with a corresponding negative c.d. curve (see Fig. 3) centered at 330 nm; the o.r.d. curve (see Fig. 3) likewise shows a negative Cotton effect. It is noteworthy that the ketone **2**, which has one more oxygen atom on an adjacent carbon atom than the ketone **4**, shows the absorption band at a wavelength that is particularly long for a saturated ketone[12].

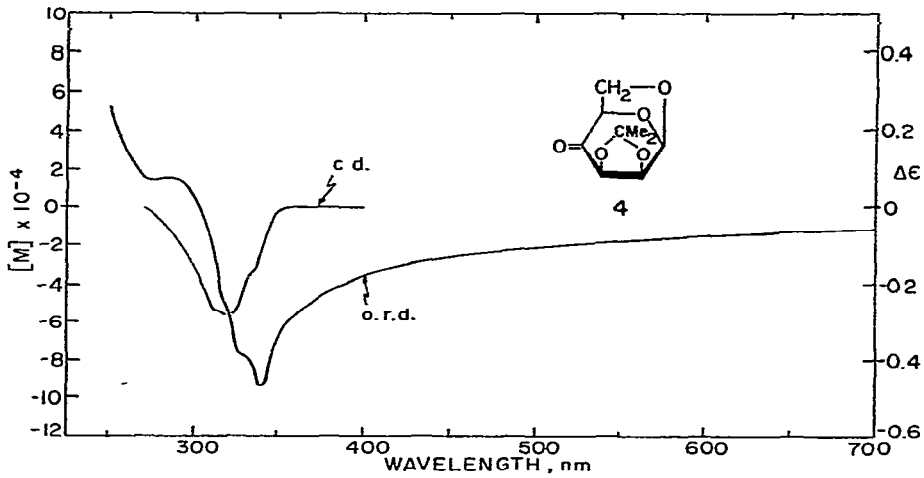


Fig. 2. The o.r.d. and c.d. spectra of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (**4** in chloroform (*c*, 0.15) at 23°.

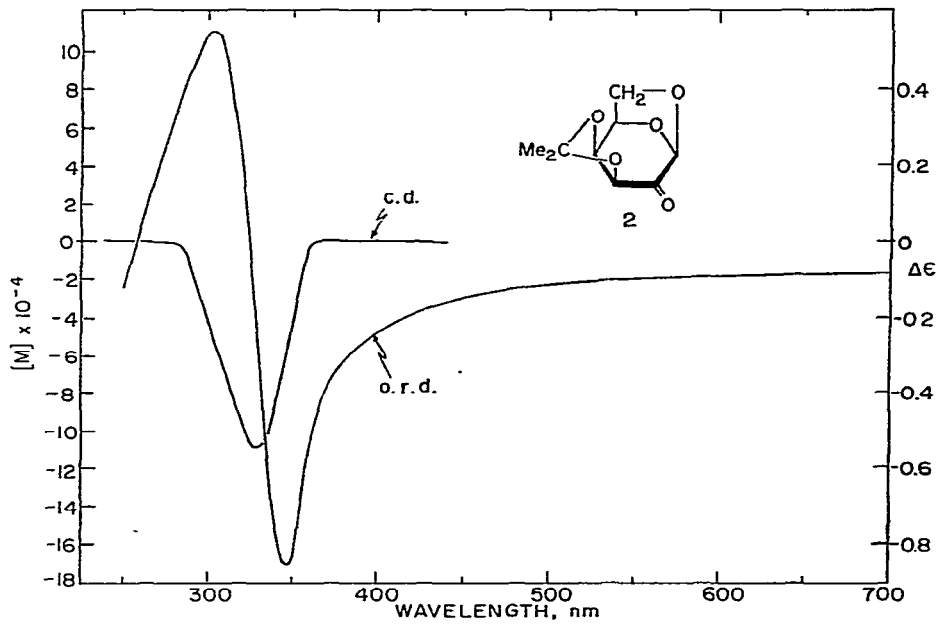


Fig. 3. The o.r.d. and c.d. spectra of 1,6-anhydro-3,4-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-2-ulose (**2**. in chloroform (*c*, 0.05) at 23°.

EXPERIMENTAL

General methods. — Solutions were evaporated below 40° under diminished pressure. Melting points were determined with a Thomas–Hoover melting-point

apparatus (Arthur H. Thomas Co., Philadelphia, Pennsylvania), and are uncorrected. Specific rotations were determined in a 2-dm polarimeter tube. Optical rotatory dispersion and circular dichroism measurements were made with a Durrum–Jasco Model ORD/UV-5 automatic spectropolarimeter–circular dichrograph (Durrum Instrument Corp., Palo Alto, California). Infrared spectra were measured with a Perkin–Elmer Model 137 "Infracord" infrared spectrometer. Ultraviolet spectra were measured with a Bausch and Lomb "Spectronic 505" spectrometer. N.m.r. spectra for routine comparison of samples were measured with a Varian A-60 n.m.r. spectrometer for solutions in chloroform-*d*. The n m.r. spectral data recorded were measured with a Varian HA-100 n.m.r. spectrometer, and the τ values recorded are relative to an internal standard of tetramethylsilane (τ 10.00). The first-order J values given are the observed line-spacings measured from spectra recorded at a sweep-width of 250 Hz, and are considered accurate to $\leqslant$ 0.5 Hz. Microanalyses were determined by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. Relative intensities were estimated visually: s, strong, m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest), and double numbers indicate approximately equal intensities. The camera diameter was 114.59 mm. Thin-layer chromatography was performed, unless otherwise stated, with Silica Gel G (E. Merck, Darmstadt, Germany), activated at 110°, as the adsorbent, and 3:1 chloroform–ether as the developing solvent. Indication was effected with sulfuric acid.

Gas liquid chromatography. — G.l.c. analyses were made with an Aerograph Autoprep gas chromatograph, Model 705 (Wilkens Instrument and Research Co., Inc., Walnut Creek, California) equipped with a flame-ionization detector. A stainless-steel column (10 ft × 0.25 in), packed with a stationary phase of 5% STAP on Chromosorb W (AW-DMCS) (Wilkens) was used, at a temperature of 150°, with nitrogen as the carrier gas at a flowrate of 80–100 ml/min.

TABLE I

G.L.C. RETENTION TIMES OF PER(TRIMETHYLSILYL) ETHERS OF SOME 1,6-ANHYDROHEXOPYRANOSES AND RELATED DERIVATIVES[a]

Per(trimethylsilyl) ether of	*Relative retention time*[b]	*Per(trimethylsilyl) ether of*	*Relative retention time*[b]
1,6-Anhydro-β-D-glucopyranose	1.0	1,6-Anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose (1)	1.25
1,6-Anhydro-β-D-galactopyranose	0.67	1,6-Anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (7)	1.25
1,6-Anhydro-β-D-mannopyranose	0.86	1,6-Anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (5)	0.98
1,6-Anhydro-β-D-talopyranose (6)	0.98	1,6-Anhydro-3,4-*O*-isopropylidene-β-D-talopyranose (3)	0.98
α-D-Talose	0.58		

[a]See Experimental section for details. [b]Relative to 1,6-anhydro-2,3,4-tri-*O*-(trimethylsilyl)-β-D-glucopyranose = 1.0.

Trimethylsilyl ethers were prepared by shaking the sample with 10–20 times its weight of a mixture of chlorotrimethylsilane and hexamethyldisilazane in pyridine (TRI-SIL, Pierce Chemical Company, Rockford, Illinois). The prepared samples were kept for at least 5 min at room temperature before being injected onto the column. The retention times of a number of per(trimethylsilyl) ethers of 1,6-anhydrohexopyranoses and derivatives are listed in Table I. The tris(trimethylsilyl) ether of 1,6-anhydro-β-D-glucopyranose, whose retention time was 12.8 min, was used as the standard, with a relative retention time of 1.0.

Oxidation of 1,6-anhydro-3,4-O-isopropylidene-β-D-galactopyranose (**1**) *to 1,6-anhydro-3,4-O-isopropylidene-β-D-lyxo-hexopyranos-2-ulose* (**2**). — *A. With methyl sulfoxide–acetic anhydride.* To a solution of **1** (1.0 g) in methyl sulfoxide (10 ml) was added acetic anhydride (3 ml), and the mixture was stirred for 36 h at 30°. The solution was evaporated in a lyophilization apparatus, and the resultant light-yellow syrup was extracted with petroleum ether (b.p. 95–110°, 300 ml). Evaporation of the extract, and repeated evaporation of carbon tetrachloride from the residue, gave crystalline **2**; recrystallization from carbon tetrachloride–petroleum ether (b.p. 35–70°) gave the product as flakes, yield 0.89 g (90%), m.p. 92–93°, $[\alpha]_D^{20}$ −89 ±1° (*c* 1, chloroform); R_F 0.35; λ_{max}^{KBr} 5.70 (C=O), 7.28 μm (CMe_2); $\lambda_{max}^{CHCl_3}$ 330 nm (ε 35); X-ray powder diffraction data: 7.65 s (2), 6.71 w, 6.43 w, 5.85 m, 5.50 m, 5.13 vs (1), 4.90 w, 4.72 m, 4.50 vw, 4.30 m, 3.80 m, 3.68 m.

Anal. Calc. for $C_9H_{12}O_5$: C, 54.00; H, 6.04. Found: C, 53.90; H, 6.20.

B. With ruthenium tetraoxide. To a solution of **1** (1.03 g) in carbon tetrachloride (500 ml) was added an approximately 2-molar excess of ruthenium tetraoxide (prepared as described previously[4]) in carbon tetrachloride. After 5 h at room temperature, the precipitate of ruthenium dioxide was filtered off, and the solution was evaporated (bath 30–32°) to give a crystalline residue of **2**, yield 0.87 g (85%), m.p. 92–93°, that was analytically pure. The product was identical with that prepared by method *A*, by mixed m.p., X-ray powder diffraction pattern, and i.r. and n.m.r. spectra.

The ketone **2** resembled the 4-ketone **4** previously described[4] in that it sublimed readily. Caution in the isolation of each ketone was required, to ensure that major losses by sublimation did not occur.

1,6-Anhydro-3,4-O-isopropylidene-β-D-talopyranose (**3**). — *A. Reduction of 1,6-anhydro-3,4-O-isopropylidene-β-D-lyxo-hexopyranos-2-ulose* (**2**) *with lithium aluminum hydride.* To a solution of the ketone **2** (504 mg) in anhydrous ether (20 ml) was added an excess of lithium aluminum hydride (200 mg). After 2 h at room temperature, the excess of reagent was decomposed by addition of ethyl acetate (5 ml). The solution was washed successively with cold 0.01N hydrochloric acid (10 ml) and saturated, aqueous sodium hydrogen carbonate (50 ml). The organic layer was removed, and the basic water layer was extracted with three 100-ml portions of chloroform. The combined organic layers were dried (magnesium sulfate), and evaporated to give a crystalline residue. Recrystallization from isopropyl alcohol gave **3** as needles, yield 506 mg (98%), m.p. 112–113°, $[\alpha]_D^{20}$ −112.5 ±1° (*c* 1, chloroform); R_F 0.35; λ_{max}^{KBr} 2.9 (OH), 7.28 μm (CMe_2); X-ray powder diffraction data: 7.79 s (2), 6.46 w, 5.57 m,

5.27 m, 5.04 vs (1), 4.29 m, 4.07 w, 3.83 vw, 3.48 m, 3.33 m, 3.22 m, and 3.11 w.

Anal. Calc. for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.74; H, 6.97.

B. Reduction of **2** *with sodium borohydride.* A solution of the ketone **2** (100 mg) in water (20 ml) was treated with an excess of sodium borohydride (50 mg). After 2 h at room temperature, the solution was cooled to 0°, and an excess of cold acetic acid (~0.3 ml) was added. After effervescence had subsided, Dowex-50 (H^+) ion-exchange resin (~0.1 ml) was added, and the entire mixture was poured quickly onto a short column (1 cm diameter) containing a 4-cm bed of Dowex-50 (H^+) resin. The column was washed rapidly with methanol (100 ml), and the combined effluent was evaporated. Methanol was evaporated several times from the residue to remove boric acid. The crystalline product was recrystallized from isopropyl alcohol to give pure **3**, yield 85 mg (84%), m.p. 112–113°, identical, by mixed m.p., X-ray powder diffraction pattern, i.r., and n.m.r. spectra, with the product formed by method *A*.

Trimethylsilylation of a sample of the crude reduction product, prepared by either method *A* or *B*, followed by gas-liquid chromatography, indicated that the reductions were completely stereospecific, because none of the D-*galacto* epimer (**1**) of the product (**3**) was detected. Experiments with pure samples indicated that less than 1% of **1** in admixture with **3** could be detected by g.l.c. of the 2-*O*-trimethylsilyl derivatives.

*Hydrogenation of 1,6-anhydro-3,4-*O*-isopropylidene-β-*D-lyxo-*hexopyranos-2-ulose* (**2**). — A solution of **2** (50 mg) in abs. ethanol (10 ml) was shaken with 5% palladium on carbon (10 mg) under hydrogen (50 lb.in^{-2}) for 48 h at room temperature. The mixture was filtered, and the filtrate was evaporated to a clear syrup. T.l.c. of this product revealed two component, R_F 0.26 and 0.35, having the mobilities and characteristics of **1** and **3**, respectively, in approximately equal proportions. G.l.c. of the trimethylsilylated product indicated that **1** and **3** were present in the ratio of 1:1. This ratio was the same in duplicate experiments. That the starting ketone **2** was uncontaminated by **1** was verified by reduction of a sample from the same batch with lithium aluminum hydride; the exclusive product was **3** (g.l.c.).

*1,6-Anhydro-2,3-*O*-isopropylidene-β-*D*-talopyranose* (**5**). — *A. Reduction of 1,6-anhydro-2,3-*O*-isopropylidene-β-*D-lyxo-*hexopyranos-4-ulose* (**4**) *with lithium aluminum hydride.* To a solution of the ketone[4] **4** (2.25 g) in anhydrous ether (150 ml) was added lithium aluminum hydride (~1.10 g). After 2 h at room temperature, an excess of ethyl acetate was added, and the product was isolated by the same procedure as that used in the reduction of the ketone **2** with lithium aluminum hydride. The crystalline, crude product was recrystallized from chloroform–petroleum ether (b.p. 30–60°) to give **5** as prisms, yield 2.27 g (98%), m.p. 104–105°, $[\alpha]_D^{20}$ −34 ±2° (*c* 1.5, chloroform); R_F 0.28; λ_{max}^{KBr} 2.90–2.99 (OH), 7.31 μm (CMe_2); X-ray powder diffraction data: 11.22 w, 7.99 s (2,2), 5.34 s (2,2), 5.09 vs (1), 4.83 m, 4.65 w, 4.46 m, 4.05 m, 4.00 vw, 3.77 m, 3.52 w, 3.15 m.

Anal.. Calc. for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.78; H, 7.20.

Six recrystallizations of the analytically pure product from chloroform–petroleum ether (b.p. 30–60°) gave **5** having m.p. 108.5–109°.

B. Reduction of **4** *with sodium borohydride.* A solution of **4** (1.00 g) in water (20 ml) was treated with sodium borohydride (~100 mg). After 2 h, the solution was cooled, acidified with acetic acid, decationized over a bed (3 × 6 cm) of Dowex-50 (H^+), and freed of boric acid as described for reduction of the ketone **2** with borohydride. The crystalline, crude product was recrystallized from chloroform–petroleum ether (b.p. 30–60°) to give prisms of **5**, yield 0.78 g (77%), m.p. 104–105°. The product was identical, by mixed m.p., X-ray powder diffraction pattern, and i.r. and n.m.r. spectra, with **5** prepared by method *A*.

Both reduction procedures, *A* and *B*, were shown to be stereospecific, since the crude product **5**, when converted into the 4-trimethylsilyl ether and the latter examined by g.l.c., was found to contain no component corresponding to the *O*-trimethylsilyl derivative of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (**7**). G.l.c. analysis of pure **5** and **7** indicated that <1% of **7** could be detected in admixture with **5**.

*Hydrogenation of 1,6-anhydro-2,3-*O*-isopropylidene-β-*D*-lyxo-hexopyranos-4-ulose* (**4**).—A solution of **4** (0.55 g) in abs. ethanol (15 ml) was shaken with 5% palladium on carbon (15 mg) under hydrogen (50 lb.in^{-2}) for 48 h at room temperature. The suspension was filtered, and the filtrate was evaporated to a syrup. T.l.c. revealed the presence of two components in the ratio of 3:1, R_F 0.28 and 0.22, having the chromatographic characteristics of **5** and **7**, respectively. The syrup was extracted with two 100-ml portions of hot petroleum ether (b.p. 95–110°), and the extracts were combined and concentrated. Prismatic crystals (0.15 g) were obtained by refrigeration of the solution; this product had m.p. 104–106° and was identical with the D-talose derivative **5**, by t.l.c. and X-ray powder diffraction pattern. The syrup that remained after the extraction was crystallized from chloroform–petroleum ether, and the product was recrystallized twice from butyl alcohol, to give the pure D-mannose derivative[4,6] **7** in low yield, m.p. 156–158°. The product had a t.l.c. mobility and an X-ray powder diffraction pattern identical with those of an authentic sample of **7**.

The observed ratio of products **5** and **7** in this reaction was verified by g.l.c. of the 4-trimethylsilyl ethers, in several independent experiments.

*1,6-Anhydro-β-*D*-talopyranose* (**6**). — *A. From 1,6-anhydro-3,4-*O*-isopropylidene-β-*D*-talopyranose* (**2**). A solution of **2** (226 mg) in N hydrochloric acid (20 ml) was stirred for 2 h at 30°, and then evaporated (codistillation with isopropyl alcohol and toluene) to a syrup that was homogeneous by t.l.c. on microcrystalline cellulose[13], R_F 0.54 (4:1:5 butyl alcohol–ethanol–water, upper phase). Crystallization from the minimal volume of hot isopropyl alcohol occurred after the inside of the flask had been scratched vigorously, and gave **6** as fine granules, yield 115 mg (65%), m.p. 185–186°, $[\alpha]_D$ −80 ±2° (c 1, water); λ_{max}^{KBr} 2.89–3.01 μm (OH); X-ray powder diffraction data: 7.69 vw, 5.41 m, 5.05 m, 4.88 m, 4.60 vs (1), 3.72 s (2), 3.12 w, 2.98 m, 2.67 m, 2.47 w, 2.41 vw, 2.33 w.

Anal. Calc. for $C_6H_{10}O_5$: C, 44.44; H, 6.21. Found: C, 44.38; H, 6.12.

*B. From 1,6-anhydro-2,3-*O*-isopropylidene-β-*D*-talopyranose* (**5**) A solution of **5** (1.02 g) in N hydrochloric acid (25 ml) was kept for 1 h at ~30°, and then evapo-

rated as in procedure *A*, to give a chromatographically homogeneous syrup. Crystallization from isopropyl alcohol gave pure **6**, yield 0.76 g (85%), m.p. 185–186°, identical, by mixed m.p., X-ray powder diffraction pattern, and comparative i.r. and n.m.r. spectra, with the product obtained by procedure *A*.

C. From 1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose (**7**). Compound **7** (1.00 g) was oxidized with ruthenium tetraoxide by the procedure[4] described for the preparation of **2** (method *B*). The crude product obtained after evaporation of the carbon tetrachloride was dissolved in water (20 ml), sodium borohydride (~50 mg) was added, and the solution was stirred for 2 h at room temperature. Hydrochloric acid (N) was added to the solution until effervescence ceased, and then Dowex-50 (H^+) resin (1 ml) was added, and the solution was kept for 1 h at 30°. Methanol (100 ml) was added, and the mixture was poured onto a column (3 × 10 cm) of Dowex-50 (H^+) resin. The column was washed with methanol (200 ml), and the combined effluent was evaporated. Methanol was evaporated several times from the residue, which was then crystallized from isopropyl alcohol to give **6**, yield 0.64 g (73%), m.p. 185–188°, identical in all respects with the product obtained by method *A* or *B*.

D. From 1,6-anhydro-3,4-O-isopropylidene-β-D-galactopyranose (**1**). A solution of **1** (0.54 g) in a mixture of methyl sulfoxide (15 ml) and acetic anhydride (3 ml) was stirred for 48 h at ~26°. The solution was evaporated in a lyophilization apparatus. The resulting syrup was dissolved in water (20 ml), and to the solution was added sodium borohydride (0.15 g). The solution was stirred for 2 h at room temperature, 30% acetic acid (5 ml) was added, and the mixture was passed through a column (3 × 10 cm) of Dowex-50 (H^+) resin. The effluent was extracted with three 100-ml portions of dichloromethane, the extract was washed with water (25 ml), dried (magnesium sulfate), and evaporated, to give a crystalline residue of **3**. This product was dissolved in N hydrochloric acid (20 ml), the solution was kept for 1 h at 35°, and the product was isolated as in procedure *A*, to give crystalline **6**, yield 0.28 g (60%), m.p. 183–186°, identical in all respects with the product prepared by the preceding methods.

This procedure could also be used for the preparation of **6** directly from **7**.

Conversion of 1,6-anhydro-β-D-talopyranose (**6**) *into α-D-talose.* — A solution of **6** (100 mg) in N hydrochloric acid (20 ml) was heated for 1.5 h at 75°, and then evaporated (codistillation with propyl alcohol) to a pale-yellow syrup. The latter was dissolved in hot ethanol, a few drops of acetone were added, and the solution was allowed to crystallize slowly at 0° to give α-D-talose, m.p. 131–133° (lit.[5] m.p. 133–134° for α-D-talose, m.p. 120–121° for β-D-talose[14]); X-ray powder diffraction data: 6.79 s (3,3), 5.64 vw, 5.10 vs (1), 4.81 s (2), 4.13 m, 3.87 m, 3.82 s (3,3), 3.50 vw, 3.34 m, 3.11 w, 2.91 w, 2.72 m, 2.68 m. The X-ray powder diffraction pattern was identical with that of an authentic sample of α-D-talose.

T.l.c. of the mother liquor from the reaction (microcrystalline cellulose[13], 4:1:5 butyl alcohol–ethanol–water, upper phase) revealed the presence of a component corresponding to a talose (R_F 0.22) and a second component corresponding to unchanged starting material **6** (R_F 0.54), in the approximate ratio of 3:1.

2,3,4-Tri-O-acetyl-1,6-anhydro-β-D-talopyranose (**8**). — Compound **6** (305 mg)

was dissolved in anhydrous pyridine (10 ml), acetic anhydride (3 ml) was added, and, after 36 h at room temperature, the solution was poured into ice and water. The mixture was extracted with three 200-ml portions of dichloromethane, and the extract was dried (magnesium sulfate) and evaporated to a syrup. Toluene was evaporated from the syrup to remove traces of pyridine, and the product was dissolved in 100 ml of hot petroleum ether (b.p. 95–105°). The solution was evaporated to a clear syrup, which was dissolved in warm ether and brought to crystallization by scratching the inside of the flask as the ether evaporated slowly; yield 325 mg (60%), m.p. 117–118°; $[\alpha]_D$ −73 ±2° (*c* 0.5, chloroform), R_F 0.51, λ_{max}^{KBr} 5.78 μm (OAc); n.m.r. data (benzene-d_6, 100 MHz, see Fig. 1): τ 4.25 (1-proton triplet of narrow triplets, $J_{2,3}$ 4.7 Hz, $J_{3,4}$ 4.7 Hz, $J_{1,3}$ 1.1 Hz, $J_{3,5}$ 1.1 Hz, H-3), τ 4.62 (1-proton narrow multiplet, W_h 3.8 Hz, H-1), τ 4.89 (1-proton triplet of narrow doublets, $J_{4,5}$ 3.9 Hz, $J_{4,6}$ 0.9 Hz, H-4), τ 5.12 (1-proton quartet, $J_{1,2}$ 1.7 Hz, H-2), τ 5.63 (1-proton doublet, $J_{6,6'}$ 7.5 Hz, $J_{5,6'}$ ~0 Hz, H-6′), τ 5.75 (1-proton broadened triplet, $J_{1,5}$ <0.5 Hz, H-5), τ 6.40 (1-proton multiplet, $J_{5,6}$ ~4.2 Hz, $J_{1,6}$ <0.5 Hz, H-6; X-ray powder diffraction data: 8.46 s (3), 7.07 m, 6.04 vw, 5.14 vw, 4.76 vs (1), 4.56 w, 4.17 s (2), 3.72 m, 3.41 m, 3.23 m.

Anal. Calc. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.98; H, 5.78.

O.r.d. and c.d. data for the ketones **2** *and* **4**. — 1,6-Anhydro-3,4-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-2-ulose (**2**) gave a negative Cotton effect (see Fig. 3) in its o.r.d. spectrum (*c* 0.05, chloroform), 23°; $[M]_{700}$ −16,400°; $[M]_{589}$ −18,100°; $[M]_{348}$ −170,400°; $[M]_{324}$ 0°; $[M]_{303}$ +109,000°; $[M]_{250}$ −24,500°. The c.d. curve for **2** (see Fig. 3) was negative (*c* 0.05, chloroform), 23°; Δε 0 (440 nm), 0 (356), −0.565 (330), 0 (284), 0 (240). 1,6-Anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (**4**) also gave a negative Cotton effect (see Fig. 2) in its o.r.d. spectrum (*c* 0.15, chloroform), 23°; $[M]_{700}$ −12,300°; $[M]_{589}$ −15,200°; $[M]_{340}$ −92,600°; $[M]_{304}$ 0°; $[M]_{280}$ +15,500°; $[M]_{250}$ +52,900°; and its c.d. curve (see Fig. 2) was negative (*c* 0.15, chloroform), 23°; Δε 0 (400 nm), 0 (348), −0.154 (shoulder, 335), −0.280 (322), −0.264 (shoulder, 313), 0 (270).

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SUMMARY

Oxidation of 1,6-anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose (**1**) with methyl sulfoxide–acetic anhydride or with ruthenium tetraoxide gave the crystalline 2-ketone (**2**) in high yield. Reduction of **2** with lithium aluminum hydride or sodium borohydride gave the D-*talo* isomer (**3**) of **1** stereospecifically. The same reducing agents

converted 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (**4**) stereospecifically into 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (**5**). Reduction of **4** with hydrogen over palladium gave a 3:1 mixture of **5** and its D-*manno* isomer (**7**); and, under these conditions, **2** was converted into a 1:1 mixture of **3** and **1**. Mild hydrolysis of **3** or **5** gave 1,6-anhydro-β-D-talopyranose (**6**), the last 1,6-anhydro-β-D-aldohexopyranose to be described; further hydrolysis gave D-talose. N.m.r. spectral data are given for the triacetate (**8**) of **6**. The ketones **2** and **4** showed optically active absorption at 330 and 300 nm, respectively, which gave rise in each case to a negative circular dichroism curve, and a Cotton effect of negative sign in the o.r.d. spectrum

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THE MASS SPECTRA OF SOME ARYL GLUCOSIDES

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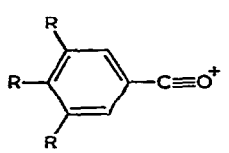
INTRODUCTION

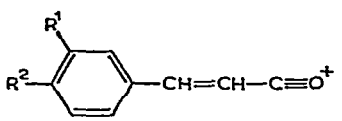
A problem of particular importance in the structural determination of natural acyl sugars and acyl glycosides is that of defining the position of acyl substitution. The usual method (permethylation, followed by removal of the acyl group and identification of the methylation pattern of the sugar) is not eminently suitable for small amounts of material. When basic reagents are used in the methylation, the results are frequently complicated by acyl migration[1,2].

Recently, we have investigated the potentialities of mass spectroscopy in elucidating this problem, and the results with a number of natural, aroyl aryl glucosides are reported here.

RESULTS AND DISCUSSION

Preliminary investigations employing various hydrolytic procedures and paper-chromatographic techniques are normally sufficient to indicate the nature of the sugar, the acyl substituent, and the aglycone[3–6]. The remaining material is then acetylated, and the product is purified by thin-layer chromatography and subjected to mass spectroscopy. The identity of the aroyl substituent is further confirmed from the mass spectrum (Figs. 3–10). Thus, the benzoyl group of populin[7] and tremuloidin[8] gives rise to a strong peak at *m/e* 105 due to ion **1** or its equivalent. In the case of galloyl esters, the analogous ion (**2**) occurs at *m/e* 279 and is accompanied by three further ions (*m/e* 237, 195, 153) produced by successive loss of three molecules of ketene[9]. Hydroxycinnamoyl esters behave similarly, giving an ion corresponding to **3** or **4**, and fragment ions caused by loss of ketene from the acetoxyl functions. In all cases, weaker ions, corresponding to loss of the individual acids, are also observed.





1 R = H, *m/e* 105
2 R = OAc, *m/e* 279→237→195→153

3 R^1 = H, R^2 = OAc, *m/e* 189→147
4 R^1 = R^2 = OAc, *m/e* 247→205→163

The mass-spectrometric behaviour of peracetylated hexopyranose derivatives has been examined in detail by Biemann[10] and Heyns[11] and their colleagues, and a number of primary pathways of fission have been formulated. As expected from this work, aryl glucosides readily lose the aryloxy radical from the molecular ion, and the latter is rarely observed. The subsequent mode of fragmentation is then predominantly as formulated in route *A* (Biemann[10]) or primary splitting *B* (Heyns[11]). Thus, independent of the nature of the aglycone, acetylated aryl glucosides give very

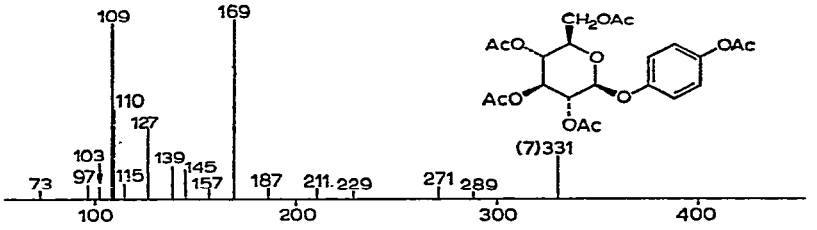


Fig. 1

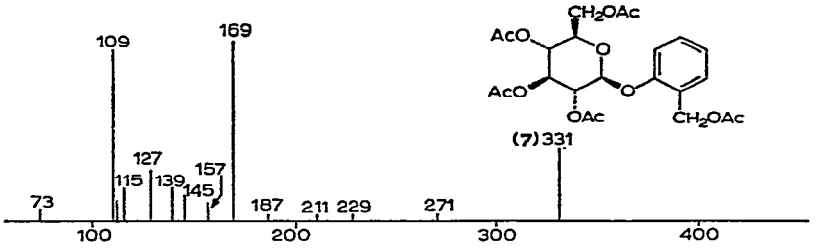


Fig. 2

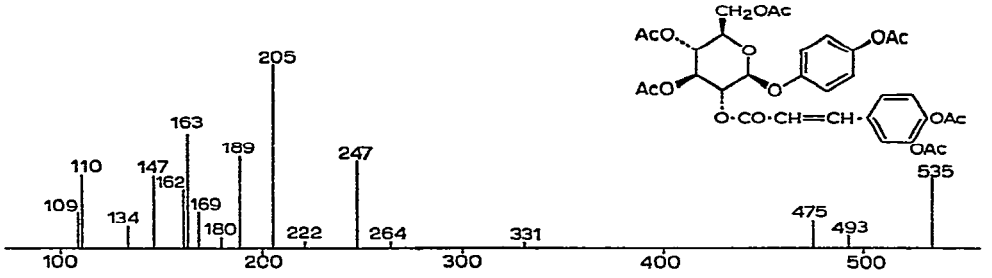


Fig. 3

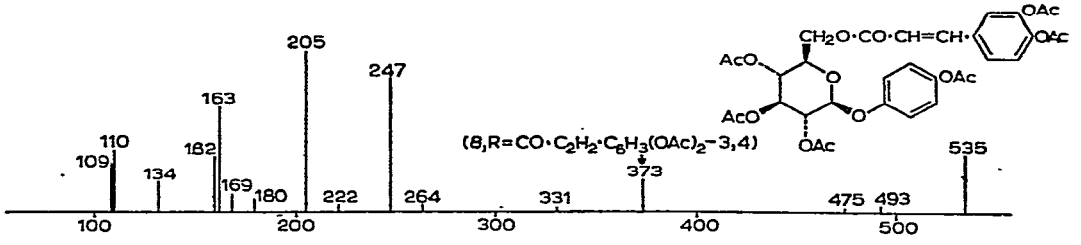


Fig. 4

Fig. 1. Arbutin penta-acetate.
Fig. 2. Salicin penta-acetate.
Fig. 3. 2-*O*-Caffeoylarbutin hexa-acetate.
Fig. 4. 6-*O*-Caffeoylarbutin hexa-acetate.

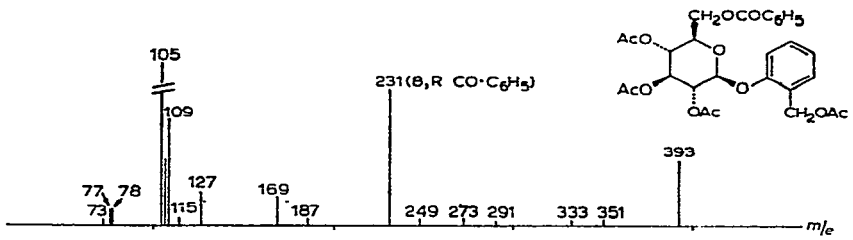


Fig. 5

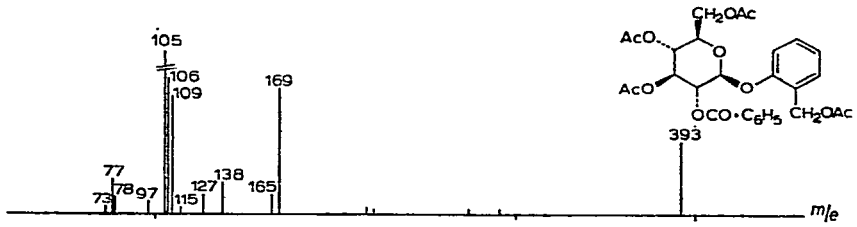


Fig. 6

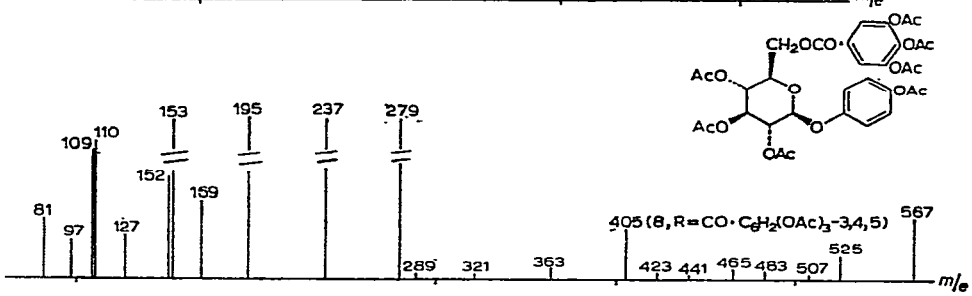


Fig. 7

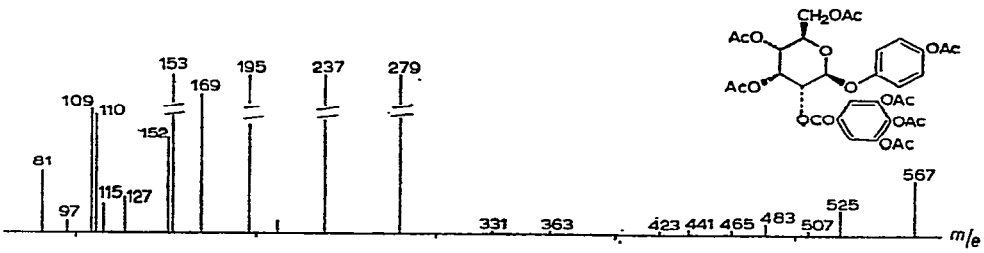


Fig. 8

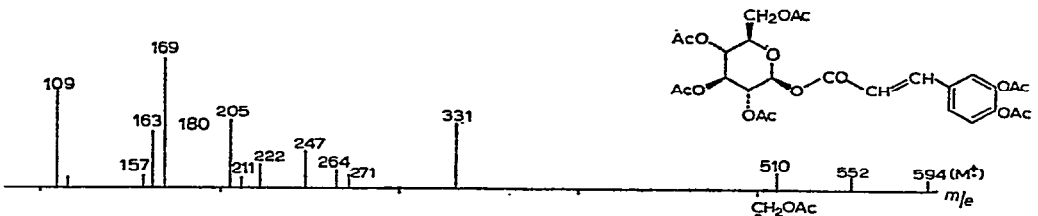


Fig. 9

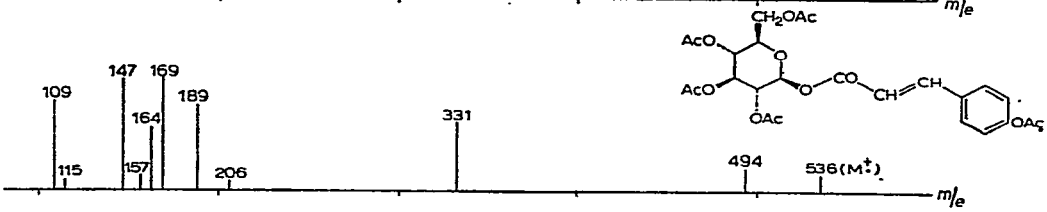
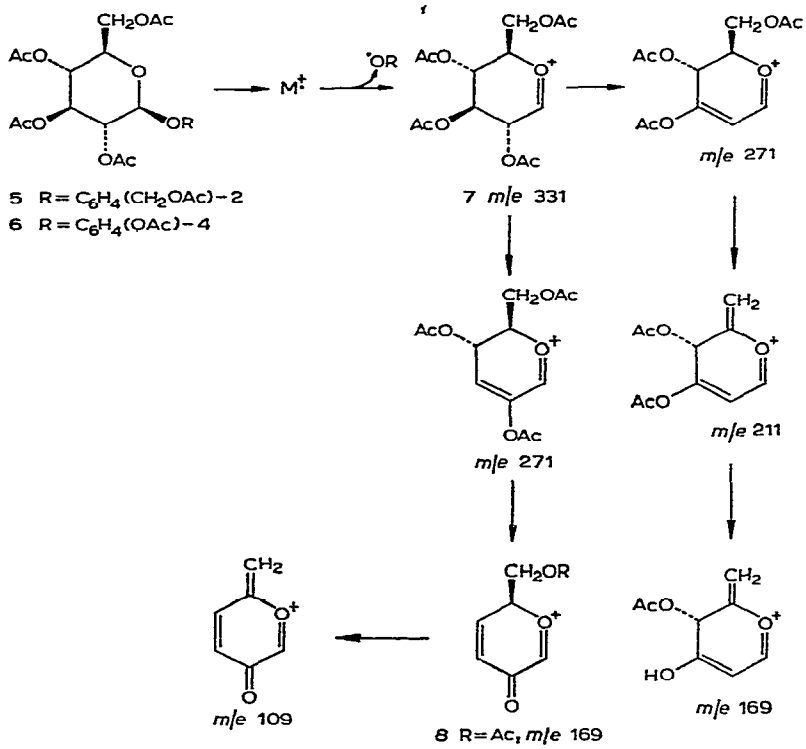


Fig. 10

Fig. 5. Populin tetra-acetate.
Fig. 6. Tremuloidin tetra-acetate.
Fig. 7. 6-*O*-Galloylarbutin hepta-acetate.
Fig. 8. 2-*O*-Galloylarbutin hepta-acetate.
Fig. 9. 1-*O*-Caffeoyl-*β*-D-glucose hexa-acetate.
Fig. 10. 1-*O*-*p*-Coumaroyl-*β*-D-glucose penta-acetate.

similar fragmentation patterns, for example, salicin and arbutin penta-acetate (**5** and **6**) (Figs. 1 and 2). Presumably in such cases, breakdown is directed predominantly from the fragment ion **7**.



The constitution of the fragment ions shown above is that suggested by Heyns and Müller[11]. In the case of aroyl aryl glucosides, the mass-spectrometric behaviour of the acetylated products, if they adhere to the general pathways indicated above, should give rise to fragmentation patterns from which the position of aroyl substitution may be determined. These ideas have been examined by using the 6- and 2-*O*-benzoyl derivatives of salicin (populin[7] and tremuloidin[8]), the 2- and 6-*O*-caffeoyl derivatives of arbutin from *Vaccinium vitis idaea*[5], and the 1′-, 2-, and 6-*O*-galloyl derivatives of arbutin from *Arctostaphylos uva-ursi*[6]. The results indicate a quite consistent pattern (Figs. 3–8). Aroyl substitution at position 6 is clearly defined. In all of the cases examined, these derivatives give rise to a strong ion corresponding to **8**, whose position is determined solely by the nature of the aroyl group (R). The ion *m/e* 169 is of decreased intensity (generally less than that of the ion *m/e* 109). In the 2-aroyl compound, the ion corresponding in *m/e* ratio to **8** (R = aroyl) is absent or of minimal significance, and the peaks *m/e* 109 and 169 are of comparable intensity once more. However, until further model compounds have been examined, it is not clear whether the fragmentation pattern of the 2-aroyl compounds is definitive for this position of substitution. Detailed discussion of this fragmentation pattern is reserved until later.

EXPERIMENTAL

The mass spectra were determined on an A.E.I. MS 9 mass spectrometer. The energy of the ion beam was 70 eV. The samples were introduced directly into the ion chamber on a probe.

ACKNOWLEDGMENTS

The author acknowledges the gift of a sample of tremuloidin tetra-acetate from Drs. I. A. Pearl and S. F. Darling of the Institute of Paper Chemistry, Wisconsin; other samples were obtained as in previous work[5,6,7,8]. He thanks Mr. S. Jones for technical assistance.

SUMMARY

A number of acetylated, naturally occurring, aroyl aryl glucosides have been subjected to mass-spectrometric examination. The identity of the aroyl group is readily determined from the mass spectrum. In the case of the 6-aroyl substituted glucosides, the fragmentation pattern has been shown to be definitive for the position of acyl substitution.

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MIGRATION OF THIOLTHIOCARBONYL GROUPS OF METHYL α-D-GLUCOPYRANOSIDE XANTHATES*

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(Received April 12th, 1967)

INTRODUCTION

Previously, we showed the effect of such reaction variables as solids content, temperature, and time upon the distribution of xanthate groups in starch xanthate[1]. We found that significant migration of the thiolthiocarbonyl groups from the secondary positions to the primary position occurred.

To verify these migrations and to provide guidelines for interpreting the mechanisms of these changes, xanthation of a model compound, methyl α-D-glucopyranoside, has now been studied. In addition, the isomeric monosubstituted 2-, 3-, and 6-(sodium xanthate) salts of the model compound have been prepared, and their behavior under alkaline conditions typical of xanthation media have been investigated.

RESULTS AND DISCUSSION

Xanthation of methyl α-D-glucopyranoside

Methyl α-D-glucopyranoside was treated with sodium hydroxide and carbon disulfide in the 2:2:1 ratios used for starch[1]. Portions of the reaction mixture were neutralized and benzylated at intervals up to 5 h. The yield of xanthate ester at each time was estimated from the u.v. absorption maximum at 355 nm. The results, presented in Table I, indicate that the increase in degree of substitution (D. S.) is almost

TABLE I

CHANGE IN DEGREE OF SUBSTITUTION (D.S.) DURING XANTHATION OF METHYL α-D-GLUCOPYRANOSIDE

Time (min)	15	30	45	60	120	180	240	300
D.S.	0.06	0.13	0.23	0.33	0.35	0.33	0.34	0.31

linear during the first h, and that the D.S. thereafter remains nearly constant. These results are closely parallel to data reported for the xanthation of starch[2].

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**This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

The xanthate esters were resolved by t.l.c., and were determined quantitatively by developing the t.l.c. plates with iodine vapor, sectioning the individual zones, evaporating the iodine, eluting the sections with methanol, and determining the u.v. absorption at λ_{max} 280–285 nm. To identify the components separated by this procedure, authentic samples of the 2-, 3-, and 6-(*S*-benzylxanthates) were prepared.

Table II shows the distributions of the various isomeric xanthates, in terms of molar contributions to the total D. S. values recorded in Table I.

TABLE II

DISTRIBUTION OF XANTHATE GROUPS DURING XANTHATION OF METHYL α-D-GLUCOPYRANOSIDE[a]

Glucoside Xanthate	*Time (min)* 15	30	45	60	120	180	240	300
2-Isomer	0.02	0.04	0.05	0.06	0.07	0.05	0.05	0.05
3-Isomer	0.01	0.01	0.02	0.03	0.03	0.02	0.02	0.02
6-Isomer	0.03	0.07	0.13	0.20	0.21	0.20	0.24	0.21
Polyxanthates	0.01	0.01	0.03	0.04	0.04	0.06	0.03	0.03

[a] Distributions of totals are presented in Table I.

Throughout the xanthation, the 6-isomer preponderates, and the 2-isomer is more abundant than the 3-isomer. Doane, Russell, and Rist[2] found similar orders of positional substitution for starch xanthated to D. S. values of 0.13 and 0.32. During the first few min of xanthation, the secondary substitution (sum of 2- and 3-isomers) and primary substitution (6-isomer) are almost the same. After several h, however, the primary substitution is about three times the secondary. In addition, there are as-yet-unidentified components having R_F values higher than those of the isomers identified. On the basis of u.v. analysis, these are believed to be polyxanthates of methyl α-D-glucopyranoside.

To permit study of the redistribution in more detail, the isomeric 2-, 3-, and 6-(sodium xanthate) salts were prepared, and each was treated with aqueous alkali under conditions typical of xanthation reactions. The resulting isomers were then benzylated, and the distributions of substituents in the products were determined.

The isomeric sodium xanthate salts were obtained from the respective *S*-benzylxanthates by treatment with sodium hydrosulfide in methyl sulfoxide:

$$ROC(=S)SCH_2Ph + NaSH \rightarrow ROC(=S)SNa + PhCH_2SH$$

Other useful solvents for the reaction were tetrahydrofuran and pyridine. A solution of the *S*-benzylxanthate was cooled to 0–5° and agitated with the theoretical amount of anhydrous sodium hydrosulfide. Some migration of substituent groups unavoidably took place during the cleavage reaction with sodium hydrosulfide. When the sodium xanthate salts were immediately dissolved in water and rebenzylated at 5°, the original

S-benzylxanthates were recoverable in yields of 70–80%. Small proportions of the other monosubstituted isomers and some polysubstituted products were also identified.

Table III gives the results obtained by incubating each of the 2-, 3-, and 6-xanthate salts in 18% sodium hydroxide for 30 min at 25° before neutralization and rebenzylation.

TABLE III

MIGRATION OF 2-, 3-, AND 6-SODIUM THIOLTHIOCARBONYL GROUPS OF METHYL α-D-GLUCOPYRANOSIDE XANTHATES[a]

Xanthate isomer treated	*Distribution of xanthate groups after treatment %*			
	2-	*3-*	*6-*	*Polyxanthates*[b]
2-	3	1	85	8
3-	11	8	77	4
6-	4	5	88	3

[a] Xanthates were treated with 18% sodium hydroxide for 30 min at 25°. [b] More than one xanthate group per molecule of methyl α-D-glucopyranoside.

With both the 2- and 3-isomers, extensive migration occurred, and gave 85 and 77%, respectively, of the 6-(sodium xanthate). The 6-isomer underwent little change, as 88% was recovered as the 6-(*S*-benzylxanthate).

To investigate the possibility of intramolecular migration of the sodium thiolthiocarbonyl group, a more detailed study was conducted with the 2-isomer. The treatment with 18% sodium hydroxide was performed at 0–5° in order to retard the rearrangement, and samples were neutralized and benzylated after 0, 30, 60, and 90 min (see Table IV). Since there was a relatively large proportion of the 3-isomer present

TABLE IV

MIGRATION OF THE 2-SODIUM THIOLTHIOCARBONYL GROUP OF METHYL α-D-GLUCOPYRANOSIDE XANTHATES[a]

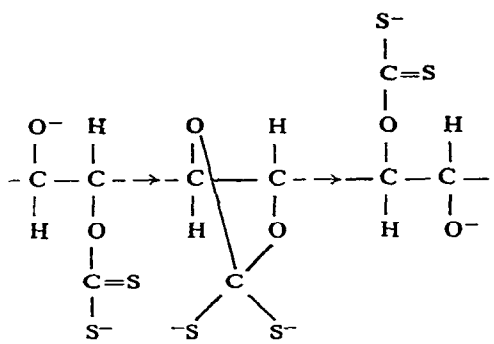
Length of treatment (min)	*Distribution of xanthate groups during treatment %*			
	2-	*3-*	*6-*	*Polyxanthates*
0	80	3	9	8
30	43	19	20[b]	18
60	13	14	62	11
90	7	12	56	25

[a] Xanthate was treated with 18% sodium hydroxide for 90 min at 5°. [b] Believed to contain some 4-isomer.

(30 min) before appreciable migration to the 6-position, transfer of substituent from the 2- to the 3- and 6-positions probably occurred intramolecularly. The presence of small proportions of polyxanthates indicates that some intermolecular transfer also occurs.

Further support for the intramolecular mechanism was obtained by use of a suitably protected derivative of methyl α-D-glucopyranoside. Methyl 3-*O*-methyl-α-D-glucopyranoside 2-(*S*-benzylxanthate) was synthesized, and cleaved to the xanthate salt; this was incubated with 18% sodium hydroxide for 2 h at 5°, the solution was neutralized, and the product was rebenzylated. A parallel experiment was performed with the unprotected 2-(*S*-benzylxanthate) of methyl α-D-glucopyranoside. T.l.c. of the rebenzylated products showed that, although extensive migration to the 6-position had taken place with the unprotected derivative, no migration was evident in the 3-*O*-methyl derivative, because only a single spot (that corresponded to the xanthate started with) was seen.

Migrations probably proceed through a series of ortho-ions as follows:



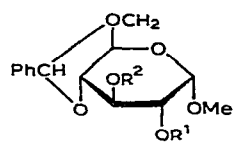
Efforts to trap and identify these ortho-ions through treatment of xanthates with alkoxide have so far been unsuccessful. Evidently, if such changes occur, they are so rapid that the intermediates are not isolable.

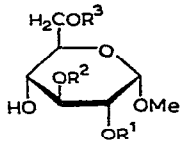
Other aspects of migration of substituents were examined. For example, if sodium thiolthiocarbonyl groups decompose to trithiocarbonate, xanthation might occur at a different position. Ingram and Toms[3] reported reactions between alcohols and trithiocarbonate in anhydrous media to form xanthates. However, treatment of ethanol or methyl α-D-glucopyranoside with sodium trithiocarbonate in 18% sodium hydroxide gave no evidence of formation of xanthate (u.v. spectra), even after several days. In view of these results and the rapidity of the migrations, the involvement of trithiocarbonate appeared unlikely. On the other hand, xanthate groups might decompose to carbon disulfide, which, in the presence of sodium hydroxide, could then re-xanthate the substrate at different positions. In control experiments, carbon disulfide was quantitatively and rapidly flushed with nitrogen from a medium of 18% sodium hydroxide into a solution of an amine in alcohol, and the carbon disulfide was determined spectrophotometrically as the dithiocarbamate salt (λ_{max} near 290 nm)[4]. Solutions of the 2-xanthate salt of methyl α-D-glucopyranoside in 18% sodium hydroxide contained small proportions of carbon disulfide, but benzylation of the nitrogen-flushed samples did not show significant differences in substituent distributions from those observed after benzylation of unflushed samples treated under similar conditions.

Apparently, the amounts of carbon disulfide involved were too low to influence the observed migrations appreciably.

Preparation of the isomeric 2-, 3-, and 6-(S-benzylxanthates) of methyl α-D-glucopyranoside

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**) was xanthated and *S*-benzylated, and the resultant mixture of xanthate esters was fractionally recrystallized





1 R^1 = H, R^2 = H
2 R^1 = CS_2CH_2Ph, R^2 = H
3 R^1 = H, R^2 = CS_2CH_2Ph
4 R^1 = CS_2CH_2Ph, R^2 = Bz
5 R^1 = Bz, R^2 = CS_2CH_2Ph
6 R^1 = $COSCH_2Ph$, R^2 = Bz
7 R^1 = Bz, R^2 = $COSCH_2Ph$
8 R^1 = CS_2CH_2Ph, R^2 = Me
9 R^1 = CS_2CH_2Ph, R^2 = H, R^3 = H
10 R^1 = H, R^2 = CS_2CH_2Ph, R^3 = H
11 R^1 = H, R^2 = H, R^3 = CS_2CH_2Ph
12 R^1 = CS_2CH_2Ph, R^2 = Me, R^3 = H
13 R^1 = CS_2Na, R^2 = Me, R^3 = H

to give methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2-(*S*-benzylxanthate) (**2**) and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 3-(*S*-benzylxanthate) (**3**). Mild, acid hydrolysis selectively removed the *O*-benzylidene groups to give methyl α-D-glucopyranoside 2-(*S*-benzylxanthate) (**9**) and methyl α-D-glucopyranoside 3-(*S*-benzylxanthate) (**10**). The identities of **2**, and **3**, were established by benzoylation to **4**, and **5**, and subsequent conversion to the known monothiolcarbonates, **6** and **7**. Methyl α-D-glucopyranoside 6-(*S*-benzylxanthate) (**11**) was prepared by direct xanthation and *S*-benzylation of methyl α-D-glucopyranoside. The 6-isomer preponderated, and could be crystallized preferentially from the ester mixture. Its identity was confirmed by comparison with the product obtained by the barium hydroxide procedure, and by preparation of the known[5] triacetate and tribenzoate.

Preparation and cleavage of methyl 3-O-methyl-α-D-glucopyranoside 2-(S-benzylxanthate)

Compound **2** was treated with diazomethane in the presence of boron trifluoride as a catalyst[6], to give methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside 2-(*S*-benzylxanthate) (**8**). Mild, acid hydrolysis removed the *O*-benzylidene group, to give methyl 3-*O*-methyl-α-D-glucopyranoside 2-(*S*-benzylxanthate) (**12**), which was cleaved with sodium hydrosulfide to yield the sodium xanthate salt **13**. Benzylation of **13** regenerated **12** exclusively.

EXPERIMENTAL

U. v. spectra between 210 and 400 nm were determined with a Perkin–Elmer*, Model 202, recording spectrophotometer equipped with 1-cm silica cuvettes. Appropriate blank corrections were made for all determinations of optical absorbance. Melting points were determined in sealed capillaries in an oil bath and are uncorrected. Optical rotations were measured with a Rudolph polarimeter.

Xanthation of methyl α-D-glucopyranoside. — A solution of methyl α-D-glucopyranoside (19.4 g) in water (20 ml) was mixed with carbon disulfide (3 ml) and sodium hydroxide solution (4 g in 5 ml of water) at room temperature, with rapid agitation. Portions (5 ml) were withdrawn at 15, 30, 45, 60, 120, 180, 240, and 300 min, neutralized with 10 ml of N acetic acid at 0–5°, and treated with 1 ml of benzyl bromide for 2–3 h. The mixtures were extracted with chloroform, dried over sodium sulfate, and diluted to 500 ml; the u.v. spectra were determined at 355 nm (the average extinction coefficient is 87 for the 2-, 3-, and 6-isomers at this wavelength).

The chloroform extracts were spotted on microscope slides coated with Silica Gel B (Brinkmann Instruments, Inc.) and developed with ethyl acetate. In later experiments, I.T.L.C. (Gelman Instrument Co.) was used and developed with 10:7:3 cyclohexane–ethyl acetate–chloroform. Charring, or staining with iodine, revealed four components, three of which corresponded to the known 2-, 3-, and 6-isomers when the latter were developed under similar conditions. For quantitative determinations, the components were revealed by the nondestructive, iodine-stain technique. The zones were sectioned, the iodine was evaporated, and the adsorbate was placed in a filter cone and eluted directly into a cuvette for determination of the u.v. absorbance at 280–285 nm. The extinction coefficients of each isomer were similar (near 12,500) in this region, and the proportion of each isomer was taken as the ratio of its absorbance to the total absorbance. To establish the validity of this method, known mixtures of the 2-, 3-, and 6-isomers were chromatographed under similar conditions, and the absorbances determined. The isomer ratios thus found agreed within 5–10% with the ratios taken by weight. It was necessary in each case to correct for absorption in the 280–285-nm region, since washing the absorbent from unspotted slides revealed slight interference in this region.

Methyl 4,6-O-benzylidene-α-D-glucopyranoside 2- and 3-(S-benzylxanthates) (**2** *and* **3**). — Compound **1** was prepared by known methods from methyl α-D-glucopyranoside[7–9]. Twenty g of **1** was xanthated by dissolving it in *p*-dioxane (50 ml) and adding carbon disulfide (50 ml) followed by 5N sodium hydroxide (12 ml). The mixture was stirred for 15 min, cooled to 5° with ice, and rendered neutral with acetic acid. The mixture was treated with benzyl bromide (7.2 ml), stirred for 4 h at 5°, and evaporated to a solid, which was extracted with several 50-ml portions of hot (75°) 0.5% sodium hydrogen carbonate solution, to give 4.23 g of **1**. The residue was dried,

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

and extracted with 1 liter of hexane, and the extract was cooled to give a solid, which was combined with the residue from this extraction; yield 17.61 g (57%). T.l.c. on Silica Gel G with 1:19 (v/v) acetone–chloroform showed two components, R_F 0.75 (85–90%), and R_F 0.61 (10–15%). These were resolved by fractional recrystallization from isopropyl alcohol. The less-soluble, minor isomer was **3**, yield 1.22 g (4%, based on **1**), m.p. 176–177°, $[\alpha]_D^{24.5}$ +38.8° (*c* 0.31, chloroform).

Anal. Calc. for $C_{22}H_{24}O_6S_2$: C, 58.9; H, 5.4; S, 14.3. Found: C, 58.9; H, 5.2; S, 14.3.

The more-soluble, major isomer was **2**, yield 10.40 g (34%, based on **1**), m.p. 132–133°, $[\alpha]_D^{24.5}$ +80.7° (*c* 0.87, chloroform).

Anal. Found: C, 59.0; H, 5.2; S, 14.4.

*Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside 3-*(S*-benzylxanthate)* (**5**). — A solution of **3** (0.45 g) in pyridine (10 ml) was treated with benzoyl chloride (2 ml), and kept overnight. The mixture was processed in the usual way, to give 0.39 g (71%) of product. Recrystallization from ethanol gave **5**, m.p. 138°, $[\alpha]_D^{25}$ +95.9° (*c* 0.37, chloroform).

Anal. Calc. for $C_{29}H_{28}O_7S_2$: C, 63.0; H, 5.1; S, 11.6. Found: C, 63.1; H, 5.3; S, 11.9.

*Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside 2-*(S*-benzylxanthate)* (**4**). — A solution of **2** (0.45 g) was benzoylated as above to give **4**, yield 0.39 g (71%). Recrystallization from ethanol gave pure **4**, m.p. 127°, $[\alpha]_D^{25}$ +68.4° (*c* 0.64, chloroform).

Anal. Found: C, 63.0; H, 5.2; S, 11.6.

*Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside 3-*(S*-benzylmonothiolcarbonate)* (**7**). — A solution of **5** (0.28 g) in 90% aqueous *p*-dioxane (10 ml) was treated with mercuric acetate solution[5] (10 ml), and kept for 5 h at 40°. Colorless crystals were obtained from ethanol, yield 0.08 g (30%), which, on recrystallization, had m.p. 132–133°, $[\alpha]_D^{24.5}$ +109.6° (*c* 0.68, chloroform); lit.[9] m.p. 133–135°, $[\alpha]_D^{25}$ +107° (*c* 2, chloroform).

*Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside 2-*(S*-benzylmonothiolcarbonate)* (**6**). — A sample of **4** (0.34 g) was treated as above to give the corresponding monothiolcarbonate (**6**), yield 0.10 g (30%), m.p. 136–137° on recrystallization from ethanol, $[\alpha]_D^{25}$ +44.5° (*c* 0.99, chloroform); lit.[9] m.p. 138–139°, $[\alpha]_D^{25}$ + 44.5° (*c* 2, chloroform).

*Methyl α-*D*-glucopyranoside 2-*(S*-benzylxanthate)* (**9**). — The *O*-benzylidene group was removed from **2** by an adaptation of the method of Capon, Overend, and Sobell[10] as follows: a solution of **2** (3.60 g) in methanol (190 ml), to which had been added concentrated hydrochloric acid (0.6 ml) and water (2 ml), was kept for 5 h at 40°. The solution was evaporated, the residue was dissolved in chloroform, and the solution was neutralized with sodium hydrogen carbonate solution, shaken with sodium hydrogen sulfite solution (to remove benzaldehyde), dried (sodium sulfate), evaporated, and the residue extracted with hot hexane, to give a colorless solid, yield 2.42 g (83.5%), m.p. 116–129°. Recrystallization from ether–hexane gave needles, m.p. 130–131°, $[\alpha]_D^{26.5}$ +97.8° (*c* 0.49, chloroform).

Anal. Calc. for $C_{15}H_{20}O_6S_2$: C, 50.0; H, 5.6; S, 17.8. Found: C, 49.9; H, 5.4; S, 17.6.

Methyl α-D-glucopyranoside 3-(S-benzylxanthate) (**10**). — A sample of **3** (1.07 g) was treated as above to remove the *O*-benzylidene group. Trituration of the final syrup with benzene gave 0.52 g of a solid (60.5%), that crystallized as needles from ether–hexane, m.p. 100–103°, $[\alpha]_D^{23}$ +150.9° (*c* 0.82, chloroform).

Anal. Found: C, 50.1; H, 5.8; S, 17.8.

Methyl α-D-glucopyranoside 6-(S-benzylxanthate) (**11**). — (*a*) *Sodium hydroxide method.* Methyl α-D-glucopyranoside (97 g) was dissolved in water (100 ml), and the solution was mixed with carbon disulfide (15 ml). The mixture was stirred rapidly, and a solution of sodium hydroxide (20 g) in water (25 ml) was added dropwise during 0.5 h, while the temperature was kept near 25°. The mixture was stirred for 5 h at 25°, rendered neutral with N acetic acid (500 ml), and cooled to 10°. Benzyl bromide (30 ml) was then added dropwise during 0.5 h, the mixture was stirred for 2 h at 10°, refrigerated overnight, extracted with chloroform, and the extract dried (sodium sulfate) and evaporated, to give 85.6 g of syrup (95% yield, based on carbon disulfide taken). The syrup was treated with warm benzene (500 ml), the suspension was filtered, and the filtrate was refrigerated overnight, to give 36.2 g of product, m.p. 77–83°, which, on repeated extraction with boiling hexane, was raised to 90–93°, yield 33.7 g (37.4%, based on carbon disulfide). The specific rotation varied somewhat with the concentration: $[\alpha]_D^{26.5}$ +69.6° (*c* 1.35, chloroform) and $[\alpha]_D^{26.5}$ +79.0° (*c* 4.82, chloroform). For this compound, $[\alpha]_D^{20}$ +79.4°, +79.8° (chloroform) have been reported[5].

(*b*) *Barium hydroxide method.* The method of Lieser, as adapted by Willard and Pacsu[5], was repeated to provide a reference for the preparation of this compound by the sodium hydroxide method. Benzylation of the xanthate solution gave a solid product, yield 14.22 g (30.6%). The yield varied according to the amount of carbon dioxide passed through the barium hydroxide solution. Crystallization of the ester from benzene, followed by boiling it with hexane, gave 11.7 g (25.2%), m.p. 91–93°, $[\alpha]_D^{26.5}$ +68.2° (*c* 1.17, chloroform) and $[\alpha]_D^{26.5}$ +79.6° (*c* 5.01, chloroform).

Products prepared by each method gave identical triacetates (84% yield) and tribenzoates (80% yield), having m.p. 106–109° and 124–127°, respectively (reported[5] 105–107° and 123–124°, 127–129°, respectively).

Sodium salts of the isomeric xanthates of methyl α-D-glucopyranoside. — The *S*-benzylxanthate isomers of methyl α-D-glucopyranoside (200–250 mg) were dissolved in a mixture of methyl sulfoxide (5 ml) and tetrahydrofuran (4 ml), the solution cooled to 0–5°, and agitated rapidly for 30–60 min with 40 mg of anhydrous sodium hydrosulfide[11]. The mixtures were neutralized with acetic acid, and the xanthate salts were precipitated as syrups on addition of ether (100 ml). The ether was decanted to remove α-toluenethiol, and the sodium salts were precipitated as solids by mixing with acetone (10 ml) and adding ether (100 ml). The products were filtered off, washed with ether, and dried immediately under vacuum over phosphorus pentaoxide. The products were deliquescent, and difficult to obtain pure. U.v. maxima were near 305 nm for the 2- and 3-xanthate salts and 303 nm for the 6-xanthate salts, and the extinction coefficients

lay between 6,000 and 10,000. The yields of the crude salts were between 90 and 100% of the theoretical.

Rebenzylation of the sodium salts of the isomeric xanthates of methyl α-D-glucopyranoside. — Portions of the xanthate salts (25–40 mg) were dissolved in water at 0–5° and treated with an excess of benzyl bromide. T.l.c. showed that, under these control conditions, the esters from which the salts had been derived were again formed. Similar amounts of salts were incubated for various time-intervals in 1 ml of 18% sodium hydroxide, and the mixtures were neutralized with 0.5N acetic acid (15 ml) at 0–5°, and benzylated similarly. The reaction mixtures were extracted with chloroform, and the extracts were dried with sodium sulfate. The yields of the rebenzylated xanthates were determined by diluting the chloroform extracts and determining the absorbance at 280–285 nm. The yields, based on the amount of xanthate salt taken, were generally quantitative. If too great an excess of benzyl bromide was used, there was some interference at this wavelength. In such instances, most of the excess benzyl bromide could be removed by evaporating the chloroform extract to a syrup and extracting this with hexane before u.v. analysis.

Conversion of methyl α-D-glucopyranoside 2-(S-benzylxanthate) into methyl α-D-glucopyranoside 6-(S-benzylxanthate). — Cleavage of the 2-(*S*-benzylxanthate) with sodium hydrosulfide, followed by incubation of the xanthate salt for 90 min in 18% sodium hydroxide at 0–5°, neutralization, and rebenzylation, gave a mixture in which the 6-(*S*-benzylxanthate) ester preponderated. The product, precipitated from benzene and hexane, melted at 75–90°, and t.l.c. showed a single component, comparable in R_F to the known 6-isomer. When the xanthate salt, in 18% sodium hydroxide at 0–5°, was neutralized immediately and then rebenzylated, the 2-(*S*-benzylxanthate) ester, m.p. 105–115°, was recovered. T.l.c. showed mainly the 2-isomer, with traces of the other isomers.

Methyl 4,6-O-benzylidene-3-O-methyl-α-D-glucopyranoside 2-(S-benzylxanthate) (**8**). — A solution of **2** (4.5 g) in dichloromethane (50 ml) was placed in a 2-l. Erlenmeyer flask and cooled to −80° in Dry Ice–acetone. A solution of diazomethane was prepared by mixing 10 g of *N*-methyl-*N*-nitrosourea with a suspension of 30 ml of 40% potassium hydroxide in 100 ml of ether and drying the resulting ether solution of diazomethane for several hours over potassium hydroxide pellets. This solution was cautiously added to the dichloromethane solution while the latter was stirred magnetically with a Teflon-covered stirring-bar. Addition of several drops of boron trifluoride etherate under these conditions caused a controlled evolution of gas, and, after 2 h, t.l.c. showed that **8** preponderated. The mixture was permitted to warm to room temperature gradually, causing slow decomposition of the excess diazomethane. The solution was then flushed with nitrogen, filtered through Celite, rendered neutral with 50 ml of 5% aqueous potassium hydrogen carbonate, and dried with sodium sulfate. The product was obtained by chromatography on Adsorbosil (Applied Science Laboratories, Inc.) by gradient elution with chloroform–cyclohexane. The product was crystallized from alcohol–water, to give crude **11**, yield 4.30 g (92%),

m.p. 15–25°. Several recrystallizations gave pure **11**, m.p. 35–37°, $[\alpha]_D^{24}$ +81.5° (*c* 1.66, chloroform).

Anal. Calc. for $C_{23}H_{26}O_6S_2$: C, 59.7; H, 5.7; S, 13.9. Found: C, 59.6; H, 5.8; S, 13.9.

Methyl 3-O-methyl-α-D-glucopyranoside 2-(S-benzylxanthate) (**12**). — Compound **8** (2.00 g) was treated for 3 h at 40° with 200 ml of 0.05M hydrochloric acid in methanol to remove the *O*-benzylidene group[10]. The product was isolated as previously described for the preparation of methyl α-D-glucopyranoside 2- and 3-(*S*-benzylxanthates). The product was separated from starting material by chromatography on Adsorbosil by using gradient elution with acetone–chloroform. Pure **12** was obtained by crystallization from benzene–hexane, yield 0.92 g (57%), m.p. 77–79°, $[\alpha]_D^{24}$ +114.6° (*c* 0.74, chloroform).

Anal. Calc. for $C_{16}H_{22}O_6S_2$: C, 51.3; H, 5.92; S, 17.1. Found: C, 51.4; H, 6.16; S, 16.5.

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SUMMARY

If similar reactant ratios are used, the xanthation of methyl α-D-glucopyranoside parallels the xanthation of starch. Degrees of substitution in the range of 0.3–0.4 were achieved, and changes in distribution from secondary to primary positions were observed. Synthesis of the individual, isomeric, xanthate salts of methyl α-D-glucopyranoside, and treatment of each with 18% sodium hydroxide, showed that the 2-, 3-, and 6-xanthate salts rearranged to mixtures in each of which the 6-isomer preponderated. Evidence is presented suggesting that the 2-isomer migrates intramolecularly to the 6-isomer by way of the 3-isomer. However, observation of the presence of minor proportions of polyxanthates in these mixtures suggests that intermolecular migration of thiolthiocarbonyl groups also occurs.

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ARYLSULFONYLHYDRAZONE UND DIALKYLTHIOACETALE DER D-*erythro*-PENTULOSE

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Aldosen unterliegen beim Behandeln mit einer Base der Lobry de Bruyn–Alberda van Ekenstein-Umlagerung[1]. Dabei entsteht ein Gemisch, das außer der eingesetzten Aldose die epimere Aldose und die zugehörige Ketose als Hauptprodukte enthält; in geringer Menge treten noch andere Substanzen, wahrscheinlich Saccharinsäuren, auf. Die Umlagerung kann zur Darstellung der D-*erythro*-Pentulose aus D-Arabinose herangezogen werden. Glatthaar und Reichstein[2] erhitzten D-Arabinose (30 g) in Pyridin, dampften die Lösung ein und trennten aus dem Rückstand die im Gemisch vorhandene D-Arabinose und D-Ribose (22 g) größtenteils durch Kristallisation ab. Der zurückbleibende Sirup (8 g) enthielt neben einer geringen Menge der beiden Aldosen die D-*erythro*-Pentulose, die in das reine, kristallisierte *o*-Nitrophenylhydrazon übergeführt wurde. Die Ausbeute an *o*-Nitrophenylhydrazon (3.6 g) betrug jedoch nur 6.3%, bezogen auf die zur Umlagerung eingesetzte D-Arabinose, oder 23.8% in bezug auf die rohe D-*erythro*-Pentulose.

Beim Nacharbeiten der genannten Untersuchungen[2] erzielten wir die gleichen Ergebnisse. Nach dem Spalten des *o*-Nitrophenylhydrazons durch Kochen mit Benzaldehyd, Wasser und etwas Benzolsäure erhielten wir schließlich 1.82 g (6.1%, bezogen auf D-Arabinose, 22.8%, bezogen auf rohe D-*erythro*-Pentulose) chromatographisch einheitliche D-*erythro*-Pentulose.

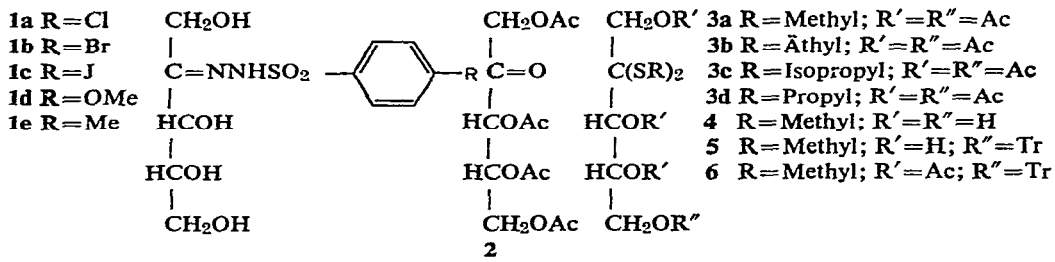
Da die Ausbeute zu wünschen übrig ließ, versuchten wir, die beiden Aldosen über ihre Isobutylthioacetale von der D-*erythro*-Pentulose abzutrennen, wie es früher für andere Aldose–Ketose-Gemische beschrieben wurde[3]. Die dünnschichtchromatographische Untersuchung der so gereinigten D-*erythro*-Pentulose (49%) ergab, daß sie zwar frei von D-Arabinose und D-Ribose war, aber in geringer Menge zwei Substanzen als Verunreinigung enthielt, die wahrscheinlich aus der D-*erythro*-Pentulose unter dem Einfluß der konz. Salzsäure bei der Thioacetalbildung entstanden waren.

Zur Reinigung der rohen D-*erythro*-Pentulose benutzten wir schließlich ein Verfahren, das von Adachi und Sugawara für die Trennung von Ketosen und Aldosen angegeben wurde[4]. Das in 75-proz. wäßrigem Propylalkohol gelöste Gemisch wird durch eine Säule filtriert, die einen mit Natriumbisulfit aktivierten Anionenaustauscher enthält. Die Aldosen werden vom Austauscher zurückgehalten, während die Ketosen die Säule passieren und durch Einengen des Filtrates gewonnen werden. Nach dem

*Because of an editorial oversight, the publication of this paper has been unduly delayed.

Passieren der Ketosen können dann die Aldosen durch Nachwaschen der Säule mit Wasser eluiert und durch Eindampfen des Eluates isoliert werden. Nach diesem Verfahren erhielten wir aus 8.0 g roher D-*erythro*-Pentulose 5.6 g (70%) gereinigte D-*erythro*-Pentulose, die frei von D-Arabinose und D-Ribose war und bei der dünnschichtchromatographischen Untersuchung nur Spuren zweier Verunreinigungen zeigte. Mit der auf diesem Wege gereinigten D-*erythro*-Pentulose wurden alle weiteren Untersuchungen durchgeführt. Die Rückgewinnung der beiden Aldosen (etwa 2.0 g), die im rohen D-*erythro*-Pentulose Sirup enthalten sind, lohnt nicht, denn man benötigt mehrere Liter Wasser, um die Aldosen vollständig aus der Säule herauszuwaschen; das Eindampfen des großen Wasservolumens ist zu aufwendig.

Da sich die Arylsulfonylhydrazone allgemein gut zur Reinigung von Aldosen eignen[5], untersuchten wir am Beispiel der D-*erythro*-Pentulose auch die Möglichkeit zur analogen Reinigung von Ketosen. Das *p*-Chlor- (**1a**), *p*-Brom- (**1b**), *p*-Jod- (**1c**) und das *p*-Methoxybenzolsulfonylhydrazon (**1d**) sowie das *p*-Toluolsulfonylhydrazon (**1e**) der D-*erythro*-Pentulose lassen sich am besten darstellen, indem man den Zucker in wenig Wasser löst, mit einer Lösung des betreffenden Arylsulfonylhydrazins in Methanol versetzt und in einem Wasserbad 3 Stunden auf 50° erwärmt. Da sich das *p*-Jodbenzolsulfonylhydrazin in Methanol schwer löst, wird es in *N,N*-Dimethylformamid mit der D-*erythro*-Pentulose zur Reaktion gebracht. Die Ausbeuten an Rohprodukt liegen bei den D-*erythro*-Pentulose-arylsulfonylhydrazonen tiefer als bei den Aldose-arylsulfonylhydrazonen. Weiterhin ist die Reinigung der Rohprodukte durch Umkristallisieren verlustreich; man gewinnt die reinen D-*erythro*-Pentulose-arylsulfonylhydrazone je nach Vertreter nur mit Ausbeuten von 12 bis 35%. Deswegen können die Arylsulfonylhydrazone für die Reinigung der rohen D-*erythro*-Pentulose nicht als geeignet angesehen werden. Wie die Arylsulfonylhydrazone der Aldosen[5] schmelzen auch die der D-*erythro*-Pentulose unter Zersetzung; die Schmelzpunkte sind



von der Erhitzungsgeschwindigkeit abhängig. In Pyridin gelöst zeigen die Hydrazone Mutarotation, was auf das Vorliegen von cyclischen *N*-Glykosid-Formen schließen läßt*.

Die Ketosen bilden bekanntlich im Gegensatz zu den Aldosen mit Thioalkoholen bei Gegenwart von Mineralsäuren keine Thioacetale. Wolfrom und Thompson[6] gelang die Synthese des Penta-*O*-acetyl-D-fructose-diäthylthioacetals durch Umset-

*Im Formelschema sind die D-*erythro*-Pentulose-arylsulfonylhydrazone (**1**) der Einfachheit wegen in der nicht cyclischen „Hydrazon-Form" wiedergegeben.

zung der Penta-*O*-acetyl-*keto*-D-fructose mit Äthylthioalkohol bei Gegenwart von Zinkchlorid. Analog wurden einige Penta-*O*-acetyl-L-sorbose-dialkylthioacetale synthetisiert[7]. Nach diesem Verfahren lassen sich auch die Tetra-*O*-acetyl-D-*erythro*-pentulose-dialkylthioacetale darstellen.

Die hierfür benötigte Tetra-*O*-acetyl-*keto*-D-*erythro*-Pentulose (**2**) erhielten wir durch Acetylieren der D-*erythro*-Pentulose mit Acetanhydrid und Zinkchlorid bei 0°. Die sirupöse Verbindung läßt sich durch Destillation im Hochvakuum reinigen; bei der Dünnschichtchromatographie erwies sie sich als einheitlich.

Die Tetra-*O*-acetyl-*keto*-D-*erythro*-Pentulose (**2**) wurde dann nach dem oben genannten Verfahren[6,7] mit einem Thioalkohol bei Gegenwart von Zinkchlorid zur Reaktion gebracht. Die dabei erhaltenen Tetra-*O*-acetyl-D-*erythro*-pentulose-dialkylthioacetale (**3**) sind Sirupe, die bisher noch nicht zur Kristallisation gebracht werden konnten. Die Thioacetale **3** lassen sich durch Destillation im Hochvakuum reinigen. Das Dimethyl-, Diäthyl- und das Diisopropylthioacetal wurden mit guten Ausbeuten als reine Verbindungen erhalten. Bei der Hochvakuumdestillation des Dipropyl-, Diisobutyl- und des Dibutylthioacetals trat eine teilweise Zersetzung auf, die Verbindungen ließen sich daher nur in geringer Ausbeute (7–19%) gewinnen. Die beiden Butylthioacetale waren nicht rein darstellbar.

Die Tetra-*O*-acetyl-D-*erythro*-pentulose-dialkylthioacetale lassen sich mit Bariummethylat in Methanol zu den sirupösen D-*erythro*-Pentulose-dialkylthioacetalen entacetylieren. Von diesen konnten wir jedoch nur das D-*erythro*-Pentulose-dimethylthioacetal (**4**) analysenrein gewinnen.

Bei der Umsetzung des D-*erythro*-Pentulose-dimethylthioacetals mit Tritylchlorid in Pyridin erhält man das 5-*O*-Trityl-D-*erythro*-pentulose-dimethylthioacetal (**5**). Obwohl die Verbindung kristallisierte, konnten wir sie nicht analysenrein darstellen, weil es nicht gelang, anhaftendes Triphenylmethanol restlos abzutrennen. Die Trityl-Verbindung wurde daher mit Acetanhydrid in Pyridin zum 1,3,4-Tri-*O*-acetyl-5-*O*-trityl-D-*erythro*-pentulose-dimethylthioacetal (**6**) acetyliert. Die kristallisierte, analysenreine Verbindung kann man einfacher darstellen, wenn man das nach der Umsetzung von D-*erythro*-Pentulose-dimethylthioacetal mit Tritylchlorid in Pyridin gelöste 5-*O*-Trityl-D-*erythro*-pentulose-dimethylthioacetal nicht als Zwischenprodukt isoliert, sondern sofort durch Zugabe von Acetanhydrid zu **6** acetyliert.

Primäre alkoholische Gruppen werden durch Tritylchlorid in Pyridin wesentlich schneller trityliert als sekundäre. Man kann daher annehmen, daß sich der Trityl-Rest im *O*-Trityl-D-*erythro*-pentulose-dimethylthioacetal (**5**) und in dessen Triacetat **6** entweder in 1- oder in 5-Stellung befindet. Da die L-Sorbose-thioacetale mit Tritylchlorid nicht die 1-*O*-Trityl-, sondern die 6-*O*-Trityl-L-sorbose-thioacetale bilden[7], ist in Analogie anzunehmen, daß sich der Trityl-Rest im *O*-Trityl-D-*erythro*-pentulose-dimethylthioacetal ebenfalls endständig, also in 5-Stellung befindet. Das konnten wir durch eine übliche[8] analytische Oxydation des 5-*O*-Trityl-D-*erythro*-pentulose-dimethylthioacetals (**5**) mit Bleitetraacetat in Benzol beweisen. Obwohl die eingesetzte Verbindung **5** nicht ganz rein war (siehe oben), fiel das Ergebnis der Oxydation eindeutig aus. Nach der Oxydation konnte kein Formaldehyd nachgewiesen werden.

Danach muß die 5-Stellung in **5** durch den Trityl-Rest blockiert sein. Der Verbrauch an Bleitetraacetat ist bei der durchgeführten Oxydation ohne analytischen Wert, da auch die Thioacetal-Gruppierung durch Bleitetraacetat oxydiert wird. Nach einer Oxydationszeit von vier Stunden stellten wir einen Verbrauch von 1.4 Mol Bleitetraacetat fest; die Theorie fordert 1.0 Mol.

EXPERIMENTELLER TEIL

Darstellung der D-erythro-*Pentulose.* — Unter Feuchtigkeitsausschluß werden 30 g D-Arabinose in 250 ml Pyridin 4 Stdn. unter Rückfluß gekocht. Dann destilliert man das Pyridin bei etwa 25 Torr ab, vermischt den Rückstand mit etwa 50 ml Wasser und dampft erneut bei 25 Torr ein. Diese Operation wird wiederholt, bis der Rückstand nicht mehr nach Pyridin riecht. Den Rückstand verreibt man anschließend mit 200 ml absol. Äthanol, läßt 16 Stdn. bei 0° stehen, saugt die auskristallisierten Aldosen ab, wäscht mit absol. Äthanol nach, engt das vereinigte Filtrat stark ein und läßt erneut bei 0° auskristallisieren. So fährt man fort, bis keine Aldosen mehr auskristallisieren. Man erhält 20–22 g eines Arabinose–Ribose-Gemisches, das wieder zur Umlagerung eingesetzt werden kann. Durch Eindampfen der letzten Mutterlauge gewinnt man 8.0 g rohe, sirupöse D-*erythro*-Pentulose.

In eine Säule von 3.0 cm Durchmesser und 45 cm Länge füllt man 100 g vorgequollenen (= 55 g lufttrockenen) Anionenaustauscher „Wofatit SBW" der Korngröße 0.3–0.7 mm*, gibt 150 ml N HCl hinzu, läßt 3 Stdn. stehen und wäscht dann die Säule mit etwa 600 ml Wasser, bis die durchgelaufene Flüssigkeit neutral reagiert. Nun ersetzt man das Wasser in der Säule durch eine 1-molare Natriumbisulfit-Lösung, indem man 250 ml der Lösung durch die Säule filtriert, bis die Flüssigkeit nur noch etwa 4 cm über dem Austauscher steht. Die Säule wird 15 Stdn. stehengelassen und anschließend mit etwa 1000 ml Wasser gewaschen, bis in der ablaufenden Flüssigkeit der Nachweis auf Chlorid-Ionen negativ ausfällt. In der Säule wird die wäßrige Phase schließlich durch Waschen mit 250 ml 75-proz. Propylalkohol verdrängt; sie ist jetzt für die Aldose-Ketose-Trennung fertig.

Die nach obiger Vorschrift gewonnene rohe D-*erythro*-Pentulose (8.0 g) wird in 150 ml 75-proz. Propylalkohol gelöst und die Lösung mit einer Durchlaufgeschwindigkeit von 1 ml pro Min. durch die Austauschersäule filtriert. Dann wäscht man mit 1000 ml 75-proz. Propylalkohol nach, dampft das Eluat (1150 ml) bei etwa 25 Torr ein, nimmt den Rückstand in 50 ml absol. Äthanol auf, schüttelt mit Aktivkohle, filtriert, dampft erneut ein und trocknet die zurückbleibende D-*erythro*-Pentulose im Vakuum. Ausbeute 5.6 g (19%, bezogen auf eingesetzte D-Arabinose, 70%, bezogen auf rohe D-*erythro*-Pentulose), gelblicher Sirup, $[\alpha]_D^{20}$ −16.5° (*c* 1.81, Wasser). Lit.[11], $[\alpha]_D^{20}$ −16.3° (Wasser).

Darstellung der D-erythro-*Pentulose-arylsulfonylhydrazone* (**1**). — D-*erythro*-Pentulose (1.50 g, 0.01 Mol) wird in 3.0 ml Wasser gelöst und mit einer Lösung von 0.01 Mol eines Arylsulfonylhydrazins[5,9] in 10 ml Methanol 3 Stdn. auf 50°

*Der Anionenaustauscher entspricht in seinen Eigenschaften dem „Amberlite IRA-400".

erwärmt. Dann läßt man etwa 24 Stdn. bei 0° und 24 Stdn. bei −20° stehen, saugt das Rohprodukt ab und kristallisiert um. Zur Herstellung des *p*-Jodbenzolsulfonylhydrazons erwärmt man D-*erythro*-Pentulose und *p*-Jodbenzolsulfonylhydrazin in 15 ml *N*,*N*-Dimethylformamid 4 Stdn. auf 50°, destilliert das Lösungsmittel im Vakuum weitgehend ab, fügt 150 ml Wasser hinzu, läßt 15 Stdn. bei 0° stehen, saugt das Rohprodukt ab und kristallisiert um. Die Schmelzpunkte der unten aufgeführten Vertreter werden bestimmt, indem man zunächst schnell bis etwa 15° unterhalb des Schmelzpunktes erhitzt und dann die Temperatur um 3–4° pro Min. erhöht.

D-erythro-*Pentulose*-p-*chlorbenzolsulfonylhydrazon* (**1a**), Ausbeute 1.12 g (33%), Nadeln aus Methanol–Wasser, Schmp. 146° (Zers.), $[\alpha]_D^{21}$ −19.0° (nach 10 Min.) → −6.4° (nach 24 Stdn.; *c* 0.95, Pyridin). ($C_{11}H_{15}ClN_2O_6S$ Ber.: C, 38.99; H, 4.46; N, 8.27. Gef.: C, 39.06; H, 4.40; N, 8.38%).

D-erythro-*Pentulose*-p-*bromobenzolsulfonylhydrazon* (**1b**), Ausbeute 1.12 g (29%), Nadeln aus Methanol–Wasser, Schmp. 148° (Zers.), $[\alpha]_D^{21}$ +43.4° (nach 10 Min.) → +6.3° (nach 24 Stdn.; *c* 0.92, Pyridin). ($C_{11}H_{15}BrN_2O_6S$ Ber.: C, 34.47; H, 3.94; N, 7.31. Gef.: C, 34.79; H, 3.94; N, 7.33%).

D-erythro-*Pentulose*-p-*jodbenzolsulfonylhydrazon* (**1c**), Ausbeute 1.50 g (35%), Nadeln aus Äthanol, Schmp. 152° (Zers.), $[\alpha]_D^{21}$ −13.5° (nach 10 Min.) → −5.3° (nach 24 Stdn.; *c* 1.31, Pyridin). ($C_{11}H_{15}JN_2O_6S$ Ber.: C, 30.70; H, 3.51; N, 6.51. Gef.: C, 31.15; H, 3.69; N, 6.69%).

D-erythro-*Pentulose*-p-*methoxybenzolsulfonylhydrazon* (**1d**), Ausbeute 0.40 g (12%), Kristallpulver aus Äthanol, Schmp. 151° (Zers.), $[\alpha]_D^{21}$ +17.3° (nach 10 Min.) → +7.4° (nach 24 Stdn.; *c* 0.96, Pyridin). ($C_{12}H_{18}N_2O_7S$ Ber.: C, 43.11; H, 5.43; N, 8.38. Gef.: C, 43.31; H, 5.50; N, 8.39%).

D-erythro-*Pentulose*-p-*toluolsulfonylhydrazon* (**1e**), Ausbeute 0.76 g (24%), Kristallpulver aus Äthanol, Schmp. 164° (Zers.), $[\alpha]_D^{21}$ +17.7° (nach 10 Min). → +3.7° (nach 24 Stdn.; *c* 0.99, Pyridin). ($C_{12}H_{18}N_2O_6S$ Ber.: C, 45.27; H, 5.70; N, 8.80. Gef.: C, 45.43; H, 5.41; N, 9.04%).

Darstellung der Tetra-O-*acetyl*-keto-D-erythro-*pentulose* (**2**). — Zu 9.00 g (0.06 Mol) D-*erythro*-Pentulose gibt man eine Lösung von 1.0 g wasserfreiem Zinkchlorid in 150 ml Acetanhydrid, rührt 8 Stdn. bei 0° (wobei die D-*erythro*-Pentulose in Lösung geht), läßt 16 Stdn. bei 0° und etwa 5 Stdn. bei 20° stehen, gießt dann in 750 ml Wasser und rührt etwa 3 Stdn. zur Zersetzung des überschüssigen Acetanhydrids. Dann gießt man die wäßrige Phase vom ausgefallenen Sirup ab, nimmt diesen in 100 ml Chloroform auf, extrahiert die wäßrige Phase zweimal mit je 50 ml Chloroform, wäscht die vereinigten Chloroform-Extrakte mit 30 ml gesättigter Natriumhydrogencarbonat-Lösung sowie mit 30 ml Wasser, trocknet über Natriumsulfat und dampft zu einem Sirup ein. Dieser wird in 50 ml Äthanol gelöst, die Lösung mit Aktivkohle geschüttelt, filtriert und bei etwa 25 Torr eingedampft. Den Rückstand destilliert man bei 10^{-4} Torr und 145–155° Badtemperatur. Ausbeute 7.6 g (40%), Sirup $[\alpha]_D^{21}$ +3.9° (*c* 2.00, Methanol), n_D^{21} 1.4502. ($C_{13}H_{18}O_9$ Ber.: C, 49.05; H, 5.70. Gef.: C, 48.95; H, 5.57%).

Darstellung der Tetra-O-*acetyl*-D-erythro-*pentulose-dialkylthioacetale* (**3**). —

Verbindung **2** (3.18 g, 0.01 Mol) wird mit 3.0 g wasserfreiem Natriumsulfat und einer auf −15° abgekühlten Lösung von 1.6 g wasserfreiem Zinkchlorid in 12 ml eines Thioalkohols versetzt. Man läßt 6 Stdn. bei 0° und anschließend 24 Stdn. (den Ansatz mit Methylthioalkohol 5 Tage) bei 20° stehen, gibt 40 ml einer gesättigten Natriumhydrogencarbonat-Lösung hinzu, schüttelt gut durch und saugt die anorganischen Bestandteile ab. Der Filterrückstand und das Filtrat werden je dreimal mit 50 ml Chloroform extrahiert, die vereinigten Extrakte mit Natriumsulfat getrocknet und zu einem Sirup eingedampft. Diesen löst man in 20 ml Äthanol, schüttelt die Lösung mit Aktivkohle, filtriert und dampft im Vakuum ein. Den Rückstand destilliert man bei 10^{-4} Torr und 150–165° Badtemperatur, wobei man die unten aufgeführten Verbindungen als Sirupe erhält.

Tetra-O-*acetyl*-D-erythro-*pentulose-dimethylthioacetal* (**3a**), Ausbeute 3.21 g (81%), $[\alpha]_D^{21}$ +37.7° (*c* 2.52, Methanol), n_D^{21} 1.5113. ($C_{15}H_{24}O_8S_2$ Ber.: C, 45.44; H, 6.10. Gef.: C, 45.89; H, 6.27%).

Tetra-O-*acetyl*-D-erythro-*pentulose-diäthylthioacetal* (**3b**), Ausbeute 2.60 g (61%), $[\alpha]_D^{21}$ +9.6° (*c* 1.78, Methanol), n_D^{21} 1.4854. ($C_{17}H_{28}O_8S_2$ Ber.: C, 48.09; H, 6.65. Gef.: C, 48.23; H,6.25%).

Tetra-O-*acetyl*-D-erythro-*pentulose-diisopropylthioacetal* (**3c**), Ausbeute 2.52 g (55%), $[\alpha]_D^{21}$ +3.4° (*c* 2.20, Methanol), n_D^{21} 1.4872. ($C_{19}H_{32}O_8S_2$ Ber.: C, 50.42; H, 7.13. Gef.: C, 50.63; H, 6.90%).

Tetra-O-*acetyl*-D-erythro-*pentulose-dipropylthioacetal* (**3d**), Ausbeute 0.85 g (19%), $[\alpha]_D^{21}$ +4.7° (*c* 1.80, Methanol), n_D^{21} 1.4888. ($C_{19}H_{32}O_8S_2$ Ber.: C, 50.42; H, 7.13. Gef.: C, 50.43; H, 7.11%).

D-erythro-*Pentulose-dimethylthioacetal* (**4**). — Man versetzt eine Lösung von 3.96 g (0.01 Mol) Tetra-*O*-acetyl-D-*erythro*-pentulose-dimethylthioacetal in 80 ml Methanol mit 10 ml 0.1N $Ba(OCH_3)_2$ in Methanol, läßt 16 Stdn. bei 20° stehen, neutralisiert mit 0.1N H_2SO_4, schüttelt etwa 5 Min. mit 0.2 g Bariumcarbonat und 0.1 g Aktivkohle, filtriert und dampft das Filtrat bei etwa 25 Torr zu einem Sirup ein, der bei 10^{-4} Torr und 35° getrocknet wird. Ausbeute 1.77 g (78%), $[\alpha]_D^{21}$ +12.8° (*c* 2.13, Methanol), n_D^{21} 1.5826. ($C_7H_{16}O_4S_2$ Ber.: C, 36.82; H, 7.06. Gef.: C, 37.04; H, 7.07%).

5-O-*Trityl*-D-erythro-*pentulose-dimethylthioacetal* (**5**). — Man löst 2.28 g (0.01 Mol) **4** und 2.78 g (0.01 Mol) Tritylchlorid in 20 ml Pyridin, läßt 24 Stdn. bei 20° stehen, verdünnt mit 40 ml Aceton, läßt die Lösung unter Rühren in 400 ml Wasser eintropfen, nimmt den ausgefallenen Sirup in 100 ml Chloroform auf, wäscht die Chloroform-Lösung mit 20 ml 10-proz. Kaliumhydrogensulfat- und 20 ml gesättigter Natriumhydrogencarbonat-Lösung sowie mit 20 ml Wasser, trocknet mit Natriumsulfat und dampft zu einem Sirup ein. Dieser wird in 40 ml Äthanol aufgenommen, die Lösung mit Aktivkohle geschüttelt, filtriert und erneut zu einem Sirup eingedampft, der in einigen Tagen durchkristallisiert. Ausbeute 3.2 g (68%), Schmp. 104°, $[\alpha]_D^{20}$ +5.0° (*c* 1.08, Chloroform). Die Verbindung ist nicht analysenrein. Bei der Oxydation mit Bleitetraacetat[8] in Benzol verbraucht die Verbindung nach 4 Stdn. 1.4 Mol des Oxydationsmittels, Formaldehyd kann nicht nachgewiesen werden.

Tri-O-*acetyl*-5-O-*trityl*-D-erythro-*pentulose-dimethylthioacetal* (**6**). — Der zuvor

angegebene Reaktionsansatz wird nach 24 Stdn. mit 8.0 ml Acetanhydrid versetzt und 16 Stdn. bei 20° stehengelassen. Dann arbeitet man auf, wie zur Darstellung von **2** angegeben. Der nach dem Eindampfen der Chloroform-Lösung erhaltene Sirup wird in 30 ml Äthanol gelöst, die Lösung mit Aktivkohle geschüttelt, filtriert und erneut eingedampft. Der zurückbleibende Sirup kristallisiert beim Stehenlassen. Man kristallisiert aus wenig Äthanol um. Ausbeute 2.60 g (44%), Schmp. 88° $[\alpha]_D^{21}$ +16.9° (*c* 2.79, Chloroform). ($C_{32}H_{36}O_7S_2$ Ber.: C, 64.40; H, 6.08. Gef.: C, 64.61; H, 6.21%).

Durchführung der Dünnschichtchromatographie. — Die Schichten werden in der üblichen Weise[10] mit einer Mischung von 30 g Kieselgel G (Merck) und 60 ml einer 0.02M wäßrigern Natriumacetat-Lösung auf Glasplatten hergestellt und 30 Min. bei 100° getrocknet.

Für die Chromatographie der D-*erythro*-Pentulose, D-Arabinose und D-Ribose benutzt man als Laufmittel ein Gemisch von 65 ml Essigsäureäthylester und 35 ml einer Lösung von Isopropylalkohol–Wasser (2:1). Die Zucker werden auf den Platten mit Naphthoresorcin–Phosphorsäure sichtbar gemacht[10]; die D-*erythro*-Pentulose gibt braune, die beiden Aldosen blaue Flecken. Der R_F-Wert für D-*erythro*-Pentulose beträgt 0.29, für D-Arabinose 0.18 und für D-Ribose 0.25.

Zur Chromatographie der Tetra-*O*-acetyl-*keto*-D-*erythro*-pentulose dient als Laufmittel ein Gemisch von 100 ml Benzol und 2 ml Methanol[10]. Die Verbindung wird auf der Platte mit der Hydroxamsäure-Reaktion nachgewiesen[10], sie gibt braune Flecken. Der R_F-Wert beträgt 0.12.

ZUSAMMENFASSUNG

D-Arabinose wird der Lobry de Bruyn–van Ekenstein-Umlagerung unterworfen. Aus dem erhaltenen Gemisch werden D-Arabinose und D-Ribose größtenteils durch Kristallisation aus Äthanol abgetrennt. Die zurückbleibende rohe, in 75-proz. Propylalkohol gelöste D-*erythro*-Pentulose wird durch eine mit Natriumbisulfit aktivierte Anionenaustauscher-Säule filtriert. Dabei hält die Säule die in der rohen D-*erythro*-Pentulose noch enthaltenen Aldosen zurück; die D-*erythro*-Pentulose passiert die Säule und wird durch Eindampfen des Eluates gewonnen. Von der D-*erythro*-Pentulose werden einige Arylsulfonylhydrazone dargestellt. Beim Acetylieren der D-*erythro*-Pentulose mit Acetanhydrid und Zinkchlorid bei 0° entsteht die Tetra-*O*-acetyl-*keto*-D-*erythro*-pentulose, die beim Behandeln mit Thioalkoholen und Zinkchlorid Tetra-*O*-acetyl-D-*erythro*-pentulose-dialkylthioacetale bildet. Das reine D-*erythro*-Pentulose-dimethylthioacetal erhält man aus dem entsprechenden Tetraacetat durch Entacetylieren mit Bariummethylat in Methanol; es wird in das kristallisierte Tri-*O*-acetyl-5-*O*-trityl-D-*erythro*-pentulose-dimethylthioacetal übergeführt.

SUMMARY

D-Arabinose has been subjected to the Lobry de Bruyn–van Ekenstein rearrangement. From the resulting mixture, D-arabinose and D-ribose were separated mainly

by crystallisation from ethanol. A solution of the residual, crude D-*erythro*-pentulose in 75% propyl alcohol was passed through an anion-exchange resin in the bisulphite form. The aldoses were retained by the column, whereas the D-*erythro*-pentulose was not retained and could be recovered from the eluate. Several arylsulphonylhydrazones have been prepared from D-*erythro*-pentulose. With acetic anhydride and zinc chloride at 0°, D-*erythro*-pentulose gave tetra-*O*-acetyl-*keto*-D-*erythro*-pentulose, which, on treatment with thiols and zinc chloride, afforded tetra-*O*-acetyl-D-*erythro*-pentulose dialkyl dithioacetals. Pure D-*erythro*-pentulose dimethyl dithioacetal was obtained by treatment of the corresponding tetra-acetate with methanolic barium methoxide and used for the preparation of tri-*O*-acetyl-5-*O*-trityl-D-*erythro*-pentulose dimethyl dithioacetal.

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ACETAL EXCHANGE REACTIONS
II. CYCLOHEXYLIDENE DERIVATIVES OF SOME GLYCOSIDES AND CYCLITOLS

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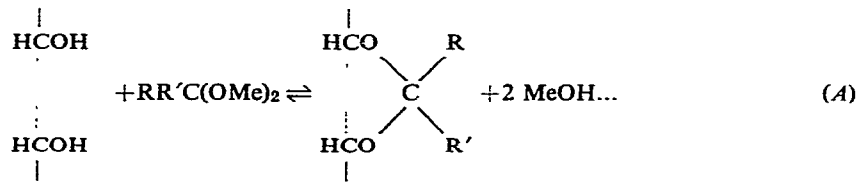
INTRODUCTION

Previously, we described the use of various dimethyl acetals in the preparation of cyclic acetals of sugars. In that work[1,2], the products of equilibrium (*A*) were isolated. The possibility of displacing reaction *A* to the right interested us, since cyclic acetals bridging vicinal, *trans* hydroxyl groups on a pyranose ring were obtained as minor products in these preparations.



(*A*)

In the present paper, we describe the results of experiments in which reaction *A* was displaced by removing methanol from the reaction mixtures, resulting in the formation of cyclic acetals, even if strained, in high yield.

RESULTS AND DISCUSSION

Attempts to eliminate methanol from isopropylidenation reactions, by performing these in boiling 2,2-dimethoxypropane, led to extensive discoloration of the solutions, and, as judged by chromatograms, little increase in the yields of cyclic acetals. Reaction of sugars with a relatively nonvolatile dimethyl acetal and an acid catalyst in boiling *N*,*N*-dimethylformamide under diminished pressure affords cyclic acetals, even if strained, in good yield. 1,1-Dimethoxycyclohexane was selected, as it cannot form cyclic acetals that are asymmetric at the methylidene carbon atom. Vigorous boiling of the reaction mixture is essential for rapid reaction; a thoroughly dry stream of air or rapid stirring are equally effective.

Reaction of methyl α-D-glucopyranoside with 1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid in *N*,*N*-dimethylformamide by boiling at 70°/15 mm gives methyl 2,3:4,6-di-*O*-cyclohexylidene-α-D-glucoside (**1**) in 93% yield. When this reaction

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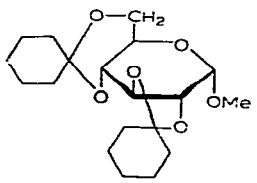
is performed at 25°/760 mm, compound **1** is obtained in only 2% yield, methyl 4,6-*O*-cyclohexylidene-α-D-glucoside (**2**) in 80% yield, and unchanged methyl glucoside in 17% yield; these results are very similar to those obtained[2] when 2,2-dimethoxypropane is used instead of 1,1-dimethoxycyclohexane. Graded hydrolysis of **1** with acid gives **2** and methyl α-D-glucopyranoside.

Reaction of *myo*-inositol with 1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid in *N*,*N*-dimethylformamide by boiling at 70°/15 mm gives a mixture of two compounds that are closely similar in chromatographic mobility. The major product appears to be the 1,2:3,4:5,6-tri-*O*-cyclohexylidene-*myo*-inositol reported by Angyal *et al.*[3], as the infrared spectra of the two compounds are indistinguishable; however, even after repeated recrystallization, we were unable to obtain a sample of our compound with a sharp melting point. We are still investigating this problem and the structure of the minor product. Cyclohexylidenation of *scyllo*-inositol by the method used for *myo*-inositol gives (presumably, 1,2:3,4:5,6)-tri-*O*-cyclohexylidene-*scyllo*-inositol in 66% yield. This appears to be the first preparation of an acetal of *scyllo*-inositol; indeed, the only other example of formation of an acetal bridging vicinal,



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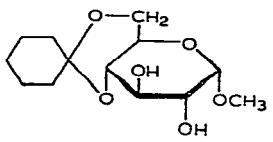

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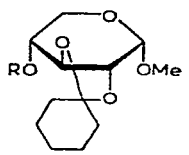
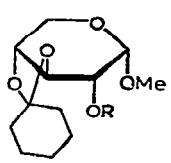
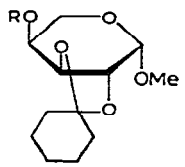


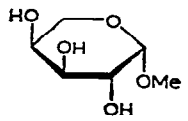
5 R = H
7 R = Ts



6 R = H
11 R = Ts



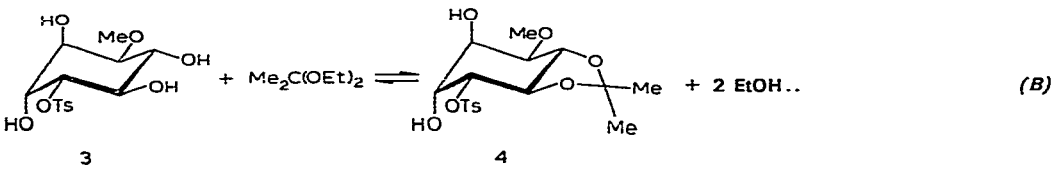
8 R = PhCO
9 R = H



10

trans hydroxyl groups on an inositol ring not already bearing at least one acetal group bridging vicinal, *cis* hydroxyl groups, is one reported by Angyal and Hoskinson[4]. These workers obtained 3,4-*O*-isopropylidene-2-*O*-methyl-5-*O*-*p*-tolylsulfonyl-(−)-inositol (**4**) by treating 2-*O*-methyl-5-*O*-*p*-tolylsulfonyl-(−)-inositol (**3**) with 2,2-diethoxypropane and *p*-toluenesulfonic acid. It is not readily apparent why **3** forms an isopropylidene acetal, although not even a monoacetal of *scyllo*-inositol has been reported, even though the latter compound has been subjected to extremely vigorous acetalating conditions by Mager and Ionescu[5]; a possible explanation is that *scyllo*-inositol is so insoluble in almost all organic solvents that insufficient material enters into solution for reaction to occur; compound **3** on the other hand, is probably some-

what soluble in 2,2-diethoxypropane, permitting equilibrium *B* to be established quite readily.



3 4

The reaction of methyl α-D-xylopyranoside with 1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid in boiling *N*,*N*-dimethylformamide at 70°/15 mm is rapid, and may conveniently be followed by g.l.c. of the trimethylsilylated reaction mixture. After 30 min, the g.l.c. pattern does not change, despite the presence of a small proportion of methyl α-D-xyloside in the reaction mixture; addition of excess 1,1-dimethoxycyclohexane and prolonged reaction do not eliminate this residual D-xyloside. The ratio of methyl 2,3-*O*-cyclohexylidene-α-D-xylopyranoside (**5**) to methyl 3,4-*O*-cyclohexylidene-α-D-xyloside (**6**) was 10:1, as judged by the peak areas of the trimethylsilyl ethers. This mixture of **5** and **6** failed to crystallize and could not be separated by column chromatography. However, *p*-toluenesulfonation gave a mixture of *p*-toluenesulfonates from which methyl 2,3-*O*-cyclohexylidene-4-*O*-*p*-tolylsulfonyl-α-D-xyloside (**7**) crystallised readily. The structure of **7** was deduced from its n.m.r. spectrum, and verified by conversion of **7** into methyl β-L-arabinopyranoside.

In compound **7**, the H-4 proton would be expected to be deshielded by the sulfonyloxy group, and H-4, H-5*e*, and H-5*a* should form an ABX system. The n.m.r. spectrum* of **7** (see Fig. 1) shows a doublet of quartets at 4.66 p.p.m. which may be analyzed as the X (H-4) part of this system. The signal from H-5e is a quartet centered at 3.89 p.p.m. ($J_{5e,5a}$ 11 Hz, $J_{4,5e}$ 4.4 Hz). H-5a gives rise to a triplet at 3.68 p.p.m. ($J_{4,5a}$ 11 Hz). H-3 gives rise to a triplet at 3.98 p.p.m. ($J_{3,4}$ 9.2 Hz, $J_{2,3}$ 9.2 Hz). The H-2 signal occurs as a doublet of doublets at 3.38 p.p.m. ($J_{1,2}$ 3.0 Hz), and the anomeric proton as a symmetrical doublet at 4.98 p.p.m. The sharp singlet (3 protons) at 3.43 p.p.m. is assigned to the methoxyl group on C-1.

If the crystalline *p*-toluenesulfonate were methyl 3,4-*O*-cyclohexylidene-2-*O*-*p*-tolylsulfonyl-α-D-xyloside (**11**), H-2 would be deshielded by approximately 1.2 p.p.m., and H-1 and H-2 would form an *AB* system—a pattern that has been observed in these laboratories for numerous 2-sulfonic esters of glycosides. Compound **11** was obtained pure and, in its n.m.r. spectrum (see Fig. 1), the H-2 signal occurs as a doublet of doublets centered at 4.54 p.p.m. ($J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.0 Hz), and H-1 gives rise to an unsymmetrical doublet at 4.86 p.p.m.

Treatment of **7** with sodium benzoate in boiling *N*,*N*-dimethylformamide caused

*N.m.r. spectra were measured in $CDCl_3$ solutions, with tetramethylsilane ($\delta=0.0$) as internal standard. Chemical shifts are in p.p.m. downfield from this standard.

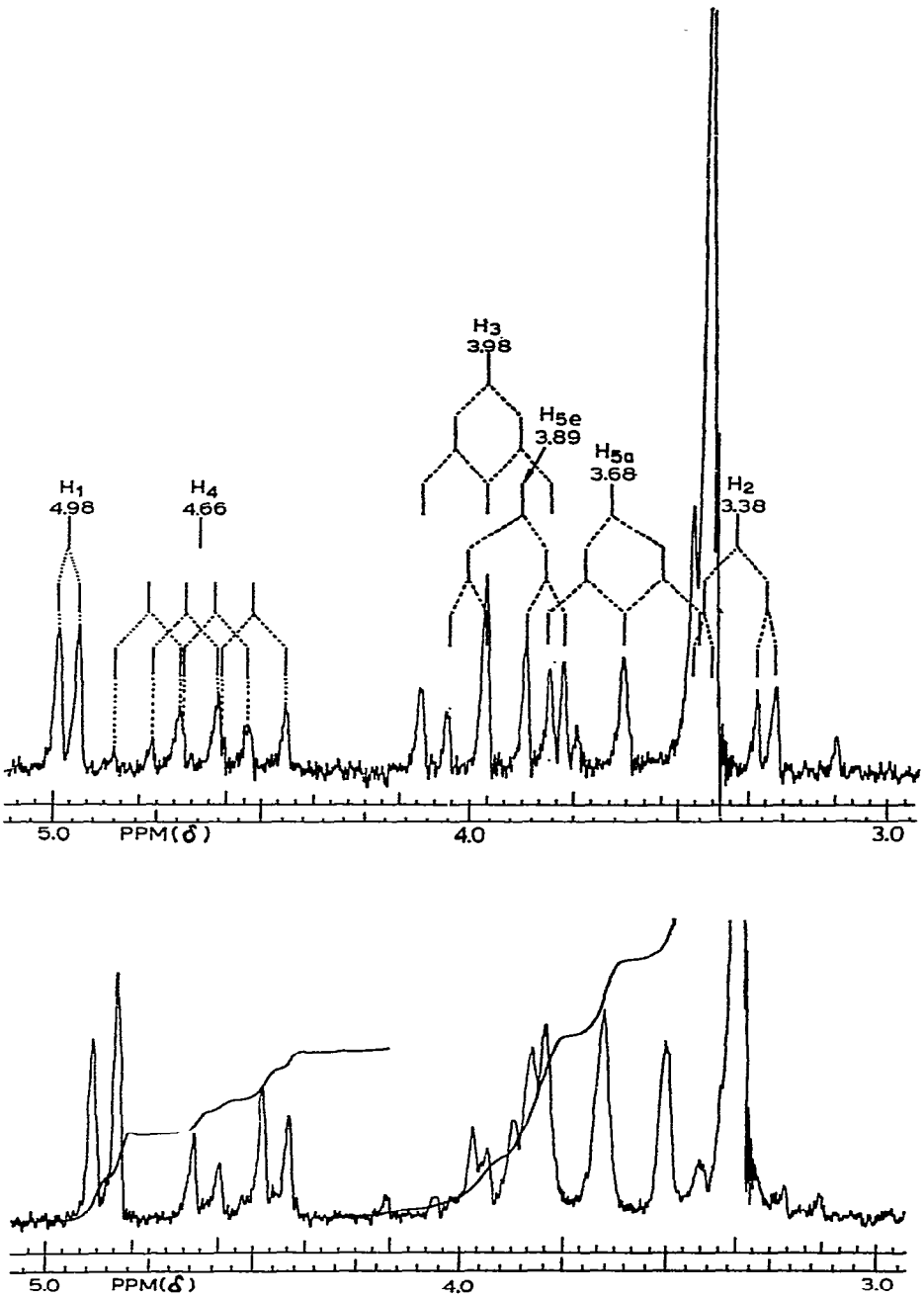


Fig. 1. N.m.r. spectra at 60 MHz of (upper) methyl 2,3-*O*-cyclohexylidene-4-*O*-*p*-tolylsulfonyl-α-D-xyloside (**7**), and (lower) methyl 3,4-*O*-cyclohexylidene-2-*O*-*p*-tolylsulfonyl-α-D-xyloside (**11**).

smooth displacement of the *p*-tolylsulfonyl group, a reaction[6] that, in the absence of neighbouring-group participation, is invariably accompanied by Walden inversion, to give methyl 4-*O*-benzoyl-2,3-*O*-cyclohexylidene-β-L-arabinoside (**8**). Successive saponification and acid hydrolysis of **8** gave the known methyl β-L-arabinopyranoside (**10**). It might be anticipated that, in the acid hydrolysis of methyl 2,3-*O*-cyclohexylidene-β-L-arabinopyranoside (**9**), the cyclic acetal group would migrate from the 2,3 positions (*trans* hydroxyl groups) to the 3,4 positions (*cis* hydroxyl groups); although this hydrolysis was followed by t.l.c., by use of a solvent system that resolves **5** and **6**, no evidence of rearrangement was seen. Foster *et al.*[7] have demonstrated the absence of apparently favorable migration during acid hydrolysis of cyclic acetals of alditols.

We were unable to obtain pure methyl 3,4-*O*-cyclohexylidene-2-*O*-*p*-tolylsulfonyl-α-D-xyloside (**11**) directly from the mother liquors of the crystallization of **7**. However, reaction of a mixture of **7** and **11** with sodium benzoate in boiling *N*,*N*-dimethylformamide left **11** unchanged, and **7** was converted into **8**. Saponification of the mixture of **11** and **8**, with sodium methoxide in methanol at 4°, converted **8** into **9** and **11** was again unchanged. The mixture of **9** and **11** was readily resolved by chromatography. Acid hydrolysis of a sample of **9**, obtained in this way, gave **10** having m.p. 167.5–169.5° (before recrystallization); as pure **10** melts at 169-170°, very little methyl α-D-lyxopyranoside could have been present in the product and, hence, very little displacement of the *p*-toluenesulfonyl group in **11** could have occurred during the benzoate exchange-reaction. We were unable to effect a separation of methyl α-D-lyxopyranoside from **10** by g.l.c., although a number of column packings and chromatographic instruments were tried.

Treatment of **11** with lithium aluminum hydride, to remove the sulfonate group, followed by methylation and acid hydrolysis, gave 2-*O*-methyl-D-xylose, identical with a specimen prepared by the method of Robertson and Speedie[8].

At no time during this work was any trouble caused by decomposition of 1,1-dimethoxycyclohexane to 1-methoxycyclohexene[9].

EXPERIMENTAL

General. — The general methods used were the same as those given in our previous paper[2]. Dowex-50W (H^+) and Rexyn-AG1 (OH^-) [Cat. No. R-252] resins were washed with methanol before use. Solvent 1 refers to benzene–chloroform–isopropyl alcohol (3:3:1). Brinkmann silica gel (0.05–0.20 mm) was used for column chromatography.

Cyclohexylidenation of methyl α-D-glucopyranoside. — *1.* Methyl α-D-glucopyranoside (0.97 g) was added to a solution of 1,1-dimethoxycyclohexane (1.6 g) and *p*-toluenesulfonic acid (0.01 g) in *N*,*N*-dimethylformamide (10 ml), and the mixture was shaken for 3 days at 25°. Dry (CaH_2) pyridine (1 ml) and excess Rexyn AG1 resin were added, and the mixture was shaken for 12 h. After evaporation of the solvents, xylene (2 × 50 ml) was evaporated from the product. Extraction of the residue with dichloromethane (3 × 100 ml) and evaporation of the combined extracts gave a mixture of

1 and **2**; extraction of the residual resin with water (100 ml), and evaporation of the extract, gave methyl α-D-glucopyranoside (0.17 g, 17%).

The mixture of **1** and **2** was fractionated by column chromatography; eluted first, with 0.2% of triethylamine in 1:1 hexane–ether, was **1** (0.04 g, 2.3%), m.p. 137–139°.

Eluted second, with 0.2% of triethylamine in ethyl acetate, was **2** (1.07 g, 78%), m.p. 118–119°. Pure **2** has m.p. 120–121° (from ethyl acetate), and $[\alpha]_D^{25}$ +122° (*c* 0.6, acetone).

Anal. Calc. for $C_{13}H_{22}O_6$: C, 56.9; H, 8.1. Found: C, 57.1; H, 8.0.

Compound **2** crystallizes readily from its syrup, but sometimes separates as a gel; on numerous occasions, crystals having m.p. 90–95° were obtained from the syrup; these appear to be a dimorphic form, as crystals of m.p. 120–121° may be obtained from the low-melting form by recrystallization from ethyl acetate. The high- and low-melting forms are indistinguishable by t.l.c.

2. A solution of methyl α-D-glucopyranoside (0.97 g), *p*-toluenesulfonic acid (0.01 g), and 1,1-dimethoxycyclohexane (1.6 g) in *N*,*N*-dimethylformamide (10 ml) was boiled under reflux at 70° (bath)/15 mm, with use of a fine stream of dry (silica gel) air, to promote steady ebullition. After 2 h, the solution was examined by t.l.c. (solvent 1) and found to contain starting D-glucoside, **1**, and **2**. 1,1-Dimethoxycyclohexane (1 g) was added to the solution, and the air stream was dried by bubbling it through concentrated sulfuric acid. After being boiled under reflux at 70° (bath)/15 mm for a further 2 h, the solution was examined as before, and found to contain only **1**. The mixture was processed as described in paragraph *1*. Evaporation of the triethylamine–ether–hexane eluate gave **1** (1.64 g, 93%), m.p. 142–143°. The pure compound has m.p. 145.5–146.5° (from triethylamine–hexane–isopropyl alcohol), and $[\alpha]_D^{25}$ +77° (*c* 1.0, acetone).

Anal. Calc. for $C_{19}H_{30}O_6$: C, 64.4; H, 8.5. Found: C, 64.6; H, 8.4.

*Tri-O-cyclohexylidene-*scyllo*-inositol.* — A solution of *p*-toluenesulfonic acid (0.01 g) and 1,1-dimethoxycyclohexane (1 g) in *N*,*N*-dimethylformamide (3 g) containing *scyllo*-inositol (0.066 g) was boiled for 8 h under reflux at 70° (bath)/15 mm. The solution was cooled, neutralized with Rexyn AG1 resin, and evaporated, and xylene (2 × 50 ml) was evaporated from the residue. The product was extracted with dichloromethane (100 ml), and the extract was filtered and evaporated, giving a crystalline product that was purified by column chromatography; elution with 0.1% of triethylamine in 1:1 hexane–dichloromethane afforded (presumably 1,2:3,4:5,6)-tri-*O*-cyclohexylidene-*scyllo*-inositol (0.092 g, 66%), m.p. 216–217°. The pure compound melts at 222° (from isopropyl alcohol). The n.m.r. spectrum of the triacetal shows a sharp singlet at 3.82 p.p.m. (integrated intensity, 38 units) and a broad band in the region of 1.2–1.9 p.p.m. (integrated intensity, 200 units).

Anal. Calc. for $C_{24}H_{36}O_6$: C, 68.5; H, 8.6. Found: C, 68.6; H, 8.3.

Elution with isopropyl alcohol afforded further crystalline material (0.029 g) that contained several components, and that could not be satisfactorily recrystallized.

Cyclohexylidenations of methyl α-D-xylopyranoside. — *1.* A vigorously stirred solution of methyl α-D-xylopyranoside (0.82 g), *p*-toluenesulfonic acid (0.01 g), and 1,1-dimethoxycyclohexane (0.86 g) in *N*,*N*-dimethylformamide (5 ml) was kept for 1 h at 25°/15 mm. A sample (0.1 ml) was removed, and added to dry pyridine (0.25 ml); hexamethyldisilazane (0.2 ml) and chlorotrimethylsilane (0.1 ml) were added successively to the mixture, and the product was analyzed with a Pye Argon gas chromatograph, by use of a column of 10% of neopentyl glycol sebacate on 80–100 mesh Chromosorb W, DMCS-A/W (Wilkens Instrument and Research, Inc.) at 150°, with a flow rate of 60 ml/min; a small peak, having a retention time 2.7 times that of the tetramethylsilyl derivative of methyl α-D-xylopyranoside, was seen. The acetalation mixture was boiled at 70° (bath)/15 mm, and analyzed, as described above, at intervals. Methyl α-D-xylopyranoside disappeared rapidly from the reaction mixture, and two acetals were formed until, after 30 min, no further change in the g.l.c. pattern was seen. Judged by the peak areas, the two acetals were formed in the approximate ratio of 10:1.

2. A vigorously stirred solution of methyl α-D-xylopyranoside (3.28 g), *p*-toluenesulfonic acid (0.01 g), and 1,1-dimethoxycyclohexane (3.2 g) in *N*,*N*-dimethylformamide (30 ml) was boiled for 4 h under reflux at 70° (bath)/15 mm. The solution was cooled, neutralized with excess Rexyn AG1 resin, and evaporated, and xylene (2 × 50 ml) was evaporated from the residue. The product was dissolved in water (120 ml) and dichloromethane (20 ml), and the mixture extracted with dichloromethane for 2 h. Evaporation of the aqueous phase gave a syrup (0.14 g, 4.3%) that crystallized on nucleation with methyl α-D-xylopyranoside. The dichloromethane extract was evaporated, benzene (100 ml) was distilled from the product, and the material was dissolved in dry pyridine (50 ml); *p*-toluenesulfonyl chloride (7.5 g) was added, and the solution was kept at 25°; after 2 days, more *p*-toluenesulfonyl chloride (2.5 g) was added, and the solution was kept for a further 24 h at 25°. The mixture was cooled to 0–4°, and water (10 ml) and sodium hydrogen carbonate (10 g) were successively added. After removal of the solvents, toluene (2 × 50 ml) was evaporated from the residue. Extraction with dichloromethane (150 ml), and evaporation of the extract, gave a syrup that was fractionated on silica gel (400 g). Elution was effected with successive 1-liter portions of 1:3 ether–hexane, 1:1 ether–hexane, 3:1 ether–hexane, and ether, all containing 0.1% of triethylamine. Fractions were examined by t.l.c., using 4 developments in 1:3 ether–hexane.

Eluted first was a mixture (0.096 g) of two compounds; this was rejected.

Eluted second was a single compound (0.076 g) that was probably a methyl *O*-cyclohexylidene-*O*-(1-methoxycyclohexyl)-α-D-xylopyranoside, as its n.m.r. spectrum indicated the presence of two methoxyl groups and twenty cyclohexylidene protons.

Eluted third was a mixture (4.7 g, 59%) of **7** and **11**; crystallization from methanol gave **7** (2.0 g), m.p. 116.5–117°, free from **11**.

Eluted fourth was **7** (1.10 g, 14%), m.p. 116.5–117°. The pure compound has m.p. 117.5–118° (from ethanol) and $[\alpha]_D^{25}$ +84° (*c* 0.7, acetone).

Anal. Calc. for $C_{19}H_{26}O_7S$: C, 57.3; H, 6.6; S, 8.1. Found: C, 57.5; H, 6.6; S, 8.0.

Eluted fifth was a mixture of **7** and a slower-moving compound; crystallization from ethanol gave **7** (0.41 g), m.p. 116.5–117°.

Eluted sixth was a mixture (0.06 g) of several compounds; this was rejected.

Eluted seventh was a mixture (0.47 g, 9.6%) of **5** and **6**, chromatographically indistinguishable from **5** and **6** obtained from preparations that were processed after cyclohexylidenation.

Benzoate exchange of methyl 2,3-O-cyclohexylidene-4-O-p-tolylsulfonyl-α-D-xyloside. — A mixture of **7** (0.40 g) and sodium benzoate (2.0 g) in *N,N*-dimethylformamide (15 ml) was stirred and boiled for 2 days under reflux. The solution was cooled, diluted with aqueous sodium hydrogen carbonate, and extracted for 6 h with dichloromethane. The extract was evaporated, and the product was dried for 1 h at 25°/0.1 mm and chromatographed on silica gel, with elution with 0.1% of triethylamine in dichloromethane.

Eluted first was a syrup (0.012 g) containing **7**.

Eluted second was a syrup (0.21 g), presumably of compound **8**, that was dissolved in methanol (20 ml), and the solution cooled to 4°, treated with a solution of sodium methoxide (0.1 g) in methanol (10 ml), and kept for 12 h at 4°. Dowex-50W resin (H^+) (2.7 g) and a few drops of water were added, and the mixture was shaken, and allowed to warm to 25°. The reaction was followed by t.l.c. (1% of isopropyl alcohol in dichloromethane); no evidence of isomerization of the cyclic acetal was seen. After 2 h, the mixture was filtered, and the filtrate was evaporated to a crystalline magma that smelled of benzoic acid. The product was washed with dichloromethane (2 × 100 ml), and passed through Amberlite-MB1 resin (25 ml) with 1:1 methanol–water (400 ml). Evaporation of the eluate gave methyl β-L-arabinoside (0.065 g, 40%), m.p. and mixed m.p. 169–170°, $[\alpha]_D^{25}$ +238° (*c* 1.4, water); the i.r. spectrum of the product was indistinguishable from that of the authentic material, prepared as described by Hudson[10].

Isolation of methyl 3,4-O-cyclohexylidene-2-O-p-tolylsulfonyl-α-D-xyloside (**11**). — A mixture (0.88 g) of **7** and **11**, dissolved in *N,N*-dimethylformamide (100 ml) containing sodium benzoate (2.0 g), was stirred and boiled for 4 days under reflux; more sodium benzoate (1.5 g) was added and the mixture was boiled for 12 h. The solution was cooled, diluted with aqueous sodium hydrogen carbonate (80 ml), and extracted for 3 h with dichloromethane. The extract was evaporated, dried for 1 h at 25°/0.1 mm, and fractionated on silica gel (50 g), with elution with 0.1 % of triethylamine in 2:3 ether–hexane. Evaporation of the eluate gave a mixture of two compounds that were closely similar in chromatographic mobility in dichloromethane. The mixture was dissolved in methanol (30 ml), cooled to 4°, and treated with a solution of sodium methoxide (0.35 g) in methanol (25 ml). After being kept for 12 h at 4°, the solution was neutralized with carbon dioxide, and evaporated, the residue was dried at 25°/0.1 mm and extracted with five 40-ml portions of dichloromethane, and the extracts were combined and evaporated. The products were separated by column chromatography; elution with 0.1% of triethylamine in 1:3 ether–hexane gave **11** (0.28 g), $[\alpha]_D^{25}$ +79° (*c* 1.27, benzene), that failed to crystallize.

Anal. Calc. for $C_{19}H_{26}O_7S$: C, 57.3; H, 6.6. Found: C, 57.4; H, 6.6.

Eluted second, with 0.1% of triethylamine in 3:1 ether–hexane, was **9** (0.16 g); this was dissolved in methanol (10 ml) containing water (0.1 ml), Dowex-50W (H^+) resin (0.2 g) was added, and the suspension was shaken for several days. After removal of the resin, the filtrate was evaporated, to give **10**, m.p. 167.5–169.5°.

Identification of methyl 3,4-O-cyclohexylidene-2-O-p-tolylsulfonyl-α-D-xyloside (**11**). — A solution of **11** in freshly distilled tetrahydrofuran (30 ml) containing lithium aluminum hydride (0.5 g) was boiled for 12 h under reflux. Ethyl acetate (5 ml) and water (sufficient to decompose the aluminum complexes) were successively added to the cooled solution. After dilution with methanol (150 ml), the solution was filtered, and the filtrate was evaporated. The residue was extracted with dichloromethane (6 × 20 ml), and evaporation of the combined extracts gave a syrup (0.08 g); this was dissolved in *N,N*-dimethylformamide (10 ml), the solution was cooled to 0–4°, and sodium hydride (0.055 g) and methyl iodide (0.1 ml) were successively added. After being stirred for 12 h at 25°, the solution was diluted with water (100 ml), and extracted for 4 h with hexane. Evaporation of the extract gave a syrup that was dried for 1 h at 25°/0.1 mm and dissolved in methanol (10 ml) containing Dowex 50W resin, and the solution was boiled for 3 h under reflux. The resin was filtered off, and the filtrate was evaporated. The product was dissolved in 0.5N sulfuric acid (8 ml), and the solution was kept for 2 days at 100°; the solution was cooled, neutralized with an excess of Amberlite-IR 45 (OH^-) resin, and evaporated. The colorless residue was extracted with methanol (30 ml); evaporation of the extract gave 2-*O*-methyl-D-xylose (0.03 g), m.p. 123–125°. Recrystallization from ethanol–ethyl acetate raised the m.p. to 133–134°; mixed m.p. 134–136°. The authentic compound, prepared according to the method of Robertson and Speedie[8], melted at 137–138°. The i.r. spectra were essentially identical.

Graded hydrolysis of methyl 2,3:4,6-di-O-cyclohexylidene-α-D-glucoside (**1**) *with acid.* — A solution of **1** (0.179 g) in glacial acetic acid (4 ml) was kept for 2 days at 25°. The acid was removed at 25°/1 mm, and toluene (20 ml) was evaporated from the product. Extraction of the residue with dichloromethane (2 × 15 ml) left a residue of methyl α-D-glucopyranoside (0.072 g), m.p. and mixed m.p. 165–167°; evaporation of the combined extracts gave a syrup that was methylated with methyl iodide and sodium hydride to give methyl 4,6-*O*-cyclohexylidene-2,3-di-*O*-methyl-α-D-glucoside, m.p. and mixed m.p. 82.5–83°, $[\alpha]_D^{25}$ +113° (*c* 0.6, acetone).

Anal. Calc. for $C_{15}H_{26}O_6$: C, 59.6; H, 8.7. Found C, 59.7; H, 8.7.

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SUMMARY

Reaction of various polyhydric alcohols with 1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid in *N,N*-dimethylformamide produces cyclic acetals in high yield, even where these acetals bridge vicinal, *trans* hydroxyl groups on a six-membered ring; methyl 2,3:4,6-di-*O*-cyclohexylidene-α-D-glucoside and 1,2:3,4:5,6-tri-*O*-cyclohexylidene-*scyllo*-inositol are both formed in high yield by use of this reaction. Application of the reaction to methyl α-D-xylopyranoside gives a mixture of the 2,3- and 3,4-*O*-cyclohexylidene acetals; *p*-toluenesulfonylation of this mixture affords crystalline methyl 2,3-*O*-cyclohexylidene-4-*O*-*p*-tolylsulfonyl-α-D-xyloside in good overall yield, and a smaller proportion of methyl 3,4-*O*-cyclohexylidene-2-*O*-*p*-tolylsulfonyl-α-D-xyloside. The former sulfonic ester undergoes reaction with sodium benzoate in boiling *N,N*-dimethylformamide with change of configuration at C-4; the latter sulfonic ester does not undergo benzoate exchange. The n.m.r. spectra of the two sulfonic esters are discussed.

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THE CONSTITUTION OF THE FRUCTAN PRODUCED BY *Leuconostoc mesenteroides* STRAIN C*

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INTRODUCTION

Extracellular fructans are synthesized by several genera of bacteria, including species of *Acetobacter*[1], *Aerobacter*[2], *Azotobacter*[3], *Bacillus*[4–10], *Corynebacterium*[11], *Leuconostoc*[12], *Pseudomonas*[6,8,13,14], *Streptococcus*[15], and *Xanthomonas*[6,14]. Structural studies[1–9, 11] on a few of these fructans have shown that they are of the levan type, comprised of (2→6)-β-D-fructofuranosyl residues. A variable degree of branching at C-1 is encountered, and most of these levans have an average chain length ($\overline{CL}$) of 10–12 units, although some having $\overline{CL}$ 6–8 have been reported[7,9].

This investigation into the structure of the fructan produced by *Leuconostoc mesenteroides* strain C was prompted by our observation that this polysaccharide gives a precipitin reaction with concanavalin A, the globulin from Jack bean (*Canavalis ensiformis*). Although it has been known for some time that some polysaccharides (glycogen[16], yeast mannan[17], and certain dextrans[18,19]) give a precipitin reaction with concanavalin A, the interaction with fructans has been reported only recently[19]. In order to gain insight into the structural requirements of this protein–polysaccharide interaction, we have investigated the chemical structures of polysaccharides that react. The present report deals with the structure of a fructan.

EXPERIMENTAL

General. — Paper chromatography was effected with Whatman No. 1 paper by the descending technique with the following solvent systems: A, pyridine–ethyl acetate–water (2:5:7, upper phase)[20]; B, same as A, (4:10:3)[21]; C, butanone–water azeotrope[22]; D, benzene–ethanol–water–ammonium hydroxide (200:47:15:1, upper phase)[23]; E, butyl alcohol–acetic acid–ethyl ether–water (9:6:3:1)[24]. Silica gel G was used for t.l.c. R_{TMG} refers to the mobility relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose as unity. Melting points were determined on a Fisher–Johns block and are uncorrected.

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Optical rotations were measured with a Bendix ETL-NPL automatic polarimeter, type 143A. All concentrations were effected *in vacuo* at 35–45° (bath temperature). The precipitin reaction with concanavalin A was performed as described previously[18], and the reactivity of the polysaccharide is expressed as glycogen value (GV), with calf-liver glycogen assigned a GV of 1.00.

Isolation of fructan. — The extracellular polysaccharides* (25 g) obtained from the culture medium of *L. mesenteroides* strain C* were dissolved in water (1 liter), and the dextran ($[\alpha]_D^{25}$ +171° in water)[25] was precipitated with 45% ethanol (aqueous). The mother liquor was concentrated to 200 ml, and poured into ethanol (1.6 liters) to precipitate the crude fructan; yield 6.5 g (26%).

Crude fructan (10.3 g), having $[\alpha]_D^{25}$ −20° (*c* 0.8, 0.5N sodium hydroxide) and GV 2.7, was dissolved in water (200 ml). A small amount of precipitate that formed on addition of ethanol (200 ml) was discarded, and the major fructan fraction was precipitated with additional ethanol (125 ml). The fructan was reprecipitated a second time with ethanol (62%) after the slight precipitate that formed in 57% ethanol had been discarded. The purified fructan was dissolved in water, and the solution poured into ethanol. The fibrous precipitate was successively washed with ethanol and petroleum ether, and dried *in vacuo*; yield 7.4 g (19% of polysaccharide mixture), $[\alpha]_D^{25}$ −40.5° (*c* 0.8, water), GV 2.2.

Acetylation of fructan. — Fructan (7.4 g) was acetylated with pyridine (400 ml) and acetic anhydride (266 ml) for 18 h at room temperature and then for 5 h at 45–60°. The product, which was precipitated from ice–water, was partially water-soluble at 25°. It was centrifuged, washed successively with 7:2 ethyl ether–ethanol and petroleum ether, and then dried, and reacetylated in the same way; yield 11.4 g (85%).

The fructan acetate (13 g) was dissolved in chloroform (500 ml), and fractionally precipitated with petroleum ether (750 ml) to give acetate **1A**, yield 4.9 g (38%), $[\alpha]_D^{25}$ +8.1° (*c* 1.0, chloroform). Additional petroleum ether (250 ml) gave acetate **1B**, yield 3.7 g (28%), $[\alpha]_D^{25}$ +16.1° (*c* 1.6, chloroform). A small amount of acetate (0.25 g) was recovered by concentration of the mother liquor.

A second batch of fructan (6 g) was acetylated and fractionated in the same manner, to give acetate **2A**; yield 1.7 g (19%), $[\alpha]_D^{25}$ +25.1° (*c* 1.3, chloroform); acetate **2B**, yield 4.2 g (47%), $[\alpha]_D^{25}$ +10.2° (*c* 1.3, chloroform); and acetate **2C**, yield 0.4 g (4%), $[\alpha]_D^{26}$ +12.2° (*c* 1.5, chloroform).

Deacetylation of fructan acetates **1A** *and* **1B**. — The acetate (1 g) was dissolved in acetone (25 ml), the solution was shaken vigorously with 20% aqueous sodium hydroxide (4 ml) and, after 1 h at room temperature, ethanol (25 ml) was added to precipitate the fructan. The precipitate was collected (centrifuge), dissolved in water (10 ml), and kept for 1 h at room temperature. After neutralization with 50% acetic acid, the solution was poured into ethanol (160 ml). The precipitate was collected by centrifugation, washed successively with ethanol and petroleum ether, and dried *in vacuo*

*Kindly provided by Dr. E. J. HEHRE. This organism has beeen designated also as NRRL B-1298.

over anhydrous calcium chloride: yield (from acetate **1A**), 0.57 g, $[\alpha]_D^{25}$ −37.5° (*c* 2.2, water), GV 1.39; from acetate **1B**, 0.70 g, $[\alpha]_D^{25}$ −29.5° (*c* 2.9, water), GV 1.74.

Hydrolysis of the fructan, and identification of the component sugars. — *A. Hydrolysis.* Fructan purified *via* the acetate was hydrolyzed under the following conditions: (*a*) 0.05N oxalic acid for 12 h at 75–80°; (*b*) 0.01N sulfuric acid, 22 h, 70–78°; (*c*) 0.05N sulfuric acid, 5 h, 77–80°; (*d*) 0.05N sulfuric acid, 1 h, 95–98°; and (*e*) N sulfuric acid, refluxed for 3 h. Oxalic acid was neutralized with calcium carbonate, and sulfuric acid with barium carbonate. Examination of hydrolyzates *a–d* by paper chromatography in solvent B showed fructose* to be the major component, together with polymeric material (composed of glucose* only) near the origin. A small amount of glucose, in addition to fructose, was observed in *e*.

B. Quantitative analysis of glucose and fructose. Fructan **1A** (0.112 g) was hydrolyzed in 0.05N sulfuric acid (20 ml) for 4 h at 75–80°, and an aliquot (*i*) of the colorless solution was removed. The remaining hydrolyzate was heated for 2 h at 95–98°, and then refluxed for 2.5 h. The solution was adjusted to N with respect to sulfuric acid, and refluxed for 3 h, after which time an aliquot (*ii*) of the yellow solution was removed. Both aliquots were neutralized with barium carbonate, and the filtrates were chromatographed in solvent A. Marginal guide-strips revealed polymeric material, glucose, and fructose in aliquot *i*; and glucose, fructose, and a faster component assumed to be a degradation product, in aliquot *ii*. The components were eluted from the paper with water, and were quantitatively determined by the phenol–sulfuric acid method[26]. The fructose: glucose ratios were calculated by assuming that the polymeric material in aliquot *i* contained glucose only, and by correcting for degradation of fructose in aliquot *ii* (see section C). For aliquot *i*, the mixture was 98% fructose and 2% glucose, and aliquot *ii* gave 97% fructose and 3% glucose. Fructan **1B** contained 96% of fructose and 4% of glucose, as revealed by analysis performed in the same manner.

C. Comparison of acid lability of glucose and fructose. D-Glucose (0.1078 g) and D-fructose (0.1057 g) were each heated with 0.05N sulfuric acid (20 ml) for 4 h at 75–80°, and aliquots were removed. Three additional aliquots were removed after: (a) heating for 2 h at 95–98°, (b) refluxing for 2.5 h, and (c) adjusting the remainder of the solution to N in sulfuric acid and refluxing for 3 h. A portion of each aliquot was analyzed directly by the phenol–sulfuric acid method. Another portion was neutralized with barium carbonate, applied to paper, and irrigated with solvent A. The components were eluted with water, and quantitatively analyzed by the phenol–sulfuric acid method.

Whereas glucose was recovered in 100% yield from all aliquots, fructose was recovered quantitatively only from hydrolyzates with 0.05N sulfuric acid. The recovery of fructose after refluxing with N sulfuric acid was 63% by direct analysis, and 34% after paper-chromatographic separation to remove degradation products.

Methylation of the fructan. — Acetylated fructan [6.1 g, consisting of fraction **1A**

*Fructose and glucose denote D(or L)-fructose and D(or L)-glucose, respectively.

(1.5 g), **2B** (4.2 g), and **2C**, (0.4 g)] was dissolved in acetone (300 ml), and the solution was stirred vigorously while 30% sodium hydroxide (390 ml) and methyl sulfate (120 ml) were added in aliquots over a period of 3 h. The temperature was kept for 1 h at 20° and then at 55°. The product, which coagulated when the reaction mixture was heated to 90°, was collected, and remethylated 4 times in the same way. After 5 of these Haworth methylations, the product was washed with water, and dissolved in acetone, and the solution was dried (anhydrous magnesium sulfate), filtered, and evaporated to dryness *in vacuo*.

The syrup was dissolved in methyl iodide (70 ml) and silver oxide (5 g) was added in portions during 12 h, while the mixture was refluxed, and stirred magnetically. After 26 h, the product was extracted with chloroform, the extract was concentrated, and the residue was twice remethylated. After the third such Purdie methylation, the chloroform phase was washed successively with 5% potassium cyanide and water, dried (anhydrous magnesium sulfate), and evaporated to a syrup; found: OCH_3, 41.32%.

The syrup was dissolved in *N,N*-dimethylformamide (60 ml), and methyl iodide (20 ml) was added. Silver oxide (20 g) was added in portions during 65 h, while the reaction was stirred. After addition of chloroform, the mixture was filtered, the filtrate was evaporated, and the product was remethylated. After the second such Kuhn methylation, the *N,N*-dimethylformamide was removed by vacuum distillation at 50–55°/1–2 mm Hg. The syrup was then remethylated twice by the Purdie method; yield 4.14 g (96%); found: OCH_3, 42.17% (Calc. 45.59%).

Fractionation of methylated fructan. — Methylated fructan was fractionally precipitated from acetone (45 ml) with petroleum ether, as shown in Table I.

TABLE I

FRACTIONATION OF *O*-METHYLATED FRUCTAN

Fraction	*Petroleum ether, (%)*	*Yield, (g)*	$[\alpha]_D^{25}$, *degrees (acetone)*
1	64	3.534	−61
2	77	0.163	−32
3	83	0.034	−39
4	93	0.019	−27
5[a]		0.386	− 8

[a]Obtained by concentration of the mother liquor from fraction 4.

Fractions 1 and 5 (50 mg) were *O*-demethylated with boron trichloride in dichloromethane (5 ml) in the cold (acetone–Dry Ice)[27]. Examination of the neutralized hydrolyzates by paper chromatography in solvent A revealed the presence of fructose and glucose in fraction 5. No glucose was detected in fraction 1.

Hydrolysis of permethylated fraction 1. — Fraction 1 (1.99 g, OCH_3 45.85%) was dissolved in methanol (200 ml), 0.94N aqueous oxalic acid (64 ml) was added,

and the mixture was refluxed for 18 h. The methanol was removed *in vacuo* (<35°) while water (200 ml) was continually added. The resulting aqueous solution was refluxed for 3 h, neutralized with calcium carbonate, the suspension was filtered, and the filtrate was evaporated to a syrup, yield 2.1 g. Examination of this syrup by paper chromatography in solvent C showed 3 components, having R_{TMG} 0.40, 0.75, and 1.05, corresponding to di-, tri- and tetra-*O*-methyl-D-fructose, respectively. No degradation products were detected.

Identification of the cleavage products from the methylated fructan. — A. Separation. The mixture of *O*-methyl sugars (1.85 g) was separated on a cellulose–hydrocellulose column[28], with butanone–water azeotrope as the eluant. The *O*-methyl sugars were located by electrophoresis, and by paper and thin-layer chromatography (solvent C), and the chromatographically pure components were combined and concentrated.

B. 3,4-Di-O-methyl-D-fructose. The fraction having R_{TMG} 0.40 (0.269 g) had $[\alpha]_D^{23}$ −56.5° (*c* 3.4, water); lit.[29] $[\alpha]_D$ −53.59° (water). Upon t.l.c. in solvents C, D, and E, a single component having a mobility identical with that of 3,4-di-*O*-methyl-D-fructose was detected.

The syrup (180 mg) was treated with periodic acid (1.15 moles/mole) for 3 h at 5°. Excess periodate was removed by precipitation with barium hydroxide, and the solution was deionized with Amberlite IR-120 (H^+) ion-exchange resin, and concentrated to a syrup (131 mg, 92%) that formed crystals at 0°. A solution of the product in methanol (10 ml) was cooled in an ice bath, and saturated with ammonia, kept for 15 h at 0°, and evaporated to dryness *in vacuo*, and a small amount of material insoluble in acetone–ethanol was removed by filtration. The filtrate was concentrated to a syrup, which crystallized on nucleation with 2,3-di-*O*-methyl-L-arabinonamide. The product was purified by t.l.c. in solvent C, and recrystallized from acetone–ethanol to give 2,3-di-*O*-methyl-D-arabinonamide, m.p. 145–147°, $[\alpha]_D^{27}$ −17.5° (*c* 1.2, water); [lit.[30] for 2,3-di-*O*-methyl-L-arabinonamide, m.p. 162; $[\alpha]_D$ +17.4° (*c* 1.2, water)].

C. 1,3,4-Tri-O-methyl-D-fructose. The fraction having R_{TMG} 0.75 (1.144 g) crystallized on nucleation with authentic 1,3,4-tri-*O*-methyl-D-fructose. After two recrystallizations from ether, the sugar had m.p. 75.5–76° (capillary tube) and 68.5–69° (block), $[\alpha]_D^{22}$ −10.7 (15 min) → −55° (equil., *c* 2.0, water); [lit.[4,5] m.p. 75°, $[\alpha]_D$ −23.8 → −51.8°(water)].

D. 1,3,4,6-Tetra-O-methyl-D-fructose. The fraction having R_{TMG} 1.05 (0.329 g) had $[\alpha]_D^{19}$ +30.6° (*c* 4.8, water) [lit. for 1,3,4,6-tetra-*O*-methyl-D-fructose, $[\alpha]_D$ +31.3° (water)[31]; for 1,3,4,5-tetra-*O*-methyl-D-fructose, $[\alpha]_D$ −121.3° (water)[32]].

Anal. Calc. for $C_{10}H_{20}O_6$: OCH_3, 52.54. Found: OCH_3, 51.67.

Quantitative analysis of methylated sugars. — A portion of the hydrolyzate (48 mg) was separated on Whatman 3 MM paper with solvent C. The components were eluted with water, and quantitatively determined by the phenol–sulfuric acid method. The molar proportions of 3,4-di-:1,3,4-tri-:1,3,4,6-tetra-*O*-methyl-D-fructose found were 1.00:3.31:0.98.

Examination of the concanavalin A–fructan complex. — A solution of fructan **1B**

(226 mg) in 2% sodium chloride (20 ml) was treated with a solution of concanavalin A, prepared as for determination[18] of GV, until no further precipitation of the protein–polysaccharide complex occurred. After 15 min, the mixture was centrifuged. The insoluble complex was washed twice with concanavalin A solution (35 ml), twice with 2% sodium chloride (30 ml), and once with water (20 ml). It was triturated twice with 10% trichloroacetic acid in water (14 ml total), and the supernatant liquor was combined, and dialyzed against water. The dialyzed material was evaporated to dryness, and hydrolyzed for 1 h in refluxing 0.05 N sulfuric acid (15 ml). Paper chromatography (solvent A) of the deionized residue (Duolite A-4, OH^-) showed fructose to be the only component.

DISCUSSION

The extracellular polysaccharides produced by *Leuconostoc mesenteroides* strain C were separated by fractionation with ethanol to give a dextran, the chemistry of which will be discussed in a separate paper[25], and a fructan. The fructan (19% of the total, extracellular polysaccharides) was purified by repeated precipitation from aqueous solution with 62% ethanol (aqueous); $[\alpha]_D^{25}$ −40.5° (*c* 0.8, water), GV 2.2.

Fractional precipitation of the fructan acetate afforded two fractions: **A** (38%), $[\alpha]_D^{25}$ +8.1°, and **B** (28%), $[\alpha]_D^{25}$ +16.1° (chloroform). Deacetylation of the fructan acetates **A** and **B** gave fructan **A** [$[\alpha]_D^{25}$ −37.5° (water), GV 1.4] and **B** [$[\alpha]_D^{25}$ −29.5° (water), GV 1.7]. On hydrolysis, the deacetylated polysaccharide **A** gave D-fructose (98%) and D-glucose (2%). Fructan **B** contained a slightly greater proportion of glucose residues (4%), and the difference in specific rotations between **A** and **B** may reflect the presence of this contaminating glucose polymer rather than a structural heterogeneity in the fructan.

The relative labilities of fructose and glucose are of interest. Fructose is relatively stable in refluxing 0.05N sulfuric acid, but 66% of this sugar is degraded by refluxing 3 h with N sulfuric acid, whereas glucose is not significantly affected. Hydrolysis of the fructan with 0.05N sulfuric acid at 80° released the fructose residues without significant degradation.

The fructan was methylated by the Haworth, Purdie, and Kuhn procedures to give a methylated product in 96% yield. Fractionation of this product afforded a major fraction (83%) having $[\alpha]_D^{25}$ −61° (acetone) and a methoxyl content of 45.8%. No glucose was detected (by paper chromatography) in this purified, methylated fructan after simultaneous demethylation and hydrolysis with boron trichloride, whereas both fructose and glucose (trace) were released from a minor fraction of the methylated polysaccharide (fraction 5, Table I).

On hydrolysis of the methylated fructan, 3,4-di-*O*-methyl-D-fructose, 1,3,4-tri-*O*-methyl-D-fructose, and 1,3,4,6-tetra-*O*-methyl-D-fructose were isolated in the molar proportions of 1.00:3.95:1.11 by hydrocellulose column chromatography. The methylated sugars were identified by thin-layer and paper chromatography, as well as

by comparison of other physical properties. 3,4-Di-*O*-methyl-D-fructose was characterized further by oxidation with periodate (1 mole per mole) to give 2,3-di-*O*-methyl-D-arabinonic acid, which was identified as 2,3-di-*O*-methyl-D-arabinonamide, m.p. 145–147°, $[\alpha]_D^{27}$ −17.5° (*c* 1.2, water).

A portion of the hydrolyzate of the methylated fructan was separated by paper chromatography, and each component was determined by the phenol–sulfuric acid method. The molar proportions of 3,4-di-:1,3,4-tri-:1,3,4,6-tetra-*O*-methyl-D-fructose of 1.00:3.31:0.98 obtained by this method are in good agreement with those calculated from the weights of the sugars isolated by column chromatography.

The identification of these methylated sugars establishes that the fructan is composed of (2→6)-linked D-fructofuranosyl residues having branches at C-1. Equimolar quantities of tetra-*O*-methyl-D-fructose and 3,4-di-*O*-methyl-D-fructose were isolated in amounts that indicated a highly branched structure, with $\overline{CL}$ 5–6, for the fructan. This fructan belongs therefore to the levan group (phlein type), as is true of all bacterial fructans that have been adequately characterized. However, the levan from *L. mesenteroides* appears to be more highly branched than any other bacterial levan so far characterized.

As the glucose appeared to arise from a contaminating polysaccharide (possibly dextran), it was of interest to determine whether the complex with concanavalin A was due to an interaction with the fructan. The polysaccharide was recovered from the concanavalin A–polysaccharide complex and found to contain fructose, proving that the reaction had, indeed, involved the fructan.

The methylation data show that the fructan produced by *L. mesenteroides* strain C is almost as highly branched ($\overline{CL}$ 5–6) as the dextran ($\overline{CL}$ 4) produced by the same organism. In view of this fact, it is interesting to note the difference in reactivity of the two polysaccharides with concanavalin A, because the fructan has a GV of 1.74, whereas the dextran has GV 8.19. This observation suggests that reactivity with concanavalin A is dependent on another factor, in addition to the degree of branching (and, consequently, the number of nonreducing end-groups); this factor, or factors, may well be the site of the branch point or the length of the branch itself.

SUMMARY

The extracellular levan elaborated by *L. mesenteroides* (strain C) when the organism is grown on a medium containing sucrose as the carbon source has been shown by methylation analysis to be composed of β-D-fructofuranosyl residues linked by (2→6)-glycosidic bonds, with branches at C-1. Methylation of the levan and subsequent hydrolysis afforded 3,4-di-, 1,3,4-tri-, and 1,3,4,6-tetra-*O*-methyl-D-fructose in the approximate mole proportions of 1.0:3.5:1.0, corresponding to an average chain-length of 5–6. This levan gives with concanavalin A a precipitin reaction of somewhat greater intensity than that displayed by the glycogens.

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THE STRUCTURE OF NASTURTIUM AMYLOID*

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INTRODUCTION

Amyloid is a name used by botanists to designate a group of cellular substances that, like starch, give a blue stain with iodine. These substances have been found in the seeds of many plants. One of these, a polysaccharide from nasturtium seed (*Tropeoleum majus*), was recognized as a reserve material by Frank[1]. No starch remains in the mature seeds, and the amyloid forms a thick layer inside the cell wall. Its characteristic staining properties are readily visible in thin sections cut from the seed. The present report gives the results of a study on the structure of nasturtium amyloid.

EXPERIMENTAL

Extraction, purification, and properties of the amyloid. — Nasturtium seed was obtained from private plantings and from a commercial source (Reuter Seed Co., 320 N. Carrollton Avenue, New Orleans, La.). Both sources yielded similar products.

After removal of the hulls, the dry seeds were ground in a Waring Blendor or a Wiley mill. Nasturtium-seed meal (100 g) was extracted with 1:1 ethyl alcohol–ether (600 ml) for 3 h under reflux. The residue was extracted four times with 1-liter portions of water for 4 to 6 h in a boiling-water bath. The aqueous extracts were centrifuged and filtered, and the filtrates were poured into 2 volumes of ethyl alcohol. The crude product was precipitated as a heavy, fibrous substance.

The precipitates were combined (7–10 g), and redissolved in water, and the solution was shaken on a machine overnight with a mixture of chloroform (400 ml) and butyl alcohol (800 ml). After centrifugation, the aqueous layer was withdrawn and retreated with the mixed organic solvents; this treatment was repeated until no white coagulum formed. The clear, aqueous solution was acidified, and the product was precipitated by pouring the solution into ethyl alcohol. The precipitate was scooped into

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1 liter of ethyl alcohol containing 8 ml of concentrated hydrochloric acid, the suspension was filtered, and the precipitate was washed with ethyl alcohol until it was free from chloride ion, and then washed successively with acetone and petroleum ether. This nasturtium amyloid (4 g) had, on an ash-free, anhydrous basis, $[\alpha]_D^{20}$ +83 ±5° (*c* 0.5, water or N hydrochloric acid). It was employed in most of the structural studies. Other preparations had $[\alpha]_D$ +67±5° and +72 ±4°. Because of the opalescence of solutions of the amyloid in water, the error in the determination of its specific rotation is estimated to be rather great.

Nasturtium amyloid is a white, fibrous substance. A typical, air-dried sample contained 12.38% of moisture, 0.84% of ash, and 0.08% of nitrogen. It was neutral after the ash had been removed by electrodialysis. After hydrolysis in N hydrochloric acid, all preparations of amyloid reached an equilibrium specific rotation of 50 ±5° based on the initial weight of anhydrous amyloid.

Amyloid solutions give a green coloration with 0.02N iodine, but, below about 0.01N iodine, no color change occurs. Concentrated solutions of iodine precipitate the amyloid from aqueous solution as a deep-blue iodine complex, from which amyloid may be recovered after removal of the iodine.

Analytical methods for sugars. — D-Glucose and D-galactose were determined by the Glucostat and Galactostat reagents (Worthington Biochem. Co.), respectively. Xylose was determined by an orcinol method[2]. The Somogyi method[3] was used for the quantitative determination of reducing sugars eluted from paper chromatograms, with use of the appropriate standard. The ferricyanide reducing-sugar method of Hanes[4] was used for determining reducing sugars after hydrolysis, in an experiment on the kinetics of hydrolysis. Quantitative determinations of total amyloid were made by the phenol–sulfuric acid method[5], with use of a 3:2:1 glucose–xylose–galactose standard.

Paper chromatography. — Whatman No. 1 and 3 MM filter papers were generally used. The solvent systems employed were (v/v): (A) 6:4:3 butyl alcohol–pyridine–water, (B) 7:1:2 isopropyl alcohol–acetic acid–water, (C) 40:10:49:1 butyl alcohol–ethanol–water–ammonia, (D) 400:9:3:2 benzene–ethanol–water–ammonia, and (E) 99:1 butanone (saturated with water)–ammonia. System C gave the best separation of methylated reducing sugars. The descending technique was used. Reducing sugars were detected with acetone–silver nitrate–alcoholic sodium hydroxide[6], aniline hydrogen phthalate[7], or *p*-anisidine hydrochloride.

For quantitative, paper chromatography of monosaccharides, the methods of Jermyn and Isherwood[8] and Dimler *et al.*[9] were employed. The sugars were streaked on 3 MM filter paper and, before application to the paper, the samples to be assayed for D-galactose were incubated with glucose oxidase to remove the D-glucose as completely as possible. A blank elution of the paper was prepared for each assay.

Methylation. — Amyloid (2 g) dissolved in water (100 ml) was methylated with methyl sulfate (2 × 12 g) and sodium hydroxide (2 × 10 g). After dialysis, the solution was concentrated to about 100 ml, and the methylation was repeated three more times. The final, dialyzed solution was evaporated to dryness under diminished pressure, and

the residue was dissolved in chloroform. The chloroform solution was filtered, and concentrated to 100 ml, and the product was precipitated by pouring the solution into petroleum ether (400 ml); yield 1.3 g; found OMe, 33.8%.

The product (0.8 g) was further methylated with methyl iodide (50 ml) and silver oxide (8 g) for 12 h under reflux. The product was extracted with chloroform, and the extract was centrifuged, filtered, and concentrated to a small volume. The product was precipitated by pouring the solution into cold petroleum ether; yield 0.5 g; found OMe, 38.8% (theoretical, 43.6%). The i.r. spectrum confirmed the presence of unmethylated hydroxyl groups, but all attempts to methylate the product further were unsuccessful; the methods used included treatments in benzene with methyl iodide and sodium, and in *N,N*-dimethylformamide with methyl iodide and silver oxide.

The methylated nasturtium amyloid was a white, fibrous powder, soluble in chloroform, acetone, benzene, xylene, *p*-dioxane, or cold water, but insoluble in hot water or ether; $[\alpha]_D^{24}$ +34° (*c* 1, water).

Methanolysis and hydrolysis of methylated amyloid. — A solution of methylated amyloid (100 mg) in 5% hydrogen chloride in methanol (10 ml) was sealed in a screw-capped tube, and heated in a boiling-water bath until the optical rotation reached a constant value ($[\alpha]_D^{24}$ changed from +25 to +105°). The solution of methyl glycosides was passed through a column of Amberlite IRA-400 (OH^-) ion-exchange resin, to remove hydrogen chloride, and the eluate was evaporated at 40° with a jet of dry air. The residue was dissolved in 4% aqueous hydrochloric acid, and hydrolyzed for 6 h at 100°; the solution was rendered neutral with silver carbonate, and was de-ionized by passing it through a column of equal parts of Amberlite IR-120 and Duolite A-4. The column was washed with water, and the effluent was filtered, and evaporated to dryness under diminished pressure. The residue was dissolved in a small volume of water, and the solution was subjected to paper chromatography.

Microscale methylation and hydrolysis of oligosaccharides. — To the disaccharide or pentasaccharide (1–2 mg), isolated by paper chromatography, was added 30% sodium hydroxide (0.6 ml) and methyl sulfate (0.3 ml). After the mixture had been stirred for 1 h at room temperature, the temperature was raised to 40°, and methyl sulfate (0.15 ml) was added dropwise. More of each reagent was added at 55°, and the solution was then boiled for 30 min. The mixture was cooled, and extracted with chloroform, and the extract was dried (anhydrous sodium sulfate), and evaporated to dryness by a stream of dry air. The residue was dissolved in cold *N,N*-dimethylformamide (0.2 ml) and methyl iodide (0.1 ml), and silver oxide (0.2 g) was added in small portions during 4 h at 30°. The mixture was stirred overnight, and the product was extracted with chloroform; the extract was dried and transferred to a small tube. After removal of the chloroform, two drops of 90% formic acid were added. The tube was sealed, heated for 4 h at 100°, cooled, and opened, and the solution was evaporated to dryness by a jet of dry air. Two drops of water were then added, the tube was sealed, heated for 1 h at 100°, cooled, and opened, and the water was evaporated in a vacuum desiccator. This hydrolysis was twice repeated. The methylated sugars were then examined by paper chromatography.

Periodate oxidation. — Nasturtium amyloid (400 mg) was dissolved in 0.025M sodium metaperiodate (500 ml), and oxidation was permitted to proceed in a refrigerator at 4°. At intervals, an aliquot (5 ml) was withdrawn for the determination of periodate consumption, and a 25-ml aliquot for the determination of formic acid. The amount of periodate consumed reached a constant value of 1.15 moles per mole of monosaccharide residue, and the formic acid produced reached 0.322 mole per mole of monosaccharide residue (assuming the ratio of hexose to pentose residues to be 2:1, equivalent weight 152 g). The periodate oxidation was also conducted at room temperature in the dark; the periodate consumed was 1.2 moles per mole of monosaccharide residue.

After the excess of periodate had been decomposed by addition of ethylene glycol, the oxidized amyloid was dialyzed, the solution was evaporated to dryness under diminished pressure, and the oxidized amyloid was reduced with sodium borohydride according to the procedure of Smith *et al.*[10]. After hydrolysis of the reduced product, less than 2% of a glucose was found.

Partial, acid hydrolysis of nasturtium amyloid, and isolation of oligosaccharides. — Nasturtium amyloid (2 g) was hydrolyzed in 30% formic acid (200 ml) under reflux for 5 h at 100°. The hydrolyzate was poured into 400 ml of alcohol, and the precipitate was centrifuged off (yield, 319 mg) and subjected to partial hydrolysis in 0.5N sulfuric acid. Cellobiose, cellotetraose, and other oligosaccharides were isolated from this hydrolyzate by paper chromatography; the oligosaccharides were not characterized, but many of them yielded cellobiose after further partial hydrolysis.

The supernatant, alcoholic solution was evaporated to small volume under diminished pressure, and the resulting syrup was placed on a column (3 × 18 cm) of 1:1 (w/w) Darco G-60 carbon and Celite No. 535. The column was washed with water until all monosaccharide had been eluted; it was then successively eluted with 5, 10, and 15% aqueous ethanol.

The 5% ethanolic eluate contained monosaccharides and a disaccharide that moved faster than cellobiose on a chromatogram. The eluate was evaporated, and the mixture was refined by elution from a column (2 × 18 cm) of Whatman cellulose powder (Standard Grade), with solvent B as the eluent. The effluent fractions that contained the disaccharide were combined, and evaporated to dryness under diminished pressure at 45°. The disaccharide, still contaminated with a galactose, was further refined on a small column (2 × 11 cm) of charcoal; yield 3.66 mg. It was then chromatographically pure; on hydrolysis, it gave equimolar proportions of glucose and xylose, and had $[\alpha]_D^{24}$ +73.8 ±2° (*c* 0.336, water).

The 10% alcoholic eluate contained cellobiose and a second compound, apparently a trisaccharide, that was not isolated in the pure state. The cellobiose was characterized by chromatography in solvents A and B, by its acid hydrolysis to a glucose, and by its cleavage to a glucose by emulsin. The latter property served to distinguish it from maltose.

The 15% alcoholic eluate contained cellobiose, the unidentified trisaccharide, a tetrasaccharide composed of two glucose residues and two xylose residues, and a

pentasaccharide. The pentasaccharide was isolated by paper chromatography on Whatman 3 MM paper; on partial hydrolysis, it gave cellobiose, glucose, and xylose. Following complete hydrolysis, quantitative paper chromatography indicated the ratio of three glucose residues to two xylose residues. Their mode of linkage was revealed by the methylation study.

Emulsin was obtained from Nutritional Biochemicals Co. It was able to cleave the β-D-glucosidic linkages of cellobiose and cellotetraose, but not the α-D-linkages of maltose and the glucose-xylose disaccharide. Nasturtium amyloid was not hydrolyzed by the emulsin.

RESULTS AND DISCUSSION

The monosaccharide components of amyloid, calculated as glycose residues, are: 55.4% of glucose, 26.7% of xylose, and 17.9% of a galactose; this corresponds to molar proportions of 3:2:1. The stereospecific reactions of D-glucose oxidase and of D-galactose oxidase with the respective components indicated that both the glucose and the galactose residues are in the D configuration. The specific rotation of the amyloid after hydrolysis, namely, $[\alpha]_D^{26}$ $+50$ $\pm 2°$, indicates that the xylose, also, is in the D configuration. (The calculated $[\alpha]_D$ for a mixture of the three sugars in the proportions of 3:2:1 is $+48.4°$ if the xylose is D-xylose, and $+38.2°$ if it is L-xylose.)

That the monosaccharides are present in the pyranoside form is indicated by the low rate of liberation of the reducing sugars on acid hydrolysis. In the early stages of the hydrolysis (0.01N HCl, 100°), a velocity constant of 80×10^{-5} (natural log) was found; this is in the range expected for pyranoside linkages. Also, galactose was the first sugar to be liberated by acid, and it was proved to have the pyranose form by subsequent methylation.

On hydrolysis of the methylated amyloid, six methylated monosaccharides were isolated; these were identified as follows: 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-D-xylose, and 2-*O*-methyl-D-glucose. The first four were identified chromatographically in solvent systems C, D, and E, and by comparing each with an authentic sample. The di-*O*-methylxylose was identified by the orcinol test, by its R_G value (0.76, relative to 2,3,4,6-tetra-*O*-methyl-D-glucose) in solvent C, and (to exclude the possibility of its being the 2,3-dimethyl ether) by a test with sodium metaperiodate. It was rapidly cleaved by periodate, under conditions similar to those of Lemieux and Bauer[11], to give an oxidized product, whereas 2,3-di-*O*-methyl-D-glucose, treated in the same way, remained unaltered. The 2-*O*-methylglucose was distinguished from the 3- and 6-methyl ethers by the spot color tests described by Schwimmer and Bevenue[12] and Bailey[13], and by the periodate-oxidation method reported by Lemieux and Bauer[11].

From the structure of the tetra-*O*-methylgalactose isolated, it is evident that D-galactose occurs in pyranoside linkage in the amyloid. It is also evident that both the galactose residues and part of the xylose residues are involved in terminal linkages at nonreducing chain-ends. That the Galactostat reagent acts on the unhydrolyzed

amyloid is also an indication that galactopyranose residues occur as nonreducing end-groups of the amyloid.

Cellotetraose was identified by correlating R_m value and n (degree of polymerization) with the fact that a plot of *Rm versus n* is linear for homologous oligosaccharides[14] [R_m is defined as log $(1/R_F - 1)$ by Bate-Smith and Westall[15]]. Solvent B was used for the paper chromatography in this study. The cellotetraose isolated was also hydrolyzed to D-glucose and cellobiose by emulsin.

The disaccharide from the 5% alcoholic extract (having $[\alpha]_D^{24}$ +73.8°) was methylated. On acid hydrolysis, the methylated product gave 2,3,4-tri-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose. Therefore, the D-xylose residues in the disaccharide are linked to the D-glucose residues by (1→6)-linkages. That these involve an α-D-glycosidic bond was indicated by the high, positive optical rotation of the disaccharide and by its resistance to the action of emulsin. This disaccharide appears to be isoprimeverose, which had been isolated by Perila and Bishop[16] from the partial hydrolyzate of a glucomannan. These workers reported $[\alpha]_D$ +70° for isoprimeverose.

The pentasaccharide from the 15% alcoholic extract contains two xylose residues and three glucose residues. After methylation and hydrolysis, it gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 3,4-di-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-glucose, and 2,3,6-tri-*O*-methyl-D-glucose. We therefore speculate that two xylose residues, joined by a (1→2)-linkage, are attached to the middle D-glucose residue of a cellotriose residue through an α-D-(1→6)-linkage. The cellotriose residue is assumed to arise from cleavage of the cellulose type of chain of the nasturtium amyloid.

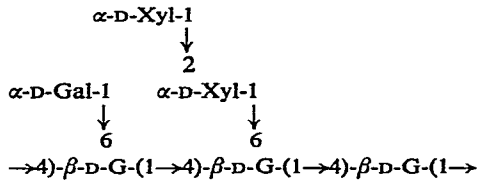
The isolation of cellobiose, cellotetraose, other higher oligosaccharides containing only D-glucose, and other oligosaccharides containing cellobiose, indicates that the main chain of the amyloid is made up of D-glucopyranose residues linked through β-D-(1→4)-linkages.

Nasturtium amyloid has one branch per 3 glycosidic residues. From the composition of the oligosaccharides (obtained by partial hydrolysis) containing two xylose residues per molecule, it appears most probable that the two xylose residues are joined together to form one side-chain. As nasturtium amyloid has two side-chains per six glycoside residues, the second must be a single D-galactose residue. That the D-xylose side-chain joins the main chain through an α-D-(1→6)-glycosidic linkage to D-glucose is evident from the structure of isoprimeverose.

The single D-galactose side-chain is, most probably, joined to the D-glucose residue by a (1→6)-linkage. Because few intact D-glucose residues remain after periodate oxidation, the hydroxyl groups at C-2 and C-3 of the D-glucose residues are, largely, unsubstituted. The 2-*O*-methyl-D-glucose is regarded as arising from undermethylation of the highly branched polysaccharide. At the branch points, the hydroxyl groups at C-3 could be expected to be very difficult to methylate[17].

The (1→6)-link between the D-galactose side-chain and the main chain, and the (1→2)-link between the two D-xylose residues, are presumed, from the specific

rotation of amyloid, to be α-D-glycosidic linkages. This conclusion is supported by a downward shift in rotation during the hydrolysis. The structure of nasturtium amyloid is, therefore, represented by a repeating unit as follows:



A polysaccharide isolated from the seed kernel of the tamarind tree (*Tamarinus indica*) has been reported to have properties similar to those of nasturtium amyloid. The tamarind polysaccharide was also classified as an amyloid[1]. It contains glucose, xylose, and galactose in the molar proportions of 3:2:1, has $[\alpha]_D$ +71.4°, and the specific rotation[18] after hydrolysis is +52 to +54°. Structural studies on the polysaccharide have been reviewed by Smith and Montgomery[19]. In a structure proposed by Khan and MukKerjee[20] for this polysaccharide, which was named tikernose, all of the glycosidic bonds are represented as β-D-linkages.

After comparing the properties of nasturtium amyloid with those reported for tikernose, we are inclined to believe that the two polysaccharides may have similar structures. The opalescence of amyloid renders difficult the determination of its exact optical activity with a visual polarimeter such as we employed, but the values observed were close to that reported for tikernose. The only manner in which the structure proposed for nasturtium amyloid differs from that advanced by Khan and MukKerjee[20] for tikernose is that the side chains in the former are represented as α-D-linked instead of β-D-linked. We have strong evidence to support one α-D-(1→6)-linkage of a D-xylose side-chain in nasturtium amyloid. The anomeric structure of tikernose was mainly deduced from the characteristic infrared absorption band at 890 cm^{-1}, allegedly indicative of β-D-linkages, and the absence of a band, supposedly characteristic of α-D-linkages, at 844 cm^{-1}. It seems doubtful that a polysaccharide having the composition of tikernose, if joined solely by β-D-linkages, would give the high dextrorotation observed and a downward shift in rotation during hydrolysis.

The fibrous character of nasturtium amyloid and the high viscosity of its aqueous solutions support the conclusion that it is a long-chain molecule. Yet it is a highly branched molecule; much more so than glycogen, for example. It has one branch per three glycosidic residues. In order that a naturally occurring macromolecule should be so highly branched and yet fiber-forming, a great regularity along its main axis might be expected. Our proposed repeating unit, consisting of six monosaccharide residues, is the simplest structure meeting all of the experimental observations, but our data do not exclude the possibility of a larger, more complicated, repeating unit.

SUMMARY

Nasturtium amyloid contains residues of D-glucose, D-xylose, and D-galactose

in the molar proportions of 3:2:1, and all of them are pyranoid. The isolation of cellobiose and cellotetraose led to the conclusion that the main chain of this amyloid is a chain of the cellulose type. There are two branches per six glycosidic residues.

If the main chain of the amyloid is, indeed, of the cellulose type, there are two branches per three D-glucose residues of the main chain. One would be a single D-galactose residue joined α(?)-D-Gal-(1→6)-D-G, and the other, α(?)-D-Xyl-(1→2)-α-D-Xyl-(1→6)-D-G.

Isoprimeverose (6-*O*-α-D-xylopyranosyl-D-glucose) was obtained by partial hydrolysis of the amyloid, together with a tetrasaccharide composed of 2 D-glucose residues and 2 D-xylose residues, and a pentasaccharide composed of 3 D-glucose residues and 2 D-xylose residues.

A repeating unit composed of six monosaccharide residues is proposed for the amyloid. The structural similarity of nasturtium amyloid and the tamarind-seed amyloid (tikernose) is discussed.

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PARTIAL TOLUENE-*p*-SULPHONYLATION STUDIES OF METHYL 6-*O*-TRITYL-*β*-D-GALACTOFURANOSIDE

PART I. SYNTHESIS OF 2,5- AND 3,5-DIMETHYL ETHERS OF D-GALACTOSE*

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INTRODUCTION

Although the di-*O*-methyl derivatives of D-galactopyranose have been known for some time, little is known about the furanoid analogues. In 1960, Siddiqui and Adams described the isolation and characterization of 3,5-di-*O*-methyl-D-galactose[1] from a methylated polysaccharide of *Gibberella fujikuroi*. The present communication deals with the synthesis of 3,5-di-*O*-methyl-D-galactose and also of 2,5-di-*O*-methyl-D-galactose, which was hitherto undescribed. The paper also describes an alternative synthesis of 3-*O*-methyl- and 2,3-di-*O*-methyl-D-galactose.

RESULTS AND DISCUSSION

The sequence of reactions which led to these syntheses was as follows. Methyl-*β*-D-galactofuranoside was tritylated to yield the crystalline methyl 6-*O*-trityl-*β*-D-galactofuranoside. The trityl derivative was partially tosylated** to give a mixture of tosyl derivatives that were separated by chromatography on silica gel and designated compounds **1**, **2**, **3** and **4**, in the order of their increasing R_F values (t.l.c.). Fraction 1 was successively methylated, detosylated, detritylated, and hydrolyzed to yield a dimethyl ether, which, on demethylation, gave galactose. The paper-chromatographic and electrophoretic behaviour and specific optical rotation of the ether agreed with those of the known 2,3-di-*O*-methyl-D-galactose. The ether gave a crystalline aniline derivative, which had the same m.p. and $[\alpha]_D$ as 2,3-di-*O*-methyl-*N*-phenyl-D-galactosylamine. Compound **1**, therefore, was methyl 5-*O*-tosyl-6-*O*-trityl-*β*-D-galactofuranoside.

Compound **2**, when subjected to the above-mentioned procedures, provided a di-*O*-methyl sugar that had $[\alpha]_D^{29}$ −10.3° (water). On demethylation, it gave galactose, and was homogeneous in paper chromatography and paper electrophoresis. None of the di-*O*-methyl-D-galactopyranoses shows a negative rotation[2]; the $[\alpha]_D$ values for 2,3- and 3,5-dimethyl ethers of D-galactose, isolated in the present work, were +94° and −25.3°, respectively. The di-*O*-methylgalactose gave an R_G value (0.42) in solvent *A* that was much higher than is to be expected for the known di-*O*-methylgalacto-

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**Tosyl=toluene-*p*-sulphonyl.

pyranoses. The comparative R_G values for 2,3- and 3,5-di-*O*-methyl-D-galactose were 0.22 and 0.40, respectively. The low M_G value (0.23) of the di-*O*-methylgalactose in borate, compared to that (0.65) for 3,5-di-*O*-methylgalactose, suggested that position 2 was substituted. Oxidation of the di-*O*-methyl-D-galactose with bromine water afforded a syrupy lactone that showed a strong C=O absorption at 1785 cm^{-1} in its infrared spectrum, indicating[3] that it was a γ-lactone. The foregoing evidence indicates that the dimethyl ether was 2,5-di-*O*-methyl-D-galactose, and, hence, the partially tosylated compound **2** was methyl 3-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside. The sugar was characterized as the crystalline 2,5-di-*O*-methyl-D-galactonamide.

The partially tosylated compound **3**, on similar methylation, detosylation, detritylation, and hydrolysis, yielded a dimethyl ether that was identical with authentic 3,5-di-*O*-methyl-D-galactose in paper chromatography and paper electrophoresis. The $[\alpha]_D$ value for the free sugar and the $[\alpha]_D$ value and m.p. for the derived hexitol were the same as for authentic samples of 3,5-di-*O*-methyl-D-galactose and 3,5-di-*O*-methyl-D-galactitol. The partially tosylated compound **3**, therefore, was methyl 2-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside.

When detritylation was performed with dry hydrogen chloride in dry chloroform, slight cleavage of the glycosidic bond invariably occurred. In order to prepare pure samples of methyl 2,3-, 2,5-, and 3,5-di-*O*-methyl-β-D-galactofuranosides, detritylations were performed with 50% aqueous acetic acid, and the glycosides were obtained as clear syrups that were chromatographically homogeneous.

The fourth, partially tosylated, compound (**4**) was identified as methyl 2,5-di-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside as follows. Compound **4**, on methylation and detosylation, yielded a methylated, tritylated glycoside that was resolved by column chromatography on silica gel, giving two crystalline fractions (4A, 4B). Fraction 4A, on detritylation, afforded a mono-*O*-methylhexofuranoside, which, on hydrolysis, was converted into a mono-*O*-methyl sugar that was identified by comparison with an authentic sample of 3-*O*-methyl-D-galactose (paper chromatography, paper electrophoresis, $[\alpha]_D$, m.p., and mixed m.p.). A freshly detritylated sample of the faster-moving fraction (4B), on immediate hydrolysis, also appeared to give mainly 3-*O*-methylgalactose. However, a sample of the detritylated sugar that had been kept in a vacuum desiccator failed to give 3-*O*-methylgalactose on hydrolysis; instead, 3 spots were observed on paper chromatography, and a single, non-complexing spot in borate electrophoresis was detected. The fastest-moving spot in paper chromatography appeared to be a hydroxymethyl-2-furaldehyde derivative. On the basis of these observations (which suggested the presence of a hydrofuranol type of ring), and on the reported behavior of 3,6-anhydro derivatives of D-galactose[4-6] and D-glucose[7], and of 2,5-anhydro-L-arabinose[8], fraction 4B is provisionally identified as a methyl 2,5-anhydro-3-*O*-methyl-6-*O*-trityl-β-D-hexofuranoside.

EXPERIMENTAL

Analytical methods. — The organic phase of the following solvent systems (v/v)

was used for paper chromatography: (*A*) butanone, saturated with water containing 2% ammonia; (*B*) ethyl acetate–pyridine–water (8:2:1). Sugars, as their borate complexes, were separated by electrophoresis[9] in 0.2M buffer (pH 10) at 800 volts for 2–3 h. Whatman No. 1 paper was used for all paper-chromatographic work, and Whatman No. 3 MM for all electrophoretic separations. R_G values refer to rates of movement relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose. Thin-layer chromatography (t.l.c.) was performed on glass plates (20 × 20 × 0.37 cm) coated with a uniform layer (250 microns) of silica gel G[10], with detection by 5% sulphuric acid in ethanol. Reactions were monitored by t.l.c. on microscope slides coated with silica gel G. Column chromatography was performed on silica gel[11]. Reducing sugars were detected on paper chromatograms and electrophoretograms with aniline hydrogen phthalate[12]. (*A*), and non-reducing sugars (glycosides) with the naphthoresorcinol reagent[13] (*B*). Evaporations were carried out at 35° on a rotary film evaporator. Melting points are corrected, and rotations are equilibrium values.

Methyl 6-O-trityl-β-D-galactofuranoside. — Methyl β-D-galactofuranoside (2.5 g, m.p. 63–65°, $[\alpha]_D$ −108°)[14] was dissolved in dry pyridine (50 ml), and chlorotriphenylmethane (3.6 g) was added. After the solution had been kept for 48 h at room temperature, the product[15] was isolated as a syrup (5.3 g, 95%). Crystallization from cold isopropyl ether containing a few drops of ethanol gave methyl 6-*O*-trityl-β-D-galactofuranoside, m.p. 91–92.5°, $[\alpha]_D^{25}$ −46.3° (*c* 3.0, chloroform).

Anal. Calc. for $C_{26}H_{28}O_6 \cdot 0.5\ C_2H_5OH$: C, 70.57; H, 6.80. Found: C, 70.51; H, 6.50.

Tosylation of Methyl 6-O-trityl-β-D-galactofuranoside. — Methyl 6-*O*-trityl β-D-galactofuranoside (5.0 g) in dry pyridine (100 ml) was tosylated by the dropwise addition of a solution of tosyl chloride (3.0 g) in dry pyridine (20 ml) at 100° during 2 h. After a further period of 8 h at 100°, the solution was cooled to room temperature and poured into a mixture of ice and water. The product was extracted with chloroform (3 × 100 ml), and the combined extracts were washed successively with water, 10% cupric sulfate solution, and water, and finally dried over sodium sulfate. Removal of chloroform gave a syrup (5.3 g) that showed, in addition to the starting material (R_F 0.20), four products when examined by t.l.c. in chloroform–acetone (85:15). The syrupy material was fractionated by column chromatography on silica gel with the same solvent. The following Table summarizes the results.

Fraction No.[a]	*Yield (g)*	$[\alpha]_D^{25}$, *degrees* (c *2-4, chloroform*)	R_F	*Found (%)* C	H	S	*Formula*	*Calc. (%)* C	H	S
1	1.1	−36.8	0.34	66.76	5.87	5.36	$C_{33}H_{34}O_8S$	67.11	5.80	5.42
2	0.6	−27.2	0.58	66 85	6.37	5.03	$C_{33}H_{34}O_8S$	67.11	5.80	5.42
3	0.7	−42.2	0.72	66.36	5.55	5.15	$C_{33}H_{34}O_8S$	67.11	5.80	5.42
4	1.2	−29	0.86	64.30	5.64	8.24	$C_{40}H_{40}O_{10}S_2$	64.50	5.41	8.61

[a] **1**, Methyl 5-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside; **2**, Methyl 3-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside; **3**, Methyl 2-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside; **4**, Methyl 2,5-di-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside.

Methyl 2,3-di-O-methyl-5-O-tosyl-6-O-trityl-β-D-galactofuranoside. — Methyl 5-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside (1.0 g) was methylated with methyl iodide (20 ml) and silver oxide (2 g). Filtration and evaporation gave a product that still contained some unmethylated material (t.l.c., chloroform–acetone, 15:1). A second methylation yielded a product (1.0 g, 95%) that was chromatographically pure and crystallized readily. Recrystallization from isopropyl ether gave the title compound, m.p. 106.5–107°, $[\alpha]_D^{25}$ −41.5° (*c* 3.3, chloroform).

Anal. Calc. for $C_{35}H_{38}O_8S$: C, 67.94; H, 6.19; S, 5.18; OCH_3, 15.05. Found: C, 67.51; H, 6.13; S, 5.14; OCH_3, 15.00.

Methyl 2,5-di-O-methyl-3-O-tosyl-6-O-trityl-β-D-galactofuranoside. — Methyl 3-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside (0.5 g) was methylated as above, yielding the title compound as a homogeneous (t.l.c.) syrup (0.5 g, 95%), $[\alpha]_D^{27}$ −42.7° (*c* 3.0, chloroform).

Found: C, 67.66; H, 5.74; S, 5.03; OCH_3, 14.82.

Methyl 3,5-di-O-methyl-2-O-tosyl-6-O-trityl-β-D-galactofuranoside. — Fraction 3 (0.5 g), on similar methylation, yielded the title compound (0.5 g, 95%), which, after crystallization from isopropyl ether, had m.p. 135–136°, $[\alpha]_D^{26}$ −51.6° (*c* 2.0, chloroform).

Found: C, 67.58; H, 5.99; S, 5.01; OCH_3, 15.20.

Methyl 3-O-methyl-2,5-di-O-tosyl-6-O-trityl-β-D-galactofuranoside. — The title compound (1 g, 95%) was prepared from Fraction 4 (1 g) by methylation as above, and, after crystallization from isopropyl ether, had m.p. 134.5°, $[\alpha]_D^{27}$ −38.4° (*c* 2.6, chloroform).

Anal. Calc. for $C_{41}H_{42}O_{10}S_2$: C, 64.89; H, 5.58; S, 8.45; OCH_3, 8.18. Found: C, 64.60; H, 5.72; S, 8.12; OCH_3, 8.46.

Methyl 2,3-di-O-methyl-6-O-trityl-β-D-galactofuranoside. — Solutions of methyl 2,3-di-*O*-methyl-5-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside (0.745 g) in benzene (10 ml) and sodium methoxide (from 1 g of sodium) in methanol (20 ml) were mixed and boiled for 7 h under reflux[16]. The mixture was cooled to room temperature, water (20 ml) was added, organic solvents were removed by aeration, and the suspension was extracted with chloroform (5 × 25 ml). The chloroform extract was dried with sodium sulfate, and the solution evaporated to a syrup (0.618 g). The product contained traces of two components besides the main product (t.l.c.), and these were removed by chromatography on a column of silica gel by using ethyl acetate–light petroleum (b.p. 65–110°) (30:70), giving the product as a homogeneous syrup (0.540 g, 96%), $[\alpha]_D^{29}$ −62.5° (*c* 2.3, chloroform).

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94; OCH_3, 20.04. Found: C, 72.06; H, 7.19; OCH_3, 19.7.

Methyl 2,5-di-O-methyl-6-O-trityl-β-D-galactofuranoside. — Methyl 2,5-di-*O*-methyl-3-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside (0.646 g) was similarly detosylated, isolated, and fractionated as above, to give a syrupy product (0.452 g, 93%), $[\alpha]_D^{29}$ −38.7° (*c* 2.57, chloroform).

Found: C, 72.13; H, 6.89; OCH_3, 19.56.

Methyl 3,5-di-O-methyl-6-O-trityl-β-D-galactofuranoside. — Methyl 3,5-di-*O*-methyl-2-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside (0.271 g) on detosylation, isolation, and fractionation as above gave the title compound as a syrup (0.20 g, *ca.* 100%), $[\alpha]_D^{27}$ −51.6° (*c* 2.015, chloroform).

Found: C, 72.64; H, 7.21; OCH_3, 19.52.

Methyl 3-O-methyl-6-O-trityl-β-D-galactofuranoside and the corresponding 2,5-anhydro derivative. — Methyl 3-*O*-methyl-2,5-di-*O*-tosyl-6-*O*-trityl-β-D-galactofuranoside (0.827 g) was detosylated as above to yield a syrup (0.510 g) that showed two components (t.l.c.). The syrup was fractionated on a column of silica gel by using acetone–chloroform (1:15). The faster-moving component (0.36 g, 76%) crystallized on trituration with ether. Recrystallization from ether–light petroleum (b.p. 65–110°) gave a product provisionally identified as a methyl 2,5-anhydro-3-*O*-methyl-6-*O*-trityl-β-D-hexofuranoside, m.p. 112–113°, $[\alpha]_D^{28}$ −63.1° (*c* 3.04, chloroform).

Anal. Calc. for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 75.02; H, 6.54.

The slower-moving fraction (0.101 g, 20%) crystallized on evaporation of the solvent. Recrystallization from isopropyl ether gave methyl 3-*O*-methyl-6-*O*-trityl-β-D-galactofuranoside, m.p. 123–124°, $[\alpha]_D^{27}$ −46.1° (*c* 1.84, chloroform).

Anal. Calc. for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 72.24; H, 6.47.

2,3-Di-O-methyl-β-D-galactose. — Methyl 2,3-di-*O*-methyl-6-*O*-trityl-β-D-galactofuranoside (0.510 g) was dissolved in dry chloroform (20 ml), and dry hydrogen chloride was bubbled through the solution under anhydrous conditions for 10 min at 0°. After 1 h at room temperature, the reaction mixture was neutralized with silver carbonate, filtered, and evaporated to a syrup that was triturated with light petroleum (b.p. 65–110°) to remove most of the triphenylmethane. The residue was dissolved in water, and the solution was filtered and evaporated to a syrup (0.227 g).

The syrup (0.223 g) was heated with 0.5N sulphuric acid (3 ml) at 100°, and the hydrolysis, which was followed by t.l.c. (chloroform–acetone, 1:1), was complete in 6 h. The hydrolysate was neutralized with barium carbonate and filtered, and the filtrate (4 ml) was extracted with chloroform (10 × 4 ml) to remove traces of a fast-moving impurity. The aqueous layer, on concentration, gave the product as a syrup (0.180 g, 82%), $[\alpha]_D^{27}$ +94° (*c* 3.05, water); lit., $[\alpha]_D$ + 80.9° (ref. 18*a*), + 116° (ref. 18*b*). Paper chromatography (solvent *A*) and paper electrophoresis showed a single component having R_F, R_G, and M_G values of 0.18, 0.22, and 0.22, respectively. (Found: OCH_3, 28.7. Di-*O*-methylhexose calc.: 29.8%). Demethylation[17] with boron trichloride gave only galactose.

2,5-Di-O-methyl-D-galactose. — Methyl 2,5-di-*O*-methyl-6-*O*-trityl-β-D-galactofuranoside (0.430 g) was detritylated, isolated, hydrolyzed, and recovered, as described above, but with hydrolysis by 0.2N sulphuric acid (3 ml) for 4.5 h at 100°. The final syrup (0.16 g, 83%), $[\alpha]_D^{27}$ −10.3° (*c* 2.46, water), on paper chromatographic (solvent *A*) and ionophoretic examination showed a single component having R_F, R_G, and M_G values of 0.32, 0.42, and 0.23, respectively. Demethylation gave galactose.

3,5-Di-O-methyl-D-galactose. — Methyl 3,5-di-*O*-methyl-6-*O*-trityl-β-D-galactofuranoside (0.271 g) was detritylated, isolated, hydrolyzed, and recovered, essentially

as described above. The product was a syrup (0.055 g, 42%), $[\alpha]_D^{29}$ −25.3° (*c* 3.75, water); lit.[1], $[\alpha]_D$ −24.8° ±0.5°, water; which showed a single component on paper chromatography and paper electrophoresis, having R_F, R_G, and M_G values of 0.31, 0.40, and 0.65, respectively. Demethylation gave galactose.

Methyl 2,3-, 2,5-, and 3,5-di-O-methyl-β-D-galactofuranosides. — Detritylation with dry hydrogen chloride produced slight cleavage of the glycosides. To overcome this difficulty, further detritylations were performed in 50% acetic acid. Methyl 2,3-di-*O*-methyl-6-*O*-trityl-β-D-galactofuranoside (0.49 g) was stirred magnetically, at room temperature, with 50% aqueous acetic acid (15 ml). After 6 h, the precipitate was removed by filtration, the solution was evaporated, and water was distilled several times from the residue. Finally, the aqueous solution was extracted once with light petroleum (b.p. 65–110°) and concentrated to give syrupy methyl 2,3-di-*O*-methyl-β-D-galactofuranoside (0.231 g, 95%), $[\alpha]_D^{28}$ −122.1° (*c* 2.12, methanol). Paper chromatography of the glycoside produced a single component R_F 0.69 (solvent *A*; a bluish red stain with spray *B*). (Found: OCH_3, 41.95. Di-*O*-methylhexoside calc.: 41.9%).
Methyl 2,5- and 3,5-di-*O*-methyl-β-D-galactofuranosides were similarly prepared in yields of 90%. The former glycoside had an R_F value of 0.71 and a bluish red stain (spray *B*); $[\alpha]_D^{29}$ −104.7° (*c* 1.6, methanol) (Found: OCH_3, 41.6). The latter had R_F (0.67, blue stain); $[\alpha]_D^{29}$ −110.8° (*c* 1.59, methanol) (Found: OCH_3, 41.9).

Methyl 3-O-methyl-β-D-galactofuranoside. — Methyl 3-*O*-methyl-6-*O*-trityl-β-D-galactofuranoside (0.090 g) was detritylated in 50% acetic acid, as described above. The syrupy product (0.038 g, 92%) showed a single component (R_F 0.43, solvent *A*) and a blue colour with spray reagent *B*. It had $[\alpha]_D^{28}$ −142.8° (*c* 1.18, methanol) (Found: OCH_3, 29.81. Mono-*O*-methylhexoside calc.: 29.66%).

2,3-Di-O-methyl-N-phenyl-D-galactosylamine. — 2,3-Di-*O*-methyl-D-galactose (0.05 g) in ethanol (2 ml) was heated under reflux with freshly distilled aniline (26 mg) for 5 h. Removal of ethanol left a syrup that crystallized from acetone at −10°, giving a product that had m.p. 151–153°, $[\alpha]_D^{27}$ −66→−13° (4 days; *c* 1.38, ethanol); lit.[19] m.p. 154–155°, $[\alpha]_D$ −56.8→+12.1° in ethanol; and[20] m.p. 152–154°, $[\alpha]_D$ −69→−13° in ethanol.

2,5-Di-O-methyl-D-galactonamide. — 2,5-Di-*O*-methyl-D-galactose (0.050 g) in water (3 ml) was oxidized in the dark for 72 h with bromine (7 drops) in the presence of barium carbonate (0.060 g). Bromine was removed by aeration, and the aqueous solution was acidified with hydrochloric acid and extracted continuously with chloroform for 24 h. Only traces of a syrup were recovered; consequently, the aqueous layer was evaporated by aeration, and the residue was extracted with chloroform–acetone (1:1). The soluble portion was fractionated on a small column of silica gel by using the same solvent. The syrup (0.020 g) thus recovered was homogeneous, as shown by t.l.c. in chloroform–acetone (1:1). It was distilled (bath temperature 150–160°/0.01 mm) to give the syrupy lactone, $\nu_{max}^{CHCl_3}$ 1785 cm^{-1}, which was dissolved in methanol saturated with ammonia. The solution was kept for 48 h at 0°, and evaporation of the solvent, with recrystallization of the residue from ethanol, gave 2,5-di-*O*-methyl-D-galactonamide, m.p. 184–186°, $[\alpha]_D^{27}$ +41.7° (*c* 1.33, water).

Anal. Calc. for $C_8H_{17}NO_6$: C, 43.04; H, 7.68; N, 6.28. Found: C, 43.40; H, 7.48; N, 6.21.

3,5-Di-O-methyl-D-galactitol. — 3,5-Di-*O*-methyl-D-galactose (0.035 g) in water (2 ml) was mixed with aqueous sodium borohydride[21] (0.07 g in 1 ml). The resulting hexitol, isolated and crystallized in the usual way[2], had m.p. and mixed m.p. 135–136°, $[\alpha]_D^{27}$ −23.7° (*c* 1.18, methanol); lit.[1], m.p. 134–135°, $[\alpha]_D^{26}$ −23.7 ± 1° (*c* 1.01, methanol).

3-O-Methyl-D-galactose. — Methyl 3-*O*-methyl-β-D-galactofuranoside (0.027 g) was hydrolyzed with 0.2N sulphuric acid (1 ml) for 6 h at 100°. Neutralization ($BaCO_3$), filtration, and evaporation of the solution gave a syrup (0.018 g). Paper chromatography and electrophoresis of this syrup showed a single component having R_F, R_G (solvent *A*), and M_G values of 0.04, 0.055, and 0.69, respectively. Demethylation[17] gave galactose. A solution of the syrupy material in absolute ethanol crystallized on seeding, and recrystallization from ethanol gave a product with m.p. 138–140° alone or in admixture with an authentic sample of 3-*O*-methyl-D-galactose; lit.[22], m.p. 144–147°.

Methyl 2,5-anhydro-3-O-methyl-β-D-hexofuranoside. — Methyl 2,5-anhydro-3-*O*-methyl-6-*O*-trityl-β-D-hexofuranoside (0.360 g) was detritylated in 50% aqueous acetic acid, and the product was recovered by the usual method. A portion (0.010 g) of the freshly detritylated material was hydrolyzed with 0.2N sulphuric acid for 6 h at 100°. Neutralization and evaporation gave a syrup that was examined by paper chromatography and electrophoresis. In addition to 3-*O*-methyl-D-galactose (R_F 0.04, R_G 0.055, M_G 0.69), appreciable proportions of a second component (R_F 0.88 and M_G 0.00) and faint traces of a third component 0.21 (R_F) were also detected. The remainder of the detritylated, unhydrolyzed material was dried overnight *in vacuo* and, when examined by paper chromatography (solvent *A*), showed a strongly reducing streak originating at the starting line. The same material, on hydrolysis (0.2N sulphuric) and examination by paper chromatography, showed 3 components [R_F 0.87, 0.208, and 0.09 (solvent *A*); and 0.79, 0.44, 0.27 (solvent *B*)]. All three spots were non-complexing on borate electrophoresis. Prolonged hydrolysis with 0.2N sulphuric acid or 50% aqueous acetic acid showed that the spot having R_F 0.87 was formed at the expense of the spot having R_F 0.09. Scarcity of material precluded further work on this fraction.

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SUMMARY

Partial toluene-*p*-sulphonylation of methyl 6-*O*-trityl-β-D-galactofuranoside produced a mixture of three mono-*O*-toluene-*p*-sulphonyl derivatives and a di-*O*-toluene-*p*-sulphonyl derivative. Successive methylation, desulphonylation, detrityl-

ation, and hydrolysis of these compounds have yielded 2,3-, 2,5-, and 3,5-dimethyl and 3-methyl ethers of D-galactose. The di-*O*-toluene-*p*-sulphonyl derivative, in addition, gave a crystalline anhydro sugar that was tentatively identified as a methyl 2,5-anhydro-3-*O*-methyl-6-*O*-trityl-β-D-hexofuranoside. A proof of structure of these methylated sugars is presented.

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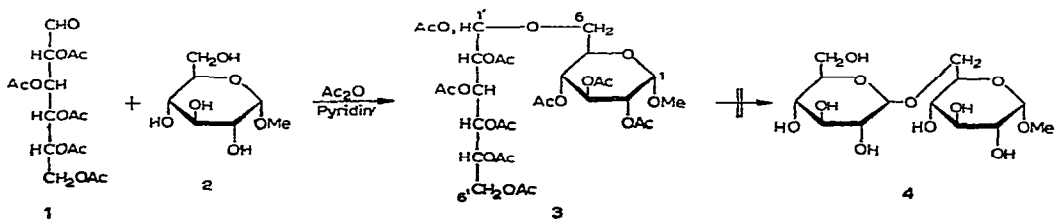
Notes

Synthese eines neuen Typs von Disacchariden[1]

Penta-*O*-acetyl-*al*-D-glucose (**1**) läßt sich mit der freien Hydroxylgruppe eines zweiten Monosaccharid-Derivates [z.B. mit Methyl-α-D-glucopyranosid (**2**)] zu einem halbacetalartigen Disaccharid-Derivat (**3**; $[\alpha]_D$ +62.6°, Chloroform) umsetzen. Dabei addiert sich eine der freien Hydroxylgruppen von **2** an die Carbonylgruppe von **1**. Da die Reaktion in Acetanhydrid–Pyridin (2:1) bei Raumtemperatur erfolgt, werden anschließend an die Additionsreaktion alle freien Hydroxylgruppen des Disaccharids einschließlich der neugebildeten acetalartigen acetyliert. Molekulargewichte, Acetyl- und Methoxylgehalte und Elementaranalysen zeigen, daß es sich nicht um cyclische Acetale handelt, die durch Kondensation der freien Aldehyd-gruppe von **1** mit 2 Hydroxylgruppen von **2** entstehen können.



(Die Verknüpfung über die Hydroxylgruppe am C-6 in **3** und die Konfiguration an C-1′ sind nicht bewiesen).

Bei der Hydrolyse mit Säuren oder bei der katalytischen Verseifung mit OH-Ionen werden die Ausgangshexosen zurückerhalten. Dies dürfte darauf zurückzuführen sein, daß die Acetoxygruppen der Zuckerkette langsamer verseift werden als Aufspaltung der Disaccharid-Bindung nach Verseifung der Acetoxygruppe am C-1′ erfolgt. Die Möglichkeit der Stabilisierung zum Pyranosid **4** scheidet dadurch aus. Zwei analoge Disaccharid-Derivate wurden durch Reaktion von **1** mit Methyl-α-D-galaktopyranosid erhalten ($[\alpha]_D$ +67.1 und +68.0°). Diese ließen sich durch Säulenchromatographie an Kieselgel mit Diisopropyläther–Pyridin (9:1) trennen. Mit Methyl-4,6-*O*-benzyliden-α-D-glucopyranosid und Methyl-6-*O*-trityl-α-D-glucopyranosid entstehen kristalline Disaccharid-Derivate vom Schmp. 155–157° bzw. 97–99° ($[\alpha]_D$ +18.5° bzw. +48.7°, Chloroform). Durch chromatographische Untersuchungen wurde nachgewiesen, daß **1** mit folgenden weiteren Monosaccharid-derivaten unter Bildung von Disaccharid-derivaten reagiert: 2-*O*-Benzyl-D-glucose, 3-*O*-Benzyl-D-glucose, 1,2-*O*-Isopropyliden-α-D-glucofuranose, 1,2-*O*-Isopropyliden-6-*O*-tosyl-α-D-glucofuranose, 2,4,6-Tri-*O*-methyl-D-glucose und Benzyl-β-D-glucosid.

Keine disaccharidartigen Reaktionsprodukte konnten bisher bei der Umsetzung von Penta-*O*-acetyl-*al*-D-glucose (**1**) mit den folgenden D-Glucose-derivaten erhalten werden: 1,2:5,6-Di-*O*-isopropyliden-α-D-glucofuranose, 2-*O*-Benzyl-4,6-*O*-benzyliden-D-glucose, 2,3,4,6-Tetra-*O*-methyl-D-glucose, Methyl-2,4,6-tri-*O*-methyl-β-D-glucosid, Methyl-3-*O*-Benzyl-4,6-*O*-benzyliden-α(β)-D-glucosid.

Ähnliche Disaccharid-Typen wurden auch aus 2-Acetamido-2-desoxy-D-glucose und D-Glucose gewonnen[2].

Additionsprodukte einfacher Alkohole (Methanol, Äthanol) an die Aldehydgruppe offenkettiger acetylierter Hexosen sind auf anderem Wege schon eher dargestellt worden[3,4]. Sie wurden beim Umkristallisieren von *al*-Hexose-acetaten aus absol. Alkohol und anschließender Acetylierung des Halbacetals erhalten. Analoge Produkte können aber auch nach unserem Verfahren z.B. mit Äthanol, Benzylalkohol und Glykol in Acetanhydrid–Pyridin erhalten werden.

BESCHREIBUNG DER VERSUCHE

Die Darstellungsmethode wird am Beispiel der Umsetzung von **1** mit **2** beschrieben: **1** (3 g) und Methyl-α-D-glucopyranosid (**2**) (3 g) werden in Essigsäureanhydrid (10 ccm) und Pyridin (5 ccm) über Nacht bei Raumtemperatur aufbewahrt. Anschließend wird mit Eiswasser versetzt und die wässrige Lösung mit Chloroform ausgeschüttelt. Die Chloroformlösung wird mit verd. Schwefelsäure, Hydrogenkarbonatlösung und Wasser gewaschen, mit Natriumsulfat getrocknet und i.V. eingedampft. Ausb. 5.3 g. Das Disaccharid-derivat wird durch Chromatographie an einer Kieselgelsäule mit Diisopropyläther–Pyridin (9:1) von den Monosaccharid-derivaten befreit. Nach dem Verdampfen des Elutionsmittels wird der Rückstand wiederholt in abs. Äthanol aufgenommen und die Lösung zur Trockne eingedampft. Sodann wird in Äther auf-

TABELLE I

ANALYTISCHE DATEN (ALLE SUBSTANZEN SIND CHROMATOGRAPHISCH REIN)

*Kondensationsprodukte aus 2,3,4,5,6-Penta-*O*-acetyl-*al*-D-glucose mit:*		*Mol. gew.*[a]	*C*	*H*	*CH_3CO*	*CH_3O*	$[\alpha]_D$ *Chloroform*
	ber.	752.6	49.47	5.89	51.48	4.12	
Methyl-α-D-glucopyranosid	gef.	745	49.44	6.04	50.87	4.45	+62.6°
Methyl-α-D-galactopyranosid Subst. (*a*)	gef.	720	49.45	6.14	51.26	4.29	+67.1°
Subst. (*b*)	gef.	780	49.29	5.88	51.49	4.61	+68.0°
Methyl-6-*O*-trityl-α-D-glucopyranosid	ber.	953	60.49	5.93	36.14	3.25	
(Schmp. 97–99°)	gef.	932	60.10	6.12	35.61	3.20	+48.7°
Methyl-4,6-*O*-benzyliden-α-D-glucosid	ber.	756.7	53.96	5.87	39.82	4.10	
(Schmp. 155–157°)	gef.	781	53.52	5.83	40.49	4.38	+18.5°

[a]Bestimmt im Dampfdruckosmometer Mechrolab Mod. 301a.

genommen und erneut eingedampft. Hierbei fällt das Disaccharid als farblose amorphe Substanz an, die mit Petroläther gewaschen wird. Ausb. 1.4 g (24.2% d. Th.)

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Carbohyd. Res., 5 (1967) 218–220

Long-chain glycosides of 3-*O*-(α-D-galactopyranosyl)-D-galactose and of maltose*

The 3-*O*-(α-D-galactopyranosyl)-D-galactopyranosyl residue is a determinant structure of glycoproteins possessing blood-group B activity[1]. In erythrocytes, glycolipids have been assumed to carry the blood-group specificity of the types A and B[2–4], but, to date, no pure glycolipid showing blood-group activity has been isolated from red-cell stroma. In order to test the validity of this assumption, the synthesis of glycolipids possessing blood-group B activity was undertaken. As a model substance, the determinant structure 3-*O*-(α-D-galactopyranosyl)-D-galactose was linked by a glycosidic bond to 1-tetradecanol (myristyl alcohol) to give 1-tetradecyl 3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside (6). In the test of hemagglutination inhibition,

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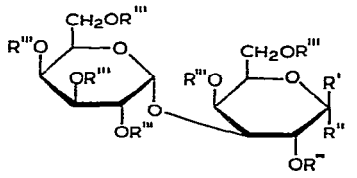
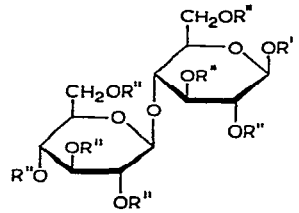
*This is publication No. 433 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. This investigation was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service (Grant AM-0364 .

the glycolipid **6** showed an enhancement of the activity over that of the disaccharide 3-*O*-(α-D-galactopyranosyl)-D-galactose, but this activity was not specific.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranose (**1**) was obtained by partial acetolysis of λ-carrageenan, followed by deacetylation, separation of the resulting product on a carbon–Celite column, and reacetylation[5]. Treatment of **1** with hydrobromic acid in glacial acetic acid for a short



	R'	R''	R'''
1	OAc	H	Ac
2	H	Br	Ac
3	OMe	H	Ac
4	OMe	H	H
5	$O(CH_2)_{13}CH_3$	H	Ac
6	$O(CH_2)_{13}CH_3$	H	H



	R'	R''
7	Ac	Ac
8	$(CH_2)_{13}CH_3$	Ac
9	$(CH_2)_{13}CH_3$	H

period of time in the cold gave the α-D-glycosyl bromide (**2**). This compound was not isolated, but was directly treated in solution with methanol to give the crystalline, fully acetylated methyl β-D-glycoside (**3**), which was directly deacetylated to give methyl 3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside (**4**). A similar sequence of reactions starting from **1**, where 1-tetradecanol replaced methanol, gave the intermediate β-D-glycoside (**5**) and thence, crystalline 1-tetradecyl 3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside (**6**). For comparison of the immunochemical properties, crystalline 1-tetradecyl 4-*O*-(α-D-glucopyranosyl)-β-D-glucopyranoside (**9**) was prepared from crystalline β-maltose octaacetate[6] (**7**), through the crystalline 1-tetradecyl glycoside heptaacetate (**8**).

EXPERIMENTAL*

Methyl 3-O-(α-D-galactopyranosyl)-β-D-galactopyranoside (**4**). — 1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranose (**1**, 294 mg), obtained by acetolysis of λ-carrageenan[5], was dissolved in a saturated solution of hydrobromic acid in glacial acetic acid (1 ml). The solution was kept for 4 h at 0° in the dark, and was then diluted with chloroform (10 ml) and ice–water (5 ml). The chloroform layer was washed successively with ice-cold water and a cold,

*For conditions, see ref. 7.

saturated solution of sodium hydrogen carbonate, and dried with calcium chloride. It was added dropwise to a suspension of silver carbonate (0.6 g), Drierite (3.0 g, dried for 2 h at 220°), chloroform (10 ml), and abs. methanol (5 ml) which had been stirred for 1 h prior to the addition of the glycosyl bromide. The mixture was stirred for 48 h, filtered through a double layer of Darco G-60 and Celite, and the filtrate was evaporated to dryness. The residue was deacetylated by the addition of 0.15% sodium methoxide in methanol (4 ml). After 20 min at room temperature, the solution was diluted with a large excess of water, and filtered through Dowex-50 (H^+) ion-exchange resin, and the filtrate was concentrated to about 1 ml. The product was chromatographed on a sheet of Whatman paper No. 3 MM for 48 h in 8:2:1 (v/v) ethyl acetate–pyridine–water. The spot corresponding to the methyl glycoside was detected by the silver nitrate reagent, and was extracted with methanol. Evaporation of the solution gave 40 mg (26%) of a crystalline residue, which was recrystallized from methanol–ether, m.p. 199°, $[\alpha]_D^{25}$ +127° (*c* 0.14, water).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.80; H, 6.74. Found: C, 43.71; H, 6.74.

1-Tetradecyl 3-O-(α-D-galactopyranosyl)-β-D-galactopyranoside (**5**). — The condensation was performed with the glycosyl bromide **2**, prepared from 152 mg of **1**, by the procedure used for the preparation of **4**, with the exception that 1.1 g of 1-tetradecanol and 20 ml of dry ether (instead of chloroform) were used. The residue obtained before deacetylation was chromatographed on silica gel (20 g). Hexane eluted a small amount of 1-tetradecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside, which was not further investigated. Benzene eluted 1-tetradecanol, and 1,2-dichloroethane eluted 95 mg of an oil, which was then deacetylated as described for **4**. Crystallization from ethyl acetate gave 20 mg (16%), m.p. 143–5°, $[\alpha]_D^{25}$ +83° (*c* 0.19 methanol).

Anal. Calc. for $C_{26}H_{50}O_{11}$: C, 57.97; H, 9.36. Found: C, 57.91; H, 9.30.

1-Tetradecyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**7**). — The reaction was performed as described for the preparation of **5**, by starting from crystalline β-maltose octaacetate[6] (370 mg), which was treated with hydrobromic acid in glacial acetic acid for 2 h at room temperature. After the condensation with 1-tetradecanol, chromatography on 50 g of silica gel gave, by elution with ethyl acetate, 379 mg of crystalline fractions. Recrystallization from methanol–water afforded 202 mg (44%), m.p. 79–82°, $[\alpha]_D^{20}$ +39° (*c* 0.50, chloroform).

Anal. Calc. for $C_{40}H_{64}O_{18}$: C, 57.68; H, 7.74; O, 34.58. Found: C, 57.64; H, 7.78; O, 34.57.

1-Tetradecyl 4-O-(α-D-glucopyranosyl)-β-D-glucopyranoside (**8**). — Compound **7** (120 mg) was treated with 0.15% sodium methoxide in methanol (2 ml) for 30 min at room temperature. After dilution with water and neutralization with Dowex-50 (H^+) ion-exchange resin, the solution was evaporated to dryness. The residue was crystallized from ethyl acetate to give 53 mg (52%), having a double m.p., 106° and 258–260°; $[\alpha]_D^{25}$ +86° (*c* 0.18, methanol).

Anal. Calc. for $C_{26}H_{50}O_{11}$: C, 57.97; H, 9.36. Found: C, 57.80; H, 9.27.

ACKNOWLEDGMENT

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Synthesis of maltulose by intestinal α-D-glucosidases

Maltulose (4-*O*-α-D-glucopyranosyl-D-fructose) is apparently not widespread in nature. It originates partly by epimerisation of maltose[1] (*e.g.*, in decoction worts) and partly by transglucosylation during the hydrolysis of sucrose by D-glucosidases from, for example, yeast[2] and honey[3]. Maltulose has also been detected in hydrolysates of waxy corn starch, and in α-amylolytic hydrolysates of rabbit-liver glycogen[4,5].

A solution containing 2 ml of a mucosal preparation from the small intestines of rats or rabbits[6], 2 ml of 0.2M sucrose in phosphate buffer (pH 6.5), and a trace of toluene (to avoid microbial growth) was incubated for 15 h at 37°. During the incubation, samples (0.5 ml) of the mixture were deproteinized[7], and examined by paper chromatography (Fig. 1). In addition to glucose and fructose, a component having the same mobility as maltulose was detected. With diphenylamine–aniline–phosphoric acid[8], this component gave the deep-purple colour that is characteristic for maltulose. With orcinol, it gave a red colour that is typical of ketoses. The saccharide was isolated and purified by paper chromatography, and had m.p. 200–202°, $[\alpha]_D^{20}$ +51.9° (*c* 1.5, water), R_G 0.65 (2 runs of the solvent system).

The formation of maltulose reached a maximum after incubation for 3 h;

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The formation of maltulose reached a maximum after incubation for 3 h;

hydrolysis proceeded simultaneously. Maltulose is formed in greater proportion than any other oligosaccharide. In similar experiments with hog intestinal mucosa, other oligosaccharides are formed in addition to maltulose. and these have been charac-

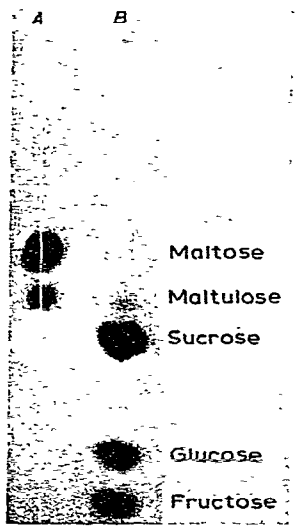

Fig. 1. Paper-chromatographic analysis of the incubation of the intestinal mucosa preparation of rats with 0.1M sucrose in phosphate buffer (pH 6.5) at 37°; descending technique on Schleicher & Schüll paper No. 2043a; run with butyl alcohol–ethanol–water (4:1:5); chromatogram developed during 4 days; after 2 days, the chromatogram was dried and developed again. *A*, reference (maltose, treated with alkali; D-glucose fermented by bakers' yeast). *B*, medium after incubation for 3 h; 2000 μg, related to the amount of sucrose used, were applied.

terized by Dahlqvist[9]. It is probable that the oligosaccharide B_1 found by Dahlqvist may be maltulose. Carnie and Porteous[10] isolated three oligosaccharides after incubation of sucrose with purified rabbit invertase (mucosa); they found a trisaccharide having a glucose : fructose ratio of 2, and two disaccharides having a glucose:fructose ratio of *ca.* 1; maltulose was not identified.

The presence of fructose results in increased formation of maltulcse in incubation solutions, whereas D-glucose has apparently an inhibitory effect.

The biosynthesis of maltulose may be due to the α-D-glucosidases located in the intestinal brush border of mammals[11], because only glucosidase (EC 3.2.1.20), but not β-D-fructosidase (EC 3.2.1.26), is able to synthesize maltulose in the course of sucrose hydrolysis.

According to the literature[12] and our results, but contrary to the findings of Carnie and Porteous[13], there is no β-D-fructofuranosidase in rat mucosa or in mucosa homogenates of hog, rabbit, cow, and man, except that of intestinal flora[14].

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Darstellung von 1-Desoxy-1-(2-sulfoäthylamino)-D-fructose

Die Umsetzung von Aldosen und Ketosen mit Aminocarbonsäuren ist von uns eingehend untersucht worden[1-3]. Danach werden aus Aldosen über eine Amadori-Umlagerung „Ketoseaminosäuren" erhalten[1,3], während Ketosen über eine Ketosylamin-Umlagerung „Aldoseaminosäuren" liefern[2]. Es reagiert z.B. D-Glucose mit Glycin zur 1-Desoxy-1-glycino-D-fructose. D-Fructose liefert mit Glycin ein Gemisch der beiden am C-Atom 2 epimeren Verbindungen 2-Desoxy-2-glycino-D-glucose und 2-Desoxy-2-glycino-D-mannose. In diesem Zusammenhang interessierte uns das Verhalten der Aminosulfonsäuren, insbesondere der in der Natur weit verbreiteten 2-Aminoäthansulfonsäure (Taurin), bei der Reaktion mit Aldosen und Ketosen.

Es zeigte sich, daß die direkte Umsetzung von D-Glucose und D-Fructose mit Aminosulfonsäuren (Taurin, Aminomethansulfonsäure) unter den bei Aminocarbonsäuren erprobten Reaktionsbedingungen nicht zu Produkten der Amadori-Umlagerung bzw. Ketosylamin-Umlagerung führt. Auch bei Neutralisation der stark sauren Sulfonsäuregruppe durch Salz- oder Amid-Bildung trat keine entsprechende Reaktion ein. Mit Erfolg ließen sich dagegen das Natrium-Salz von Taurin und Tauramid mit 4,6-*O*-Benzyliden-D-glucopyranose umsetzen, die nach Micheel *et al.*[4] mit Aminen besonders leicht unter Amadori-Umlagerung reagiert.

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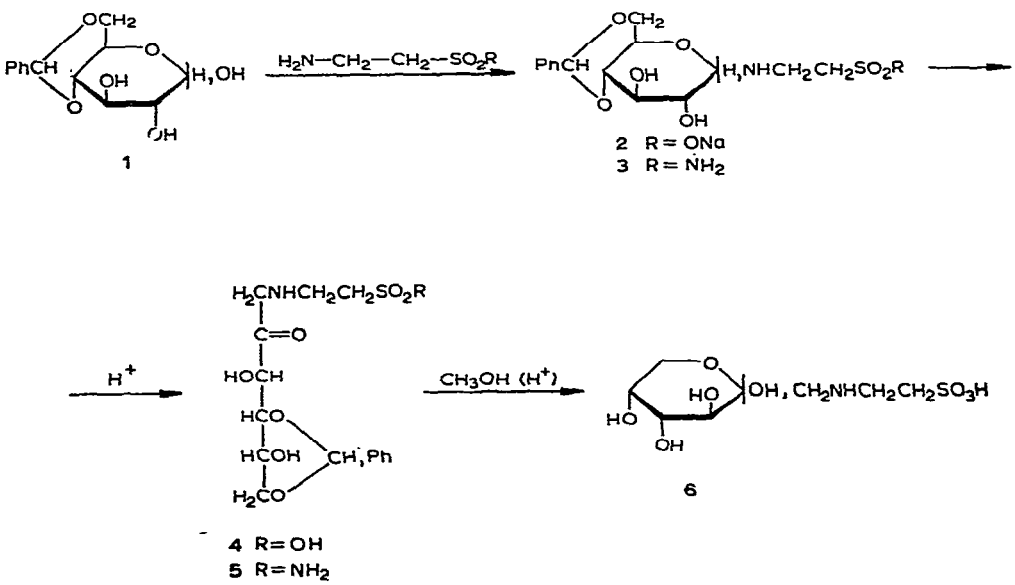
Die Umsetzung von 4,6-*O*-Benzyliden-D-glucopyranose (**1**) mit Natrium-2-aminoäthansulfonat in Methanol führt zunächst zum Natrium-*N*-(4,6-*O*-benzyliden-D-glucosyl)-2-aminoäthansulfonat (**2**), das bei Abwesenheit von Säure in 90% Ausbeute kristallin isolierbar ist. Mit wasserfreier Oxalsäure läßt sich das *N*-Glycosid **2** unter Amadori-Umlagerung in 4,6-*O*-Benzyliden-1-desoxy-1-(2-sulfoäthylamino)-*keto*-D-fructose (**4**) überführen. Das Amadori-Produkt **4** kann auch ohne Isolierung des *N*-Glycosids **2** unmittelbar aus den Komponenten bei Zusatz von Oxalsäure dargestellt werden. Es wird in kristalliner Form erhalten und ist frei von Na^+- und Oxalat-Ionen. Eine scharfe C=O-Bande bei 1720 cm^{-1} im IR-Spektrum der Verbindung und die leichte Überführbarkeit in ein Oxim beweisen die angegebene offenkettige Struktur von **4**. Im Oxim von **4** tritt die C=O-Bande im IR-Spektrum nicht mehr auf.

Aus **4** erhält man nach vorsichtiger Abspaltung der Benzyliden-Gruppe durch Umacetalisierung mit 0.1N H_2SO_4 in absolutem Methanol die freie Amadori-Verbindung 1-Desoxy-1-(2-sulfoäthylamino)-D-fructose (**6**). Die Verbindung reagiert schwach mit Ninhydrin und zeigt gegenüber Silbernitrat und *o*-Dinitrobenzol die charakteristischen Reduktionseigenschaften. In wäßriger Lösung ist **6** recht labil und zersetzt sich beim Stehen der Lösung, wobei Taurin und eine Reihe unübersichtlicher, schneller laufender Komponenten, jedoch keine D-Glucose, als Spaltprodukte chromatographisch nachweisbar sind. Aus dem Fehlen der C=O-Bande im IR-Spektrum von **6** kann auf die angegebene cyclische Halbketal-Form geschlossen werden.



Das Tauramid läßt sich ebenfalls in hoher Ausbeute mit **1** umsetzen zum *N*-(4,6-*O*-Benzyliden-D-glucosyl)-2-aminoäthansulfonamid (**3**). Das *N*-Glycosid **3** kann mit wasserfreier Oxalsäure zur Amadori-Verbindung 4,6-*O*-Benzyliden-1-desoxy-1-

(2-sulfamoyläthylamino)-*keto*-D-fructose (**5**) umgelagert werden, die als kristallines Oxalat erhalten wird. Bei dieser Umlagerung tritt eine beträchtliche Rückspaltung in die Komponenten ein, wobei das Tauramid als sehr schwer lösliches Oxalat ausfällt. Zum Beweis der offenkettigen Form in **5** wurde aus einem Teil des Oxalats mit Natriummethylat die amorphe freie Base **5** gewonnen, bei der im IR-Spektrum eine C=O-Bande zu erkennen ist.

EXPERIMENTELLER TEIL

Natrium-N-(*4,6*-O-*benzyliden*-D-*glucosyl*)-*2-aminoäthansulfonat* (**2**). — Jeweils heiß-gesättigte Lösungen von 4,6-*O*-Benzyliden-D-glucopyranose[5] (**1**) (20.3 g; 75 mMol) in abs. Methanol (50 ml) und Natrium-2-aminoäthansulfonat (11.1 g; 75 mMol) in abs. Methanol (250 ml) wurden vereinigt und 2–3 Min. am Rückfluß gekocht. Das *N*-Glycosid **2** kristallisiert sofort aus. Die Lösung wird auf Zimmertemperatur abgekühlt, der Niederschlag abgesaugt und mit abs. Methanol und abs. Äther gewaschen. Ausb. 90%; **2** ist löslich in Methylsulfoxid, schwer- bis unlöslich in den anderen organischen Lösungsmitteln, hydrolyseempfindlich und reduziert *o*-Dinitrobenzol nicht. Schmp. 205–207° (Zers.); $[\alpha]_D^{20}$ −45.0° (*c* 1, Methylsulfoxid; nach 5 Min.) → −24.0° (Endwert; nach 2 Tagen; Lösung hellgelb).

$C_{15}H_{20}NO_8SNa$ (397.4) Ber.: C, 45.34; H, 5.07; N, 3.52; S, 8.07. Gef.: C, 44.87; H, 5.23; N, 3.44; S, 8.18.

N-(*4,6*-O-*Benzyliden*-D-*glucosyl*)-*2-aminoäthansulfonamid* (**3**). — Das *N*-Glycosid **3** wird analog dargestellt aus 4,6-*O*-Benzyliden-D-glucopyranose[5] (**1**) und Tauramid[6,7,8]. Ausb. 75%; **3** kann aus abs. Isopropylalkohol umkristallisiert werden. Schmp. 131° (Zers.). $[\alpha]_D^{20}$ −43.0° (*c* 1, Methylsulfoxid; nach 5 Min.) → −24.0° (Endwert; nach 2 Tagen).

$C_{15}H_{22}N_2O_7S$ (374.4) Ber.: C, 48.12; H, 5.92; N, 7.48. Gef.: C, 47.81; H, 5.89; N, 7.09.

4,6-O-*Benzyliden-1-desoxy-1*-(*2-sulfoäthylamino*)-keto-D-*fructose* (**4**). — Eine Suspension von *N*-Glycosid **2** (20 g; 50 mMol) in abs. Methanol (750 ml) wird zum Sieden erhitzt, mit wasserfreier Oxalsäure (4.5 g; 50 mMol), gelöst in wenig abs. Methanol, versetzt und *ca.* 5 Min. am Rückfluß gekocht. Das *N*-Glycosid **2** geht vollständig in Lösung, die Reaktionslösung färbt sich orangegelb und es beginnt die Kristallisation eines farblosen Produktes, das ein Gemisch aus dem Amadori-Produkt **4** und Natriumoxalat darstellt. Durch mehrfache fraktionierte Kristallisation aus Wasser–Äthanol wird **4** in reiner, kristalliner Form erhalten. Farblose Nadeln, Ausb. 51% d. Th. an reinem Produkt. Schmp. 176–177° (Zers.); $[\alpha]_D^{20}$ −62.0° (*c* 1, Methylsulfoxid); IR: 1720 cm^{-1}.

$C_{15}H_{21}NO_8S$ (375.4) Ber.: C, 47.99; H, 5.64; N, 3.73; S, 8.54. Gef.: C, 48.56; H, 5.70; N, 3.49; S, 8.38.

4,6-O-*Benzyliden-1-desoxy-1*-(*2-sulfamoyläthylamino*)-keto-D-*fructose-oxalat* (**5** als Oxalat). — Das Amadori-Produkt **5** wird analog aus **3** dargestellt. Es wird zunächst ein Gemisch aus **5** (als Oxalat) und Tauramid-oxalat (entstanden durch Rückspaltung)

erhalten, aus dem **5** (als Oxalat) durch fraktionierte Kristallisation aus abs. Methanol in reiner, kristalliner Form erhalten wird. Ausb. 34% d. Th. an reinem Produkt, Schmp. 165–166.5° (Zers.), $[\alpha]_D^{20}$ −55.8° (*c* 1, Methylsulfoxid); IR: 1720 cm^{-1} (nach Abspaltung des Oxalat-Restes mit Natrium-methylat).

$C_{15}H_{22}N_2O_7S(COOH)_2$ (464.4) Ber.: C, 43.97; H, 5.21; N, 6.03; S, 6.90. Gef.: C, 43.00; H, 5.17; N, 6.06; S, 7.21.

*1-Desoxy-1-(2-sulfoäthylamino)-*D*-fructose* (**6**). — Verbindung **4** (750 mg; 2 mMol) wird in 40 ml einer 0.1N Lösung von H_2SO_4 in abs. Methanol suspendiert und 20 Min. unter Stickstoff am Rückfluß gekocht. Die Reaktionslösung wird auf Zimmertemperatur gekühlt, und mit Äther ein farbloses, amorphes Produkt gefällt. Man löst das Produkt in Wasser, entfernt die SO_4^{2-}-Ionen mit $Ba(OH)_2$ als $BaSO_4$ und befreit die Lösung von überschüssigen Ba^{2+}-Ionen durch Behandlung mit einem sauren Ionenaustauscher. Nach dem Abdest. des Lösungsmittels wird das freie Amadori-Produkt **6** in amorpher Form erhalten. In wäßriger Lösung ist **6** recht labil. $[\alpha]_D$ −41.6° (*c* 1,Methylsulfoxid; nach 5 Min.) → −10.3° (Endwert; nach 4 Tagen; Lösung gelb).

$C_8H_{17}NO_8S$ (287.3) Ber.: C, 33.44; H, 5.96; N, 4.88; S, 11.16. Gef.: C, 33.10; H, 6.03; N, 4.63; S, 11.09.

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Hydrogen bonding in crystalline β-D-glucopyranose and methyl β-D-xylopyranoside

Examination of available crystal structures[1] of carbohydrates reveals that the usual pattern is for all of the hydroxyl groups to be involved in hydrogen bonding. However, the results of recent X-ray investigations of the crystal structures of β-D-glucopyranose[2] and methyl β-D-xylopyranoside[3] indicate the presence of free hydroxyl groups. In this work, the hydroxyl-stretching frequencies of the above compounds have been obtained and found to be lower than those normally attributed to unbonded groups. Bands arising from free hydroxyl groups, if present, should be detectable in the spectra, even though such bands are normally less intense than those associated with bonded hydroxyl groups, since, in β-D-glucopyranose, the free hydroxyl groups are said to comprise one-fifth[2] of the total number and, in methyl β-D-xylopyranoside, one-third[3].

EXPERIMENTAL

The infrared spectra for β-D-glucopyranose (m.p. 150°, $[\alpha]_D$ +18.6° in water) and methyl β-D-xylopyranoside (m.p. 157°, $[\alpha]_D$ −65.3° in water) were recorded on a Grubb–Parsons double-beam S4 spectrometer equipped with silica and sodium chloride prisms. The materials were dispersed in potassium chloride discs, and their spectra were obtained at room temperature, and, since bands in the spectra of carbohydrates at low temperatures have been reported[4] to be sharper and better resolved, at −180° also.

RESULTS AND DISCUSSION

The spectra are shown in Fig. 1. The most obvious effect of cooling the samples was seen in band sharpening and increased absorbance, often accompanied by shifts to lower frequencies. Changes in other regions of the spectrum (3000–670 cm^{-1}) were only slight, and there were no indications that phase changes had occurred during cooling.

The highest frequency peaks in the spectra of β-D-glucopyranose and methyl β-D-xylopyranoside were found at 3550 and 3465 cm^{-1} (room temperature values), respectively. There was also a shoulder at 3520 cm^{-1} on the side of the band envelope for methyl β-D-xylopyranoside. These frequencies are much lower than those reported for free hydroxyl groups in studies of the spectra of tetrahydropyranols[5,6] and of cyclohexane and triterpenoid alcohols[7] in dilute solution in carbon tetrachloride. Free, primary hydroxyl groups in these compounds were found to absorb in the range 3641–3640 cm^{-1} and secondary groups in the range 3635–3620 cm^{-1}. The observed frequencies are also somewhat lower than the range 3615–3570 cm^{-1} found for equatorial hydroxyl groups involved in intramolecular hydrogen-bonds to oxygen atoms

gauche on an adjacent carbon atom in *O*-methyl derivatives of D-glucose, D-xylose, and related glycosides[8]. The extent to which the stretching frequencies were modified by the change in the environment of the groups from solution to crystal is difficult to ascertain,

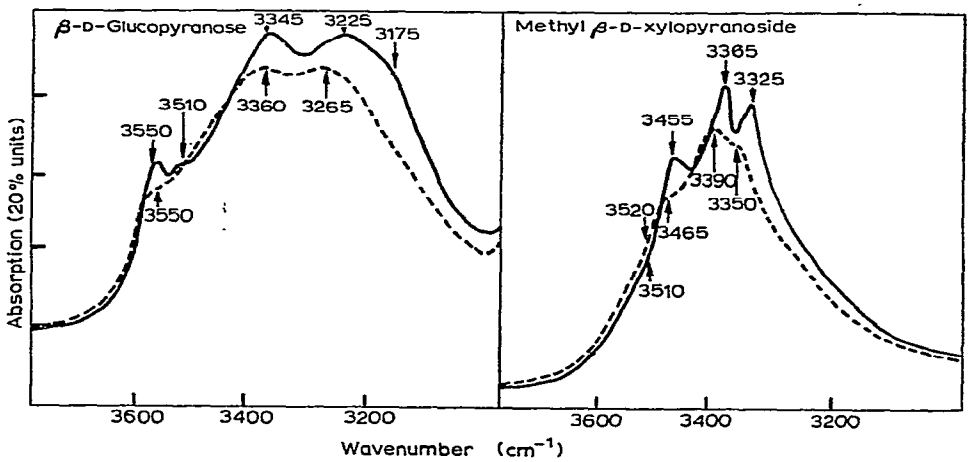

Fig. 1. Spectra in the hydroxyl-stretching region of β-D-glucopyranose and methyl β-D-xylopyranoside at two temperatures: — — — — — —, 22°; ————, −180°.

since such a change is usually accompanied by the formation of new hydrogen bonds that also lead to lower frequencies. Studies[9] of the effect of such changes on the frequencies of hydroxyl groups hindered in respect to rotation and hydrogen-bond formation, in both alcohols and phenols, indicated that such effects were probably quite small. For example, in 2,4,6-tri-*tert*-butylphenol, there was no change in frequency, and, in tricyclohexylmethanol, bands at 3632 and 3614 cm^{-1} in the solution spectra coalesced to form a band at 3610 cm^{-1} in the solid. However, in monosaccharides, the hydroxyl-stretching frequencies might also be affected by non-bonded interactions with neighbouring oxygen atoms. Nevertheless, the lower hydroxyl frequencies observed for β-D-glucopyranose and methyl β-D-xylopyranoside do not seem to be compatible with the presence of free hydroxyl groups in the crystals. Bellamy and Pace[10] have shown recently that the frequencies of bonded hydroxyl groups, in which the oxygen atom serves as an acceptor, are appreciably lowered in comparison with the frequencies of groups that serve only as donors, but the frequencies of non-bonded hydroxyl groups are known[11] to be little affected. Thus, it may be that the observed frequencies can be attributed to the presence of hydroxyl groups that act as acceptors and are involved in intramolecular hydrogen-bonds to oxygen atoms of equatorial hydroxyl groups on adjacent carbon atoms. Evidence for the formation of such bonds in individual molecules has been found in a wide variety of cyclohexane[7,11] and tetrahydropyran derivatives[5,6,8], and their energy has been estimated[12] to be ~0.8 kcal. $mole^{-1}$.

The X-ray evidence that certain hydroxyl groups in crystalline β-D-glucopyranose and methyl β-D-xylopyranoside were not involved in hydrogen bonds was of a different

nature for the two compounds. In the first case, it was found that the hydrogen atoms of such groups could not be located as precisely as those of other hydroxyl groups[2], and, in the second case, those atoms that had been located did not lie near the direct line between the oxygen atoms[3]. However, a hydrogen atom could still participate in a hydrogen bond and yet not appear fixed in position if the bond was sufficiently weak as to be disrupted periodically by thermal vibrations. The energies of intramolecular hydrogen-bonds of the type discussed above seem to be low enough to allow such disruption to occur. Although the O-H...O angle involved in the formation of an intramolecular hydrogen-bond in the structure of methyl β-D-xylopyranoside is much less than 180°, it is almost the same (to within 10°) as that for an O-H...O bond involving equatorial hydroxyl groups on adjacent carbon atoms in the ideal chair forms of cyclohexane and tetrahydropyran rings. Such bonds certainly seem to form in individual molecules (*cf.* cyclohexane-*trans*-1,2-diol[11] and 1,5-anhydro-2-deoxy-D-*threo*-pentitol[5]), although the possibility of some deviations from the ideal chair shape in these molecules has to be admitted. Nevertheless, the occurrence of such an O-H...O angle in the structure would not necessarily preclude the formation of a hydrogen bond.

The observed frequency for β-D-glucopyranose would also seem to be compatible with the long, intermolecular hydrogen-bond suggested initially by Ferrier[13], since Hordvik[14,15] has proposed similar long bonds in the structures of crystalline β-L-arabinopyranose and β-D-lyxopyranose; in the spectra of these pentoses, peaks occur near 3530 and 3525 cm^{-1}, respectively.

In conclusion, it would appear that the relevant hydroxyl groups are not free but are involved in very weak hydrogen bonds that may be disrupted by thermal vibrations.

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The optical rotations of trehalose derivatives

Staněk[1] has applied Klyne's rules[2] to calculate the optical rotations of the three isomeric trehaloses and their octaacetates. Good agreement between calculated and observed values was found in five out of the six cases, particularly if the calculated value was compared with the least positive, optical rotation observed in each case. In one case (α,α-trehalose octaacetate), however, the observed and calculated figures differed considerably. Because this anomaly was not explained, it was considered desirable to apply Klyne's rules to further trehalose derivatives, the physical constants of which have now become available[3,4]. This type of calculation can only be carried out if literature values for the rotations of the trehalose derivatives and corresponding methyl glucosides apply to a common solvent.

Group *A* in Table I includes those trehalose derivatives where good agreement between the observed and calculated optical rotations has been found, and Group *B* contains examples where conflicting values have been reported. In each case, the published figures fall into two groups for each compound, and the calculated figure is in reasonable agreement with one of the groups, not always the least positive. Thus, it is reasonable to use the calculated rotation to discriminate between conflicting, published observations.

The case of α,β-trehalose octaacetate is interesting, in that the two "high" determinations, which are presumably in error, appear to be in good agreement with each other and form a group whose mean is significantly different statistically from that of the "low" determinations.

Group *C* contains those compounds for which the calculated and observed optical rotations differ. It is open to question whether these differences are due to a breakdown of the theory on which the calculation is based, or because of errors

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Group *C* contains those compounds for which the calculated and observed optical rotations differ. It is open to question whether these differences are due to a breakdown of the theory on which the calculation is based, or because of errors

TABLE I

Compound	$[\alpha]_D$, *degrees* *Calc.*	*Obs.*	*References*
Group A			
α,α-Trehalose	+181	+179	1, 3
Hexa-*O*-acetyl-	+161	+159	7, 12
6,6′-Di-*O*-tosyl-[a]	+111	+110	8, 12
Hexa-*O*-acetyl-6,6′-di-*O*-tosyl-	+133-6	+136	7, 12
Hexa-*O*-acetyl-6,6′-di-*O*-mesyl-	+147	+143	9, 12
6,6′-Dideoxy-	+175	+175	4, 12
4,6:4′,6′-Di-*O*-ethylidene-	+141	+143	4, 12
4,6:4′,6′-Di-*O*-ethylidene-tetra-*O*-tosyl-	+53	+52	4, 12
Tetrachloro-tetradeoxy-, disulphate	+152	+152	10, 12
2-Amino-2-deoxy-, hydrochloride	+175	+176	11, 12
β,β-Trehalose octaacetate	−19.4	−18.6	1, 3
Group B			
6,6′-Di-*O*-trityl-α,α-trehalose	+92	+62 +94	7, 9, 12
β,β-Trehalose	−38.5	−18.4 −38.2 −39.4 −40.23 −41.5	1, 3
α,β-Trehalose	+71	+67.1 +70.0 +95	1, 3
α,β-Trehalose octaacetate	+60	+64 +67 +68.1 +80 +82	1, 3
Group C			
α,α-Trehalose octaacetate	+139*	+163	1, 3
Hexa-*O*-acetyl-6,6′-di-*O*-trityl-α,α-trehalose	+143	+115	5, 6, 7, 9
Hexa-*O*-acetyl-6,6′-dideoxy-α,α-trehalose	+172*	+206*	4, 12
4,6:4′,6′-Di-*O*-ethylidene-2,3:2′,3′-di-*O*-(oxidodiethylidene)-α,α-trehalose	+91	+103*	4, 12
Hexa-*O*-acetyl-6,6′-dideoxy-6,6′-diiodo-α,α-trehalose	+123*	+92*	7, 12

[a]Tosyl = toluene-*p*-sulphonyl. *An uncorroborated value.

in determining the optical rotation of the compound or its corresponding methyl glucoside. No plausible chemical explanation for the differences is obvious, and, in every case except one, the comparisons in Group *C* rest on a single, uncorroborated report of an optical rotation (these figures are indicated by an asterisk). In the other case, the calculated value is based on two independent investigations[5,6] of the corresponding glucoside, which agreed concerning the optical rotations ($[\alpha]_D$ +137° in pyridine) but disagreed concerning the melting points (136° and 143–5°). It would

therefore appear that further evidence is required before the apparent differences in Group *C* can be attributed to the inapplicability of Klyne's rules to these compounds.

It should be pointed out that Klyne's rules can only be valid provided that conformational analogy exists between the trehalose derivatives and the corresponding glucosides. Up to now, no conformational evidence has been published for trehaloses or their derivatives, and although the *C1* form of the D-glucose rings is preferred, fully substituted derivatives may possibly adopt other conformations. For example, 4,6:4′,6′-di-*O*-ethylidene-2,3:2′,3′-di-*O*-(oxidodiethylidene)-α,α-trehalose contains two trioxacycloheptane rings[4] that can be puckered in several ways.

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Synthèse d'un D-*galacto*-heptos-6-ulose protégé

La méthode d'extension par le diazométhane, due à Wolfrom, a été surtout appliquée à des chlorures d'acides aldoniques peracétylés[1] et au chlorure de tétra-*O*-acétylgalactonyle[2]. Nous l'avons employée ici avec le chlorure d'un acide uronique protégé.

Nous préparons une solution du chlorure de l'acide 1,2:3,4-di-*O*-isopropylidène-α-D-galacturonique par action du chlorure d'oxalyle sur une suspension du sel de potassium dans le benzène anhydre. Sa présence en solution est prouvée par la précipitation directe de l'anilide cristallisé connu[3] par addition d'aniline (rendement 50% après recristallisation). Sans isoler le chlorure d'acide présumé instable, nous ajoutons

therefore appear that further evidence is required before the apparent differences in Group *C* can be attributed to the inapplicability of Klyne's rules to these compounds.

It should be pointed out that Klyne's rules can only be valid provided that conformational analogy exists between the trehalose derivatives and the corresponding glucosides. Up to now, no conformational evidence has been published for trehaloses or their derivatives, and although the *C1* form of the D-glucose rings is preferred, fully substituted derivatives may possibly adopt other conformations. For example, 4,6:4′,6′-di-*O*-ethylidene-2,3:2′,3′-di-*O*-(oxidodiethylidene)-α,α-trehalose contains two trioxacycloheptane rings[4] that can be puckered in several ways.

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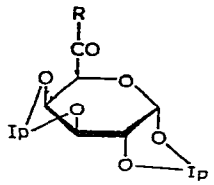
Synthèse d'un D-*galacto*-heptos-6-ulose protégé

La méthode d'extension par le diazométhane, due à Wolfrom, a été surtout appliquée à des chlorures d'acides aldoniques peracétylés[1] et au chlorure de tétra-*O*-acétylgalactonyle[2]. Nous l'avons employée ici avec le chlorure d'un acide uronique protégé.

Nous préparons une solution du chlorure de l'acide 1,2:3,4-di-*O*-isopropylidène-α-D-galacturonique par action du chlorure d'oxalyle sur une suspension du sel de potassium dans le benzène anhydre. Sa présence en solution est prouvée par la précipitation directe de l'anilide cristallisé connu[3] par addition d'aniline (rendement 50% après recristallisation). Sans isoler le chlorure d'acide présumé instable, nous ajoutons

deux équivalents de diazométhane dans l'éther. Le traitement habituel conduit à un résidu cristallisé jaune pâle, où l'analyse sur couche mince révèle deux constituants, la diazocétone **1** et la chlorocétone **2**. Toutefois, la diazocétone ainsi purifiée contient encore 4% de 1,2:3,4-di-*O*-isopropylidène-α-D-galacturonate de méthyle qui persiste après recristallisation dans l'éther. On peut cependant observer, sur cet échantillon,



1 $R = CHN_2$
2 $R = CH_2Cl$
3 $R = CH{=}N{-}N{=}P(Ph)_3$
4 $R = CH_2OAc$
5 $R = CH_2OCOPhNO_2$

la bande infrarouge à 2100 cm^{-1} caractéristique du groupement C=N=N et un spectre ultraviolet dépendant du solvant identique à celui des diazocétones simples[4]. On obtient aisément, avec la triphénylphosphine (*cf.* réf. 5), un dérivé pur hautement cristallin **3**, dont le spectre ultraviolet est très caractéristique.

L'acétolyse, selon Wolfrom et Wood[6], de la diazocétone purifiée par l'acide acétique anhydre en présence d'acétate de cuivre, donne une huile séparable en deux constituants par chromatographie de vapeur (g.l.c.) préparative. Le premier (rdt. 4%) est l'ester méthylique contaminant, et le second (rdt. 95%) est le 7-*O*-acétyl-1,2:3,4-di-*O*-isopropylidène-α-D-*galacto*-heptos-6-ulose (**4**) qui a même temps de rétention et spectre n.m.r. que le composé décrit ci-dessous. Les difficultés de purification à plus grande échelle à toutes les étapes nous ont engagés à chercher une autre route.

Après la réaction avec le diazométhane, le traitement du mélange brut par une solution éthérée de gaz chlorhydrique, suivi d'une évaporation à sec et de la recristallisation du résidu dans l'éther permet d'obtenir la chlorocétone **2** pure avec 66% de rendement à partir du galacturonate protégé. L'attaque de **2** par le *p*-nitrobenzoate de sodium dans la *N,N*-diméthylformamide donne avec 8% de rendement le 7-*p*-nitrobenzoate (**5**) de l'heptosulose protégé. Sur le spectre de n.m.r. de **5**, par ailleurs compatible avec la structure attendue, on observe les deux protons méthyléniques du groupement $-CH_2OCOC_6H_4NO_2$ à δ 5,20 et 5,31 p.p.m.

Avec l'acétate de sodium dans la *N,N*-diméthylformamide, la conversion de la chlorocétone (mesurée par g.l.c.) est aussi peu efficace. Nous avons réalisé une synthèse satisfaisante de l'acétate **4**, en passant par l'iodocétone **6** (non isolée) qui est traitée par un excès d'acétate d'argent dans l'acide acétique anhydre. On obtient, après distillation avec un rendement de 55%, une huile incolore, qui donne un seul pic en g.l.c. et se prend en masse, lentement, à la température ambiante. On peut poursuivre la purification par recristallisation dans le mélange éther–éther de pétrole (rendement définitif : 46%). Sur le spectre de n.m.r. de l'acétoxycétone **4**, les deux protons méthyléniques du groupement $-CH_2OCOCH_3$ apparaissent à δ 4,77 et 4,90 p.p.m.

On observe des dichroïsmes circulaires négatifs comparables avec les composés carbonylés **2** et **4**, avec un minimum à 282 mμ, et des valeurs $\Delta\varepsilon$ −2,21 pour la chlorocétone et $\Delta\varepsilon$ −1,87 pour l'acétoxycétone.

Le 1,2:3,4-di-*O*-isopropylidène-α-D-galacturonate de méthyle, décrit jusqu'à présent comme une huile[7], a cristallisé spontanément et facilement entre nos mains. Il se fragmente dans le spectromètre de masse comme le 1,2:3,4-di-*O*-isopropylidène-α-D-galactose[8].

PARTIE EXPÉRIMENTALE

On a déterminé les points de fusion avec le microscope Reichert, les spectres infrarouges en solution dans le dichlorométhane ou le chloroforme avec le spectrophotomètre Infracord Perkin–Elmer, les spectres ultraviolets en solution voisine de 0,1 mM avec le spectrophotomètre Jobin–Yvon. Les spectres de n.m.r. sont mesurés à 60 Hz et rapportés en p.p.m. (δ) à partir du tétraméthylsilane. On a fait les chromatographies sur couche mince sur plaque de gel de silice fluorescent, avec le chloroforme comme phase mobile, les chromatographies de vapeur avec une colonne Chromosorb W, 5%, SE 30 de 150 cm (Aerograph Co.), avec l'azote comme gaz porteur.

*Chlorure de 1,2:3,4-di-*O*-isopropylidène-α-D-galacturonyle.* — A une suspension dans du benzène anhydre de 1,2:3,4-di-*O*-isopropylidène-α-D-galacturonate de potassium (4 g) rendu parfaitement anhydre par évaporations successives à sec de benzène anhydre, est ajoutée par petites quantités en 1 h, à l'abri de l'humidité de l'air, une solution de chlorure d'oxalyle (2 ml) dans du benzène. Le mélange réactionnel est gardé la nuit à l'abri de l'humidité. Le benzène est évaporé, puis l'excès de chlorure d'oxalyle est complètement entraîné par évaporations successives de benzène. L'huile résultante est reprise par le benzène sec (20 ml). A cette étape, un dosage avec NaOH N indique la présence en solution de 11,5 mmoles de chlorure d'acide (rdt 90%).

*1,2:3,4-Di-*O*-isopropylidène-α-D-galacturonanilide.* — On ajoute à la solution benzénique (0.5 ml) de l'aniline refroidie (0.1 ml). On recueille le précipité immédiat, sèche 1 h sous vide poussé, triture avec HCl 1%, filtre, lave à l'eau et recristallise dans l'alcool chaud (3,5 ml). On obtient ainsi 57 mg (50%) par rapport au galacturonate protégé, p. f. 174°.

*7-Désoxy-7-diazo-1,2:3,4-di-*O*-isopropylidène-α-D-galacto-heptos-6-ulose* (**1**). — Au diazométhane (25,6 mmoles) dans l'éther à −15°, on ajoute en 1 h le chlorure d'acide (préparé à partir de 4 g ou 12,8 mmoles de sel de potassium) avec agitation manuelle, et à l'abri de l'humidité. On laisse une nuit à −15°, on filtre les polyméthylènes et l'on évapore l'éther à froid. On obtient 3,5 g de cristaux jaunes, principalement la diazocétone avec un peu de chlorocétone et d'ester méthylique.

On sépare la diazocétone de la chlorocétone par chromatographie sur couche mince (révélation par $AgNO_3$ à 0,7% dans l'acétone, puis la soude alcoolique à 2%), suivie de cristallisation dans l'éther, p.f. 118–128°; ν_{max} 2100 cm^{-1} (C=N=N), $\lambda_{max}^{cyclohexane}$ 249 mμ, ε 6750; λ_{min} 211 mμ, ε 1100; λ_{max}^{MeOH} 248, 284 mμ, ε 4900, 6100; λ_{min} 215, 255mμ, ε 900, 4800.

Anal. Calc. pour $C_{13}H_{18}N_2O_6$: C, 52,34; H, 6,08; N, 9,39 Trouvé: C, 52,28; H, 6,28; N, 8,40.

La présence de 4% d'ester méthylique résulte de l'acétolyse décrite ci-dessous.

Dérivé avec la triphénylphosphine (**3**). — On dissout la diazocétone **1** (149,4 mg; 0,5 mmole) dans le minimum d'éther et on ajoute une solution de triphénylphosphine (170 mg; 0,65 mmole) dans 0,5 ml d'éther. On laisse reposer jusqu'à la cristallisation (rapide) du dérivé, qui est recristallisé dans l'alcool, p.f. 163–165°; $\lambda_{max}^{cyclohexane}$ 253, 259, 265, 271 mμ, ε 1900, 2250, 2550, 2100; λ_{min} 255, 262, 269; ε 1900, 2050, 1500.

Anal. Calc. pour $C_{31}H_{33}N_2O_6P$: C, 66,43; H, 5,89; N, 5,00; P, 5,54. Trouvé: C, 66,93; H, 5,76; N, 5,95; P, 5,71.

7-O-Acétyl-1,2:3,4-di-O-isopropylidène-α-D-galacto-heptos-6-ulose (**4**) *à partir de* **1**. — On chauffe à 100°, à l'abri de l'humidité, une solution de diazocétone (231 mg) purifiée comme ci-dessus, dans l'acide acétique anhydre[9] (18 ml) avec de l'acétate de cuivre (3 mg), jusqu'à virage du vert au jaune. On fait alors bouillir 5 min à reflux, on évapore à sec et chasse les dernières traces d'acide par addition et évaporation d'alcool. On sèche sur KOH, ajoute une solution 0.2M de $NaHCO_3$ et extrait à l'éther. Par distillation de l'extrait éthéré lavé et séché, on recueille une huile, p.éb. 125°/0.2 mm, qui, selon l'analyse par g.l.c. (184°, 720 mm), contient deux composés: le premier passant à 2 min 30 sec est le 1,2:3,4-di-*O*-isopropylidène-α-D-galacturonate de méthyle (4%); il est identique à un échantillon authentique (temps de rétention et spectre de n.m.r.) et possède un spectre de masse compatible. Le second passant à 5 min 3 sec est le composé **4** (95%). Il est trouvé identique (temps de rétention, spectre de n.m.r.) à l'acétate décrit ci-dessous.

7-Chloro-7-désoxy-1,2:3,4-di-O-isopropylidène-α-D-galacto-heptos-6-ulose (**2**). — On dissout dans le minimum d'éther la diazocétone brute **1** provenant de 4 g de sel de potassium protégé et on ajoute une solution d'HCl (26 mmoles) sec dans l'éther anhydre (10 ml). Après la fin du dégagement d'azote (15 min) on évapore à sec, sèche sur KOH et recristallise dans l'éther. Les cristaux obtenus (2,6 g, 67%), p.f. 150°, apparaissent homogènes en g.l.c. (temps de rétention 2 min 31 sec à 203° et 730 mm); ν_{max} 1745 cm^{-1} (C=O); dichroïsme circulaire $\lambda_{min}^{dioxane}$ 282 mμ, $\Delta\varepsilon$ −2,21.

Anal. Calc. pour $C_{13}H_{19}O_6Cl$: C, 50,89; H, 6,19; Cl, 11,58. Trouvé : C, 50,71; H, 6,02; Cl, 11,67.

1,2:5,6-Di-O-isopropylidène-7-O-p-nitrobenzoyl-α-D-galacto-heptos-6-ulose (**5**). — On chauffe 11 h à 100° sous azote un mélange de la chlorocétone **2** (1,2 g) dans la *N,N*-diméthylformamide (20 ml) avec du paranitrobenzoate de sodium (1,4 g). On essore la solution très brune obtenue, et évapore à sec le filtrat, qui est soigneusement desséché en présence de KOH solide et de P_2O_5 et ensuite extrait à l'éther anhydre. Après évaporation, l'extrait éthéré est dissous dans le chloroforme (100 ml) et purifié sur une colonne de gel de silice (15 g), en éluant au chloroforme jusqu'à la sortie de la première zone brune, également recueillie. On recristallise cette fraction de tête dans l'éther. On obtient en tout 150 mg (8,6%) de cristaux, p.f. 168–170°; ν_{max} 1730, 1750 cm^{-1} (C=O), 1530 cm^{-1} (NO_2); $\lambda_{max}^{cyclohexane}$ 256 mμ, ε 14000; spectre de n.m.r. (chloroforme-*d*); δ 1,29 (6 protons), δ 1,47 (6 protons), δ 4,38 (2 protons), δ 4,58 (2 protons), δ 5,20 (1 proton), δ 5,31 (1 proton), δ 5,57, 5,65 (1 proton doublet, *J* 5 Hz, H-1), δ 8,27 (4 protons).

Anal. Calc. pour $C_{20}H_{23}NO_{10}$: C, 54,92; H, 5,30; N. 3,20; Trouvé : C, 54,99; H, 5,32; N, 3,21.

7-O-Acétyl-1,2:3,4-di-O-isopropylidène-α-D-galacto-heptos-6-ulose à partir de **2**. — La solution, initialement limpide, obtenue en mélangeant la chlorocétone **2** (1,89 g) dans l'acétone (50 ml) à l'iodure de sodium (1,57 g) dans l'acétone (35 ml) se trouble rapidement à froid par précipitation de NaCl. On termine la réaction par 45 min. d'ébullition à reflux. On filtre, évapore à sec, reprend à l'éther, lave à l'eau, puis sèche sur Na_2SO_4 l'extrait éthéré. On obtient par évaporation à sec une huile (2 g), probablement surtout l'iodocétone.

A la solution de cette huile (2 g) dans l'acide acétique anhydre (30 ml), on ajoute une suspension d'acétate d'argent (4,2 g) dans l'acide acétique anhydre (250 ml). On fait bouillir à reflux 3 h, filtre, évapore à sec, puis on entraîne les dernières traces d'acide par deux additions et évaporations d'éthanol. On sèche sur KOH, et on reprend le résidu par l'éther et $NaHCO_3$ 0.2M. On lave à l'eau, sèche sur Na_2SO_4 et distille l'extrait éthéré. On recueille une huile faiblement jaune, (1,122 g, 55%), p.éb. 128–134°/0.2 mm, qui, en quelques semaines, se prend lentement en masse cristalline, p.f. 83–89°, s'élevant à 92–94° par recristallisation dans l'éther et l'éther de pétrole (rendement global : 46%); temps de rétention en g.l.c. (184°, 720 mm) : 4 min 58 sec; ν_{max} 1740, 1752 cm^{-1} (C=O); dichroïsme circulaire : $\lambda_{min}^{dioxane}$ 283 mμ, $\Delta\varepsilon$ −1,87; spectre de n.m.r. (tétrachlorure de carbone) : δ 1,30 (6 protons), δ 1,43 (3 protons), δ 1,50 (3 protons), δ 2,08 (3 protons), δ 4,20 (multiplet, 2 protons), δ 4,50 (triplet, 2 protons), δ 4,77 (1 proton), δ 4,90 (1 proton), δ 5,47, 5,55 (1 proton, doublet, J 5 Hz).

Anal. Calc. pour $C_{15}H_{22}O_8$: C, 54,54; H, 6,71. Trouvé : C, 54,68; H, 6,88.

1,2:3,4-Di-O-isopropylidène-α-D-galacturonate de méthyle. — En répétant le procédé décrit[7], on a obtenu d'emblée un produit cristallisé, lavé par trituration dans l'éther de pétrole, p.f. 54–8°, temps de rétention en g.l.c. (184°, 720 mm) : 2 min 30 sec; spectre de n.m.r. : δ 1,31 (6 protons), δ 1,41 (3 protons), δ 1,50 (3 protons), δ 3,75 (3 protons), δ 4,28 (quadruplet, 2 protons), δ 4,53 (triplet, 2 protons), δ 5,50, 5,58 (doublet, J 5 Hz., 1 proton).

Le spectre de masse semble tout à fait comparable à celui du 1,2:3,4-di-*O*-isopropylidène-α-D-galactose[8] et nous le présentons avec la même classification des fragments : A 273 (M-15) (pic de base); B 215 (M-15-58); C 213 (M-15-60); D 155 (M-15-60-58); E 113; F_1 100; F_2 85; G 127; 59 ($CH_3C{=}O^+HCH_3$); 43 (Ac).

Anal. Calc. pour $C_{13}H_{20}O_7$: C, 54,16; H, 6,99; O, 38,85. Trouvé : C, 54,33; H, 7,00; O, 38,48.

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MARIE-ODILE POPOT

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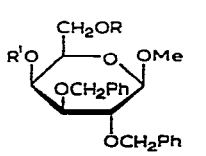
Carbohyd. Res., 5 (1967) 234–239

Preliminary communications

The solvolysis of methyl 2,3-di-*O*-benzyl-6-*O*-methanesulphonyl-*β*-D-galactopyranoside: participation by a benzyloxy group

In connection with work on sulphonate migrations, we have examined the solvolysis of methyl 2,3-di-*O*-benzyl-6-*O*-methanesulphonyl-*β*-D-galactopyranoside* {**1**, m.p. 88–89°, $[\alpha]_D$ +4 ±1° (*c* 2.0, chloroform)} and the isomeric 4-methanesulphonate* {**2**, m.p. 78–80°, $[\alpha]_D$ +38° (*c*, 1.0, chloroform)} under various conditions. It was noticed that sulphonate **1** was transformed in boiling 50% aqueous methanol into at least three products, and that the solution became distinctly acidic. When sulphonate **1** was heated in aqueous methanol buffered with sodium acetate, almost complete conversion into a single product (*A*) occurred within 17 h; sulphonate **2** was unaffected by these conditions.

The crystalline product *A* (*ca.* 50% yield), m.p. 68–70°, $[\alpha]_D$ −16° (*c* 0.9, chloroform), had a molecular weight and elemental analyses corresponding to the formula $C_{14}H_{18}O_5$. Its n.m.r. spectrum (measured in deuteriochloroform) showed the presence of only one benzyloxy group and confirmed the loss of the sulphonic ester group. Removal of the remaining benzyl group by catalytic hydrogenolysis gave methyl 3,6-



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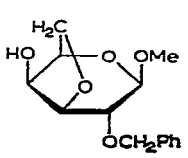

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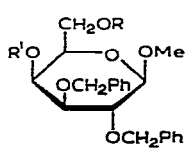

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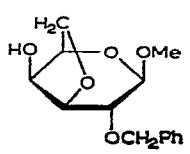

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anhydro-β-D-galactopyranoside[1], m.p. 116–117°, identified by comparison with an authentic specimen. On this evidence, the product of the solvolysis can reasonably be identified as methyl 3,6-anhydro-2-*O*-benzyl-β-D-galactopyranoside (3).

The facile conversion **1**→**3** under these conditions points to neighbouring-group participation by the benzyloxy group at C-3 in the solvolysis of the sulphonic ester*. Although benzyl ethers[3] have found extensive use as protecting groups in the carbohydrate field, in only one other instance, so far as we are aware, has participation by a benzyloxy group been observed. Thus, the benzyloxy group was found[4] to render powerful anchimeric assistance in the solvolysis of a series of 2,3,4-tri-*O*-benzyl-1,5-di-*O*-toluene-*p*-sulphonylpentitols, and the products are 1,4-anhydro derivatives. On the other hand, it has been noted[5] that the benzyloxy group provides little or no assistance in the reaction of methyl 2-*O*-benzyl-5-deoxy-3-*O*-methanesulphonyl-α-D-xylofuranoside with sodium benzoate in boiling *N*,*N*-dimethylformamide, whereas a benzoyloxy group at C-2 is highly effective.

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*We are informed[2] that methyl 6-*O*-toluene-*p*-sulphonyl-β-D-galactopyranoside solvolyses slowly ($t_{\frac{1}{2}}$ *ca.* 16 h) in aqueous solution at 50° to give a 3,6-anhydro derivative.

Cleavage of oligosaccharide glycosides by means of dihalomethyl methyl ethers: a novel preparation of rutinose

The isolation of oligosaccharides from glycosides is of considerable importance as a method of preparation of these compounds, and in determining the structure of the sugar components of natural products.

Selective cleavage of the linkage between an oligosaccharide and an aglycon group has been effected by enzymes, by partial hydrolysis or acetolysis, and by reduction or hydrogenolysis[1]. Another useful method is ozonolysis, which affords oligosaccharides without affecting the linkages between the monosaccharide units[2]. Hydrogen bromide in glacial acetic acid was also recommended by Zemplén *et al.*[3] for the cleavage of acetylated glycosides.

This paper describes a new process that appears to be of general applicability for the selective cleavage of the glycosidic linkage of oligosaccharide glycosides.

Gross and Farkas[4] were the first to report the conversion of methyl β-D-glucopyranoside tetraacetate into tetra-*O*-acetyl-α-D-glucopyranosyl chloride by means of dichloromethyl methyl ether. It has now been found that a similar reaction may be effected with aliphatic or aromatic glycosides of disaccharides. Treatment with dichloro- or dibromo-methyl methyl ether in the presence of zinc chloride affords the sugar component as the *O*-acetylglycosyl halide. By this method, the heptaacetates of methyl β-cellobioside and phenyl β-cellobioside gave hepta-*O*-acetyl-α-cellobiosyl chloride in yields of 82 and 49%, respectively.

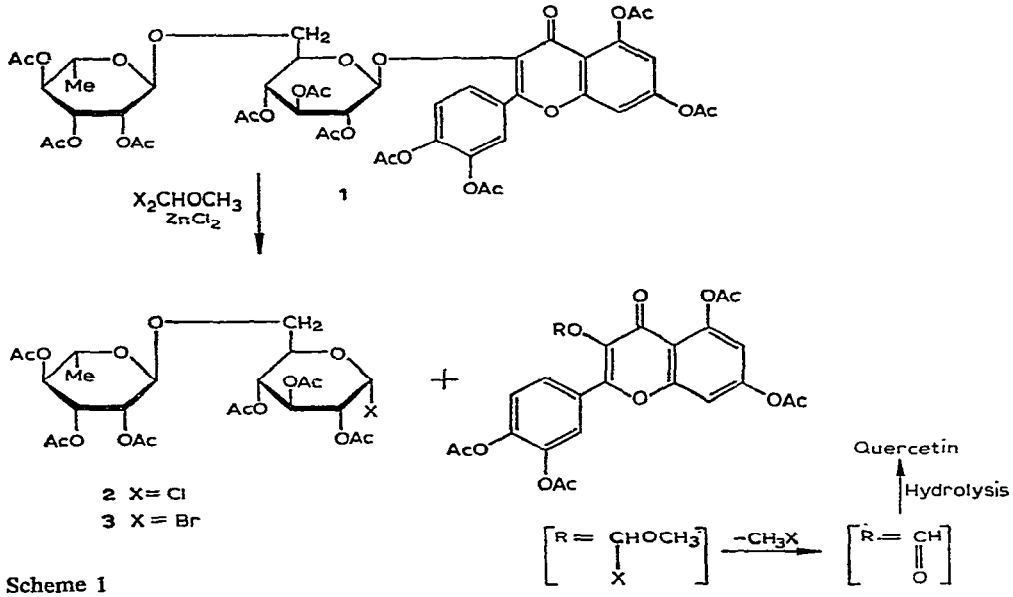
An example of the application of the method to flavonol glycosides is the cleavage of rutin to give rutinose, (6-*O*-α-L-rhamnopyranosyl-D-glucose), a disaccharide of frequent occurrence in natural products. Treatment of a solution of rutin decaacetate (**1**, 5g) in chloroform (20 ml) for 2 h at 75° with dichloromethyl methyl ether (5 ml) and zinc chloride (1 g) gave hexa-*O*-acetyl-α-rutinosyl chloride (**2**, 82%), m.p. 149°, $[\alpha]_D$ +68.2° (*c* 1.0, chloroform); lit.[5], m.p. 150–150.5°, $[\alpha]_D$ +66.5° in chloroform (Found: Cl, 6.03. $C_{24}H_{33}ClO_{15}$ calc.: Cl, 5.94%).

The reaction was also performed with dibromomethyl methyl ether[6]. Thus, rutin acetate (**1**, 1 g) in chloroform (10 ml) was heated at 75° with dibromomethyl methyl ether (1 ml) and anhydrous zinc chloride (0.4 g) to give chromatographically homogeneous hexa-*O*-acetyl-α-rutinosyl bromide (**3**) (52–57%), m.p. 127–128° (from ether), $[\alpha]_D$ +91–94° (*c* 0.5, chloroform); lit.[7], m.p. 127–128°, $[\alpha]_D$ +89.9° in chloroform (Found: Br, 12.39. $C_{24}H_{33}BrO_{15}$ calc.: Br, 12.46%).

The *O*-acetylrutinosyl halides were converted into hepta-*O*-acetyl-β-rutinose in the usual way[8].

The primary, aglycon product of the cleavage reactions was not homogeneous. On acid hydrolysis, however, the free aglycon, quercetin (65–70%), was formed and, with acetic anhydride in pyridine, yielded quercetin pentaacetate (38–45%). A possible, reaction pathway is shown in Scheme 1. The specific attack at the link between the

aglycon group and the disaccharide needs further investigation; experiments to elucidate the details are in progress.



Scheme 1

It should be pointed out that rutinose is a disaccharide of widespread occurrence in the vegetable kingdom and is mainly found as a component of flavonoid glycosides. The method described in this paper is therefore of interest, since it utilises natural rutin, an industrial product, and provides a much more convenient route to rutinose or *O*-acetylrutinosyl halides than does a multistage synthesis of the disaccharide.

The authors are grateful to the Hungarian Academy of Sciences for support of this work.

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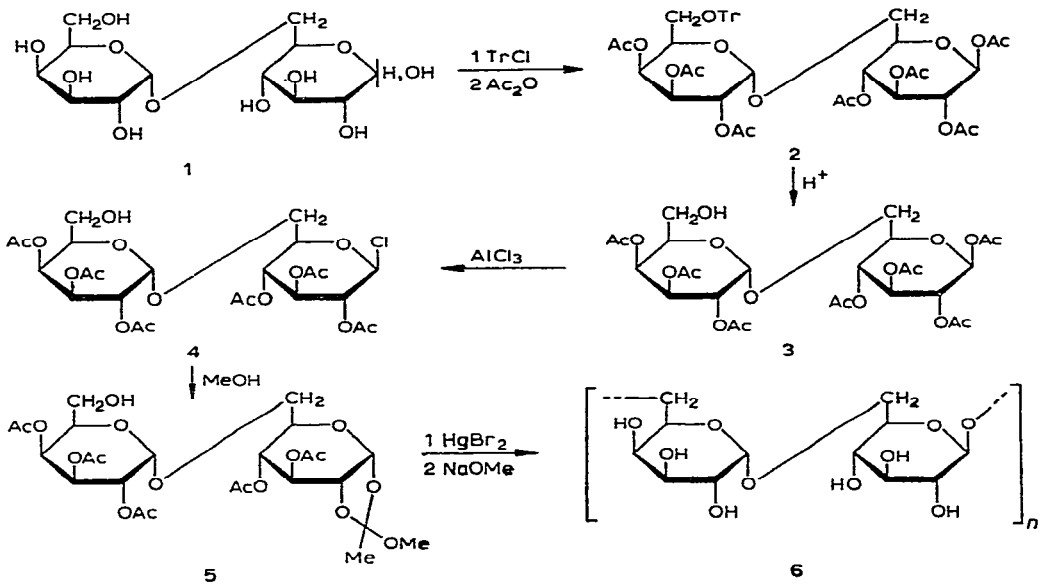
Synthesis of a galacto-glucan of regular structure*

In spite of the significant amount of work that has been carried out on the chemical synthesis of polysaccharides[1–3], until recently, a general method of synthesis, giving polysaccharides of predicted and definite structure, was not available. Based on the orthoester method of glycosylation[4], we suggested[5,6] a method of synthesis of regular homopolysaccharides by the polymerisation of tricyclic orthoesters of sugars. In the present work, we describe the first synthesis of a regular heteropolysaccharide by the orthoester method, involving the polycondensation of a disaccharide orthoester that contains a free hydroxyl group of known location.

The starting orthoester (**5**) was synthesized in the following way. Tritylation and subsequent acetylation of melibiose (**1**) gave a high yield of hepta-*O*-acetyl-6-*O*-trityl-β-melibiose (**2**), m.p. 165–7°, $[\alpha]_D$ +55° (chloroform). Detritylation of **2**, either



*This work was presented in the Symposium on the Chemistry of Natural Compounds held at Stockholm in June, 1966.

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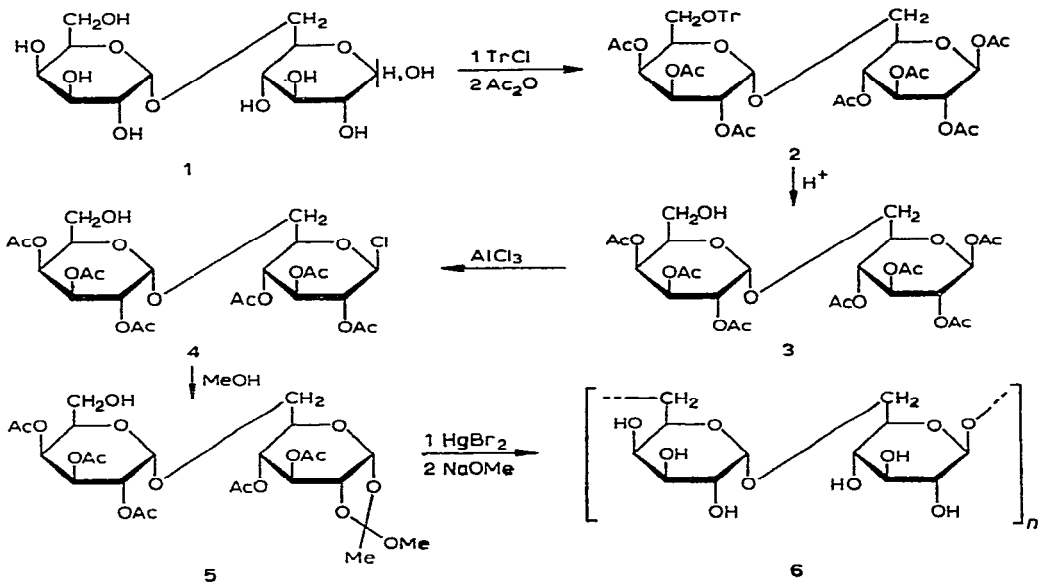

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by the action of hydrogen bromide in ether or of aqueous acetic acid, gave the heptaacetate (3), m.p. 204–7°, $[\alpha]_D$ +110° (chloroform). Treatment of 3 with aluminium chloride in absolute chloroform gave the β-chloride 4, which, without isolation, was treated with methanol in the presence of 2,6-dimethylpyridine, leading to the desired orthoester (5), $[\alpha]_D$ +118° (chloroform).

Polycondensation of orthoester 5 under the standard conditions of glycosylation by orthoesters[4] (but during a longer period), followed by saponification with sodium methoxide and chromatography on Sephadex G-25, gave a polysaccharide fraction that was free of lower oligosaccharides. Final purification by precipitation from water with ethanol yielded the polysaccharide (6), which we call galacto-glucan S-1 (S stands for synthetic preparation) and the structure of which follows from the following data. By complete hydrolysis of 6, D-glucose and D-galactose, in the ratio of 1:1, were obtained. The polysaccharide consumed 1.97 mol. of periodate and gave 0.99 mol. of formic acid per anhydrohexose unit. Only traces of glucose (<1%), galactose (<2%), and threitol (<1%) were detected in the products of periodate oxidation followed by reduction and hydrolysis. The specific rotation of the polysaccharide {$[\alpha]_D$ +112° (water)} agrees with the value expected, from Klyne's rule[7], for structure 6. It is therefore concluded that galacto-glucan S-1 is a regular heteropolysaccharide consisting of alternating, (1→6)-linked residues of β-D-glucopyranose and α-D-galactopyranose.

It is probable that the orthoester method will be of general applicability for the synthesis of other, regular heteropolysaccharides.

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THE POLYCONDENSATION OF ALDOHEXOSES AND RELATED DERIVATIVES

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INTRODUCTION

The monosaccharides undergo intermolecular and intramolecular reactions when heated, forming polymers and anhydro sugars, and the conditions that favor one or the other of these reactions have been studied[1-4]. Mora and co-workers[1,5] have reported methods for acid-catalyzed polycondensation of aldohexoses, together with data on the characterization of the resulting polysaccharides. In their work, reactions were taken to a solidification point, and the temperature at which the sugars were heated differed appreciably from one sugar to another. Consequently, no correlation of the structure of the monomer and of the end product could be made. Micheel *et al.*[6] also studied the acid-catalyzed polycondensation of aldohexoses; although they compared the behavior of aldohexoses under the same conditions of temperature, significant differences in the products were not observed, presumably because the reaction was conducted for an extended period of time. Thus, the molecular weights of their polyglucose, polymannose, and polygalactose (formed under similar conditions) were 24,000, 24,400, and 24,100, respectively.

The purpose of the present investigation was comparison of the behavior of the anomers of D-glucose, D-mannose, and D-galactose when heated (in the absence of catalyst and solvent) under identical conditions, to determine how variation in the structure of the aldohexose influences the intermolecular and intramolecular reactions. By differential thermal analysis, Morita[7] had found slight differences in behavior between the anomers of D-glucose; alumina was mixed with the sample, and had a marked effect on the course of the reaction, but no attempt was made to interpret the differences observed. It has now been found that differences in the loss in weight associated with the intermolecular and intramolecular reactions of the three monosaccharides may be observed directly with a thermobalance. The scanning that it provides indicated immediately that differences in the condensation products may be readily discerned, provided that the analyses are performed for the initial stages of the reaction. The end products were characterized by paper chromatography, reducing values, polarimetry, and end-group determinations. Chromatographic analysis showed

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the presence of large molecules in the reaction mixture, and gave a quantitative measure of the amount of unreacted monosaccharide. The number-average molecular weight of each reaction mixture was determined from the reducing value.

EXPERIMENTAL

Equipment. — The thermogravimetric measurements were made with a vacuum, semimicro, automatic, recording balance (Ainsworth Model RV-AU-I) attached to a vacuum system (0.1 μm of Hg). The furnace was a Pyrex vessel (positioned below the balance) which was heated with insulated Briskeat heating-tape wrapped around it. Thermal insulation of this furnace was achieved with two layers of glass wool, separated by aluminum foil, placed around the heating tape. Further to lessen the heat losses resulting from radiation, the outside of the furnace was enclosed in aluminum foil.

A thermocouple for measuring and monitoring the temperature of the furnace was connected either to a pyrometric, proportion controller (Gardsman Model JP) for single temperatures, or to a pyrometric, stepless controller (Gardsman Model JSG-2) for linear increase of the temperature. Components for both programmers were obtained from the West Instrument Co., Chicago, Illinois. The sample was placed in a Coors porcelain crucible (O_5), which was then placed in the furnace.

Paper chromatography was conducted in a thermally insulated cabinet.

Materials. — Anhydrous D-glucose (reagent grade) was obtained from Fisher Scientific Co., Fairlawn, New Jersey, and the Mallinkrodt Chemical Co., New York, New York; methyl α-D-mannopyranoside (**1**), D-galactose, and D-mannose from the Nutritional Biochemicals Corp., Cleveland, Ohio; and 4,6-*O*-ethylidene-α-D-glucopyranose (**2**) from the California Biochemical Research Corp., Los Angeles, California. Methyl α-D-glucopyranoside (**3**) was kindly supplied by Dr. G. N. Bollenback of Corn Products Refining Co. These derivatives were found to be pure enough for use without further treatment.

The procedure of Hudson and Dale[8] was used for preparation of the anomers of D-glucose from anhydrous dextrose. Each anomer was recrystallized twice from glacial acetic acid. To remove traces of acid, the β-D anomer (**4**) was then recrystallized twice from cold absolute ethanol; the α-D anomer (**5**) was washed with cold, 95% ethanol.

β-D-Galactose (**6**) was prepared from a mixture of the anomers of D-galactose by the method of Hudson and Yanovsky[9]. The β-D anomer was precipitated from water by addition of absolute ethanol at 0°. Judging from measurements of the optical rotation, four fractional precipitations were required before the β-D-galactose was obtained in pure form. Pure α-D-galactose (**7**) was obtained by allowing it to crystallize slowly from aqueous ethanol during a few days; two such recrystallizations were required.

The method of Hudson and Sawyer[10] was used for preparing the anomers of D-mannose. Seed crystals of β-D-mannose were supplied by Dr. N. K. Richtmyer of

the National Institutes of Health, Bethesda, Md. Polarimetry, determinations of melting point, and paper-chromatographic analysis were used to ascertain the purity of the anomers; these showed that the "β-D-mannose" (**8**) consisted of 90% of the β- and 10% of the α-D anomer; the "α-D-mannose" (**9**) consisted of 90% of the α- and 10% of the β-D anomer. Repeated recrystallizations did not appreciably alter these compositions. Paper-chromatographic analysis showed the absence of impurities, and pH measurements revealed that no trace of acetic acid (used in the preparation of the anomers) remained.

Heating of the monosaccharides. — Method A. The initial weight of the sample was 100 mg. The furnace was completely flooded with argon before heating of the sample was started. The temperature was raised at a uniform rate of 6.5°/min, under a continuous flow of argon at the rate of 0.08 ft^3 /h.

Method B. A 500-mg sample was used, and the temperature was kept constant. As the result of exploratory work, the temperature of 176° was selected, and the experiments were conducted *in vacuo*.

Paper chromatography. — Butyl alcohol–pyridine–water (6.4:3 v/v)[11] was used in descending, paper-chromatographic analysis of the reaction products on Whatman No. 1 filter paper. The developing reagent[12] of silver nitrate–sodium hydroxide developed not only the reducing sugars but also the nonreducing sugars. By modifying the developing technique, the intensity of the developed spots was increased, so that trace amounts of nonreducing sugars in the reaction mixtures could be observed. The amount of silver nitrate deposited on the paper was twice that used by Trevelyan *et al.*[12], and heat was applied to the wet chromatogram bearing the developer; this resulted in the immediate appearance of the spots corresponding to the nonreducing sugars. The spots could be readily discerned, and the intensity of the spots estimated, if the chromatograms were examined immediately after having been washed with 6N ammonia.

Tailing of the spots could be lessened by allowing them to move in a direction at right angles to the machining direction of the paper. The chromatogram was equilibrated in the chromatocabinet in the presence of the solvent system for 24 h. The solvent was then added to the chromatogram. It was found that, to effect a good separation of the substances in the reaction mixtures, 44 h was required. Because the solvent front moved off the paper, R_G values were used instead of R_F values. So that spots could be identified, known compounds were run on the same chromatograms as the reaction mixtures.

The procedure of Dimler *et al.*[13] was used for determining the amount of monomer in a reaction mixture. The concentration of the sugar in the eluate was determined colorimetrically by the modified anthrone method[14]. If the concentration of sugar was kept below 45 μg/ml, a linear relationship existed between the intensity of the color and the concentration of the sugar. The intensity of color for the same concentration of sugar in the eluate was different for D-glucose, D-mannose, and D-galactose; therefore, a separate calibration curve was prepared for each.

End-group determination. — The colorimetric procedure of Nussenbaum and Hassid[15] was used for determining the end-group values of the polymers formed on heating the various monosaccharides. The number-average molecular weight of the polymers was calculated from the number of end groups in the reaction mixture.

RESULTS

Weight losses associated with the condensation of the monosaccharides. — The percent loss in weight associated with the condensation reactions of the anomers of D-glucose, D-mannose, and D-galactose is given in Table I. Anomers **6**, **8**, and **9** were

TABLE I

ANALYSIS OF CONDENSATION PRODUCTS

Compound heated	*Upper temp. limit, degrees*	*Loss in wt., %*	*Number-average mol. wt. of reaction products*[a]	*Unreacted monomer in reaction product (%)*
A. Heating rate[b] *6.5°/min*				
4	220	6.0		
5	220	6.0	580	13
8	220	8.0	940	13
9	220	9.6	1260	8.7
6	220	13.3	2680	6.0
7	220	8.7	3830, 4050	6.0
4	260	13.5	5230, 5450	5.6
5	260	12.9	5550	4.9
7	260	11.4	7210	3.6
B. Temperature[c] *maintained at 176°*				
	Time heated (min)		*Number-average mol. wt. of reaction products*[a]	
4	250		650	
5	250		630	
8	250		1260	
9	250		1500, 1620	
7	250		4150	
6	120		2220	
7	120		2620	

[a]Based on end-group analysis. [b]Under 1 atmosphere of argon. [c]At 1 μm of Hg.

not heated above 220°, because of the formation of water-insoluble products that complicated the analysis. Very little material, other than water, was distilled off at this temperature. When the monosaccharides were heated isothermally at 1 μm of Hg, a considerable amount of carbohydrate material distilled during the first 60 min.

Analysis of reaction products. — The results of the paper-chromatographic analysis of the reaction products from the experiments described in Table I are given in Table II; these indicate a wide variation in the molecular size of the products,

because the R_G values vary from 0.01 to 1.70. The R_G value for maltose is 0.65. The intensities of spots indicate that there is a considerable difference in the amounts of the various compounds in the reaction mixtures. Comparing the results in Columns A and B of Table II, it may be seen that heating of the monosaccharides above 220° results

TABLE II

CHROMATOGRAPHY OF CONDENSATION PRODUCTS[a]

Aldohexose heated	*R_G values*[b] *A*	*B*	*C*	*D*
β-D-Glucose (4)		1.7 [s] 1.0 [m] 0.52 [w] 0.39 [w] 0.27 [w[0.03 [s]		2.1 [vw] 1.0 [s] 0.55 [vw] 0.34 [w] 0.10 [s]
α-D-Glucose (5)	1.6 [m] 1.0 [vs] 0.73 [m] 0.51 [s] 0.36 [ms] 0.24 [ms] 0.04 [vs]	1.7 [s] 1.0 [m] 0.52 [m] 0.25 [m] 0.03 [s]		2.1 [vw] 1.0 [s] 0.54 [m] 0.31 [s] 0.10 [s]
β-D-Mannose (8)	1.6 [vw] 1.1 [s] 0.86 [w] 0.76 [w] 0.66 [w] 0.42 [m] 0.16 [s] 0.02 [vs]			1.2 [vs] 1.0 [w] 0.80 [w] 0.74 [s] 0.57 [s] 0.42 [ms] 0.32 [ms] 0.01 [s]
α-D-Mannose (9)	1.2 [s] 1.1 [vw] 0.90 [vw] 0.77 [vw] 0.65 [vw] 0.52 [vw] 0.19 [m] 0.04 [s]			1.2 [s] 1.0 [w] 0.70 [w] 0.58 [w] 0.44 [w] 0.34 [w] 0.02 [s]
β-D-Galactose (6)	1.3 [vw] 1.2 [vw] 0.85 [s] 0.74 [w] 0.63 [w] 0.52 [mw] 0.34 [m[0.10 [s]		0.86 [s] 0.49 [w] 0.39 [w] 0.23 [w] 0.02 [w]	
α-D-Galactose (7)	0.86 [s] 0.63 [vw] 0.51 [w] 0.32 [w] 0.05 [s]	1.7 [ms] 0.89 [w] 0.04 [s]	0.86 [s] 0.48 [vw] 0.36 [vw] 0.23 [vw] 0.01 [s]	0.86 [m] 0.48 [vw] 0.38 [vw] 0.20 [vw] 0.05 [s]

TABLE II *(cont.)*

R_G *values of standards*	
D-Glucose	1.0
D-Mannose	1.1, 1.2
D-Galactose	0.88
Cellobiose	0.65
Maltose	0.65
Melibiose	0.43, 0.44

[a]Key: A = sample heated to 220° at 6.5° min^{-1}; B = sample heated to 260° at 6.5° min^{-1}; C = sample heated isothermally at 176° for 120 min; D = sample heated isothermally at 176° for 250 min. [b]Intensity of spots: m, moderate; s, strong; v, very; w, weak.

in an increase in molecular size of the products, this increase occurring at the expense of molecules of both the intermediate and monomeric size.

The concentration of the compounds having R_G values of about 1.7 increases when the monosaccharides are heated to 260°, as shown by the results for **5** and **7**. The product from heating of **5** to 220° gives a spot having R_G 1.7, but it is of lower intensity. No spot having R_G 1.7 was produced from **7**.

When the monosaccharides are heated at 176°/1μm of Hg, more than 60% of the reaction mixture is distilled off during the first 60 min of heating. Chromatographic analysis and polarimetry show that this distillate consists mainly of unreacted monosaccharides. Compound **6** was heated for only 2 h, because a gel is formed when it is heated for longer periods of time.

The data for the chromatographic analysis of the residues resulting from isothermal heating at 176° of the monosaccharides is presented in columns C and D of Table II; they indicate that the residues are a complex mixture of molecules of various sizes. The R_G values of the spots for various components in the reaction mixtures range from 0.01 to 2.1. The reaction products do not contain compounds having R_G values of 1.7, indicating the absence of anhydro sugar*.

Effect, on the condensation reaction, of protecting various hydroxyl groups in a monosaccharide. — To ascertain the positions of those hydroxyl groups that are essential for the intermolecular condensation, the thermal behavior of a series of derivatives was investigated. Compounds **1**, **2**, **3**, and **5** were each heated nonisothermally under argon at a heating rate of 6.5°/min.

The thermogravimetric curves of these derivatives are shown in Fig. 1, from which it is apparent that, above 200°, **5** is more reactive than the derivatives. When the loss of weight at 220° is used as the basis of comparison, the curve representing **1** shows the lowest loss in weight, followed by **3**, **2**, and **5**. Compounds **1** and **3** have rather similar thermogravimetric curves. Compound **2** was heated to only 220°; above

*The spot of R_G 1.7 may be associated with the anhydro sugars in accordance with the work of Peat *et al.*[16], who showed that the R_F value of 1,6-anhydro-D-glucose is almost twice that of D-glucose.

this temperature, a considerable amount of water-insoluble material is formed, and charring occurs.

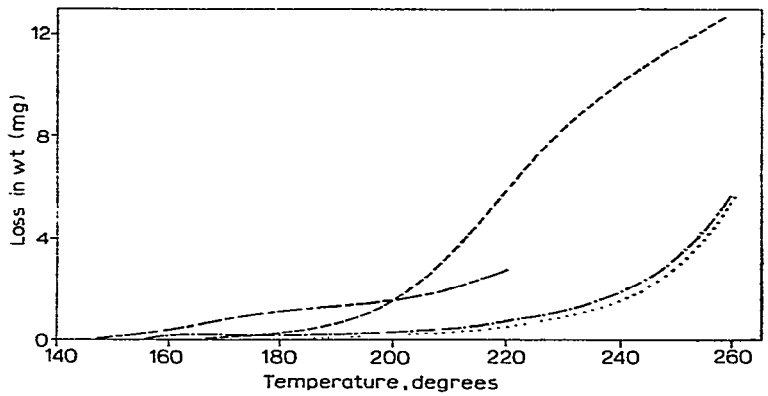

Fig. 1. Thermogravimetric behavior of derivatives of D-glucose and D-mannose (loss in weight *versus* temperature). Initial weight of samples, ·556 mM; the samples were heated at 6.5° min^{-1} in an atmosphere of argon. (. . . . ., methyl α-D-mannopyranoside;—·—·—·—·, methyl α-D-glucopyranoside; — — — — —, 4,6-*O*-ethylidene-α-D-glucopyranose; and ----------, α-D-glucose.)

The chromatographic results for the residues of the heat-treated derivatives of D-glucose and D-mannose are shown in Table III. The chromatographic results of the product obtained by heating **3** to 220° indicate that only the unreacted D-glucoside is present. Analysis of the products obtained by heating **2** shows the presence of the unreacted derivative and D-glucose, as indicated by two spots having R_G values of 2.0 and 1.0, respectively. The D-glucose amounted to 6% of the reaction mixture, as shown by eluting the D-glucose off the chromatogram and determining the quantity colorimetrically.

The results for **3** and **1** heated at 260° indicate that condensation occurs; this may readily be seen from the range of R_G (from 1.6 to 0.15).

The movement of a sugar on a paper chromatogram is a function not only of the molecular size but also of the number of free hydroxyl groups present in the molecule[17]. As is well known, protection of the hydroxyl groups increases the rate of migration. Comparison with standards shows that **3** and **1**, having one free hydroxyl group less than the corresponding aldohexoses, have R_G values of 1.3 and 1.5, respectively, and **2** (R_G 2.2), having two hydroxyl groups less than the corresponding aldohexose, travels more than twice the distance of D-glucose.

Relative reactivity of the three aldohexoses in intermolecular condensation. — The number-average molecular weight of the polymers of **4** and **5** are the same (see Table I), in agreement with the percent loss in weight associated with the condensation reactions of the anomers of D-glucose. An estimate of the reproducibility of these experiments (including heating of the monosaccharide, and colorimetric determination) is indicated by two number-average molecular weights determined for each sugar.

The molecular weights associated with the polymers may be divided into three groups. The largest polymers are obtained from the condensation reaction of D-galactose, followed by those of D-mannose and D-glucose in decreasing order. This order of reactivity of the aldoses is corroborated by the percentage of unreacted hexose (see the last column in Table I). From Table I, it may be seen that a second-order effect is operative. The number-average molecular weights of the polymers formed by heating **7** and **9** are larger than those of the polymers obtained by heating **6** and **8**.

TABLE III

CHROMATOGRAPHIC RESULTS ON THE RESIDUES FROM THE HEAT-TREATED DERIVATIVES

Compound heated		*Upper limit of temperature, degrees*	R_G *values*	
Methyl α-D-glucopyranoside	(3)	220	1.4	[vs]
4,6-*O*-Ethylidene-α-D-glucopyranose	(2)	220	2.0	[vs]
			1.0	[s]
Methyl α-D-glucopyranoside	(3)	260	1.6	[s]
			1.3	[vs]
			1.0	[m]
			0.86	[m]
			0.73	[s]
			0.50	[m]
			0.39	[ms]
			0.15	[ms]
Methyl α-D-mannopyranoside	(1)	260	1.5	[vs]
			1.0	[m]
			0.94	[vs]
			0.77	[ms]
			0.53	[m]
			0.41	[m]
			0.30	[mw]
Standards				
Methyl α-D-glucopyranoside	(3)		1.3	
Methyl α-D-mannopyranoside	(1)		1.5	
4,6-*O*-Ethylidene-α-D-glucopyranose	(2)		2.2	
D-Glucose			1.0	
D-Mannose			1.1	
Cellobiose			0.64	
Melibiose			0.44	

When each aldohexose is heated to 260°, the number-average molecular weight of the polymers is increased markedly (see Table I), in general agreement with the results of Mora and co-workers[5] and Micheel[6]. However, there is a decrease in the differences between the molecular weights of the polymers from **7** and the anomers of D-glucose, **4** and **5**.

Higher temperatures are necessary for the intramolecular than for the inter-

molecular reaction, as is shown by the data in Table II; these indicate that significant proportions of anhydro sugars are formed at 260° (R_G 1.7). In contrast, little or no anhydro sugar is formed in the reaction mixtures heated to 220°, or isothermally at 176°.

DISCUSSION

The thermal behavior[18] of the derivatives of the three aldohexoses indicates that the hydroxyl group on C-1 of the pyranose is essential for intermolecular condensation at temperatures below 220°. Fig. 1 shows that heating of **3** and **1** to 220° results in little loss of weight. Chromatographic analysis of the reaction products of **3** indicated the presence of unreacted starting material only. Replacement of the hydroxyl group at C-1 by a methoxyl group does not inhibit the condensation above 220°. The loss in weight shown in Fig. 1, and the variety of components having R_G values less than 1.0 in the reaction products from the glycosides heated to 260°, indicate the occurrence of intermolecular condensation.

Two possible explanations are suggested for this behavior. One is that heating above 220° is sufficiently drastic to cause hydroxyl groups other than those on C-1 to participate in the condensation reaction; this does not seem likely, as D-glucitol does not undergo condensation when heated to 260°. The other possibility is that the linkage between the methoxyl group and C-1 is broken. In support of this mechanism is the chromatographic analysis (see Table III), which reveals spots having R_G values (1.0) sufficiently close to that of D-glucose and D-mannose to suggest the presence of aldohexose in the reaction mixture. These monosaccharides could be formed, after removal of the methoxyl group, by the addition of a hydroxyl group from the water formed as a result of intermolecular condensation.

The thermal behavior of **2** indicates that the intermolecular condensation of D-glucose heated to 220° involves primarily[3,4] the hydroxyl groups on C-1 and C-6. The 1,6-anhydro linkage has also been found to preponderate in the structure of the polyglucose from the acid-catalyzed, intermolecular condensation of D-glucose[19]. Chromatographic analysis of the products obtained by heating **2** to 220° indicated that this derivative does not polymerize. As the hydroxyl group on C-4 of D-glucose has been alleged[20] to exhibit essentially the same reactivity as those on C-2 and C-3, the absence of the hydroxyl group on C-4 in **2** should not influence its condensation reaction. Apparently, the hydroxyl group on C-6 is critical for occurrence of the intermolecular condensation.

The position of axial hydroxyl groups on the pyranose ring of the aldohexopyranoses appears to influence their intermolecular condensability. If it is assumed that the aldohexoses investigated exist in the *C1* (D) conformation[21], it appears that the axial hydroxyl group on C-4 of D-galactopyranose influences the intramolecular condensation reaction to a greater extent than the axial hydroxyl group on C-2 of D-mannopyranose. The D-glucose configuration, in which the hydroxyl groups on C-2, C-3, and C-4 of the *C1* (D) conformation are equatorial, appears to be the least

reactive. The difference in reactivity of the anomers of D-galactose and D-mannose may be due to the fact that the α-D anomers contain an additional axial hydroxyl group (on C-1).

Recently, investigators[22] studying D-glucose near its melting point observed no differences in the behavior of the anomers, and found that mutarotation occurs more rapidly than condensation. In the present study, it has been shown that heating of aldohexoses to higher temperatures, that is from 176° to 220°, reveals differences in behavior attributable to the anomers of the sugar.

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SUMMARY

The condensation reactions of the anomers of D-glucose, D-mannose, and D-galactose, as well as of certain of their derivatives, have been investigated in the molten state in the absence of catalysts. The reaction was followed thermogravimetrically, the products being analyzed by means of chromatography, polarimetry, and end-group determinations.

The loss in weight associated with the condensation reactions was found to be different for each of the monosaccharides heated under a variety of conditions, provided that analyses were performed well before completion of the reaction. At temperatures below 220°, intermolecular condensation predominated; above 220°, intramolecular condensation became more apparent. Study of the behavior of derivatives indicated that the hydroxyl group on C-1 is essential to condensation. As already known, for D-glucose, the intermolecular condensation reaction occurred primarily between C-1 and C-6. The extent of polymerization under standardized conditions was greatest for the D-galactoses, followed by the D-mannoses; the D-glucoses yielded the smallest polymers. It was also observed that a higher degree of polymerization is obtained for the α-D anomers of D-mannose and D-galactose than for the β-D anomers. A possible explanation of this behavior is given on the basis of the conformations of the aldohexoses employed.

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THE GUM EXUDATE OF *Encephalartos longifolius* LEHM. (FEMALE) (FAMILY CYCADACEAE)

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INTRODUCTION

The Cycads constitute a family of great interest to botanists on account of their having remained relatively unchanged morphologically since the Mesozoic age. The chemistry of components of Cycads has been reviewed[1]. A preliminary communication[2] described some properties of the gum found in radial canals and on the bark of stems of a female specimen of *Encephalartos longifolius* Lehm. (order Cycadales, family Cycadaceae), including the nature of the sugar residues of this complex polysaccharide material, and the results of a g.l.c. assay of the methanolysis products of the methylated gum. This study has been extended by examining in detail the acidic components of the polysaccharide produced on graded hydrolysis.

EXPERIMENTAL

General experimental conditions. — Paper (Whatman No. 1) chromatography of sugars and their methyl ethers was performed by using solvent systems chosen from the following (all v/v): (*a*) butyl alcohol–ethanol–water (4:1:5, upper layer), (*b*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), (*c*) ethyl acetate–pyridine–water (10:4:3), (*d*) butyl alcohol–acetic acid–water (2:1:1). Thin-layer chromatography (t.l.c.) of methylated sugars, methyl glycosides, and glycitols was performed on Kieselgel G (E. Merck, Darmstadt, Germany) with chloroform–methanol (5:1, unless otherwise stated), and detection by 2N sulphuric acid at 110°. Paper ionophoresis was for 3 h at 10 volts/cm in 0.1M borate buffer (pH 9.2). R_{Gal} and M_G refer to rates of movement relative to galactose and glucose, respectively. Sugars were detected on the paper sheets with *p*-anisidine hydrochloride or triphenyltetrazolium hydroxide. Analytical gas–liquid chromatography (g.l.c.) was carried out with a Beckman GC-2A instrument, and helium as carrier gas. For glycosides of methylated sugars, a 3-foot, 0.25-in o.d., copper column of 14% poly(ethylene glycol succinate) on 80–100 mesh Chromosorb W at 155° was used with a flame-ionisation detector; retention times (T) are relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside[3]. For glycitol acetates, the column packing was 3% ECNSS-M on 100–120 mesh Gas Chrom Q at 190°. Identification was achieved by addition of authentic, standard substances[4].

Solutions were concentrated at 35–40°/20 mm in rotary evaporators. Methyl-

ation was effected by the Haworth and Purdie procedures. Melting points (uncorrected) were determined with the Fisher–Johns apparatus (Fisher Scientific Company, U.S.A.). Specific rotations (unless otherwise stated) are equilibrium values for aqueous solutions at *ca.* 25°. Infrared spectra were measured with a Perkin–Elmer Infracord spectrometer. X-ray powder diffraction data, obtained by using a Philips model P.W. 1050 diffractometer, give interplanar spacings (Å) for Cu Kα radiation (peak intensities: vs, very strong; s, strong; m, moderate; w, weak; vw, very weak).

Acid hydrolysis (stage one) of the polysaccharide from E. longifolius. — The polysaccharide sample[2] had $[\alpha]_D$ −28° and equiv. wt. 600. The air-dried polysaccharide, after being reprecipitated with ethanol from aqueous solution, had N, 0.6; ash, 2.8; OMe, 2%. After hydrolysis (0.5N sulphuric acid for 11 h at 96°) of the polysaccharide (8.4 g), the neutralised ($BaCO_3$), syrupy product (7.0 g) was transferred to a column (98 × 4.5 cm) of powdered cellulose (kept at 30° by means of a water jacket) and eluted with water-saturated mixtures of light petroleum (b.p. 100–120°) and butyl alcohol, wet butyl alcohol, and finally, after elution of neutral sugars, with water. Fractions were collected, and monitored by paper chromatography (solvents *a*, *b*, and *c*). Combinations of fractions that contained similar components gave nine fractions, which were characterised as follows. All paper-chromatographic comparisons with known compounds were in solvents *a*, *b*, and *c*.

Fraction I. Eluted with light petroleum–butyl alcohol (7:40), this fraction (142 mg) had m.p. 112° (from water), $[\alpha]_D$ +33° (*c* 3.15), and was chromatographically and ionophoretically identical with 3-*O*-methyl-L-rhamnose. Admixture with an authentic specimen did not depress the m.p. The i.r. spectrum and X-ray powder diffractogram were identical with those of 3-*O*-methyl-L-rhamnose. The *d*-spacings found were: 13.8w, 10.2m, 7.22s, 6.17s, 5.15s, 4.82m, 4.47s, 4.11m, 3.67m, 3.59m, 3.26w, 3.17s, 3.12m, 2.96w, 2.81w, 2.61w, 2.48w, 2.38w, 2.23m, 2.10w, 2.02vw, 1.97vw, 1.93vw, 1.88vw. De-*O*-methylation of the sugar with hydrobromic acid gave rhamnose (paper chromatography), and borohydride reduction afforded 3-*O*-methyl-L-rhamnitol, which was identical (t.l.c.) with an authentic specimen and gave formaldehyde on periodate oxidation.

Fraction II. L-Rhamnose hydrate (666 mg), m.p. 92–94° (from aqueous methanol), $[\alpha]_D$ +6.6° (*c* 3.3), chromatographically homogeneous and identical with an authentic specimen; this sugar was characterised as the phenylosazone, m.p. and mixed m.p. 176–178° (decomp.).

Fraction III. Two components, chromatographically similar to rhamnose and fucose, were present in this fraction (17 mg), which had $[\alpha]_D$ −38° (*c* 0.42) indicative of L-rhamnose and L-fucose in the approximate ratio of 3:4 by weight.

Fraction IV. D-Xylose (147 mg), m.p. 144–145°, $[\alpha]_D$ +19° (*c* 3.3), chromatographically identical with an authentic specimen. The derived di-*O*-benzylidene dimethyl acetal had m.p. and mixed m.p. 210–210.5°.

Fraction V. Two components, chromatographically similar to xylose and arabinose; yield, 141 mg; $[\alpha]_D$ +79° (*c* 3.1), corresponding to D-xylose and L-arabinose in the ratio of *ca.* 1:2 by weight.

Fraction VI. L-Arabinose (242 mg), $[\alpha]_D$ +104.5→97° (equil., *c* 2.4), containing about 10% by wt. of xylose. Recrystallisation from methanol gave the sugar, m.p. 156–156.5°, characterised as the benzoylhydrazone, m.p. and mixed m.p. 198–200° (decomp.).

Fraction VII. Two components, chromatographically similar to arabinose and mannose; yield, 112 mg; $[\alpha]_D$ +80° (*c* 2.24), corresponding to L-arabinose and D-mannose in the ratio of 8:3 by wt.

Fraction VIII. D-Galactose (367 mg), $[\alpha]_D$ +78.5° (*c* 1.5), chromatographically identical with an authentic specimen, and characterised as the 2-methyl-2-phenylhydrazone, m.p. and mixed m.p. 186–188.5°.

Fraction IX. Eluted with water, this fraction (3.50 g, as barium salts) contained acidic material that was slower-moving than galactose on paper chromatograms, the bulk travelling concurrently with 6-*O*-(barium β-D-glucopyranosyluronate)-D-galactose. In view of the presence of a considerable proportion of material having molecular weight higher than that of the biouronic acid, portions of Fraction IX were submitted to further acid hydrolysis as described below.

Details of the performance of the cellulose column and the yields of sugar components are summarised in Table I.

TABLE I

ACID HYDROLYSIS (STAGE ONE) OF THE POLYSACCHARIDE FROM *E. longifolius*

Combined fraction	*Sugar components*	*Fraction numbers*	*Yield (mg)*
I	3-*O*-Methyl-L-rhamnose	57–88	142
II	L-Rhamnose	131–178	666
III	L-Rhamnose + L-fucose	179–203	17
IV	D-Xylose	204–240	147
V	D-Xylose + L-arabinose	241–262	141
VI	L-Arabinose (+ D-xylose)	263–284	242
VII	L-Arabinose + D-mannose	285–348	112
VIII	D-Galactose	373–538	367
IX	Acidic residues (as Ba salts)	539–625	3500

Acid hydrolysis (stage two) of the polysaccharide from E. longifolius. — A portion (70 mg) of fraction IX was heated in 0.5N sulphuric acid at 100°, and samples were neutralised and chromatographed at 5, 6, 13, and 18 h; at 18 h, the slowest-moving material corresponded to 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. The bulk (3.34 g) of fraction IX was hydrolysed under the same conditions for 8 h, neutralised ($BaCO_3$), concentrated, and separated into neutral and acid components by successive treatments with the resins Amberlite IR-120 (H^+) and Duolite A4 (OH^-) followed by elution from the latter, first with water and then with N sodium hydroxide.

Neutral sugars. This fraction (0.39 g), $[\alpha]_D$ +65° (*c* 2.4), crystallised, and by paper chromatography was shown to contain galactose, mannose, arabinose, and

xylose (trace). A portion (0.12 g) of the mixture was reduced (aqueous sodium borohydride), acetylated (acetic anhydride and sodium acetate), and analysed[4] by g.l.c.; found, in terms of mol. of sugar present in the mixture subjected to reduction and acetylation: galactose 8, mannose 4, arabinose 2, and xylose 1. Galactitol hexaacetate, m.p. 168–9°, was recovered from the mixed acetates by recrystallisation from ethanol.

Acidic components. The neutralised, acidic fraction (2.88 g) was shown by paper chromatography to contain numerous components that were separated by transferring the bulk of this fraction (2.24 g) to a cellulose column (56 × 3 cm), eluting with butyl alcohol–acetic acid–water (3:1:1), and collecting fractions at 2-h intervals. As elution proceeded, the content of butyl alcohol in the solvent mixture was reduced to 2 parts and finally to 1 part; after fraction 225, elution was effected with aqueous ethanol. Fractions containing chromatographically similar components were combined to give the following fractions (the column performance and the nature of the separated products are summarised in Table II).

TABLE II

ACID HYDROLYSIS (STAGE TWO) OF THE POLYSACCHARIDE FROM *E. longifolius*. ACIDIC COMPONENTS

Combined fractions	*Fraction numbers*	*Yield*[a] *(mg)*	$[\alpha]_D$, *degrees*	R_{Gal}[b]			*Identity*[c]
1	24–29	30		1.2,	0.85	(1.7)	A+B
2	30–32	13		1.2,	0.85	(1.7)	A+B
3	33–41	100	+15	0.85,	(1.7),	1.2	B+A
4	42–48	124	+3	0.85,	0.6		B+C
5	49–54	145	−12	0.85,	0.6,	0.5	B+C+D
6	56–59	118	−29	0.6,	0.5		C+D
7	60–68	138	−29	0.5			D
8	70–118	800	−6	0.4			E
9	126–144	50	−15	0.25			F+trace of E
10	151–170	28	−3	0.21			G+trace of F
11	171–184	97	−22	0.15			H+trace of G
12	185–210	31	−4	0.15			H+I
13	211–224	100	−23	0.07			J
14	Washings	108	−14	<0.05			K

[a]As Ba salts, except for combined fractions 1 to 3.
[b]Solvent mixture *d*; R_{Gal} values fluctuate with age of solvent mixture and duration of run.
[c]A=4-*O*-methyl-D-glucuronic acid; B=D-glucuronic acid (D-glucurono-6,3-lactone); C= 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose; D= 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose; E=6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose; F=dimer of acid D; G=acid E linked to D-galactose; H=trimer of acid D; I=acid E linked to D-galactose; J=tetramer of acid D, plus acid E linked to D-galactose; K=mainly polymeric acid D. The identifications of F to K are tentative.

Fraction 1. The syrup (30 mg), $[\alpha]_D$ +25° (*c* 2.5), recovered in acid form, showed two components on paper chromatography and ionophoresis, corresponding in mobility to D-glucurono-6,3-lactone and 4-*O*-methyl-D-glucuronic acid. Treatment with boiling methanolic hydrogen chloride, reduction with aqueous borohydride, and

hydrolysis with aqueous acid gave two components having paper-chromatographic and -ionophoretic mobilities identical with those of D-glucose and 4-*O*-methyl-D-glucose (major component). De-*O*-methylation with hydrobromic acid (20 min at100°) caused enrichment of the glucose fraction (paper chromatography). Periodate oxidation of the sugar mixture, followed by paper chromatography, gave, as the major product, a substance (presumably 2-*O*-methylerythrose) which corresponded to that obtainable[5] from 4-*O*-methyl-D-glucose by similar treatment.

Fraction 2. This fraction (13 mg) was similar in composition to Fraction 1, and, upon formation of the methyl ester methyl glycosides, reduction, and hydrolysis, it yielded glucose and 4-*O*-methylglucose (major component).

Fraction 3. The syrup (100 mg), $[\alpha]_D$ *ca.* +15° (*c* 1.9), consisted largely of glucuronic acid; treatment as for Fraction 1 gave a mixture of glucose and a trace of 4-*O*-methylglucose, $[\alpha]_D$ +40° (*c* 1.6).

Fraction 4. Recovered as the barium salt (124 mg), $[\alpha]_D$ +3° (*c* 1.5), this fraction was an approximately equimolar mixture (paper chromatography and ionophoresis) of glucuronic acid and a less-mobile component. Acid hydrolysis (20 h, N H_2SO_4 at 100°) of a portion of the mixture yielded glucurono-6,3-lactone and glucuronic acid together with galactose and 4-*O*-methylglucuronic acid (paper chromatography). Identification of the substances in Fractions 4, 5, and 6 (see Table II) was achieved after combination of the fractions and re-chromatography on cellulose.

Fraction 5. In addition to the two components in Fraction 4, this fraction (145 mg, as Ba salts), $[\alpha]_D$ −12° (*c* 3.1), contained a third acid which was less mobile both on paper chromatography and ionophoresis.

Fraction 6. Recovered as the barium salts (118 mg), $[\alpha]_D$ −29° (*c* 3.0), this fraction was shown by paper chromatography and ionophoresis to contain the two components other than glucuronic acid present in Fraction 5. Acid hydrolysis yielded glucurono-6,3-lactone, glucuronic acid, galactose, mannose, and 4-*O*-methylglucuronic acid.

Fraction 7. This fraction (138 mg as barium salt), $[\alpha]_D$ −29° (*c* 3.45), was chromatographically and ionophoretically homogeneous, and identical with one of the components present in Fractions 5 and 6. Acid hydrolysis afforded glucurono-6,3-lactone, mannose, and glucuronic acid, and chromatographic comparison of the parent compound with the barium salt of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose indicated their identity. Methylation gave a chromatographically homogeneous (t.l.c.) product, $[\alpha]_D$ −16° (*c* 2.5 in methanol), which crystallised from ether–light petroleum (b.p. 40–60°) as prisms, m.p. 138–139° alone or in admixture with methyl 2-*O*-(methyl 2,3,4-tri-*O*-methyl-β-D-glucosyluronate)-3,4,6-tri-*O*-methyl-D-mannoside[6]. Hydrolysis of this product gave 3,4,6-tri-*O*-methylmannose and methylated uronic acid, while methanolysis and g.l.c.[3] gave methyl glycosides of 3,4,6-tri-*O*-methylmannose (*T* 3.71) and of methyl 2,3,4-tri-*O*-methylglucuronate (*T* 2.75, 3.71) in equimolar proportions.

Fraction 8. Recovered as the barium salt (800 mg), $[\alpha]_D$ −6° (*c* 5.1), this fraction was homogeneous on paper chromatography and ionophoresis and identical with the barium salt of 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. Acid hydrolysis gave

glucurono-6,3-lactone, galactose, and glucuronic acid. The methylated derivative (472 mg from 575 mg of barium salt), $[\alpha]_D -26°$ (*c* 3.5, chloroform), appeared to be homogeneous on t.l.c. Recrystallisation from light petroleum (b.p. 60–80°) gave the methyl ester of methyl 6-*O*-(2,3,4-tri-*O*-methyl-β-D-glucosyluronic acid)-2,3,4-tri-*O*-methyl-D-galactoside as prismatic needles, m.p. 80–83°, which, on hydrolysis, gave 2,3,4-tri-*O*-methylgalactose and methylated uronic acid. Methanolysis and g.l.c. of a sample of the total methylated product gave methyl glycosides of methyl 2,3,4-tri-*O*-methylglucuronate (*T* 2.79, 3.73), 2,3,5-tri-*O*-methylgalactose[7,8] (*T* 5.52), and 2,3,4-tri-*O*-methylgalactose (*T* 9.8).

Fraction 9. The barium salt (50 mg), $[\alpha]_D$ *ca.* −15° (*c* 0.6), of this fraction had an equiv. wt. of 320 [determined by passage through Amberlite IR-120 (H^+) and titration with sodium hydroxide (phenolphthalein indicator)], R_{Gal} 0.32, 0.25 (solvents *b* and *d*), and M_G 0.55. Some contamination by Fraction 8 was apparent from the chromatograms. Hydrolysis (N sulphuric acid for 6 h and 25 h at 100°) gave initially 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose in major proportion, and ultimately mannose, glucurono-6,3-lactone, and glucuronic acid, together with a small proportion of galactose.

Fraction 10. As the barium salt (28 mg), $[\alpha]_D$ *ca.* −3° (*c* 1.0), this fraction had R_{Gal} 0.21 (solvent *d*, strong response to the triphenyltetrazolium reagent) and M_G 0.70. Acid hydrolysis (as for Fraction 9) liberated mainly galactose, together with glucurono-6,3-lactone, mannose, and glucuronic acid. Galactose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose were preponderant at the intermediate stage of hydrolysis.

Fraction 11. As the barium salt (97 mg), this fraction had $[\alpha]_D$ −22° (*c* 2.4); equiv. wt., 380, R_{Gal} 0.18, 0.15 (solvents *b* and *d*); and M_G 0.60; but was slightly contaminated with the substance in the previous fraction. Acid hydrolysis gave a pattern identical with that for Fraction 9.

Fraction 12. This fraction (31 mg as barium salts) resembled Fraction 11 in chromatographic behaviour but had $[\alpha]_D$ −4° (*c* 1.1), gave a strong positive reaction with the triphenyltetrazolium reagent, and liberated more galactose upon hydrolysis.

Fraction 13. As the barium salts (100 mg), $[\alpha]_D$ −23° (*c* 3.0), this material had equiv. wt. 347; R_{Gal} 0.07, 0.07 (solvents *b* and *d*); and M_G 0.60. Acid hydrolysis gave a pattern similar to that of Fraction 11. Methylation of Fraction 13 yielded an amorphous product (56 mg), $[\alpha]_D$ −15° (*c* 2.8, chloroform) and −16° (*c* 2.2, methanol), which was homogeneous on t.l.c. (chloroform–methanol, 20:1). The i.r. spectrum showed strong absorption at 1750 cm^{-1} (CO_2Me) relative to that at 1100 cm^{-1} (OMe region), thus resembling the methylated aldobiouronic acids. Hydrolysis of a portion gave mainly methylated uronic acid and 3,4,6-tri-*O*-methylmannose, with some di-*O*-methylmannose (?) and a trace of 2,3,4-tri-*O*-methylgalactose. Further methylation and hydrolysis gave similar sugars, whilst methanolysis gave glycosides of methyl 2,3,4-tri- (*T* 2.77, 3.75) and methyl 2,3-di-*O*-methylglucuronates (*T* 11.3, 15.1), and of 3,4,6-tri-*O*-methylmannose (*T* 3.75) in large proportion, together with that of 2,3,4-tri-*O*-methylgalactose (*T* 9.2) in about one-tenth of this quantity. Unidentified di-*O*-methyl sugars (*T* 12.4 and 22.7) were also detected.

Fraction 14. The material from the washings from the column (108 mg, barium salts); $[\alpha]_D$ −14°(*c* 1.1); equiv. wt., 386; was chromatographically heterogeneous, having R_{Gal} <0.05 (solvent *d*), M_G *ca.* 0.55. Acid hydrolysis gave a pattern similar to that for Fraction 9.

Re-fractionation of Fractions 4-6. — Fractions 4 (109 mg), 5 (122 mg), and 6 (123 mg) were combined, and the bulk (336 mg) of this mixture was separated on a cellulose column (68 × 3 cm) by using butyl alcohol–acetic acid–water mixtures [6:1:1, changed stepwise to 3:1:1 (v/v)]. Two-hourly fractions were collected and monitored as before, giving three major sub-fractions.

Sub-fraction i. This was recovered as barium D-glucuronate (70 mg), $[\alpha]_D$ +11.5° (*c* 1.4), chromatographically and ionophoretically identical with the authentic material. Reduction of the methyl ester methyl glycoside with borohydride, followed by hydrolysis, gave syrupy D-glucose, $[\alpha]_D$ +49° (*c* 1.28), which was identified by paper chromatography and ionophoresis but contained traces of 4-*O*-methylglucose and mannose. The *p*-nitroaniline derivative[9] had m.p. 175° (with softening at 170°) alone or in admixture with *N*-*p*-nitrophenyl-D-glucosylamine[10]; the i.r. spectra of the compounds were identical.

The X-ray diffractogram of *N*-*p*-nitrophenyl-D-glucosylamine (*a*) is markedly different from that of the D-galactose analogue (*b*), as the following *d*-spacings indicate: (*a*) 16.98w, 6.03w, 5.61m, 5.07s, 4.93w, 4.53w, 4.44vw, 4.32m, 4.14w, 3.53 vw, 3.44w, 3.35m, 3.29m, 3.19vw, 3.04vw, 2.93vw, 2.80w, 2.67w, 2.54vw, 2.37vw. (*b*) 16.1w, 7.40vs, 5.78vs, 5.31s, 4.80m, 4.48w, 4.15vs, 4.04vs, 3.96m, 3.90vs, 3.71w, 3.53vw, 3.34w, 3.18m, 3.15w, 3.03w, 2.97s, 2.88w, 2.65w, 2.53m, 2.41w, 2.37w, 2.13vw, 2.10vw, 1.98vw.

Sub-fraction ii. The barium salt (100 mg); $[\alpha]_D$ −6° (*c* 2.2); had R_{Gal} 0.80, 0.60 (solvents *b* and *d*), and M_G 0.97. Its chromatographic and ionophoretic behaviour was identical with that of the barium salt of 6-*O*-(4-*O*-methyl-β-D-glucosyluronic acid)-D-galactose[11], and acid hydrolysis afforded the same products. Methylation gave a product, $[\alpha]_D$ −25° (*c* 0.2, chloroform), similar to methylated Fraction 8 on t.l.c. (chloroform–methanol, 20:1), which crystallised on being seeded. Acid hydrolysis gave 2,3,4-tri-*O*-methylgalactose and methylated uronic acid, and methanolysis gave methyl glycosides of methyl 2,3,4-tri-*O*-methylglucuronate (*T* 2.80, 3.73), 2,3,5-tri-*O*-methylgalactose (*T* 5.52), and 2,3,4-tri-*O*-methylgalactose (*T* 9.8). There was evidence of contamination of this fraction by the acid of Sub-fraction iii.

Sub-fraction iii. As the barium salt (114 mg), $[\alpha]_D$ −33° (*c* 2.5), this material was identical by paper chromatography and ionophoresis with the barium salt of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose, as identified in Fraction 7.

RESULTS AND DISCUSSION

Inspection of the products of acid hydrolysis of the polysaccharide gum of *Encephalartos longifolius* enables the main structural features of this highly complex material to be discerned. A more-detailed picture emerges when the proportions of sugar residues bound in specific ways, as determined by analysis of the methylated

polysaccharide[2], are taken into consideration. It is by no means certain that the polysaccharide is homogeneous, and, in fact, there are indications from this study of ethanol-precipitated material that, as has recently been demonstrated for the gum exudate of the North African tree *Anogeissus leiocarpus*[12] (formerly *A. schimperi*)[13], there may be fundamentally different types of constituent molecule present. This aspect of the structural problem is under investigation.

Acid hydrolysis (0.5N acid for 11 h on the boiling water-bath) of the polysaccharide cleaves the glycosidic bonds between neutral sugars but only a minor proportion of bonds between uronic acid and sugar residues. In this way, the following monosaccharides are released in the proportions indicated as *approximate* percentages (by wt.) of total carbohydrate in the polysaccharide: 3-*O*-methyl-L-rhamnose (3), L-rhamnose (14), L-fucose (0.2), D-xylose (4), L-arabinose (8), D-mannose (0.5), and D-galactose (8). The variety in nature and amount of these sugars, which mainly represent units attached to the periphery of the structural chains, suggests the presence of more than one type of polysaccharide. The occurrence of 3-*O*-methyl-L-rhamnose in the polysaccharide and the possibility of its being present in other polysaccharides from botanically related sources have been commented upon[2]. The D-mannose units released represent such a small proportion of the total D-mannose in the gum that most, if not all, of the D-mannose must be linked by glycosidic bonds from uronic acid residues. This is not so for the D-galactose units, which constitute nearly one-third of the total D-galactose, and are presumably end groups, or chain units and branch points to which acids are not attached.

Most of the residual material (comprising 60% of the total carbohydrate in the gum) consists of D-glucuronic acid linked β-(1→6) to D-galactose, plus mannose-containing, acidic oligosaccharides of differing complexity. Further acid hydrolysis (under similar conditions for 8 h) of this residue releases traces of xylose (0.5) and arabinose (1) (in each case, a small fraction of the sugar released up to this point), and of mannose (2) and galactose (4) (much of which probably comes from cleavage of biouronic acid residues). 4-*O*-Methyl-D-glucuronic acid (1) and D-glucuronic acid (5) are also obtained free at this stage. The acidic oligosaccharides present (comprising over 40% of total carbohydrate in the gum) are as follows: 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose (4), 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose (8), and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (23), together with fractions that appear to contain in similar quantities the dimer, trimer, and tetramer of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose (a total of 8). Acid oligosaccharides containing D-glucuronic acid and more than one D-galactose residue, together with material of higher molecular weight, composed of D-glucuronic acid and D-mannose residues, constitute the rest (4.5) of the hydrolytic products. The characterisation of the polymeric series involving D-glucuronic acid and D-mannose residues depends upon: the linear relationship, for two acid-solvent systems, of $[\log 1/R_{monomer}]-1$ against suspected degree of polymerisation[14]; the constancy of ionophoretic mobility, specific rotation, and equivalent weight (less than that of the biouronic acid); and partial hydrolysis of each member of the series to give, predominantly,

the biouronic acid. To test the hypothesis, methylation and hydrolysis of the fourth member of the series was performed; 3,4,6-tri-*O*-methylmannose was the major, neutral-sugar product. Methanolysis showed high proportions of 2,3,4-tri-*O*-methylglucuronic acid, 2,3-di-*O*-methylglucuronic acid, and 3,4,6-tri-*O*-methylmannose, and, in addition, a small proportion of 2,3,4-tri-*O*-methylgalactose, as expected from the acid hydrolysis results. The presence of other sugars, unidentified at present but probably representing branch points, makes it impossible to describe the composition of this material more fully. This result approximates, however, to that found (by an alternative procedure) for the repetitive sequence of D-glucuronic acid and D-mannose in the polysaccharide from *A. leiocarpus* (which also contains L-arabinose and D-xylose residues), and it is possible that a polysaccharide component of similar type may be present in the gum from *E. longifolius*. The bulk of the polysaccharide material, however, consists of D-glucuronic acid bound to galactan, with peripheral sugars attached.

Assay of the methanolysis products of the methylated gum from *E. longifolius* gives methylated sugars in the approximate proportions recorded earlier[2]. The total sugars found thereby agree well with those observed in the present study, and the equivalent weight of the gum is likewise consistent with the uronic acids found by both procedures. It may be tentatively concluded that there are present: 3-*O*-methyl-L-rhamnopyranose, L-rhamnopyranose, and L-arabinofuranose residues as end groups, L-arabinopyranose and (probably) D-xylopyranose residues also in the periphery of the molecules, chain units of D-galactopyranose linked through C-3 or C-6, branch points consisting of D-galactopyranose linked through C-3 and C-6, D-mannopyranose in part as end groups but also as branch points linked through C-2 and C-3. The high proportion of uronic acid residues, in pyranoid form, comprise end groups or chain units linked through C-4, presumably by D-mannose as in the gum from *A. leiocarpus*, and L-rhamnose as in *Acacia*[15] gums. The modes of union of the acid residues to D-galactose and D-mannose have been established as β-(1→6) and β-(1→2), respectively. The degree of branching is thus very high, unsubstituted chain-units constituting less than one third of the total sugar residues.

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SUMMARY

The structural components of a polysaccharide gum from *Encephalartos longifolius*, earlier characterised by isolating the monosaccharides released on graded

hydrolysis and by g.l.c. assay of the methanolysis products from the methylated polysaccharide, has been submitted to further acid hydrolysis in order to identify the acidic components and the sugars linked thereto. The biouronic acids 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose, thus isolated, together with what appears to be a linear series of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose polymers linked through C-4 of the D-glucuronic acid residues, constitute a high proportion of the total polysaccharide. This evidence, taken with the methylation data, is used to describe the structural units and their methods of linkage in the complex polysaccharide material.

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ACID-CATALYZED DEGRADATION OF D-FRUCTOSE

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An attempt to study the nonenzymic browning reactions occurring in stored, dehydrated orange juice suggested that preliminary studies on a simple model system might lead to the development of more efficient methods. Such a study would also provide background information for interpretation of data obtained with the more complex system present in powdered, dehydrated citrus juice.

A number of different approaches have been used to study nonenzymic browning, which is usually initiated in foods by reactions of reducing sugars with amines or acids, especially basic amino acids[1]. One approach has been the examination of changes that carbohydrates undergo in presence of the amines or acids[2,3]. Another has been the study of the products formed when acidic solutions of sugars are heated. Sugisawa[4] used column-chromatographic separation to identify several acids and esters formed when acidic solutions of D-glucose are heated. Livingston[5] reported that malic acid causes more-rapid browning of D-fructose than does hydrochloric acid at the same pH. In another approach, involving studies on syrups and on fruit-juice concentrates, Braverman[6] observed darkening on heating, and a relationship between pH and rate of reaction; a pH of 3–4 was found optimal for this browning reaction, and a correlation between darkening and appearance of 5-(hydroxymethyl)-2-furaldehyde was demonstrated. Berry and Tatum[7] isolated 5-(hydroxymethyl)-2-furaldehyde from dehydrated orange juice that had been stored at elevated temperatures. Further investigations of these findings indicated that one of the principal precursors of substances formed in dehydrated orange powders during storage is D-fructose.

To find out if D-fructose is a principal precursor of off-flavors that may develop in citrus juices on storage, the products of thermal degradation of D-fructose in acid solution were studied at three pH values: pH 3.5 (in the range commonly encountered in citrus juice), pH 2.15, and pH 1.0. The highest acidity permitted more rapid accumulation of materials in quantities sufficient for complete identification, and provided information concerning the effect of pH on the course of acid-catalyzed, nonenzymic browning.

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RESULTS AND DISCUSSION

The products of thermal degradation of D-fructose in acid solution are given in Table I. These include the two major components, 5-(hydroxymethyl)-2-furaldehyde and 2-(2-hydroxyacetyl)furan, previously reported[8,9] as products from the acid-catalyzed degradation of sugars. In addition, eleven other compounds were isolated and identified.

TABLE I

PRODUCTS ISOLATED FROM ACID-CATALYZED DEGRADATION OF D-FRUCTOSE

Compound	*Retention time, min* Carbowax 20M	SE-30	R_F (t.l.c.)	*Color with anisaldehyde spray*	λ_{max}^{EtOH}, *nm* (ε)
Acetic acid (1)	9.5	2.5			
Formic acid (2)	—	2.5			
α-Angelica lactone (3)	9.5	6.6	0.93	green, then purple	
Furfural (4)	10.9	6.2	0.93	blue	
4-Hydroxy-2,3,5-hexanetrione (acetylformoin) (5)	12.5	8.4	0.73	red-orange	300[a,b], 354 in $CHCl_3$[a,b]
5-Methyl-2-furaldehyde (6)	15.0	—	0.97	blue	
Isomaltol (7)	17.4	8.4	0.80	yellow	281 (14,600)[c]
β-Angelica lactone (8)	20.7	7.8	0.70	blue	
2-(2-Hydroxyacetyl)furan (9)	31.2	11.0	0.50	red, then blue	270 (14,000)[d] 225 (1,900)
2-(2-Hydroxyacetyl)furan formate (10)	43.7	14.2	0.90	red, then blue	271 (18,000) 223 (4,600)
4-Hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (11)	50.0	13.0	0.29	yellow	298 (6,300)
Levulinic acid (12)	52.8	—	0.36	purple	
5-(Hydroxymethyl)-2-furaldehyde (13)	62.3	15.8	0.33	blue-green	281 (15,400)[e] 226 (2,700)

[a]The quantity of material collected was insufficient for determination of an accurate ε value. [b]Ref. 12 reported, for **5** in chloroform, λ_{max} 355 nm (ε 15,000); but, in ethanol, form **5a** preponderated, and had λ_{max} 303 nm (ε 7,500). [c]Ref. 11 reported λ_{max}^{EtOH} 280 nm (ε 16,000). [d]Ref. 8 reported $\lambda_{max}^{H_2O}$ 275 nm (ε 14,000), 225 nm (ε 2,790). [e]B. SING, G. R. DEAN, AND S. M. CANTOR, *J. Am. Chem. Soc.*, 70 (1948) 517, found $\lambda_{max}^{H_2O}$ 284 nm (ε 16,700), 230 nm (ε 3,080).

When the acid-catalyzed degradation of D-fructose was effected at pH 3.5 with citric acid, all compounds listed in Table I except **6** and **10** were found. In addition to 5-(hydroxymethyl)-2-furaldehyde (**13**) and 2-(2-hydroxyacetyl)furan (**9**), the following compounds were identified: α-angelica lactone (**3**), furfural (**4**), 4-hydroxy-2,3,5-hexanetrione (**5**), isomaltol (**7**), β-angelica lactone (**8**), levulinic acid (**12**), and compound **11**, namely, 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone. The latter is a new compound, and its structural assignment is discussed later. The presence of acetic acid (**1**) and formic acid (**2**) was indicated by g.l.c. retention times, but positive

identification was not made until we studied the reaction at pH 1.0, in which much greater proportions of these acids were formed.

At pH 2.15, all compounds found at pH 3.5 were present, together with 2-(2-hydroxyacetyl)furan formate (**10**). This compound (previously unreported) was identified by comparison with a sample synthesized from **9** and formic acid.

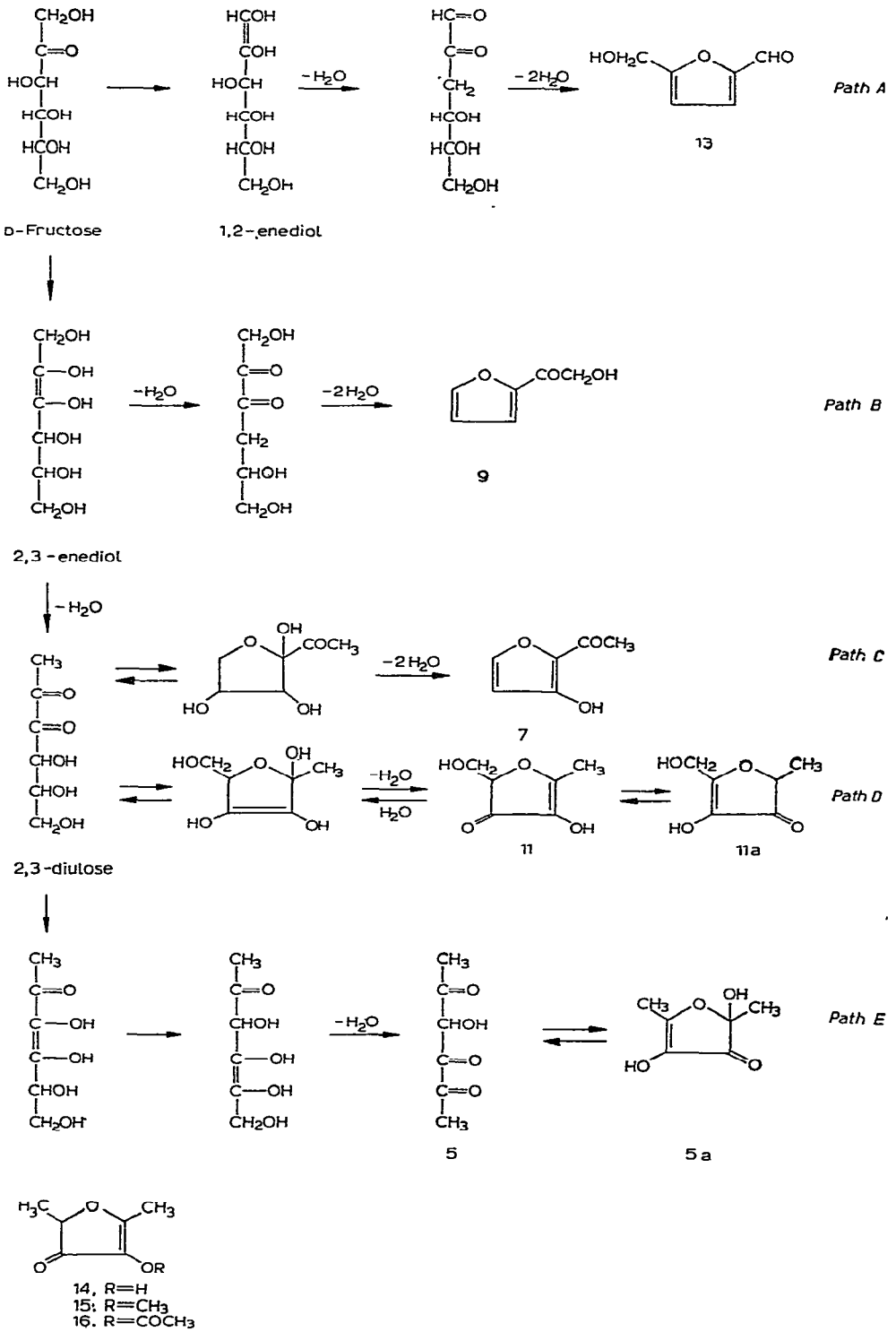
Degradation of D-fructose at pH 1.0 with hydrochloric acid afforded all of the compounds in Table I except **11**. 5-Methyl-2-furaldehyde (**6**) was the only compound present at this pH that was not found at either of the higher pH levels. Identity in degradation products when either citric or hydrochloric acid was used would appear to exclude citric acid as a contributing source for such products as formic or acetic acid.

Identification of isomaltol (**7**) is of special interest, because it had previously been found as a degradation product of maltose and of lactose [10,11]. It has not previously been identified as a degradation product of D-fructose.

The structural assignment for the previously unreported compound **11** was based on its spectra. The ultraviolet maximum at 298 nm (ε 6,300) is similar to that of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (**14**) [λ_{max}^{EtOH} 289 nm (ε 6,700)][12] and also to that of 4-hydroxy-2,3,5-hexanetrione, which exists in the cyclic form **5a** in ethanol [λ_{max}^{EtOH} 303 nm (ε 7,500)][13]. The infrared spectrum showed a strong, broad hydroxyl band at 3320 cm^{-1}; a strong band at 1660 cm^{-1} and a very strong band at 1610 cm^{-1} indicated a conjugated carbonyl group. Similar bands were noted[12,13] for related compounds **14** and **5a**, although the overall spectra of the latter substance and compound **11** (which have the same molecular weight) were quite different. The mass spectrum indicated a molecular weight of 144 for **11**, and the cracking pattern was similar to, but not identical with, that of 4-hydroxy-2,3,5-hexanetrione (form **5a**).

The n.m.r. spectrum of compound **11** could be interpreted in the methyl region only, because of the small quantity of material available. A methyl singlet at τ 7.88 was observed with the use of a time-averaging computer, indicating structure **11** rather than its tautomer **11a**. This value is in good agreement with the methyl singlets of **14**, **15**, and **16**, found at τ 7.80, 7.87, and 7.88, respectively[12,14].

Consideration of mechanisms of formation indicates that most of these products can be formed from D-fructose by a simple combination of enolization and dehydration steps. Anet[2,9,15] has shown (Chart I) that acid-catalyzed degradation of D-fructose occurs primarily through 1,2-enolization (*Path A*) with loss of the 3-hydroxyl group by dehydration and then further dehydration to give 5-(hydroxymethyl)-2-furaldehyde (**13**). When the less-favorable 2,3-enolization occurs, elimination of the 4-hydroxyl group (*Path B*) leads to 2-(hydroxyacetyl)furan[2,15] (**9**). Elimination of the 1-hydroxyl group and dehydration of a furanose form of the 2,3-diulose thus formed (*Path C*) can lead to isomaltol[10]. However, dehydration of an alternative furanose form of the 2,3-diulose (*Path D*) can produce compound **11**. On several occasions, the spontaneous transformation of **11** into isomaltol (**7**) has been observed in chloroform. This could be explained by assuming that a trace of acid and water could reverse *Path D* to form the 2,3-diulose, which could give isomaltol by *Path C*. 4-Hydroxy-2,3,5-hexanetrione (**5**) can be formed from the 2,3-diulose by further enolization, followed by


D-Fructose
1,2-enediol
13
Path A
2,3-enediol
9
Path B
7
Path C
11
11a
Path D
2,3-diulose
5
5a
Path E
14, R=H
15: R=CH3
16. R=COCH3

dehydration with loss of the 6-hydroxyl group (*Path E*)[10,16]. The fact that the quantities of products **5**, **7**, and **11** isolated were small, relative to 2-(2-hydroxyacetyl)furan (**9**), indicates that elimination of a C-4 hydroxyl group is favored over a C-1 hydroxyl group, following 2,3-enolization[2] under these conditions.

The three carboxylic acids and two lactones that were identified were probably produced by further degradation of some of the compounds already mentioned. Thus, acetic acid and formic acid can be formed by acid-catalyzed degradation of isomaltol[11]. Levulinic acid is a known degradation product of 5-(hydroxymethyl)-2-furaldehyde[17]. The α- and β-angelica lactones are probably formed from levulinic acid during the distillation step[18].

Currently, stored dehydrated citrus powders are being examined for the presence of the above mentioned and related compounds, which might have been formed by nonenzymic browning. Methods of analysis that have been developed in the present study are being applied to stored citrus-juice powders, and a report on these results will be forthcoming[19].

EXPERIMENTAL

Melting points are uncorrected. U.v. spectra were recorded in 95% ethanol with a Cary-14 spectrophotometer, i.r. spectra were obtained with a Perkin–Elmer 137 Infracord spectrophotometer, and mass spectra were determined with a Bendix Model 12-100 Time-of-Flight mass spectrometer. Mass-spectral data give *m/e* values, and peak intensities expressed, in parentheses, as percent of the most intense peak. G.l.c. analyses were performed with an F & M Model 810 instrument on both a silicone column (G.E. SE-30, 23% on Gas-Chrom Z; 9.5 ft × 0.25 in. stainless-steel column; 83 ml of helium per min; injection port at 190°, on-column injection; column oven initially at 65° and programmed at 15° per min to 150°; 5:1 splitter at oven temperature; and flame-ionization detector at 210°) and on a poly(oxyethylene) column (Carbowax 20M, 20% on 60–80 mesh Gas-Chrom P; 4 ft × 0.25 in. stainless-steel column; 130 ml of helium per min; injection port at 190°, on-column injection; column oven at 50° for 4 min, and then programmed at 60° per min to 130°, isothermal until 20 min into run, and then programmed at 60° per min to 170°, isothermal until 48 min into run, and then programmed at 60° per min to 200°, and then isothermal; 5:1 splitter at oven temperature; and flame-ionization detector at 210°). G.l.c. samples were collected in capillary tubes cooled with liquid nitrogen.

Thin-layer chromatograms[7] were developed in 200:47:15:1 benzene–ethanol–water–conc. ammonia[20] (upper phase).

Footnotes to each compound indicate the method of preparation for comparison samples.

Acid-catalyzed degradation of D-*fructose*[9]. — *A. At pH 3.5.* A solution of 240 g of D-fructose (Fisher Scientific Co., Fair Lawn, N. J.) in 440 ml of water, adjusted to pH 3.5 by the addition of 0.044 g of citric acid, was heated for 5 h at 100°. Sodium chloride (50 g) was added, and the mixture was cooled, and extracted with one 300-ml

portion and three 200-ml portions of ether. The extracts were combined, and evaporated to dryness under diminished pressure at room temperature. At this point, 5-(hydroxymethyl)-2-furaldehyde (**13**) could be crystallized by dissolving the residue in ether and cooling the solution in a freezer. As the distillation described below separated the minor components from **13**, this crystallization step was usually omitted. Compound **13** was recrystallized from ether, and identified by its i.r. spectrum, its mass spectrum [*m/e* 126 (59), 109 (8), 97 (68), 69 (30), 53 (20), 52 (11), 51 (18), 50 (12), 41 (100), and 39 (60%)], and by t.l.c. comparison, with an authentic sample (Fluka AG, Buchs S G, Switzerland).

The total residue from the combined ether extracts was distilled for 1 h at 60°, with a trap cooled in liquid nitrogen as the receiver. The yellow solid that collected in the receiver was dissolved in ether, and the ether solution was decanted from a small amount of water that was usually present. The ether solution was dried, concentrated to a small volume, and cooled, and the precipitate that formed was collected to give 2-(2-hydroxyacetyl)furan (**9**), m.p. 80–81°, *m/e* 126 (22), 95 (100), 39 (18%), identical (by i.r., mass-spectral, and t.l.c. comparison) with an authentic sample[8]. The mother liquor was analyzed by g.l.c., and the following compounds were collected in sufficient quantities to permit positive identification by i.r., mass-spectral, and t.l.c. comparison with authentic samples: furfural (**4**, Fisher Scientific Co.), *m/e* 99 (6), 96 (100), 95 (79), 62 (2), 51 (3), 50 (4), 42 (8), 40 (12), 39 (81%); α-angelica lactone (**3**, K & K Laboratories Inc., Plainview, N. J.), *m/e* 98 (87), 55 (100), 43 (85%); β-angelica lactone (**8**, K & K Laboratories, Inc.), *m/e* 98 (83), 83 (57), 69 (19), 55 (100), 43 (81%); isomaltol* (**7**), *m/e* 126 (63), 111 (100), 84 (7), 55 (21), 43 (32%); 4-hydroxy-2,3,5-hexanetrione[21] (**5**), m.p. 78–79°, *m/e* 144 (33), 101 (59), 73 (33), 55 (36), 43 (100%); levulinic acid (**12**, Eastman Organic Chemicals, Rochester, N. Y.), *m/e* 116 (10), 56 (23), 55 (15), 45 (13), 43 (100), 31 (46%); 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (**11**), λ_{max} 298 nm (ε 6,300), $\nu_{max}^{oil\ film}$ 3320 s, 2870 w, 1660 s, 1610 vs, 1460 w, 1420 m, 1380 w, 1270 w, 1205 s, 1180 m, 1080 m, 1060 m, 1020 m, 965 m, 910 m, 880 w, 810 w, 780 w, cm^{-1}; *m/e* 144 (37), 101 (31), 73 (14), 72 (20), 55 (25), 45 (25), 44 (75), 43 (100%). Compounds **9** and **13** were also isolated from the g.l.c. column.

B. At pH 2.15. The amount of citric acid added was 6.10 g. One additional compound was isolated, which was found to be 2-(2-hydroxyacetyl)furan formate (**10**), m.p. 55.0–55.5°, λ_{max} 271 nm (ε 18,000), 223 nm (ε 4,600), $\nu_{max}^{oil\ film}$ 3090 w, 2900 w, 1725 s, 1680 s, 1570 m, 1465 s, 1390 m, 1265 m, 1255 w, 1220 w, 1175 s, 1155 s, 1080 m, 1045 m, 1035 m, 1015 m, 945 m, 910 m, 895 w, 883 m, 770 s, cm^{-1}; *m/e* 154 (12), 95 (100), 40 (62%), identical (by i.r., mass-spectral, and t.l.c. comparison) with the sample prepared from **9** and formic acid.

Authentic **10** was prepared as follows: a solution of **9** (0.04 g) in 0.4 ml of 90% formic acid was kept for 22 h at room temperature. The formic acid was removed by warming under a nitrogen stream, and the residue was dissolved in 0.5 ml of chloroform and spotted onto an 8 × 8-inch plate coated with a 1-mm layer of silica gel

*J. E. Hodge kindly provided us with an authentic sample.

HF-254 (Brinkmann Instruments, Inc., Westbury, L.I., N.Y.). The less-polar, absorbing band was scraped off, and eluted with acetone. Concentration of the eluate, and crystallization of the residue from ether–pentane gave **10**, m.p. 55–55.5°. Three recrystallizations from ether–pentane afforded an analytical sample, m.p. 55.5–56.0°.

Anal. Calc. for $C_7H_6O_4$: C, 54.55; H, 3.92. Found: C, 54.36; H, 4.04.

An indication of the relative proportions of products formed from 240 g of D-fructose at pH 2.15 was obtained by g.l.c. analysis of the total crude extract, and of the distillate, on a silicone column by the cut-and-weigh method. From 1.16 g of crude extract (87% of **13**, 9% of **9**, and 4% of lower-boiling material) there was obtained 0.30 g of distilllate (72% of **9**, 17% of a mixture of 7 and **5**, 4% of **11**, and 7% of lower-boiling material).

At pH 1.0. Hydrochloric acid was added to pH 1.0. Analysis of the D-fructose degradation mixture showed the presence of all compounds in Table I except **11**. In addition, 5-methyl-2-furaldehyde (**6**) was produced; *m/e* 110 (100), 109 (85), 81 (13), 53 (69), 51 (22), 50 (16), 43 (15), 39 (18%); identical (by i.r., mass-spectral and t.l.c. comparison) with an authentic sample (K & K Laboratories, Inc.).

Formic acid and acetic acid came off the silicone column together, as a single, broad peak in quantities larger than were found at the other two levels of acidity. The leading half of the peak was collected, and shown by infrared analysis to be formic acid. The infrared spectrum of the material collected from the trailing half of the peak revealed it to be mostly acetic acid together with some formic acid. Acetic acid, but not formic acid, could be isolated on the Carbowax column.

SUMMARY

Interest in nonenzymic browning of dehydrated food-products led to this study of the acid-catalyzed degradation of D-fructose. The two major furan derivatives formed in this degradation are 5-(hydroxymethyl)-2-furaldehyde and 2-(2-hydroxyacetyl)furan. Several minor products were identified. These included furfural, 5-methyl-2-furaldehyde, isomaltol, 2-(2-hydroxyacetyl)furan formate, and 4-hydroxy-2,3,5-hexanetrione (acetylformoin). Formic acid, acetic acid, and levulinic acid were also present. A new hydroxyfuranone has been isolated, and evidence is presented supporting its structure. Degradation studies at pH 1.0, 2.15, and 3.5 showed a variation in the minor components formed. The mechanism for formation of the furan derivatives is discussed.

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POLYSACCHARIDE–GELATIN COMPLEXES IN ACIDIFIED ETHANOL SOLUTIONS

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INTRODUCTION

D-Glucan–gelatin complexes have been characterized as biopolymeric complexes that are highly soluble in trichloroacetic acid (TCA)–ethanol. Dextrans, glycogen, and soluble starch remain soluble in TCA–ethanol at certain definite concentrations of gelatin[1–3], as well as of acid-soluble collagens[4]. Although the stoichiometry of the binding of D-glucans to gelatin has been reported to be independent of the molecular weight of the D-glucan, it was noted that the proportion of protein needed in order to maintain solubility in TCA–ethanol was dependent on the nature of the internal glycosidic linkages in the D-glucan employed (soluble starch, glycogen, and dextrans)[3].

The present study involved attempts to determine the reactivity of gelatin with dextrans having known internal glycosidic linkages and degrees of branching. In addition, several polysaccharides having monomeric constituents other than D-glucose and having various anomeric configurations were tested for interactions with gelatin in TCA–ethanol. The influence of acid environments on formation of a glycogen–gelatin complex was also investigated.

EXPERIMENTAL

Dextrans having known linkages (B-1298, B-1254, B-1355, B-742, B-1225, and B-1255, in order of decreasing degree of branching; and B-1208 and B-1351, as representative linear polymers) and levans were kindly supplied by Dr. Allene R. Jeanes, Northern Regional Research Laboratories, Peoria, Illinois. Galactan, laminaran, nigeran, yeast mannan, and lichenan were purchased from Pierce Chemical Co., Rockford, Illinois. Inulin was obtained from Mann Research Laboratories, New York, N. Y. Isolichenan, pullulan, and yeast D-glucan were gifts from Dr. I. J. Goldstein, University of Michigan, Ann Arbor, Michigan. Dr. G. Roberts, Baxter Laboratories Glendale, California, provided the *O*-(2-hydroxyethyl)starch (degree of substitution, 0.85). The monomeric and oligomeric sugars used in the inhibition studies (D-ribose, D-galactose, D-mannose, D-glucose, α-D-glucose pentaacetate, maltose, isomaltose, melibiose, raffinose, and stachyose) were of the highest purity obtainable from Mann Research Laboratories, New York.

The D-glucans (dextran having average mol. wt. of 80,000, glycogen, and

erythrodextrin-free, soluble starch) of which derivatives were prepared were those of a previous study[1]. D-Glucan acetates and formates were prepared by the method of Roberts[5], and Wurzburg[6], respectively. D-Glucan phosphate was prepared by the orthophosphate procedure described by Paschall[7]. The method of Wolff[8] was used for preparing carbanilates of D-glucans. All of these derivatives were dialyzed exhaustively against distilled water (4°), and the resulting solutions were lyophilized prior to studies of gelatin binding.

Binding determinations were effected according to a previous publication[1]. To 1.0 ml of a polysaccharide–gelatin solution was added 1 ml of 10% TCA and 10 ml of 95% ethanol. The solutions were shaken, and kept overnight at 4°. Insoluble (unbound) polysaccharide was removed by centrifugation at 16,000 *g*, washed twice with 95% ethanol, and analyzed for carbohydrate content by the anthrone method of Mokrasch[9]. It may be pointed out that solutions of soluble starch gave variable results unless precautions were taken to standardize the conditions. Heating of aqueous solutions of soluble starch until the turbidity just disappeared was adequate to yield reproducible results in gelatin-binding studies.

In studying the competitive effects of monosaccharides and oligosaccharides on glucan–gelatin binding in TCA–ethanol, 2.0 mg of either D-ribose, D-galactose, D-mannose, D-glucose, α-D-glucose pentaacetate, maltose, isomaltose, melibiose, raffinose, or stachyose was added to stoichiometrically bound glucan–gelatin solutions that contained 0.5 mg of gelatin and either 2.0 mg of dextran, 0.5 mg of glycogen, or 0.25 mg of soluble starch. The glucan–gelatin mono- or oligo-saccharide mixtures were subsequently fractionated by the standard partitioning procedure with 0.83% TCA–83% ethanol.

RESULTS AND DISCUSSION

The data in Table I reveal that the proportion of polysaccharide material that can be retained in the phase soluble in TCA–ethanol is a function of the concentration of the gelatin. Furthermore, the solubilization of a specific polysaccharide by gelatin is dependent on the nature of the respective neutral homo- or hetero-polysaccharide. Such stoichiometric interactions have previously been described in terms of complex-formation[1].

It was previously noted that polymers of D-glucose having high contents of α-D-(1→6)-glycosidic linkages bind more effectively with gelatin than do D-glucans having mainly α-D-(1→4)-linked, D-glucose residues[1]. The data in Table I show that dextran B-1355, which had the lowest content[22] of α-D-(1→6)-linkages (57%), bound less effectively with gelatin than did dextrans B-1255 and B-1351 [89 and 85% of α-D-(1→6)-linkages, respectively][22]. Dextrans B-1298, B-1254, B-1225, B-742, and B-1208, which contained between 64 and 95% of α-D-(1→6)-linkages[22], bound gelatin in a manner similar to that of the dextran B-1255 listed in Table I. Although these dextrans showed wide ranges in their binding capabilities, it should be noted that the dextran least effectively bound (B-1355 in Table I) still exhibited a higher weight-

TABLE I

SPECIFICITY OF POLYSACCHARIDE COMPLEX-FORMATION WITH GELATIN IN 0.83% TCA–83% ETHANOL[a]

Polysaccharide[b]	*Principal monomers*	*Principal linkages*	*Linear (L) or branched (B)*	*Reference*	*Gelatin concentration (mg)* 0.05	0.1	0.25
Dextran B-1355	D-glucose	α-(1→6), α-(1→4), α-(1→3)	B	10, 22	5.4 ±2.0	72.8 ±4.1	92.8 ±1.1
Dextran B-1351	D-glucose	α-(1→6), α-(1→4), α-(1→3)	L	10, 22	83.1 ±1.7	94.0 ±0.9	96.3 ±1.0
Dextran B-1255	D-glucose	α-(1→6), α-(1→3)	B	10, 22	89.6 ±0.9	95.5 ±1.1	93.0 ±0.9
D-Galactan	D-galactose	β-(1→4)	L	13	6.2 ±2.3	13.3 ±1.4	91.7 ±2.6
Inulin	D-fructose	β-(2→1)	L	14	0.0 0.0	0.0 0.0	0.0 0.0
Isolichenan	D-glucose	β-(1→3), β-(1→4)	L	15	0.0 0.0	69.4 ±6.1	90.4 ±3.1
Laminaran	D-glucose	β-(1→3)	L	16	0.0 0.0	8.2 ±3.9	50.1 ±4.1
Levan NRRL B-1662-M	D-fructose, D-glucose	β-(2→6)	B	17	89.1 ±4.8	94.3 ±4.0	98.6 ±4.1
Levan NRRL B-512	D-fructose, D-glucose	β-(2→6)	B	17	77.0 ±1.9	89.3 ±2.6	94.7 ±1.2
Lichenan	D-glucose	β-(1→3), β-(1→4)	L	15	11.6 ±4.5	18.4 ±3.7	54.4 ±2.8
Nigeran	D-glucose	α-(1→3), α-(1→4)	L	18	0.0 0.0	0.0 0.0	0.0 0.0
Pullulan	D-glucose	β-(1→4), β-(1→6)	L	19	84.6 ±4.1	90.2 ±3.1	— —
Yeast D-glucan	D-glucose	β-(1→6), β-(1→3)	B	20	0.0 0.0	0.0 0.0	0.0 0.0
Yeast D-mannan	D-mannose	α-(1→6), α-(1→2), α-(1→3)	B	21	77.9 ±3.1	95.8 ±2.6	97.1 ±1.9

[a] Results are expressed as % of polysaccharide bound. Each value represents the average of 4–16 determinations, with standard error. [b] Polysaccharide concentrations were 0.5 mg per reaction mixture.

binding ratio than glycogen [α-D-(1→4)-, α-D-(1→6)-linked] or soluble starch [mainly α-D-(1→4)-linked].

The data in Table I also indicate that *a priori* predictions cannot be made regarding the reactivity of polysaccharides with gelatin in 0.83% TCA–83% ethanol. Polysaccharides having diverse monomeric constituents and modes of linkage, such as yeast mannan, pullulan, and the levans B-1662-M and B-512, bind gelatin just as effectively as do the dextrans having a high proportion of α-D-(1→6)-linkages. D-Galactan and isolichenan approach the weight-binding ratio previously reported for glycogen, whereas the weight-binding ratios for laminaran and lichenan resemble those of soluble starch[1]. In contrast, inulin [a linear β-D-(2→1)-linked D-fructan], nigeran [an α-D-(1→3)-linked D-glucan], and a yeast glucan (a highly branched β-D-linked D-glucan) show no reactivity with gelatin. Thus, the presence of high concentrations of α-D-(1→6)-linked residues of D-glucose are not, *per se*, essential for enhanced reactivity of polysaccharides with gelatin, and the anomeric configuration does not appear to be the sole factor controlling polysaccharide–gelatin interactions.

The ability of the branched-chain fructans (levans) to complex with gelatin, and the lack of complex-formation with the linear fructan, inulin (see Table I) suggests that both branching and internal linkages may be governing factors in interactions between gelatin and ketohexose polymer. Conversely, linear polymers of D-glucose may (isolichenan, laminaran, lichenan, and pullulan) or may not (nigeran) exhibit complex-formation with gelatin (see Table I).

With respect to the dextrans, the highly branched polymers did not bind with gelatin so effectively as the linear polymers. Based on the work of Goldstein and coworkers with concanavalin A–dextran precipitin reactions[10], the most highly branched dextrans are, in order: B-1298, B-1254, B-1355, B-1225, and B-1255, whereas the remainder are linear. With one exception (B-1351, linear), the four dextrans having the highest degree of branching showed concomitant, lower proportions of α-D-(1→6)-glycosidic linkages[22] and lower gelatin-binding capacities, than either the linear polymers or the dextrans (B-1225 and B-1255) having lower degrees of branching. The data suggest that higher degrees of branching in D-glucans may contribute to less binding of gelatin by the respective D-glucans.

In a previous study, it was noted that formation of a glycogen–gelatin complex is not inhibited when the reactants are initially suspended in a platelet-preservation medium that contains 5% of D-glucose[2]. Incorporation of other mono- or oligosaccharides (such as D-glucose pentaacetate, D-galactose, D-mannose, maltose, isomaltose, melibiose, raffinose, D-ribose, or stachyose) into gelatin–glucan solutions did not result in any significant inhibition of glucan–gelatin complex-formation. These findings indicated that terminal groups of the polysaccharides are probably not the active binding-sites. This conclusion places gelatin–polysaccharide complex-formation in a unique position with respect to most protein–polysaccharide interactions. For example, formation of the concanavalin A–polysaccharide complex depends on the terminal, nonreducing sugar residues of highly branched polysaccharides[11].

In an attempt to ascertain the specific, protein-binding-sites in glucans, various

chemically modified D-glucans (see Table II) were tested for their ability to bind gelatin. With the exception of starch acetate, the introduction of acetate, formate, or phosphate groups onto the residues in the glucan either had no effect on, or enhanced,

TABLE II

D-GLUCAN DERIVATIVE–GELATIN BINDING IN 0.83% TCA–83% ETHANOL[a]

Polysaccharide[b]	*Gelatin concentration (mg/reaction mixture)*			
	0.05	*0.10*	*0.25*	*0.50*
Dextran (unmodified)	57.8 ±2.7	89.9 ±1.4	94.9 ±1.8	
acetate	49.8 ±1.4	90.9 ±1.0	95.4 ±2.4	
formate	54.3 ±1.8	87.8 ±1.1	96.3 ±2.0	
carbanilate	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Glycogen (unmodified)	2.5 ±1.4	25.6 ±1.8	85.5 ±2.2	96.9 ±2.1
acetate	4.0 ±1.4	42.5 ±3.9	87.7 ±1.1	100.0 ±0.0
formate	7.7 ±1.0	67.7 ±1.2	83.1 ±4.4	95.7 ±2.4
carbanilate	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
phosphate	29.4 ±3.6	66.0 ±4.2	94.7 ±2.2	96.3 ±2.0
Soluble starch (unmodified)	18.3 ±2.0	57.1 ±1.1	75.9 ±1.7	83.8 ±3.0
acetate	7.8 ±1.0	42.5 ±1.3	56.0 ±2.4	60.0 1.0
formate	71.6 ±3.7	89.1 ±0.8	98.4 ±1.0	97.2 ±1.2
carbanilate	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
phosphate	15.2 ±2.8	77.2 ±1.2	87.1 ±2.2	86.0 1.8
O-(2-Hydroxyethyl)starch	60.8 ±0.6	76.3 ±2.3	86.1 ±0.8	92.1 ±0.6

[a]Values represent % of polysaccharide bound. Each value represents the average of 4–16 determinations, with standard error. [b]Concentrations were: dextran, 1.0 mg; glycogen, 0.5 mg; and starches, 0.25 per reaction mixture.

complex-formation. Formic and phosphoric esters possess slightly acidic hydrogen atoms (acetate, to a much lesser degree) in aqueous solution. Because acid characteristics are greatly suppressed in 83% ethanol, the "active" hydrogen atom of such groups can be considered to be closely associated with adjacent, electron-rich atoms; therefore, hydrogen-bonding capabilities should be enhanced in 83% ethanol. Such an interpretation would partially support the findings presented in Table II.

In contrast to the enhancement of complex-formation observed with the formate, acetate, and phosphate derivatives of D-glucan, and *O*-(2-hydroxyethyl)starch, the D-glucan carbanilates, which are insoluble in water as well as in TCA, were incapable of forming stable complexes with gelatin (see Table II). The most plausible explanation of this result is that the spatial arrangement of the bulky phenyl groups of the carbanilates caused steric inhibition of interactions between the active protein and binding sites on the D-glucan.

As regards the protein moiety, it has also been shown that complex-formation arises from the formation of stable hydrogen-bonds between the respective biopolymers[4]. In addition to the hydrogen-donating ability of polysaccharides, the ring oxygen-atom probably participates in these gelatin–polysaccharide interactions[4].

Thus, the specificity of the reaction may depend on the conformation of the polysaccharides, that is, on the availability of the hydroxyl groups and of the ring oxygen-atom for interaction with binding sites on the protein.

Although complex-formation has been tested routinely in acidified ethanol solutions, the necessity for an acid environment for detection of these biopolymeric interactions has not yet been investigated. On varying the TCA concentration from 0.0 to 0.83%, we found that glycogen–gelatin binding occurs to almost the same extent (*ca.* 80–90%). At TCA concentrations of 4% or above, the gelatin and glycogen are precipitated concomitantly. Although the complex may still persist in these precipitated biopolymers, it could not be characterized directly.

In Fig. 1 are shown gelatin–D-glucan binding curves obtained in the absence of TCA. With the exception of soluble starch, the stoichiometry of these reactions does not differ markedly from results obtained in 0.83% TCA–83% ethanol. The data in Fig. 1 reveal that concentrations of gelatin greater than a 2:1 protein:starch ratio were

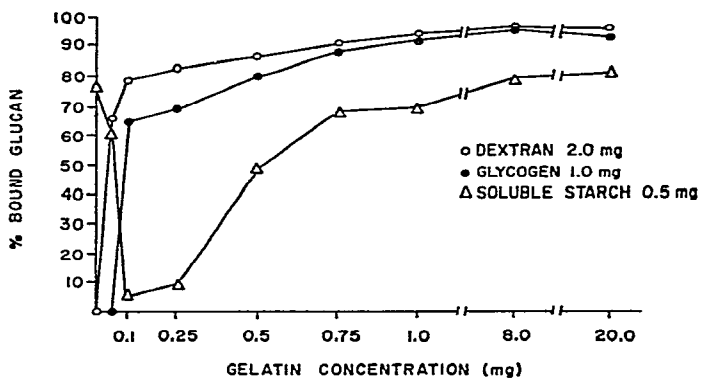

Fig. 1. D-Glucan–gelatin binding in 83% ethanol.

necessary in order to solubilize 80% or more of the soluble starch in the absence of TCA (see Table II). Thus, although TCA, *per se*, had no apparent effect on glycogen– or dextran–gelatin interactions, the absence of TCA resulted in significant decreases in formation of soluble starch–gelatin complex.

Although TCA is not essential for the solubilization of D-glucan–gelatin complexes in 83% ethanol, soluble starch could not be precipitated from 83% ethanolic solutions in the absence of both TCA and the binding protein (see Fig. 1). This "anomalous" solubility characteristic of the preponderantly α-D-(1→4)-linked D-glucan—as compared to the D-glucans having α-D-(1→6), α-D-(1→4), and α-D-(1→6) linkages—may be governed by specific, multiple interactions between the solute molecules (soluble starch) and the binary solvent system (water and ethanol). Conversely, it follows that the specific, structural conformations of glycogen and dextran are not conducive to solubilization of these D-glucans in the presence of this binary solvent.

The mechanisms whereby TCA influences the solubility characteristics of

soluble starch have not yet been identified. Although 5% TCA has been routinely used to solubilize and, simultaneously, partition D-glucans from other major cellular biopolymers (DNA, RNA, and protein), the modes of action responsible for these partitioning effects still remain obscure. In any event, these results further exemplify the advantages of incorporating TCA in reaction mixtures when comparative, quantitative studies on formation of glucan–protein complex are conducted.

When other acids were substituted for TCA in the glycogen–gelatin reaction, the binding varied with the strength of the acid. In general, those acids having a higher pK than that of TCA (pK 0.7) give more binding, whereas the acids having low pK inhibit binding. Acetic acid (pK 4.7) and monochloroacetic acid (pK 2.9) exhibit slightly more binding than does TCA at the same concentration, whereas hydrochloric acid (negative pK) essentially inhibits formation of complex. One notable exception is the formation of glycogen–gelatin complexes in the presence of trifluoroacetic acid (pK −0.7), where no significant differences in binding were observed in the presence of either TCA or trifluoroacetic acid; however, trifluoroacetic acid possesses unusual properties in that it is a good protein solvent, and is apparently devoid of hydrolytic properties[12]. Thus, a conformation dependent on hydrogen ion is not required of the protein for solubilization of D-glucan by gelatin in 83% ethanol. It is generally accepted that polysaccharides dissolve in aqueous solutions by virtue of extensive hydrogen-bonding with water molecules. Analogous to this are the interactions reported herein, where it has been shown that gelatin solubilizes polysaccharides both in acidified and nonacidified 83% ethanol solutions.

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SUMMARY

Numerous polysaccharides of diverse monomeric constituents and modes of linkage (such as galactan, isolichenan, laminaran, levans, lichenan, pullulan, and yeast mannan) combine with gelatin to form macromolecular complexes soluble in 0.83% trichloroacetic acid–83% ethanol. In contrast, inulin, nigeran, and yeast D-glucan do not complex with gelatin. Dextrans having various contents of α-D-(1→6), α-D-(1→4), and α-D-(1→3) linkages appear to bind gelatin more effectively than either glycogen or soluble starch. Decreased stoichiometric binding has been correlated with increased branching of the dextran polymers; conversely, the branched-chain levans bind gelatin, whereas inulin, a linear polymer, does not. With the exception of starch acetate, esterification of neutral glycans with formic, acetic, or phosphoric acid either enhances, or has no effect on, formation of complex.

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UNSATURATED SUGARS

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INTRODUCTION AND DISCUSSION

The unsaturated sugars have been reviewed recently[1], and a recent publication[2] prompted the author to report the following results.

In an attempt to form sugars containing selenium in the pyranoid ring, 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose was treated with potassium selenocyanate in methanol at room temperature. The expected episelenide was not formed, but selenium separated and 1,2-*O*-isopropylidene-5,6-dideoxy-α-D-*xylo*-hex-5-enofuranose was isolated in good yield. The same product was obtained from 5,6-anhydro-1,2-*O*-isopropylidene-α-L-idofuranose. Under similar conditions, 5,6-dideoxy-5,6-epithio-1,2-*O*-isopropylidene-α-D-glucofuranose did not react. When the reaction was repeated with potassium selenocyanate in the presence of ammonium chloride, no selenium separated. The crude product contained neither the 5-enose nor any selenocyanate group as shown by t.l.c. and infrared spectroscopy, respectively. When 1,2-*O*-isopropylidene-5,6-di-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose was treated with a boiling solution of potassium selenocyanate in methanol for six hours, no reaction occurred. When *N*,*N*-dimethylformamide was used, the 5-enose was formed in good yield, but some 3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose was also isolated.

It was shown[3] that methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside (**1**) reacted with potassium thiocyanate to give a 10% yield of methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epithio-α-D-glucopyranoside; when ammonium chloride was added, the yield of episulphide was increased to 20%. With ammonium thiocyanate in 2-methoxyethanol, compound **1** gave a mixture of the 2- and 3-thiocyanato compounds. Treatment of the methanesulphonic ester of this crude mixture with base gave the episulphide[4]. When **1** was treated with a boiling solution of potassium selenocyanate in aqueous 2-methoxyethanol, methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside was obtained. When the reaction was repeated in the presence of ammonium chloride, a mixture of two compounds, probably methyl 4,6-*O*-benzylidene-2-deoxy-2-selenocyanato-α-D-glucopyranoside and methyl 4,6-*O*-benzylidene-3-deoxy-3-selenocyanato-α-D-altropyranoside was formed. The latter compound was preponderant, and its structure was assumed by analogy with the findings of Christensen and Goodman[4]. The crude mixture of selenocyanates was treated with toluene-*p*-

sulphonyl chloride, and the resulting ester, on treatment with alkali, gave the 2-enoside and selenium. However, when methyl 4,6-*O*-benzylidene-2,3-di-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside was treated with a boiling solution of potassium selenocyanate in *N*,*N*-dimethylformamide or 2-methoxyethanol, no reaction occurred.

Formation of unsaturated compounds by means of the selenocyanate reagent was also applied to an acyclic system. It has been shown[5] that 1,2:5,6-di-*O*-isopropylidene-D-mannitol 3,4-thionocarbonate, with trimethyl phosphite, gave 1,2: 5,6-di-*O*-isopropylidene-*trans*-3-hexene-D-*threo*-1,2,5,6-tetrol, and the corresponding *cis* compound was also prepared. Further, 1,2:5,6-di-*O*-isopropylidene-3,4-di-*O*-toluene-*p*-sulphonyl-D-mannitol, on treatment with boiling *N*,*N*-dimethylformamide, sodium iodide, and zinc dust, has been shown[6] to give the *trans* compound. Treatment of the above disulphonate with potassium selenocyanate in refluxing *N*,*N*-dimethylformamide or of 3,4-anhydro-1,2:5,6-di-*O*-isopropylidene-D-talitol with potassium selenocyanate in methanol at room temperature gave 1,2:5,6-di-*O*-isopropylidene-*cis*-3-hexene-D-*threo*-1,2,5,6-tetrol. The above anhydro compound, with potassium selenocyanate in refluxing 2-methoxyethanol, gave a better yield of the *cis* 3-ene. 5,6-Anhydro-1,2:3,4-di-*O*-isopropylidene-D-mannitol gave 3,4:5,6-di-*O*-isopropylidene-1-hexene-D-*arabino*-3,4,5,6-tetrol on treatment with potassium selenocyanate in methanol.

EXPERIMENTAL

Melting points are uncorrected. Known compounds were identified by melting points, mixed melting points, and infrared spectra. T.l.c. was performed on Merck Kieselgel G with benzene–methanol (9:1) as irrigant. The plates were sprayed with 10% ethanolic sulphuric acid and heated.

*5,6-Dideoxy-1,2-*O*-isopropylidene-α-*D*-xylo-hex-5-enofuranose.*—(*a*) A mixture of 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose[7] (or the corresponding L-*ido*[8] compound; 2.02 g, 0.01 mole), methanol (50 ml), and potassium selenocyanate[9] (2.88 g, 0.02 mole) was kept at room temperature overnight. Selenium started to separate immediately. The solution was filtered, diluted with water, and repeatedly extracted with chloroform. The chloroform extracts were dried (Na_2SO_4) and evaporated. The residue crystallised and was recrystallised from hexane to give the title compound (1.2 g, 64%), m.p. 64° (lit.[10] m.p. 58–60°). T.l.c. of the crude compound showed a weak spot at R_F 0.18 and an intense spot of the 5-enose (R_F 0.40).

When the reaction was repeated in the presence of ammonium chloride (1.07 g) dissolved in water (5 ml), no selenium separated. T.l.c. showed a weak spot at R_F 0.32 and a strong spot at R_F 0.18.

(*b*) 1,2-*O*-Isopropylidene-5,6-di-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose[11] (1.06 g, 0.002 mole), potassium selenocyanate (1.0 g), and *N*,*N*-dimethylformamide (10 ml) were refluxed for 2 h. The reaction mixture was poured into water (40 ml), and 3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose[12] (0.10 g), m.p. 132–133°, separated. The filtrate was extracted with chloroform, and evaporation of the dried extracts gave a syrup (0.27 g, 73%) that crystallised on standing. Recrystallisation from hexane gave the 5-enose, m.p. 64°.

Reactions of methyl 2,3-anhydro-4,6-O-benzylidene-α-D-mannopyranoside. — (*a*) *Potassium selenocyanate.* The 2,3-anhydro compound[13] (2.64 g, 0.01 mole), potassium selenocyanate (3.0 g), 2-methoxyethanol (25 ml), and water (3 ml) were refluxed for 6 h. Selenium separated. The reaction mixture was poured into water, and the solid which separated was crystallised from dilute, aqueous alcohol to give methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (1.90 g, 81%), m.p. 119–120° (lit.[4] m.p. 117–119°).

(*b*) *Potassium selenocyanate in the presence of ammonium chloride.* The reaction mixture was prepared as in (*a*), except that ammonium chloride (1.1 g) was added. Water was added to the reaction mixture to give a solid (2.4 g, 65%), m.p. 207–220°; λ_{max}^{KBr} 2.98 (OH), 4.69 (SeCN), 13.03 and 14.19 μ (monosubst. phenyl). T.l.c. showed a minor component at R_F 0.42 and a major component at R_F 0.26.

Anal. Calc. for $C_{15}H_{16}O_5NSe$: Se, 21.4. Found: Se, 21.2%.

The crude mixture (1.0 g), dry pyridine (5 ml), and toluene-*p*-sulphonyl chloride (0.7 g) were kept for 60 h at 40°. Water (0.5 ml) was added, and, after 0.5 h, the reaction mixture was diluted with excess water. The solid (1.1 g), m.p. 192–194°, was filtered off. Its infrared spectrum (KBr) showed the absence of hydroxyl absorption and had λ_{max}^{KBr} 4.69 (SeCN), 7.32 and 8.44 μ (>SO_2).

The crude sulphonate (512 mg) was dissolved in warm methanol (10 ml), and (3 ml) sodium methoxide (100 mg) in methanol (3 ml) was added. Selenium separated immediately. After 1 h, the solution was filtered, and diluted with water to give the 2-enoside (200 mg, 85%), m.p. 119–121° (from dilute alcohol).

1,2:5,6-Di-O-isopropylidene-cis-3-hexene-D-threo-1,2,5,6-tetrol. — (*a*) 1,2:5,6-Di-*O*-isopropylidene-3,4-di-*O*-toluene-*p*-sulphonyl-D-mannitol[14] (1.12 g, 0.002 mole), potassium selenocyanate (1.0 g), and *N*,*N*-dimethylformamide (10 ml) were refluxed for 4 h. The solution was diluted with water and extracted with chloroform. The extracts were washed with water, dried, and evaporated. The syrup was taken up in light petroleum (15 ml, b.p. 40–60°); starting material (0.10 g) crystallised. The mother liquor was evaporated to give a syrup that was chromatographed on silica gel to give the title compound (0.20 g, 45%); $[\alpha]_D^{20}$ −6° (*c* 2.0, chloroform); $b.p._5$ 100–103° {lit.[5], $[\alpha]_D^{20}$ −8.1° (chloroform), $b.p._1$ 84–86°}; $\lambda_{max}^{CHCl_3}$ 6.23 (*cis* C=C), 8.69 and 9.28μ (isopropylidene).

Anal. Calc. for $C_{12}H_{20}O_4$: C, 63.2; H, 8.8. Found: C, 62.9; H, 8.8%.

(*b*) 3,4-Anhydro-1,2:5,6-di-*O*-isopropylidene-D-talitol[15] (1.22 g, 0.005 mole), potassium selenocyanate, and methanol (15 ml) were kept at room temperature for two days. Selenium slowly separated. By the usual isolation procedure and chromatography, the title compound (0.35 g, 32%) was isolated.

The experiment was repeated, but 2-methoxyethanol (12 ml) and water (1.5 ml) replaced the methanol. The solution was refluxed for 6 h. A syrup (1.20 g) was isolated, which, after chromatography, gave the title compound (1.0 g, 88%).

3,4:5,6-Di-O-isopropylidene-1-hexene-D-arabino-3,4,5,6-tetrol. — 5,6-Anhydro-1,2:3,4-di-*O*-isopropylidene-D-mannitol[16] (1.22 g, 0.005 mole), methanol (20 ml), and potassium selenocyanate (2.0 g) were kept at room temperature overnight. The

usual isolation procedure yielded a syrup (1.39 g). T.l.c. showed the presence of a major component (R_F 0.80; the desired olefin) and a minor component (R_F 0.70). The olefin (0.87 g, 76%), isolated by column chromatography on silica gel, had $[\alpha]_D^{20}$ −5° (*c* 1.0, chloroform).

The compound was hydrolysed by heating it on a boiling-water bath for 7 h with 0.3N sulphuric acid to give 1-hexene-D-*arabino*-3,4,5,6-tetrol[16], m.p. 145–147°, λ_{max}^{KBr} 2.98 (OH), 5.43, 6.07, 7.05, 9.94, and 10.83 μ ($CH_2=C\langle^H$).

SUMMARY

Terminal epoxides of sugars react readily with potassium selenocyanate in methanol at room temperature to give unsaturated sugars. Non-terminal epoxides of sugars with potassium selenocyanate in boiling, aqueous 2-methoxyethanol also gave the corresponding unsaturated sugar. Terminal or acyclic, vicinal di-*O*-toluene-*p*-sulphonyl esters gave the unsaturated sugar when treated with potassium selenocyanate in boiling *N*,*N*-dimethylformamide. Vicinal, *trans* toluene-*p*-sulphonate and selenocyanate groups eliminate selenium, on treatment with base, to give the unsaturated sugar.

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PREPARATIVE SEPARATIONS OF SUGARS ON BISULPHITE RESINS

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INTRODUCTION

The use of strongly basic, ion-exchange resins in the bisulphite form for the preparative separation of aldehydes and ketones was first introduced by Samuelson[1]. The procedure is based on the greater stability of aldehyde bisulphite complexes in comparison with complexes of ketones, which enables the more-facile removal of the ketone from a bisulphite column. Recent work by Samuelson *et al.*[2] has shown that ion-exchange resins can be employed for ketose–aldose separations on a semimicro scale. Bisulphite resin has been used for the isolation of ketoses from catalytic-oxidation mixtures[3] and from mixtures formed after oxidation with methyl sulphoxide[4].

In spite of the ease with which aldoses are equilibrated to aldose–ketose mixtures by bases, the isolation of the ketose from such mixtures is generally an inconvenient and time-consuming process. Existing procedures involve, for instance, chromatography on thick paper in the isolation of D-*threo*-pentulose (D-xylulose)[5], fermentation with a galactose-adapted yeast in the isolation of D-*xylo*-hexulose (D-tagatose)[6], and bromine oxidation of the remaining aldose in the preparation of D-*gluco*-heptulose[7].

The preparation of pure samples of such common sugars as D-fructose and D-galactose is difficult to achieve, even though sucrose and lactose are readily available as precursors. D-Fructose is generally prepared by the hydrolysis of inulin, rather than of sucrose, due to the problem of removal of the D-glucose from the hydrolysate[8]. D-Galactose may be isolated only after fermentation of a lactose hydrolysate with bakers' yeast to remove the contaminating D-glucose[9].

We now present our studies on the application of preparative chromatography, involving a column of resin in the bisulphite form, to the problems of separation of ketoses and isolation of pure sugars.

RESULTS AND DISCUSSION

Paper electrophoresis of various sugars in bisulphite buffer[10] indicates that ketohexoses do not migrate and thus possess little or no tendency to form bisulphite complexes. On the other hand, all of the aldohexoses investigated migrate at various rates, with glucose being the slowest. The electrophoretic mobilities of aldoses indicate that those having the more-stable hemialdal rings are less likely to form a

bisulphite complex. Aldoses possessing a *xylo*-pyranoid configuration and having all substituents equatorial would therefore be expected to show the least tendency to form bisulphite complexes.

Glucose, having the lowest electrophoretic mobility of the aldoses studied and thus the least tendency to form bisulphite complexes, will be the most difficult aldohexose to separate from the ketohexoses. As a test of the resolving power of the bisulphite column, an equimolar mixture of D-glucose and D-fructose, such as one would obtain from the hydrolysis of sucrose, was fractionated on the column at 30°. The elution pattern obtained is shown in Fig. 1. Complete separation was achieved, and the sugars obtained were chromatographically pure.

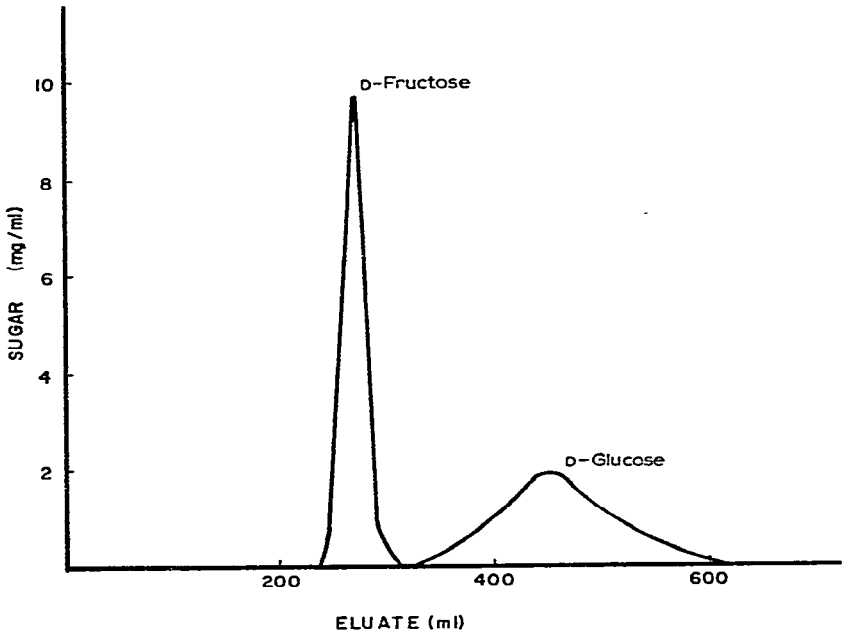

Fig. 1. Separation of D-fructose and D-glucose on Dowex-1 (HSO_3^-) at 30°.

The other common sugar possessing a *xylo*-pyranoid configuration is xylose. Although xylose exhibits the slowest electrophoretic mobility of the aldopentoses, as would be expected from the preceding discussion, each aldopentose exhibits a much higher mobility than that of the corresponding aldohexose. As pentuloses do not migrate, they are thus easily separated from the aldopentoses. Pyridine–water isomerisation of L-arabinose provided a mixture of L-*erythro*-pentulose and the parent sugar. After crystallisation of the mixture to remove excess of L-arabinose, the mother liquor was fractionated on the bisulphite column. L-*erythro*-Pentulose was eluted from the column in Fractions 40–49 (Table I). Although L-arabinose could not be removed from the column under the conditions used, a small amount of unknown material was eluted in Fractions 50–58.

A similar procedure was applied to D-xylose, from which pure D-*threo*-pentulose

TABLE I

SEPARATION OF KETOSES FROM ALDOSES ON DOWEX-1 (HSO_3^-) AT 30°

Ketose	*Aldose epimerised (8 g)*	*Aldose recovered (g)*	*Ketose containing eluate (ml)*	*Comments*	*Overall yield, %*
D-*threo*-Pentulose (D-xylulose)	D-xylose	5.5	441–520	unknown material, 391–430 ml, D-xylose retained	5
L-*erythro*-Pentulose (L-ribulose)	L-arabinose	6.0	401–490	unknown material, 501–580 ml, L-arabinose retained	5
D-*lyxo*-Hexulose (D-tagatose)	D-galactose	6.5	251–320	D-galactose retained	4
D-*gluco*-Heptulose	D-*glycero*-D-*gulo*-heptose	—	231–280	heptose retained	35

was isolated (Table I). The isomerisation of D-xylose, as followed by gas–liquid chromatography of the trimethylsilyl derivatives, showed a slow formation of D-*threo*-pentulose in refluxing, dry pyridine. Small proportions of water were found to increase the rate of formation of the ketose, and good results could be obtained by carrying out the isomerisations in refluxing pyridine–water (99:1) for 8 hours. Longer reaction times resulted in the formation of significant amounts of unidentified compounds (other ketoses?), as indicated by paper chromatography. No ketose formation was detected under the isolation conditions, which contradicts an earlier report[11] that all rearrangement of the aldose occurs during this step.

The application of the bisulphite-column method to a heptose–heptulose mixture was clearly demonstrated by the isolation of D-*gluco*-heptulose after isomerisation of D-*glycero*-D-*gulo*-heptose. D-*lyxo*-Hexulose was isolated as a chromatographically pure material after isomerisation of D-galactose. The duration of the isomerisation is critical in this case, since prolonged refluxing converts the D-*lyxo*-hexulose (D-tagatose) into the more-stable D-*xylo*-hexulose (D-sorbose). The two hexuloses have the same retention characteristics and thus are not separated on the bisulphite column. Furthermore, they are very difficult to separate by means of crystallisation, and this has led to some confusion over the physical properties of D-*lyxo*-hexulose[12]. Table I indicates that D-*lyxo*-hexulose is easily separated from its parent aldose, D-galactose. A careful fractionation is still necessary, however, as D-glucose is a contaminant of commercial D-galactose and is eluted from the column immediately after the hexulose (see Fig. 1 for example).

All examples presented so far have illustrated separations of ketoses from aldoses. In several preparations of D-*lyxo*-hexulose, it was observed that D-glucose was easily separable from D-galactose. Such a preparative separation would allow the isolation of pure D-galactose from lactose hydrolysates or purification of commercial D-galactose to remove the D-glucose contaminant. A typical separation is shown in Fig. 2, in which an equimolar mixture of D-glucose and D-galactose was fractionated to

provide chromatographically pure materials. There is some evidence that, on extended contact with the bisulphite resin at elevated temperatures, sugars may be irreversibly absorbed (Table II).

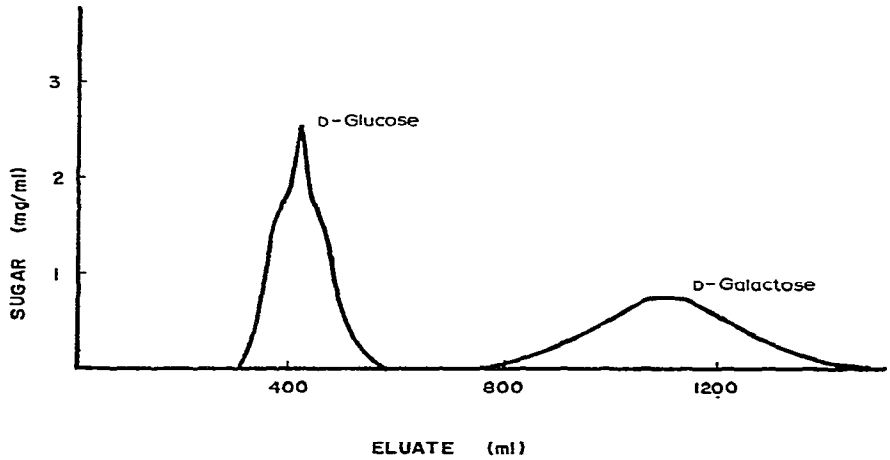

Fig. 2. Separation of D-glucose and D-galactose on Dowex-1 (HSO_3^-) at 45°.

TABLE II

SEPARATION OF SUGARS ON DOWEX-1 (HSO_3^-)

Separation	*Eluate volume (ml)*	*Material applied to column (mg)*	*Material recovered from column (mg)*	*Recovery, %*	*Column temperature, °*
D-Fructose from D-glucose	231–320	250	245	98	30
	331–650	250	243	97	
D-Glucose from D-galactose	291–580	250	254	102	45
	801–1400	250	232	93	

No attempt was made to establish conditions of maximal yield of uloses in the pyridine isomerisations, although conditions were chosen that gave the purest product. An advantage of the bisulphite-column method is that the sugar is obtained in an essentially pure, aqueous solution. Fractionations seldom take longer than twenty-four hours when an automatic, fraction-collecting apparatus is employed. The isolation of several pure sugars on a preparative scale indicates the versatility of the bisulphite-column method.

EXPERIMENTAL

Evaporations were carried out at bath temperatures below 45°. Quantitative

determination of the sugar present in column eluates was made by the phenol–sulphuric acid method[17]. Melting points are uncorrected.

General epimerisation procedure. — Epimerisation of aldose (8 g) with refluxing pyridine–water (99:1; 400 ml) for 8 h gave a solution that was evaporated to a syrup. Water (100 ml) was added, and the solution re-evaporated to give a syrup that did not smell of pyridine. This material was dissolved in water (40 ml), decolourizing charcoal (1 g) was added, and the mixture was heated to 50–60° and filtered. The residue was washed with warm water (2 × 15 ml), and the combined filtrates were evaporated. The resulting syrup was thinned with the minimal amount of hot ethanol and seeded with the starting aldose. Crystallisation was allowed to proceed first for 2–3 h at room temperature and finally overnight at 3–5°. After filtration, the recovered aldose was washed with ethanol, and the combined filtrates were evaporated to a syrup. This material was dissolved in water (5 ml) and applied to the top of a jacketed column (5 × 32 cm) of Dowex-1 (HSO_3^-; 200–400 mesh) maintained at 30°. The column was eluted with water at a rate of 0.5 ml/min, and fractions (10 ml) were collected (see Table I). All fractions showing positive spot-tests on paper (Ag^+/EtO^- reagent) were chromatographed on paper. Fractions to be evaporated were combined and passed through Dowex-3 (free base) before evaporation.

D-threo-*Pentulose* p-*bromophenylhydrazone.* — The hydrazone, prepared from the syrupy pentulose by the method of Hough and Theobald[13], had m.p. 127.5–128.5°, $[\alpha]_D^{25}$ +25° (*c* 2.3, pyridine) after 15 min; lit.[13], m.p. 128–129°, $[\alpha]_D^{25}$ +24° in pyridine after 15 min.

L-erythro-*Pentulose* o-*nitrophenylhydrazone.* — The hydrazone, prepared from the syrupy pentulose in the same manner as described for the D-isomer by Glatthaar and Reichstein[14], had m.p. 167–169, $[\alpha]_D^{20}$ +48° (*c* 1, methanol); lit.[14] for D-isomer, m.p. 168–169.5°, $[\alpha]_D^{20}$ −48.3° (methanol).

D-lyxo-*Hexulose* (D-*tagatose*). — The hexulose was recrystallised from ethanol to give material free from D-*xylo*-hexulose, as indicated by paper chromatography (ethyl acetate–pyridine–water, 8:2:1). M.p. 131–132°, $[\alpha]_D^{25}$ −3° (*c* 3.7, water); lit.[6], m.p. 132°, $[\alpha]_D$ −5° (water)[15].

D-gluco-*Heptulose.* — D-*gluco*-Heptulose was recrystallised from methanol to give crystals, m.p. 169–172°, $[\alpha]_D^{25}$ +66° (*c* 2.4, water); lit.[16], m.p. 170–173°, $[\alpha]_D$ +66° (water).

Fructose–D-glucose separation. — A solution of D-fructose (250 mg) and D-glucose (250 mg) in water (5 ml) was applied to the top of the resin in the column (see previous experiment for column details). The temperature was maintained at 30°, and both sugars were recovered in good yield and high purity (see Fig. 1 and Table II).

D-*Glucose–D-galactose separation.* — A similar experiment to the previous one was carried out with D-glucose (250 mg) and D-galactose (250 mg) in water (5 ml). The temperature was maintained at 45° (see Fig. 2 and Table II).

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SUMMARY

The use of a strongly basic, anion-exchange resin in the bisulphite form for the preparative separation of sugars has been studied. The preparation of D-*threo*-pentulose, L-*erythro*-pentulose, D-*lyxo*-hexulose, and D-*gluco*-heptulose by epimerisation of the more-common, parent aldose and subsequent separation on a bisulphite column is described. The utility of the bisulphite-column method is demonstrated not only by the separation of ketoses from aldoses but also by the preparation of chromatographically pure D-fructose, D-glucose, and D-galactose from commercial products.

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FLUORINATED CARBOHYDRATES

PART I. 3-DEOXY-3-FLUORO-D-GLUCOSE[1]

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INTRODUCTION

The knowledge[2] that replacement of hydrogen atoms or functional groups in an organic compound by fluorine atoms may cause a dramatic change in biological activity is undoubtedly one reason for interest in the synthesis of deoxyfluoro sugars*. Until recently and in spite of considerable efforts[3], deoxyfluoro sugars, as a class of carbohydrate derivative, were poorly exemplified, and, in the case of secondary fluorides having the *gluco* configuration, they are still relatively inaccessible synthetically.

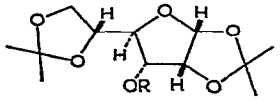
Primary deoxyfluoro sugars are readily obtained by nucleophilic displacement of suitably substituted sugar derivatives by fluoride salts in such solvents as ethylene glycol. In this way, derivatives of 6-deoxy-6-fluoro-D-galactose[4,5], 6-deoxy-6-fluoro-D-glucose[6], and 5-deoxy-5-fluoro-D-ribose[4] have been prepared. The only examples of carbohydrate geminal difluorides, 2,5-anhydro-1-deoxy-1,1-difluoro-D-ribitol and 2,5-anhydro-1-deoxy-1,1-difluoro-D-mannitol, were obtained by treatment of 3,4-di-*O*-acetyl-D-arabinal and 3,4,6-tri-*O*-acetyl-D-glucal with lead tetra-acetate–hydrogen fluoride followed by de-acetylation[7].

There have been several approaches to the synthesis of carbohydrate secondary fluorides. Total synthesis from simple aliphatic precursors has given (±)-2-deoxy-2-fluoroglyceraldehyde[3,8] and 2-deoxy-2-fluoro-DL-ribitol[9], but this approach is limited because of the formation of racemic products. The cleavage of suitably protected sugar epoxides with hydrogen fluoride has permitted the synthesis of 3-deoxy-3-fluoro-L-xylose[10a] (hydrogen fluoride–*p*-dioxane at 120°), 3-deoxy-3-fluoro-D-arabinose[10b] (potassium hydrogen fluoride in ethylene glycol), 3-deoxy-3-fluoro-D-xylose[11], (potassium hydrogen fluoride–diethylene glycol, potassium fluoride–molten acetamide), and 2-deoxy-2-fluoro-D-altrose and 3-deoxy-3-fluoro-D-glucose[12] (hydrogen fluoride–boron trifluoride at −70°). 3-Deoxy-3-fluoro-D-xylose has been converted into the D-arabinose and D-ribose analogues[11], and 2-deoxy-2-fluoro-D-allose has been

*The term deoxyfluoro sugars connotes carbohydrates in which one or more of the hydroxyl functions, other than the glycosidic centre [glycosyl fluorides, see F. Micheel and A. Klemer, *Advan. Carbohydrate Chem.*, 16 (1961) 85], have been replaced by a fluorine atom.

obtained by epimerisation[12] of the D-altrose analogue. Syntheses of 2-deoxy-2-fluorouridine[13] and 2-deoxy-2-fluorothymidine[14] have been described, and from the former compound, 2-deoxy-2-fluoro-D-ribose has been obtained[15].

Several methods that have been used successfully in the synthesis of aliphatic secondary fluorides failed when applied in the carbohydrate field[16]. Although direct displacement of carbohydrate primary sulphonates with fluoride ion proceeds readily[4], the method has not been successfully applied to secondary sulphonates[5]. Because of solvation, fluoride ion in a protic solvent is a relatively poor nucleophile, but, in dipolar aprotic solvents, only cations are strongly solvated[17], and the nucleophilicity of fluoride ion is significantly enhanced. Thus, Henbest and Jackson[18] have shown that sulphonates attached to five-membered carbocyclic compounds (testosterone toluene-*p*-sulphonate) and axially or equatorially to six-membered rings (cholestan-3-α- and 3-β-yl toluene-*p*-sulphonate) can be converted into fluorides, in good yield with inversion of configuration, by treatment with tetrabutylammonium fluoride in acetone or ethyl methyl ketone. In seeking new routes to deoxyfluoro derivatives of D-glucose, we were thus prompted to examine the response of sulphonates of 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (*e.g.*, **1**) towards tetra-alkylammonium fluorides in dipolar aprotic solvents. 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose[19] (**2**) is now readily[19–21] available, and it appeared from molecular models that, in a nucleophilic displacement of a 3-sulphonate group, the attack of fluoride ion would be from the *exo*-side of the *cis*-fused tri-oxabicyclo[3.3.0]octane ring-system formed by the tetrahydrofuran ring and the 1,2-*O*-isopropylidene group, and steric hindrance would be minimal. The opposite situation obtains for 3-sulphonates of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, which are strongly resistant towards attack by nucleophiles[22].



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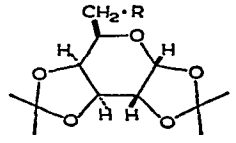
2,R = H



3, R = OTs
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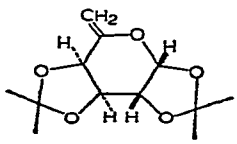
1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**2**) was prepared by oxidation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose with phosphorus pentaoxide in methyl sulphoxide[20], followed by reduction of the resulting ketone with sodium borohydride[19]. A lower yield of ketone was obtained with methyl sulphoxide–acetic anhydride[21], but this reagent was more convenient to use. Sulphonylation of the allose derivative (**2**) proceeded readily on treatment with toluene-*p*-sulphonyl chloride in pyridine at room temperature.

In preliminary experiments, 1,2:3,4-di-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-galactopyranose (**3**) was used as a model compound and was treated with tetrabutylammonium fluoride variously in ethylene glycol (reaction temperature, 100°), tetrahydrofuran (80°), acetone (56°), *N*,*N*-dimethylformamide (37°, 50–60°), and acetonitrile (37°, 63–67°, 81–83°). Surprisingly, no reaction occurred in ethylene glycol

after 22 h, but, in all other cases, a mixture of products was obtained which contained components having retention times in gas–liquid chromatography identical with those of 6-deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[4] (**4**) and 6-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*arabino*-hex-5-enopyranose[23a] (**5**). In those cases where reaction temperature was varied, the proportion of olefin (**5**) in the product mixture was higher at the elevated temperature; the fluoro derivative (**4**) was stable under the reaction conditions. Acetonitrile appeared to be the most suitable solvent; thus, after reaction for 10 h at 81–83° with a six-fold excess of tetrabutylammonium fluoride, the yield of fluoride **4** was 69%, and the ratio of olefin to fluoride was *ca.* 1:2.2.



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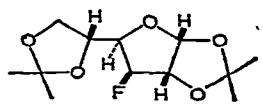

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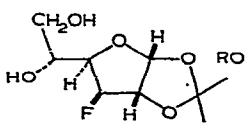
1,2:5,6-Di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose (**1**) reacted slowly with tetrabutylammonium fluoride in acetonitrile at 70–80° (completion of reaction required 3.5 days), but 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**) was subsequently isolated in 74% yield after column chromatography and distillation. Under essentially similar conditions of reaction, 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose gave 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose[23b] (**10**) as the preponderant product. Thus, in devising syntheses of carbohydrate secondary fluorides based on fluoride displacements, careful attention must be paid to the steric environment of the carbon atom carrying the leaving group. Other examples of fluoride displacements are being studied.



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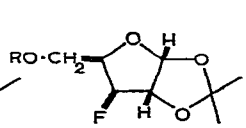
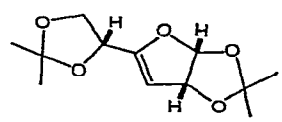


8, R = H
9, R = Ts



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On mechanistic grounds and by analogy, compound **6** would be expected to have the D-*gluco* configuration. Thus, the toluene-*p*-sulphonate **1** reacts with sodium azide in *N*,*N*-dimethylformamide to give 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose[24]. The ^{19}F n.m.r. spectrum (Perkin–Elmer R-10, $CDCl_3$) of 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, which was consistent with the assigned structure, contained 8 signals (centre-of-gravity ∅, +207.8 p.p.m. with respect to CCl_3F) having the following coupling constants J_{H_3F} 49.8, J_{H_4F} 29.8, and J_{H_2F} 10.8 Hz. The ^1H-spectrum ($CHCl_3$, internal tetramethylsilane)

showed, *inter alia*, a doublet at τ 4.06 (J 3.8 Hz) for the anomeric proton, and the low-field part of the signal for H-3 appeared at τ 4.60.*

The D-*gluco* configuration of compound **6** was established unequivocally by the following sequence of reactions. Graded hydrolysis of compound **6** with sulphuric acid in *p*-dioxane–methanol cleaved the 5,6-acetal group and gave syrupy 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (**7**) which was characterised as the 5,6-benzeneboronate and the 5,6-carbonate. Treatment of the diol (**7**), in sequence, with sodium periodate and sodium borohydride gave syrupy 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranose (**8**) which was characterised as the 5-toluene-*p*-sulphonate (**9**). Acidic hydrolysis of compound **8** afforded 3-deoxy-3-fluoro-D-xylose, which was identical with the authentic compound[11], and the derived 2,6-dichlorophenylhydrazone was identical with the compound reported by Taylor *et al.*[10b]. The establishment of the D-*xylo* configuration for compound **8** requires that compound **6** has the D-*gluco* configuration.

Hydrolysis of compound **6** with hot N sulphuric acid or, preferably, with a hot, aqueous suspension of Amberlite IR-120 (H^+) gave 3-deoxy-3-fluoro-D-glucose. This was non-crystalline but was homogeneous in paper chromatography, and its mobility and detection characteristics were identical with those of the authentic compound described by Johansson and Lindberg[12]. 3-Deoxy-3-fluoro-D-glucose gave a crystalline β-tetra-acetate on treatment with sodium acetate and hot acetic anhydride. The stability of the fluorine atom under other reaction conditions common in carbohydrate chemistry was exemplified by the fact that acid-catalysed acetonation of 3-deoxy-3-fluoro-D-glucose, followed by graded acidic hydrolysis of the resulting diacetal, gave 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (**7**). When a similar reaction sequence was performed with cyclohexanone, a mono-acetal was obtained, which is provisionally assigned the structure 1,2-*O*-cyclohexylidene-3-deoxy-3-fluoro-α-D-glucofuranose.

The yields in the conversion[19–21] of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose into the D-allose isomer (**2**) and in the various stages of the subsequent reaction sequence **2**→**1**→**6**→3-deoxy-3-fluoro-D-glucose are uniformly high (overall yield from D-glucose, 15–18%), and the route provides a more ready access to the final product than does the original method[12]. The key stages in the latter route involved treatment of methyl 2,3-anhydro-4,6-di-*O*-methyl-α-D-allopyranoside with boron trifluoride–hydrogen fluoride at −70° to give mainly 2-deoxy-2-fluoro-4,6-di-*O*-methyl-D-altropyranosyl fluoride, together with a small proportion of 3-deoxy-3-fluoro-4,6-di-*O*-methyl-D-glucopyranosyl fluoride, from which the deoxyfluoro sugars were subsequently obtained.

The biological properties of 3-deoxy-3-fluoro-D-xylose and 3-deoxy-3-fluoro-D-glucose are being investigated and will be reported elsewhere.

*A detailed analysis of the n.m.r. spectra of the fluorine-containing sugar derivatives described in this paper will be published elsewhere.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was performed on glass plates (3.25 in sq.) with Kieselgel G (Merck, 7731) and detection with iodine vapour or conc. sulphuric acid.

Treatment of 1,2:3,4-di-O-isopropylidene-6-O-toluene-p-sulphonyl-α-D-galactopyranose with fluoride. — A solution of the title compound (**3**, 0.5 g) and tetrabutylammonium fluoride (2 g, *ca.* 6 mol.) in acetonitrile (2 ml) was boiled under reflux, and monitored by t.l.c. (carbon tetrachloride–ether, 9:1). After 10 h, reaction was complete, and a product having an R_F value identical with that of 6-deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[4] was detected. The mixture was diluted with water and extracted with ether in the usual manner. The combined and dried ($MgSO_4$) extracts were concentrated, and the residue was distilled to give a product (0.32 g), b.p. 95–140° (bath)/0.1 mm, ν_{max}^{liquid} 1660 cm^{-1} (C=C), which, on examination by g.l.c. [Pye-Argon chromatograph, poly(ethyleneglycol adipate) on Celite, 150°], showed two components, with retention times of *ca.* 15 and 19 min, corresponding to the olefin **5** and the fluoride **4**.

A series of experiments was then carried out with variation of solvents, temperature, and reaction time. The products were isolated essentially as described above and analysed by g.l.c. by comparison with mixtures, of known composition, containing compounds **4** and **5**. The results are shown in Table I.

TABLE I

TREATMENT OF 1,2:3,4-DI-*O*-ISOPROPYLIDENE-6-*O*-TOLUENE-*p*-SULPHONYL-α-D-GALACTOPYRANOSE WITH TETRABUTYLAMMONIUM FLUORIDE

Solvent	*Temp. (degrees)*	*Reaction time*	*Molar excess of Bu_4NF*	*Ratio of fluoride (4): olefin (5)*	*Yield of fluoride 4 (%)*
Ethylene glycol	100	22 h	5	No reaction	No reaction
Tetrahydrofuran	80	24 h	5	1.4:1	29
Acetone	56	16 h	9	1.6:1	[a]
Acetonitrile	81–83	10 h	6	2.2:1	69
	63–67	3.5 days	9	3.7:1	61
	37	9 days	6	5.5:1	39
	37	23 days	15	3.2:1	64
N,N-Dimethylformamide	50–60	3.5 days	6	3.2:1	53
	37	13.5 days	6	3.0:1	45

[a]Additional products formed.

A mixture of 6-deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (0.17 g), tetrabutylammonium fluoride (2 g), and acetonitrile (2 ml) was stored for 11 days at 37°. The mixture was then processed as described above, and the product was examined by g.l.c.; no trace of the olefin **5** could be detected.

1,2:5,6-Di-O-isopropylidene-3-O-toluene-p-sulphonyl-α-D-allofuranose. — 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose[19,20] [7.7 g, m.p. 74–75°, $[\alpha]_D$ +37° (chloroform)]

was sulphonylated in the usual manner with toluene-*p*-sulphonyl chloride (7.7 g, 1.24 mol.) and pyridine (77 ml, dried by repeated distillation from phosphorus pentaoxide) for 21 h at room temperature. Ice–water (10 ml) was added, and, after a few min, the mixture was poured into ice–water (1.5 l). Recrystallisation of the product from ethanol–hexane yielded the title compound (8.8 g, 72%), m.p. 120–121°, $[\alpha]_D$ +87° (*c* 1.0, chloroform) (Found: C, 54.75; H, 6.3; S, 8.0. $C_{19}H_{26}O_8S$ calc.: C, 55.1; H, 6.3; S, 7.7%).

*3-Deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-*D*-glucofuranose.* — Tetrabutylammonium fluoride[25] was prepared by titration of 40% aqueous tetrabutylammonium hydroxide with 20% aqueous hydrofluoric acid to pH 7. The solution was concentrated under reduced pressure, and the resulting syrup was dried and stored over phosphorus pentaoxide at 0.1 mm. Dehydration of the fluoride by prolonged azeotropic distillation with benzene causes decomposition.

A mixture of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose (40 g), tetrabutylammonium fluoride (136 g, 5.4 mol.), and acetonitrile (200 ml, distilled from phosphorus pentaoxide) was kept under reflux at 70–80°, and monitored by t.l.c. (benzene–ether, 9:1). After 3.5 days, reaction appeared to be complete, and the mixture was poured into ether (500 ml) and washed with water (2 × 200 ml). The ethereal layer was dried ($MgSO_4$), and concentrated under diminished pressure, and the syrupy residue was submitted to column chromatography on silica gel (Hopkin & Williams). Elution with benzene–ether (9:1) yielded 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (18.4 g, 71%), b.p. 66–70°/0.03 mm, $[\alpha]_D^{30}$ −22° (*c* 1, chloroform) (Found: C, 55.5; H, 7.3; F, 7.6. $C_{12}H_{19}FO_5$ calc.: C, 55.0; H, 7.3; F, 7.3%).

3-Deoxy-3-fluoro-D-glucose. — 3-Deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (1 g) was heated under reflux with N sulphuric acid (50 ml) for 1 h; t.l.c. then showed reaction to be complete. The hydrolysate was neutralised ($BaCO_3$), and concentrated under diminished pressure, and the residue was eluted from Kieselgel (Merck, 7734) with ethyl acetate–ethanol (9:1) to yield syrupy, chromatographically homogeneous 3-deoxy-3-fluoro-D-glucose (0.4 g, 57%), $[\alpha]_D$ +47° (*c* 0.7, water), R_F 0.4 (t.l.c., ethyl acetate–ethanol, 4:1) and R_G 2.1 (chromatography on Whatman No. 1 paper, organic phase of butanol–ethanol–water, 4:1:5). The mobility in paper chromatography and response to aniline hydrogen phthalate[26] were identical with those of 3-deoxy-3-fluoro-D-glucose described by Johansson and Lindberg[12].

An alternative and more convenient procedure for hydrolysis was as follows. A solution of the diacetal (1.2 g) in ethanol (10 ml) and water (50 ml) was stirred at 60–70° with Amberlite IR-120 (H^+, *ca.* 15 ml). The hydrolysis was monitored by t.l.c. (ethyl acetate) and shown to be complete in 8 h. Concentration of the filtered solution gave chromatographically homogeneous 3-deoxy-3-fluoro-D-glucose (0.8 g, 96%). The foregoing product (0.39 g) was treated with a boiling solution of sodium acetate (0.5 g) in acetic anhydride (7 ml) for 10 min. The product, isolated in the usual manner[27], was twice recrystallised from benzene–light petroleum (b.p. 60–80°) to give 1,2,4,6-tetra-*O*-acetyl-3-deoxy-3-fluoro-β-D-glucose, m.p. 119–120°, $[\alpha]_D^{20}$ −12°

(*c* 0.9, chloroform) (Found: C, 48.2; H, 5.4; F, 5.4. $C_{14}H_{19}FO_9$ calc.: C, 48.0; H, 5.4; F, 5.4%).

1,2-O-Cyclohexylidene-3-deoxy-3-fluoro-α-D-glucofuranose. — A mixture of 3-deoxy-3-fluoro-D-glucose (0.3 g), cyclohexanone (5 ml), and conc. sulphuric acid (0.25 ml) was stored at room temperature and monitored by t.l.c.; reaction was complete in 12 h. The mixture was extracted with heptane (10 ml), and the extract was concentrated under diminished pressure. The residue was subjected to graded hydrolysis by treatment with *p*-dioxane–methanol–5N sulphuric acid (6:3:1, 10 ml) for 5 h at room temperature and then neutralised with barium carbonate. The hydrolysate was concentrated under diminished pressure, and the residue was chromatographed on Kieselgel (Merck, 7734). Elution with benzene–methanol (9:1) yielded the title compound (0.25 g, 58%), m.p. 121–122° [from ethanol–light petroleum (b.p. 60–80°)], $[\alpha]_D^{25}$ −12° (*c* 0.7, chloroform) (Found: C, 54.9; H, 7.4; F, 7.3. $C_{12}H_{19}FO_5$ calc.: C, 55.0; H, 7.25; F, 7.3%).

3-Deoxy-3-fluoro-1,2-O-isopropylidene-α-D-glucose. — (*a*) A solution of 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucose (4.45 g) in *p*-dioxane–methanol–5N sulphuric acid (6:3:1, 126 ml) was stored at room temperature and monitored by t.l.c. (benzene–ether, 9:1). After 4 h, no starting material remained. The hydrolysate was neutralised ($BaCO_3$), and concentrated under diminished pressure, and the residue was distilled to yield 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (3.77 g, 83%), b.p. 124–126°/0.3–0.4 mm, m.p. 50–52° [from toluene–light petroleum (b.p. 60–80°)], $[\alpha]_D$ −18° (*c* 0.8, chloroform) (Found: C, 48.4; H, 7.0. $C_9H_{15}FO_5$ calc.: C, 48.6; H, 6.8%).

(*b*) A mixture of 3-deoxy-3-fluoro-D-glucose (0.5 g), dry acetone (20 ml), and conc. sulphuric acid (0.8 ml) was stored at room temperature; reaction appeared to be complete in 20 h (t.l.c.). The mixture was neutralised (K_2CO_3) and concentrated, and the syrupy residue was subjected to graded hydrolysis by storage at room temperature for 4 h with *p*-dioxane–methanol–5N sulphuric acid (6:3:1, 15 ml). After neutralisation ($BaCO_3$), and concentration under diminished pressure, the residue was eluted from Kieselgel (35 g, Merck, 7734) with benzene–methanol (9:1) to yield 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (0.38 g, 62%), m.p. 51–52° alone or in admixture with the product described in (*a*).

3-Deoxy-3-fluoro-1,2-O-isopropylidene-α-D-glucofuranose 5,6-benzeneboronate. — 3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (159 mg), benzeneboronic acid (87.5 mg, 1 mol.), and benzene (50 ml) were refluxed for 2 h in a Dean & Stark apparatus. Evaporation of the benzene, followed by recrystallisation of the product from light petroleum (b.p. 40–60°), yielded the title compound (150 mg, 68%), m.p. 115–116°, $[\alpha]_D$ −2° (*c* 2.1, chloroform) (Found: C, 58.2; H, 5.7; F, 6.4. $C_{15}H_{18}BFO_5$ calc.: C, 58.5; H, 5.8; F, 6.2%).

3-Deoxy-3-fluoro-1,2-O-isopropylidene-α-D-glucofuranose 5,6-carbonate. — Phosgene was bubbled for 1 h through a solution of 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (300 mg) in pyridine (15 ml) at 0°. Excess of phosgene was then destroyed with water (50 ml), the mixture was extracted with chloroform

(3 × 50 ml), and the combined extracts were dried ($MgSO_4$) and concentrated. Recrystallisation of the residue from ethanol–light petroleum (b.p. 60–80°) gave the title compound (300 mg, 90%), m.p. 84–85°, $[\alpha]_D^{25}$ −27° (*c* 1.0, chloroform) (Found: C, 48.7; H, 5.5; F, 8.0. $C_{10}H_{13}FO_6$ calc.: C, 48.4; H, 5.2; F, 7.7%).

3-Deoxy-3-fluoro-1,2-O-isopropylidene-α-D-xylofuranose. — To a solution of 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (6.5 g) and sodium hydrogen carbonate (2 g) in water (50 ml) was added sodium metaperiodate (8 g), portionwise with stirring during 1 h. After addition of excess of barium chloride solution and barium carbonate, the filtered solution was treated portionwise with sodium borohydride (10 g). After 1 h, the solution was extracted with chloroform (4 × 50 ml), the combined extracts were dried ($MgSO_4$), and concentrated under diminished pressure, and the residue was distilled to give 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranose (4.0 g, 71%), b.p. 95°/3–4 mm, $[\alpha]_D^{20}$ −20° (*c* 1.0, chloroform) (Found: C, 49.8; H, 6.8. $C_8H_{13}FO_4$ calc.: C, 50.0; H, 6.8%).

The toluene-*p*-sulphonate, prepared in the usual manner, had m.p. 60–61°, [from ethanol–light petroleum (b.p. 40–60°)], $[\alpha]_D$ −27° (*c* 2, chloroform) (Found: C, 52.1; H, 5.7; F, 5.7. $C_{15}H_{19}FO_6S$ calc.: C, 52.0; H, 5.5; F, 5.5%).

3-Deoxy-3-fluoro-D-xylose. — 3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranose (1 g) was heated under reflux with N sulphuric acid (25 ml) for 1 h. The cooled solution was neutralised ($BaCO_3$), and concentrated under diminished pressure. The residue was eluted from Kieselgel (Merck, 7734) with ethyl acetate, to give a chromatographically homogeneous syrup (0.53 g, 67%) that crystallised on seeding. Recrystallisation from ethanol gave 3-deoxy-3-fluoro-D-xylose, m.p. 134–136°, $[\alpha]_D^{20}$ +37° (*c* 0.3, water, equil.); lit.[11], m.p. 125–127°, $[\alpha]_D^{25}$ +70 → +38° (water). The i.r. spectra (Nujol) of the compounds were indistinguishable.

3-Deoxy-3-fluoro-D-xylose (100 mg) and 2,5-dichlorophenylhydrazine (130 mg) were dissolved in methanol (50 ml), and the solution was concentrated using a steam bath. Chromatography of the residue on Kieselgel (25 g, Merck, 7734), with elution by ethyl acetate, yielded the chromatographically homogeneous 2,5-dichlorophenylhydrazone, m.p. 64–66° (from methanol–water). A sample of the 2,5-dichlorophenylhydrazone[10b], kindly provided by Dr. N. F. Taylor and which had partially decomposed on storage, was purified by chromatography and recrystallised from methanol–water to give a product having m.p. 64–67°, alone or in admixture with the product described above. The i.r. spectra (Nujol) of the two 2,5-dichlorophenylhydrazones were indistinguishable. The 2,5-dichlorophenylhydrazone underwent partial decomposition on storage at room temperature for a few days.

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SUMMARY

Treatment of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose with tetrabutylammonium fluoride in acetonitrile gave 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**) in 74% yield. The structure of **6** was established by a multistage conversion into known 3-deoxy-3-fluoro-D-xylose. Graded hydrolysis of **6** with acid gave 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (**7**), and total hydrolysis gave 3-deoxy-3-fluoro-D-glucose (15–18% overall yield from D-glucose).

3-Deoxy-3-fluoro-D-glucose gave a crystalline β-tetra-acetate and was reconverted into **7** on treatment with acetone in the presence of acid, followed by graded hydrolysis with acid. In a similar manner, 1,2-*O*-cyclohexylidene-3-deoxy-3-fluoro-α-D-glucofuranose was obtained.

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PHENYLHYDRAZONO–PHENYLAZO TAUTOMERISM.

PART II. STRUCTURES OF 2-OXO-1,3-BIS(PHENYLHYDRAZONO) COMPOUNDS* AND RELATED COMPOUNDS

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INTRODUCTION

In prior publications[1,2], we reported the preparation, from *myo*-inositol, of *xylo*-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane (**1**) and showed that it exists in a red form and in a yellow form. In the preceding paper[2], we pointed out that certain previously known, crystalline, oxo-bis(phenylhydrazono) compounds, when prepared under mild conditions, are yellow, but change to dark red on standing or on heating in toluene, and we suggested that **1** and also these other compounds undergo a tautomeric reaction such as that depicted in Fig. 1. We further suggested

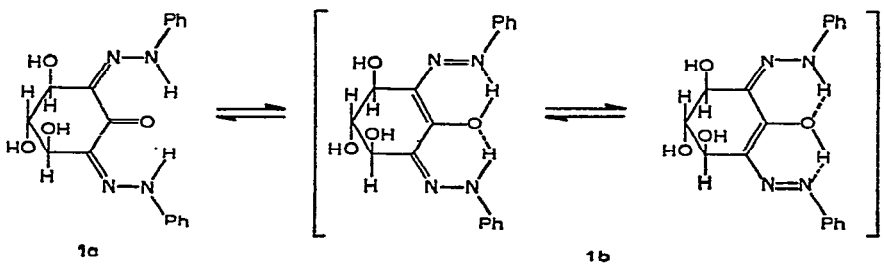


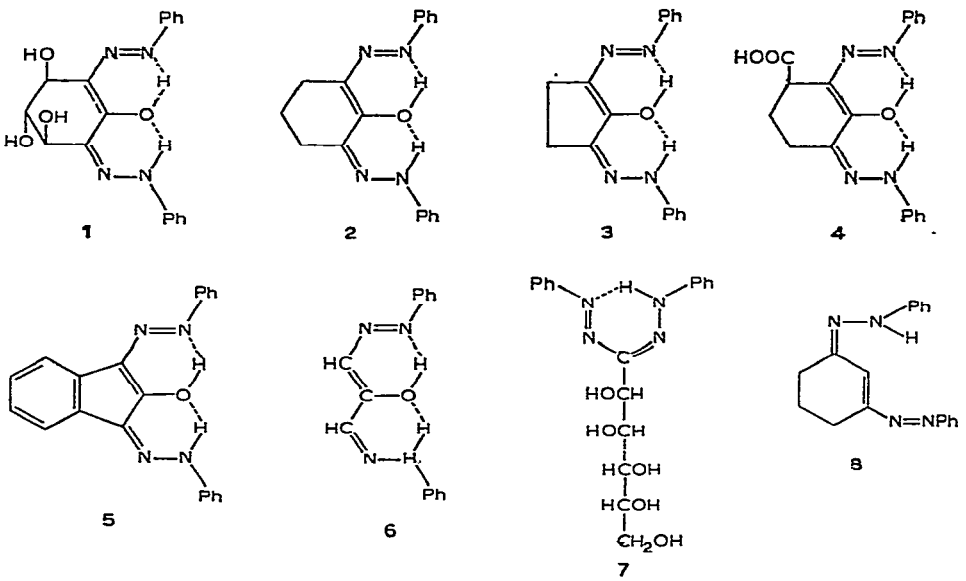
Fig. 1. Tautomerism of 2-oxo-1,3-bis(phenylhydrazones).

that the red forms have an enolic, phenylhydrazono–phenylazo resonance-structure (**1b**), whereas the yellow forms have a normal phenylhydrazono structure (**1a**).

We have now prepared and studied the following, previously known oxo-bis(phenylhydrazono) compounds: 2-oxo-1,3-bis(phenylhydrazono)cyclohexane[3] (**2**), 2-oxo-1,3-bis(phenylhydrazono)cyclopentane[4] (**3**), 3-oxo-2,4-bis(phenylhydrazono)-cyclohexane-1-carboxylic acid[5] (**4**), 2-oxo-1,3-bis(phenylhydrazono)indan[6] (**5**), and 2-oxo-1,3-bis(phenylhydrazono)propane[7,8,9] (**6**). The structures and properties of oxo-bis(phenylhydrazono) compounds have been the subject of numerous prior investigations[3–18], but heretofore, the structures of **1–6** have not been rigorously determined.

*For purposes of comparison, the compounds are considered as 2-oxo-1,3-bis(phenylhydrazono) derivatives, even though, in their systematic names, the groups may be otherwise designated.

In fact, **3** has been reported[19], without proof, to be a 3-oxo-1,2-bis(phenylhydrazono) compound, rather than a 2-oxo-1,3-bis(phenylhydrazono) compound. In the present paper, the structures of the compounds were studied by measurement of n.m.r. spectra and by comparison of their u.v. and visible spectra with those of compounds of known structure. Certain parameters for correlation of spectra and structure have been found, and specific structures have been assigned to the compounds of interest. For simplicity of representation, only one enolic structure for each of the compounds **1–6** is shown, although all possible enolic resonance forms corresponding to **1b** should be considered possible for the compounds.



DISCUSSION

Red and yellow forms of 2-oxo-1,3-bis(phenylhydrazono) compounds

The u. v. and visible spectra of the red forms of compounds **1–6**, given in Table I, show three main regions of absorption*, namely, at 243–251 nm (ε ~20,000), 282–289 nm (ε ~5,000), and 432–488 nm (ε ~35,000). The similarity of the absorption spectra indicates that all of the compounds have like chromophores, and consequently, similar structures for the phenylhydrazono moieties.

The deep-red color of the compounds, and especially the high intensity of the absorption band at 432–488 nm cannot be explained by the presence of a phenylhydrazono, or a simple phenylazo, chromophore[20]. The spectra suggest that each of the compounds has a phenylhydrazono group and a phenylazo group in a conjugated resonance structure. The diphenylformazans of sugars[21] have a phenylhydrazono

*The ultraviolet peaks are given as the wavelength (λ) in nanometers (nm), formerly called millimicrons (mμ); 1 nm = 10^{-9} m.

TABLE I

ULTRAVIOLET AND VISIBLE ABSORPTION BANDS[a], AND THEIR INTENSITIES, FOR PHENYLHYDRAZONO–PHENYLAZO COMPOUNDS AND RELATED COMPOUNDS

Compound	*Solvent*	λ_{max} (*in nm*) *and* ε_{max}[b]			
		Phenylhydrazono–phenylazo compounds			
1	EtOH	251 (18,800)		286 (sh) (6,000)	487 (34,300)
2	MeOH	249 (24,000)		289 (sh) (5,000)	470 (36,800)
3	MeOH	243 (18,000)	262 (sh) (12,500)	285 (sh) (4,200)	462 (54,800)
4	MeOH	250 (21,500)		282 (sh) (5,500)	470 (34,000)
5[c]	MeOH	233 (24,000)	263 (sh) (18,900)	288 (15,800)	488 (28,700)
6	MeOH	250 (20,200)		288 (sh) (6,000)	432 (3,300)
7	EtOH	257 (16,700)		292 (sh) (7,200)	441 (18,500)
8	EtOH	253 (22,000)		292 (sh) (8,100)	487 (30,000)
		Phenylhydrazono compounds			
9	EtOH	278 (23,500); 302 (sh) (10,200)			
10	MeOH	247 (sh) (9,700); 302 (10,500); 372 (48,600)			
11	MeOH	259 (24,200); 318 (10,800); 399 (21,900)			
14	MeOH	249 (16,700); 255 (sh) (15,100); 295 (24,500); 315 (28,700); 418 (23,500)			
		Phenylazo compounds			
12	MeOH	361 (4,500); 375 (4,400); 398 (sh) (2,250); 466 (sh) (1,500)			
13	MeOH	233 (11,000); 227 (12,600); 233 (10,800); 302 (23,800); 425 (350)			
		Tris(phenylhydrazono) compounds			
18	EtOH	238 (sh) (20,200); 261 (27,000); 296 (30,000); 322 (sh) (21,900); 392 (27,500); 455 (sh) (9,400)			
19	EtOH	258 (28,300); 293 (31,200); 316 (sh) (18,700); 418 (37,700); 465 (sh) (16,700)			
20	EtOH	237 (sh) (22,300); 261 (20,300); 291 (21,200); 324 (sh) (15,600); 390 (16,400); 452 (sh) (6,700)			
21	EtOH	237 (sh) (17,200); 251 (17,900); 302 (22,700); 344 (24,000); 408 (29,400)			

[a]The spectra were recorded 30 min after the sample had dissolved. [b]The molecular extinction coefficient is given in parentheses. [c]The spectrum of **5** is complicated by the existence of the polycyclic indan ring, but the bands at 233–263, 288, and 488 nm clearly indicate that the compound has the characteristic phenylhydrazono–phenylazo structure. In addition to the bands listed, the compound shows bands at 355 ($\varepsilon \sim 30{,}700$) and 430 nm (sh) ($\varepsilon \sim 15{,}100$).

group and a phenylazo group attached to the *same* carbon atom, presumably in a resonance state. Because of the similarity of the resonance structures, the 2-oxo-1,3-bis(phenylhydrazones) and the diphenylformazans of sugars would be expected to show similar u.v. and visible absorption. In agreement with this expectation, D-mannose *N,N*-diphenylformazan (**7**) gave three intense absorption bands in the regions characteristic of the red forms of compounds **1–6** (see Table I). The marked similarity in the absorption spectra of the 2-oxo-1,3-bis(phenylhydrazono) compounds and of the formazans clearly supports the enolic, phenylhydrazono–phenylazo structure proposed. The structure was further supported by comparison of the u. v. and visible spectra of the compounds with the spectrum of 1-phenylazo-3-phenylhydrazono-1-cyclohexene (**8**). This compound was originally prepared by Merling[22], who suggested that it has a phenylhydrazono group in conjugation with a phenylazo group. We have repeated the preparation of **8** and find that it shows u. v. and visible absorption bands similar to those listed for D-mannose *N,N*-diphenylformazan and for the red forms of compounds **1–6**.

Product **2**, prepared from 1,2,3-cyclohexanetrione and phenylhydrazine under mild conditions, crystallized from aqueous ethyl alcohol in the form of a yellow hydrate (**2a**). A freshly prepared, ice-cold solution of **2a** in aqueous ethyl alcohol shows an absorption band at about 400 nm that shifts rapidly to a longer wavelength (about 470 nm). The solution, after equilibration and evaporation, yields red crystals of **2b**. We could not obtain crystalline hydrates of other 2-oxo-bis(phenylhydrazono) compounds, but, in each instance, the product prepared under mild conditions was yellow or orange, and became dark red on recrystallization. The relative stability of the red and yellow forms of the several 2-oxo-1,3-bis(phenylhydrazones) seems to vary widely.

The bis(phenylhydrazone) of 1,2,3-cyclopentanetrione (**3**) separated from aqueous ethyl alcohol in orange-colored, anhydrous crystals, slightly soluble in toluene. The freshly prepared toluene solution is yellow-orange, but turns red on standing. Evaporation of the red solution gives dark-red crystals of the enolic form, **3b**. The products obtained by recrystallization from ethyl alcohol varied widely and appeared to be mixtures.

It has been reported by others[23–25] that the phenylhydrazono structure is ordinarily favored over the phenylazo structure. In solution, however, the relative stability of the two forms may depend on the formation of complexes and on the polarity of the solvent[26].

A comparison of the infrared spectra of the yellow hydrate **2a** and the red form **2b** reveals significant differences; thus, **2a** shows the following characteristic absorption bands*: 2.82 μm (O-H); 3.00 μm (N-H); 6.10 μm (C=N); and 6.48 μm (N-H bending). In marked contrast, **2b** does not show these bands, but shows a new band at 6.36 μm which may be attributed to the -N=N- vibration[27,30]. This absorp-

*The infrared absorption bands are given as the wavelength (λ) in micrometers (μm), formerly called microns (μ); 1 μm = 10^{-6} m.

tion band and the lack of absorption bands characteristic of C=O, C=N, and N-H (bending) support the enolic, phenylhydrazono–phenylazo structure proposed for **2b**.

The infrared spectra of the red forms of **1** and **3–6** all show a band at 6.32 to 6.44 μm, attributed to the -N=N- vibration, but they also show weak bands at 2.88 to 3.00 μm, corresponding to N-H vibrations, at 6.45 to 6.58 μm attributed to N-H bending[31,33], and a band at 6.10 μm (C=N). These bands are not shown by **2b** (in which the normal N-H vibrations may be restricted by the highly stable, chelate structure). It thus appears that **2b** was isolated in the pure, chelate form, whereas compounds **1** and **3–6** contain minute proportions of the hydrazono form.

The structure of compound **2** is particularly favorable for chelation, because the phenylhydrazono and oxo groups are in position to form a resonance structure without steric hindrance or ring strain. The steric factors are not quite so favorable for compounds **1**, **3**, **4**, **5**, and **6**, and this fact may account for the presence of N-H bands in their i.r. spectra. With compound **2**, the resonance energy may be sufficient to

TABLE II

NUCLEAR MAGNETIC RESONANCE SPECTRA OF 2-OXO-1,3-BIS(PHENYLHYDRAZONO) COMPOUNDS AND RELATED COMPOUNDS[a]

Compound	*Solvent*	*Chelated* N-H	*Nonchelated* N-H	*Other pertinent signals*
1	*p*-dioxane	14.30 12.32		
1	Me_2SO	14.40 14.28	10.86 11.42	
2	$CDCl_3$	13.98		
2	Me_2SO	14.46 14.12	10.00	
3	Me_2SO	13.70	10.30	
4	Me_2SO	14.30 14.00	10.43	10.20 (CO_2H)
5	Me_2SO	12.80		
6	*p*-dioxane	13.90	9.91	7.90 (methine)
7	*p*-dioxane	12.64		
10	Me_2SO	(12.60)[b]	10.45 (11.50)[b]	7.83 (methine)
11	Me_2SO	12.86	10.40	
14	*p*-dioxane	13.20	9.40	8.10 (methine) 9.50 (aldehyde)
14	Me_2SO	13.25	10.90	8.18 (methine) 9.50 (aldehyde)
18	$CDCl_3$	13.54 12.75		
19	*p*-dioxane	12.55 11.85	10.80	
20	Me_2SO	13.75 12.80	10.80	9.91 (CO_2H)
21	$CDCl_3$	(13.34)[b]		8.20 (methine)

[a]All resonance positions are given as p.p.m. downfield from internal tetramethylsilane (δ-scale); the average deviation is $\pm$0.1 p.p.m. [b]Signal observed after equilibration.

hybridize the phenylhydrazono–phenylazo moieties completely. Presumably, the electron-withdrawing power of the carbonyl group is a driving force for the formation of the resonance structure depicted in **1b**.

To obtain additional information concerning the structures, the n.m.r. spectra of the 2-oxo-1,3-bis(phenylhydrazono) compounds were compared with the spectra of compounds having somewhat similar features (see Table II). In each instance, the spectrum in *p*-dioxane, chloroform-*d*, or methyl sulfoxide shows a strong signal (12.55 to 14.46 p.p.m.) indicative of a chelated N-H group. Compound **2** was studied in considerable detail, because it does not have substituent groups that complicate interpretation of the spectrum, and it is readily prepared and purified.

The n.m.r. spectrum of compound **2** in chloroform-*d* (see Fig. 2) shows multiplets centered at 1.9 p.p.m. (the four protons on C-4 and C-6), at 2.62 p.p.m. (the two nonequivalent protons on C-5), and at 7.28 p.p.m. (the protons on the phenyl ring). The nonequivalence of protons on C-5 probably arises from axial and equatorial attachment and the spin–spin interaction with the protons on neighboring atoms C-4 and C-6. The spectrum also shows a strong signal at 13.98 p.p.m., indicative of an intramolecular bond between the imino hydrogen atom of the phenylhydrazono group and the carbonyl oxygen atom. The spectrum does not, however, show any signals typical of a free imino proton. This fact and the absence, in the infrared spectrum of **2**, of absorption characteristic of N-H, C=O, or O-H groups support the enolic phenylhydrazono–phenylazo structure in which the imino hydrogen atoms of the phenylhydrazono groups are alternately joined with the oxygen atom. An approximate calculation of the intensity of the n.m.r. signal of **2** at 13.98 p.p.m. in chloroform-*d* suggests that the two protons are involved but cannot be resolved. In a polar solvent, however, the two forms of the compound may exist as separate entities. In fact, the n.m.r. spectrum of **2** in methyl sulfoxide shows two low-field signals; a relatively strong signal at 14.46 and a weaker signal at 14.12 p.p.m. This result is in accordance with the chelate rings depicted in the structures shown in Fig. 1. Addition of a few drops of methyl sulfoxide to a probe of **2** in chloroform-*d* produces (by means of a solvent shift) two distinct peaks from the (otherwise submerged) low-field signal observed at 13.98 p.p.m. for **2** in chloroform-*d*.

Related phenylhydrazono and phenylazo compounds

Further insight into the structures of compounds **1–6** was obtained by study of certain compounds having related phenylhydrazono and phenylazo structures (formulas **9–14**).

myo-Inosose-2 phenylhydrazone (**9**) (a typical phenylhydrazone) shows two absorption bands in the u.v., and none in the visible region. Glyoxal bis(phenylhydrazone) (**10**), the simplest acyclic bis(phenylhydrazone), shows two bands in the u.v. region and one in the visible region at 372 nm[33,34]. The cyclic compound, D-*myo*-inosose-1 phenylosazone (**11**) also shows three bands, but at somewhat longer wavelengths than those for the acyclic compound **10**.

Simple phenylazo compounds give a low-intensity band at 380 to 415 nm

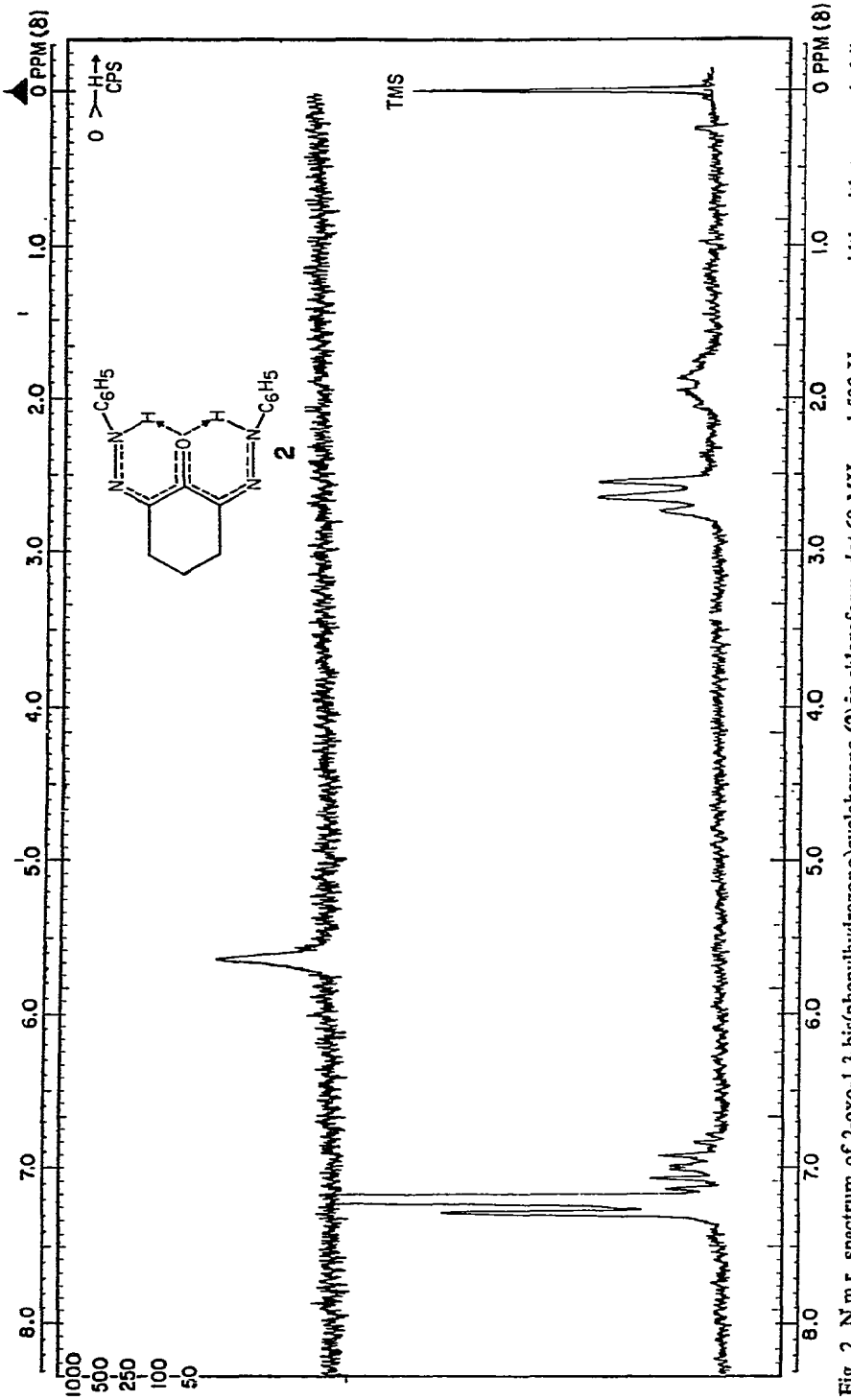
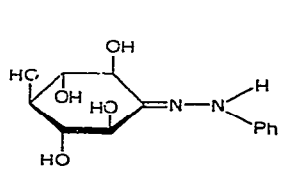


Fig. 2. N.m.r. spectrum of 2-oxo-1,3-bis(phenylhydrazono)cyclohexane (2) in chloroform-*d* at 60 MHz and 500-Hz sweep-width, with tetramethylsilane as internal standard. The upper trace is at a sweep-offset of 500 Hz.

($\varepsilon \sim 10$–250), but the presence of a conjugated double bond causes a bathochromic shift and an increase in the extinction coefficient. Thus, phenylazomethane and 1,2-diphenyl-1,2-bis(phenylazo)ethane[35] absorb at 403 and 404 nm, respectively. In



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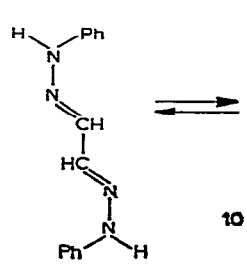

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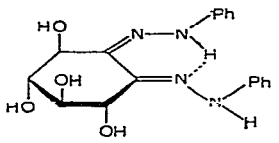

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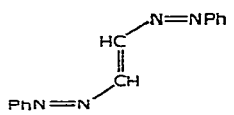

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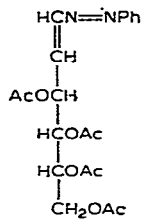

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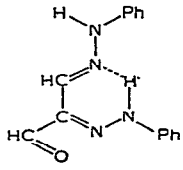


14

marked contrast, 1,2-bis(phenylazo)ethylene[36] (**12**) gives a high-intensity band at 466 nm ($\varepsilon \sim 1{,}500$). This is in accordance with the observations that the azo group is iso-π-electronic with the vinyl group[37] and that the conjugation of two electronically equivalent chromophores gives rise to a high-intensity band (K band)[38]. A conjugated phenylazo chromophore in compounds **1**–**6** may account, in like manner, for the high-intensity absorption band at 430–490 nm.

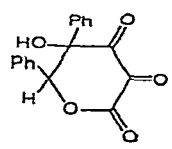
Comparison of the u.v. spectrum of the acyclic compound **6** with the spectra of the cyclic compounds **1**–**5** reveals that conjugation of a phenylazo group in the acyclic compound **6** produces less bathochromic shift and a lower extinction coefficient than conjugation in the cyclic compounds. Other conjugated azo structures show similar effects. Thus, the acyclic compound **13**, first prepared by Wolfrom and co-workers[39], shows a band at 425 nm ($\varepsilon \sim 350$), whereas the cyclic compound **8** shows a band at 487 nm ($\varepsilon \sim 30{,}000$). Likewise, in the visible region, compound **6** shows lower absorption than cyclic compounds **1**–**5**. In the latter compounds, the phenylhydrazono groups are held in a fixed and favorable position for forming a resonance structure, whereas, in the acyclic compound **6**, the phenylhydrazono groups may assume a less favorable position. Hence, the effect of conjugation is greater for the cyclic compounds.

In addition to a u.v. absorption band at 302 nm (previously reported for **13** by Wolfrom and co-workers[39]) and the band at 425 nm reported here, compound **13** shows a characteristic triplet: λ_{max}^{MeOH} 222.5 ($\varepsilon \sim 11{,}000$), 227.5 ($\varepsilon \sim 12{,}600$), and 233 nm ($\varepsilon \sim 10{,}800$). These characteristic bands may be used for detection of the structure $-C=C-N=N-Ph$ in other compounds.

The n.m.r. spectrum of **10** after equilibration (see Table II), in agreement with the observations of Chapman and co-workers[40], gave evidence for the existence of a chelated and a nonchelated N-H group. The n.m.r. spectrum of **11** in methyl sulfoxide shows a signal at 12.86 p.p.m., thus confirming the chelate ring-structure, analogous to the structures reported for the phenylosazones of the sugars[40–42].

The points of attachment of the phenylhydrazono groups in compounds **1** and **6** had previously been established[2,8,9] by reliable chemical methods; the phenylhydrazono groups in compounds **2**, **3**, **4**, and **5** are now assigned to C-1 and C-3 from the similarity of the spectra of the compounds to the spectra of compounds **1** and **6**. The compounds were prepared by treatment of the triones with phenylhydrazine and, in each instance, the phenylhydrazono groups could have entered at C-1 and C-2, or at C-1 and C-3. It was, therefore, of interest to study the spectra of a 3-oxo-1,2-bis(phenylhydrazono) derivative. For this purpose, we prepared mesoxalaldehyde 1,2-bis(phenylhydrazone) (**14**), a compound originally reported by Chargaff and Magasanik[43] and investigated by El Khadem and Abdel Rahman[44]. The u.v. and visible spectra of **14** were found to be in agreement with those reported by the above authors, except for a broad band at 251 nm not reported by them [44]. The spectrum of **14** in the visible region shows absorption bands at 315 and 418 nm, whereas the spectra of the 2-oxo-1,3-bis(phenylhydrazono) derivatives show a single band at 432–487 nm. The two bands at lower wavelength, instead of one band at higher wavelength, serve to distinguish the 3-oxo-1,2-bis(phenylhydrazono) from the 2-oxo-1,3-bis(phenylhydrazono) derivatives. The n.m.r. spectrum of **14** in methyl sulfoxide (and in *p*-dioxane) agreed with that of El Khadem and Abdel Rahman[44].

The properties of **14** are of particular interest with reference to certain phenylhydrazono compounds of unknown structure prepared from the following heterocyclic triones: tetrahydro-5-hydroxy-5,6-diphenylpyran-2,3,4-trione (**15**), alloxan **16**), and dehydro-L-ascorbic acid (**17**).



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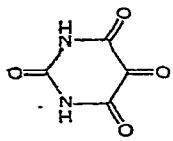

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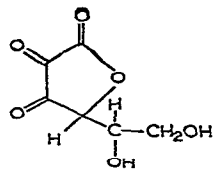

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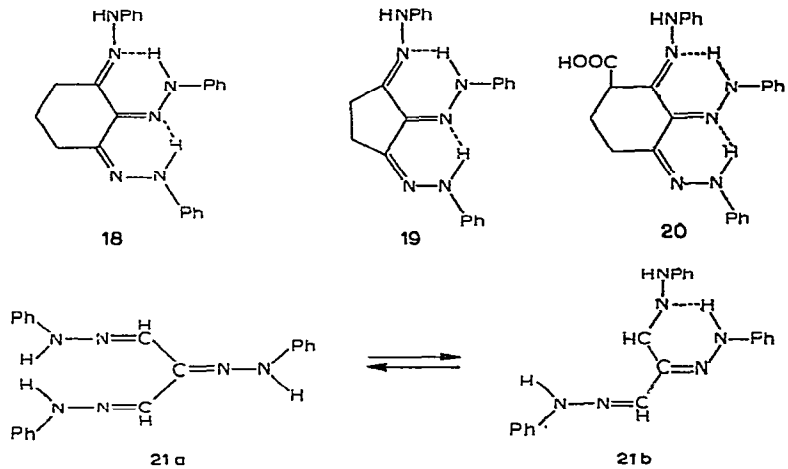
The red bis(phenylhydrazone) of **15** melted at 231–232°, as reported[16]; it showed λ_{max}^{EtOH} 238 (ε ~19,300), 261 (ε ~21,600), 293 (ε ~18,700), 356 (ε ~9,800), and 456 nm (ε ~ 16,500). However its i.r. spectrum showed strong carbonyl absorption at 5.82 and 5.98 μm. Treatment of the bis(phenylhydrazone) with phenylhydrazine at 130° produced a slightly pinkish, crystalline product, m.p. 268–270°, that differed widely from the yellow tris(phenylhydrazones) prepared from 1,2,3-triones (compounds **18–21**). Thus, the bis(phenylhydrazone) of **15** must have a structure different from the enolic phenylhydrazono–phenylazo structure of the red forms of compounds **1–6**.

Treatment of alloxan with phenylhydrazine gave a rearranged product presumably similar to the ring-contraction products reported to result from the reaction of alloxan with aliphatic or aromatic amines[45]. Treatment of dehydro-L-ascorbic acid with phenylhydrazine gave two products, in agreement with reported work[46]. Neither of these was analogous to the 1,3-bis(phenylhydrazones) **1**–**6**.

Tris(phenylhydrazono) compounds

To obtain a more complete understanding of chelated phenylhydrazono structures, the tris(phenylhydrazono) derivatives depicted were prepared. These derivatives are related, respectively, to the 2-oxo-1,3-bis(phenylhydrazono) compounds **2**–**4**, and **6**.



The n.m.r. spectrum of **18** in chloroform-*d* (see Fig. 3) shows two low-field signals, at 13.54 and 12.75 p.p.m., that are slowly exchangeable with deuterium (D_2O); these results indicate the presence of two chelated rings, as depicted in the formula. In chloroform-*d*, the signal for the free imino proton was submerged with the signals for the protons of the phenyl group.

The n.m.r. spectrum of **19** in *p*-dioxane shows signals at 12.55 and 11.85 p.p.m., ascribed to two chelate rings, and a signal at 10.80 p.p.m. readily exchangeable with deuterium at room temperature, which is ascribed to the nonchelated, imino proton. Compound **20** in methyl sulfoxide shows signals at 13.75 and 12.80 p.p.m. ascribed to the presence of two chelate rings, and a signal at 10.80 p.p.m. ascribed to a nonchelated, imino proton. The signal at 9.91 p.p.m. readily exchangeable with deuterium (D_2O) is ascribed to the proton of the carboxyl group.

El Khadem and Abdel Rahman[44] have reported that 1,2,3-tris(phenylhydrazono)propane (**21**) gives n.m.r. signals in methyl sulfoxide at 10.22, 10.83, and 12.51 p.p.m. corresponding, respectively, to the presence of two nonchelated, imino protons and one chelated proton. We find that the n.m.r. spectrum of **21** in chloroform-*d* does not show any low-field signals immediately after dissolution, but, after

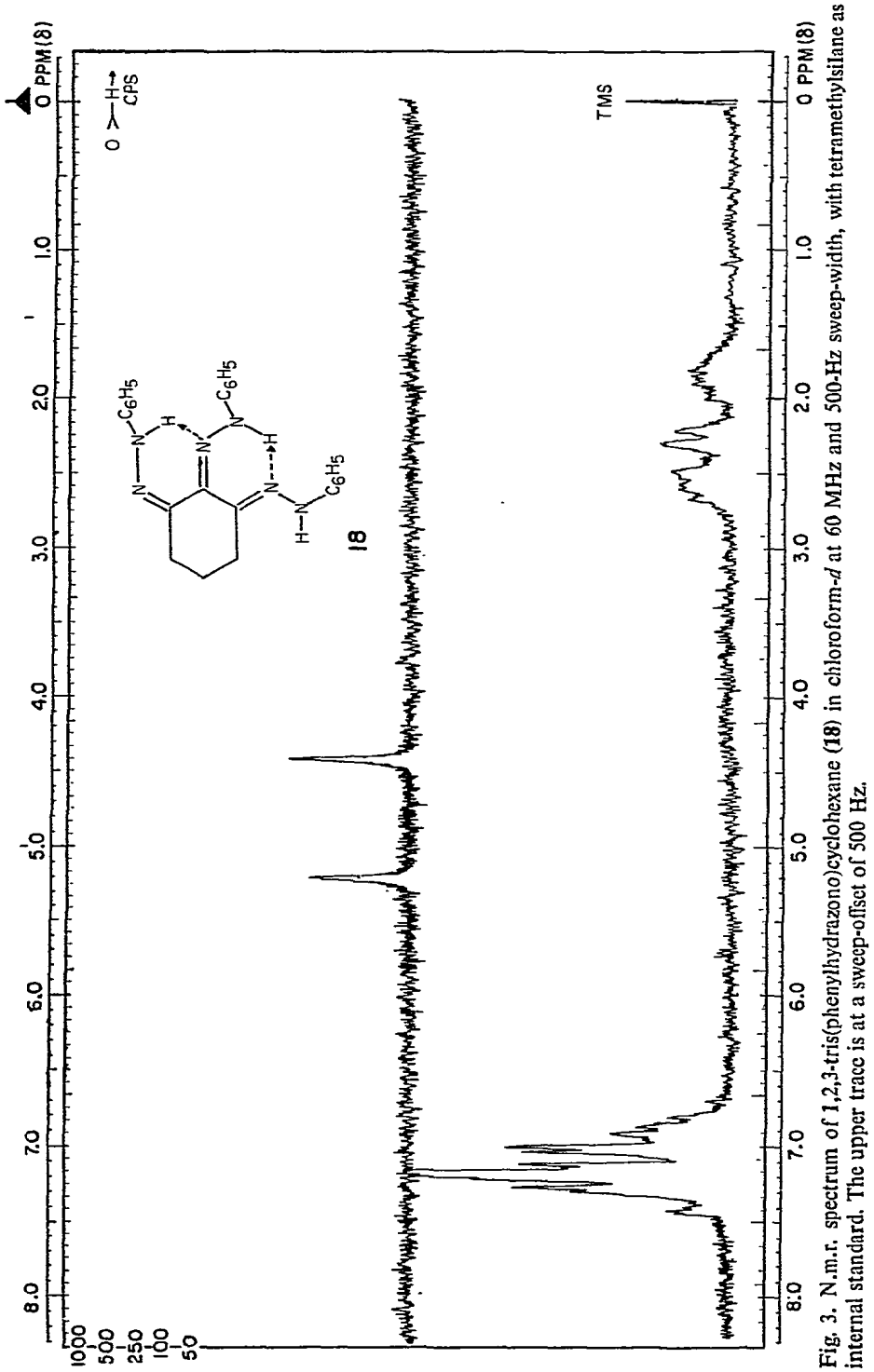


Fig. 3. N.m.r. spectrum of 1,2,3-tris(phenylhydrazono)cyclohexane (**18**) in chloroform-*d* at 60 MHz and 500-Hz sweep-width, with tetramethylsilane as internal standard. The upper trace is at a sweep-offset of 500 Hz.

a few minutes of equilibration, a weak, low-field signal develops at 13.34 p.p.m., indicating chelate-ring formation. The signal is not readily exchangeable with deuterium (D_2O) at room temperature; this suggests that **21** in chloroform-*d* may establish an equilibrium containing forms **21a** and **21b**, analogous to equilibrium mixtures reported by Chapman and co-workers[40] for certain 1,2-bis(phenylhydrazones).

The visible spectrum of **21** in ethanol shows a strong absorption band at 408 nm (see Table II) that may indicate that the compound undergoes further isomerization in polar solvents.

EXPERIMENTAL

Prior to analysis, all compounds were dried for 4 h at 25°/0.1 mm over phosphorus pentaoxide, unless otherwise stated. Melting points were observed in Pyrex* capillary tubes and are uncorrected. Ultraviolet and visible spectra were recorded with a Beckman DK-2 recording spectrophotometer*. Infrared spectra were determined in potassium chloride pellets, and in Nujol* or fluorocarbon mulls by use of a Perkin–Elmer "Infracord" Spectrophotometer, Model 137*; they are reproduced in a separate publication[47].

The n.m.r. spectra were measured with a Varian A-60 spectrophotometer* (sample temperature, 25–35°); all resonances are expressed as p.p.m. downfield from internal-reference tetramethylsilane (TMS) (δ-scale). *p*-Dioxane (commercial grade) was distilled from a small proportion of lithium aluminum hydride, and stored over molecular sieves prior to use. Methyl sulfoxide (reagent grade) was dried over molecular sieves for at least a week prior to use. Chloroform-*d* (commercial, spectrographic grade) and deuterium oxide were used without purification.

xylo-*4,5,6-Trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane* (**1**). — Compound **1** was prepared from "*xylo*-4,5,6-trihydroxycyclohexenediolic acid" by a procedure reported elsewhere[1,2]. Compound **1** failed to produce the expected tris-(phenylhydrazone) when heated with phenylhydrazine for 90 min at 145° by the procedure described for compound **18**.

2-Oxo-1,3-bis(phenylhydrazono)cyclohexane (red form) (**2b**). — To a solution of dihydropyrogallol (2,3-dihydroxy-2-cyclohexen-1-one, prepared by catalytic reduction of pyrogallol[3,48]) in 300 ml of 4% aqueous acetic acid was added a saturated solution of iodine [prepared from iodine and 1:4 (v/v) glacial acetic acid–methanol] until a trace of free iodine remained. The excess of iodine was removed by cautious addition of a small quantity of the parent compound, and the solution was filtered through a thin layer of a decolorizing carbon. The colorless solution, containing 1,2,3-cyclohexanetrione, was treated with 10 ml of phenylhydrazine in 10 ml of methanol at 60–70°. The resulting, cherry-red product was twice recrystallized from warm methanol.

*Mention in this article of certain commercial instruments or chemical compounds does not constitute endorsement by the National Bureau of Standards.

The yield of **2b**, as lustrous, dark-red prisms, was 0.35 g, m.p. 132–133°; lit.[3] m.p. 131–132.5°.

Anal. Calc. for $C_{18}H_{18}N_4O$: C, 70.56; H, 5.92; N, 18.29. Found: C, 70.28; H, 5.85; N, 18.09.

Hydrated 2-oxo-1,3-bis(phenylhydrazono)cyclohexane (**2a**). — Dihydropyrogallol (5 g) was dissolved in 300 ml of 4% aqueous acetic acid and oxidized with iodine by the procedure given for the preparation of **2b**. The colorless solution containing 1,2,3-cyclohexanetrione was cooled to 0°, and 15 ml of phenylhydrazine in 15 ml of methanol, and 8 g of sodium acetate triacetate in 50 ml of cold water, were added with stirring. After 30 min at 0°, the light-orange precipitate was removed, and washed with cold water. The damp, crude product was quickly dissolved in warm, 95% ethanol, and the solution was filtered, and cooled quickly. The resulting, light-yellow needles were collected by filtration, washed with cold, 50% aqueous ethanol, and dried for 3 h at 25°/0.1 mm; yield 7 g; m.p. 130° (red melt, apparently anhydrous **2b**).

Anal. Calc. for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.22; N, 17.27. Found: C, 66.67; H, 6.18; N, 17.40.

Recrystallization of the hydrate **2a** from hot ethanol yielded red, anhydrous **2b**. Stored in a closed bottle, the crystalline hydrate retained its yellow color for months without appreciable loss of water of crystallization or change in i.r. spectrum.

1,2,3-Tris(phenylhydrazono)cyclohexane (**18**). — A suspension of 0.15 g of crude **2b** and 2 ml of phenylhydrazine, in a test tube protected from the atmosphere, was heated in an oil bath for 1 h at 130–140°. The brownish yellow solution was then cooled, diluted with 2 ml of ethanol, and poured (with stirring) into 150 ml of cold, 4% aqueous acetic acid. The resulting bright-yellow precipitate was stirred for 1 h, filtered off, and washed with cold, 25% aqueous ethanol, to yield 100–200 mg of **18**; it was recrystallized from ethanol, or aqueous ethanol, and dried in a vacuum desiccator to constant weight; yellow needles, m.p. 185–186°, lit[3]. m.p. 186°.

Anal. Calc. for $C_{24}H_{24}N_6$: C, 72.70; H, 6.10; N, 21.20. Found: C, 72.77; H, 6.02; N, 21.04.

2-Oxo-1,3-bis(phenylhydrazono)cyclopentane (red form) (**3b**). — Reductic acid (2,3-dihydroxycyclopenten-1-one) (1 g) in 100 ml of 5% aqueous acetic acid was oxidized with iodine by essentially the procedure given for preparing **2**. The colorless solution containing 1,2,3-cyclopentanetrione was treated at 65–75° with 2 g of sodium acetate trihydrate and 2 ml of phenylhydrazine in 2 ml of methanol. The resulting 2-oxo-1,3-bis(phenylhydrazono)cyclopentane, brownish red leaflets, was recrystallized twice from hot ethanol and once from hot toluene. The product was dried for 2 h at 100°/0.1 mm; yield 1.1 g of lustrous, reddish bronze leaflets, m.p. 247–249°; lit.[4] m.p. 247.5–248.5°.

Anal. Calc. for $C_{17}H_{16}N_4O$: C, 69.84; H, 5.52; N, 19.17. Found: C, 69.70; H, 5.62; N, 19.42.

On heating compound **3b** with phenylhydrazine for 1 h at 145° by the procedure described for the preparation of **18**, 1,2,3-tris(phenylhydrazono)cyclopentane (**19**) was formed; yellow-orange prisms, m.p. 206–208°; lit.[4] m.p. 207–208°.

2-Oxo-1,3-bis(phenylhydrazono)cyclopentane (orange-yellow form) (**3***). — Reductic acid (2 g) in 75 ml of 4% aqueous acetic acid was oxidized with iodine by the procedure used for preparing the red form, **3b**. The clear solution containing 1,2,3-cyclopentanetrione was cooled to 0°, and 150 ml of crushed ice, 3 ml of phenylhydrazine in 3 ml of methanol, and 3 g of sodium acetate trihydrate, were successively added with continuous stirring. After 15 min at 0°, the orange-yellow crystals were separated by filtration, washed with aqueous methanol, and dried for 3 h at 25°/0.1 mm; the yield of **3*** was 3–3.5 g.

Anal. Calc. for $C_{17}H_{16}N_4O$: C, 69.84; H, 5.52; N, 19.17. Found: C, 69.68; H, 5.47; N, 19.35.

The i.r. spectrum of the orange-yellow form of **3*** was found to be essentially the same as that of the reddish bronze form **3b** (see Table II).

3-Oxo-2,4-bis(phenylhydrazono)cyclohexane-1-carboxylic acid (**4**). — The starting material, dihydrogallic acid, was prepared according to the procedure of Mayer and co-workers[5], except for the isolation of the product, which was conducted as follows. The crude product (80–85 g) obtained by reduction of 100 g of gallic acid was suspended in 150 ml of ice–water, and the suspension was vigorously stirred for 20 min at 3–5°, and filtered. The filtrate, on being kept for 24 h at 3°, deposited crystals. The process was repeated with the original, crude mixture in 150 ml of ice–water. The crystalline product from two extractions was combined, and dissolved in 150–175 ml of warm water; the solution was decolorized with carbon, the suspension filtered, and the filtrate kept for 24–48 h at room temperature. The thick prisms of dihydrogallic acid that separated were washed with ice-cold water, and dried *in vacuo* at room temperature; yield 6–8 g, m.p. 192–193°; lit.[5] m.p. 192–193°. By concentration of the mother liquor and further extraction, the yield of product was raised to 10–12 g.

For the preparation of **4**, 1 g of dihydrogallic acid was oxidized with iodine, and the resulting trione was condensed with phenylhydrazine at 80–90° by the procedure given for the preparation of **2**. The resulting, orange-red product (1.3 g) was recrystallized twice from warm ethyl acetate; m.p. 222–223°; lit.[5] m.p. 221–223°.

Anal. Calc. for $C_{19}H_{18}N_4O_3$: C, 65.15; H, 5.18; N, 15.98. Found: C, 65.20; H, 5.10; N, 15.88.

On heating compound **4** with phenylhydrazine for 90 min at 155–160°, 2,3,4-tris(phenylhydrazono)cyclohexanecarboxylic acid (**20**) was formed; yellow prisms (ethanol), m.p. 232–234°; lit.[5] m.p. 234–235°.

Anal. Calc. for $C_{25}H_{24}N_6O_2$: C, 68.16; H, 5.49; N, 19.08. Found: C, 68.28; H, 5.66; N. 19.32.

(2-Oxo-1,3-bis(phenylhydrazono)indan (**5**). — Compound **5** was prepared by the reaction of ninhydrin (1,2,3-indantrione monohydrate) with phenylhydrazine in 75% aqueous acetic acid by the method of Ruhemann[6]. Crude **5** was twice recrystallized from hot ethanol (950 ml per g); orange-red needles, m.p. 207–209°; lit.[6] 207–208°.

*Possibly a mixture of two anhydrous forms.

Anal. Calc. for $C_{21}H_{16}N_4O$: C, 74.10; H, 4.74; N, 16.46. Found: 74.04; H, 4.60; N, 16.60.

When heated with phenylhydrazine for 90 min. at 155°, compound **5** failed to give the expected tris(phenylhydrazone).

2-Oxo-1,3-bis(phenylhydrazono)propane (**6**). — 3-Oxoglutaric acid, prepared by the method of Adams and co-workers[49], was oxidized with benzenediazonium chloride by published procedures with slight modification. An ice-cold solution of benzenediazonium chloride (from 18.7 g of aniline, 80 ml of 6N hydrochloric acid, and 14 g of sodium nitrite in 400 ml of water) was added dropwise, with stirring at 0°, to 16 g of 3-oxoglutaric acid in 80 ml of water. Sodium acetate trihydrate (55 g) in 100 ml of water was then added, and the temperature was slowly raised to 50°. Rapid evolution of carbon dioxide occurred, followed by crystallization of **6**. For analysis, and study of its spectra, the compound was recrystallized from acetone; m.p. 174–175°; lit.[7] m.p. 171–173°, lit.[9] m.p. 175–176°.

Anal. Calc. for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.85; H 5.45; N, 21.20.

When compound **6** was heated with phenylhydrazine for 1 h at 130°, 1,2,3-tris-(phenylhydrazono)propane (**21**) was formed; after recrystallization from ethanol, it had m.p. 166°; lit.[43] m.p. 167°, lit.[44] m.p. 168°.

D-*Mannose diphenylformazan* (**7**). — This compound, m.p. 174–175°, was prepared and purified according to the procedure of Mester[21].

1-Phenylazo-3-phenylhydrazonocyclohexene (**8**). — The compound was prepared by the procedure of Merling[22], and purified by column chromatography [silica gel, 1:1 (v/v) acetic acid–benzene].

myo-*Inosose-2 phenylhydrazone* (**9**). — The compound was prepared by treatment of *myo*-inosose-2 with phenylhydrazine by the procedure of Posternak[50]; recrystallization from methanol yielded colorless crystals, m.p. 174–176°; lit.[51] m.p. 174–176°.

Glyoxal 1,2-bis(phenylhydrazone) (**10**). — The compound was prepared by the standard method from commercial, 50% aqueous glyoxal and phenylhydrazine at room temperature. The crude product was recrystallized several times from methanol; lustrous crystals, m.p. 169–170°; lit.[51] m.p. 169–170°.

D-myo-*Inosose-1 phenylosazone* (**11**). — D-*myo*-Inosose-1 was prepared by catalytic oxidation of *levo*-inositol[52], and converted into D-*myo*-inosose-1 phenylhydrazone. From the latter compound, the phenylosazone was prepared by oxidation with air in the presence of phenylhydrazinium acetate, as described by Carter and co-workers[53] and by Magasanik and Chargaff[54] for the preparation of the corresponding L isomer. The crude product was purified by recrystallization from 2:1:5 (v/v) water–pyridine–2-methoxyethanol, to give yellow needles, m.p. 207–209°; lit.[54] m.p. 217°; 210°.

1,2-Bis(phenylazo)ethylene (**12**). — Compound **12** was prepared by a slight modification of the procedure of von Pechmann[55]. A mixture of 5 g of **10**, 6 g of potassium dichromate, and 5 ml of glacial acetic acid in 50 ml of water was rapidly

heated to 95°, and kept at this temperature for 5 min. Heating was discontinued, and the reaction mixture was kept for 24 h at room temperature. The dark product was filtered off, washed with water, and dried. It was triturated with cold acetone (25 ml), and the suspension was filtered, to yield 0.8–1.0 g of crude product. Purification by column chromatography (neutral alumina, chloroform), followed by recrystallization from methanol and acetone, yielded lustrous, dark-red plates, m.p. 149–150°; lit.[55] m.p. 152°, lit.[36] m.p. 150°.

6-Phenylazo-trans-*5-hexene*-D-lyxo-*1,2,3,4-tetrol tetraacetate* (**13**) (D-arabino-*3,4,5,6-tetraacetoxy-1-phenylazo*-trans-*1-hexene*). — This compound was prepared by the procedure of Wolfrom and co-workers[28].

Mesoxalaldehyde 1,2-bis(phenylhydrazone) (**14**). — The compound was prepared according to the procedure of Chargaff and Magasanik[43], and was recrystallized from aqueous ethanol, to give light-orange–yellow needles, m.p. 196–198° (dec), lit.[43] m.p. 198°.

Bis(phenylhydrazone) of tetrahydro-5-hydroxy-5,6-diphenylpyran-2,3,4-trione. — The bis(phenylhydrazone) of **15**, having an unknown structure, was prepared, by the method of Dahn and Hauth[16], from **15** and phenylhydrazine. The reddish orange product was purified by recrystallization from absolute ethanol, to give orange-red crystals, m.p. 230–231° (dec); lit.[16] m.p. 230–232°.

Anal. Calc. for $C_{29}H_{24}N_4O_3$: C, 73.09; H, 5.08; N, 11.76. Found: C, 72.90; H, 5.22; N, 11.70.

SUMMARY

The structures of *xylo*-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane (**1**), 2-oxo-1,3-bis(phenylhydrazono)cyclohexane (**2**), 2-oxo-1,3-bis(phenylhydrazono)cyclopentane (**3**), 3-oxo-2,4-bis(phenylhydrazono)cyclohexane-1-carboxylic acid (**4**), 2-oxo-1,3-bis(phenylhydrazono)indan (**5**), and 2-oxo-1,3-bis(phenylhydrazono)propane (**6**) were studied by comparison of their n.m.r., u.v., visible, and i.r. spectra with the spectra of reference compounds of known structures. The results showed that compounds **1–6** exist in two forms. The red forms have an enolic (chelated) phenylhydrazono–phenylazo structure. A similarity of the compounds to the diphenylformazans is noted, and substantiated by a comparison of absorption spectra. Compound **2** crystallizes from aqueous ethyl alcohol in the form of a yellow hydrate shown to have a phenylhydrazono structure. On dehydration, the yellow hydrate yields the red enolic form.

The n.m.r. spectra of 1,2,3-tris(phenylhydrazono) derivatives of cyclohexane, cyclopentane, and cyclohexanecarboxylic acid show two low-field signals indicative of two chelated rings. Tris(phenylhydrazono)propane in chloroform-*d* shows no low-field n.m.r. signals immediately after dissolution, but, after equilibration, shows a weak, low-field signal; this suggests that the substance may establish an equilibrium analogous to the equilibria reported by others for phenylosazones.

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N-GLYKOSYL-DERIVATE

TEIL XIII. DER NACHTRÄGLICHE AUSBAU DES AGLYKONS. SYNTHESE VON *N*-GLYKOSYL-DERIVATEN DES 2-AMINO-THIAZOLS, 2-AMINO-1,3,4-THIADIAZOLS UND 5-AMINO-1,2,3,4-THIATRIAZOLS

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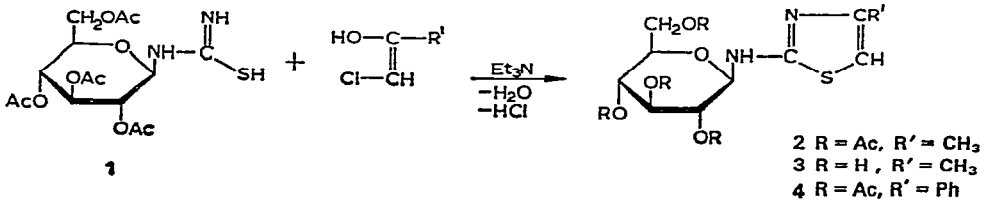
Die Herstellung der *N*-Glykosyl-Derivate lässt sich im allgemeinen mit Methoden durchführen, bei denen freie Kohlenhydrate bzw. ihre entsprechenden Abkömmlinge mit primären oder sekunderen Aminen kondensiert werden[1,2].

In manchen Fällen ist aber der Ablauf der Kondensation nicht eindeutig, oder die Ausbeuten sind unbefriedigend, zumal es oft unmöglich ist, auf diesem Wege das gewünschte *N*-Glykosyl-Derivat zu synthetisieren. Verhältnismässig gering ist die Zahl jener Verfahren, bei denen die Glykosylierung nicht mit dem fertigen Aglykon, sondern mit einer einfacheren Verbindung durchgeführt wird, und der Aglykon-Teil der so erhaltenen Verbindung sich dann mit darauffolgendem Ringschluss zum gewünschten Produkt umsetzen lässt, wobei die *N*-Glykosyl-Bindung unberührt bleibt. Als hervorragendes Ergebnis dieses Prinzips gilt die Synthese des Adenosins, die Todd und seine Mitarbeiter in 1949 verwirklicht haben[3]. In jüngster Zeit haben Rousseau *et al.* über die Synthese des 7-β-D-Ribofuranosyl-adenins berichtet[4]. Todd und seine Mitarbeiter haben von Ribofuranosyl-amino-pyrimidin, Rousseau *et al.* von Imidazol-Ribofuranosid ausgehend das Aglykon ausgebaut, so sind sie zum 9-, bzw. 7-β-D-Ribofuranosyl-adenin gekommen.

Der nachträgliche Ausbau des Aglykons kann aus Tetra-*O*-acetyl-D-glucopyranosyl-isocyanat[5], Tetra-*O*-acetyl-D-glucopyranosyl-isothiocyanat[6], Glykosyl-harnstoff[7], Glykosyl-thioharnstoff[8] usw. geschehen, sogar auch durch Substitution bzw. durch weitere Umsetzung von Glykosylaminen mit freier Aminogruppe[9–11].

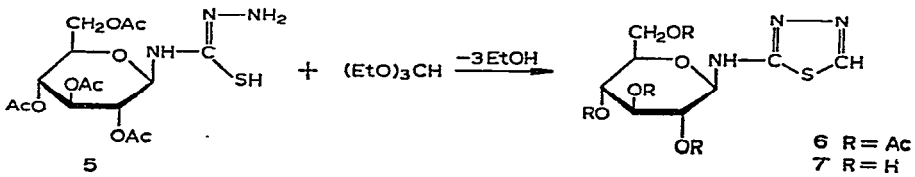
Mit der Ausbreitung des obigen Prinzips, mit dem nachträglichen Ausbau des Aglykon-Teiles der *N*-Glykosyl-Derivate gelang es uns, die *N*-Glykosyl-Derivate der Amino-Abkömmlinge des Thiazols, des 1,3,4-Thiadiazols und des 1,2,3,4-Thiatriazols herzustellen. Weiterhin war es möglich, mehrere, bisher unbekannte Glykosylamin-Abkömmlinge zu synthetisieren, die wir mit der unmittelbaren Kondensation der Komponenten nicht darstellen konnten.

Unsere Ergebnisse geben wir im folgenden bekannt. Zur Herstellung des 2-Glykosylamino-4-methyl-thiazols kondensierten wir Tetra-*O*-acetyl-D-glucopyranosyl-thioharnstoff (**1**) mit Chloraceton in Anwesenheit von Triäthylamin in Äthylacetat-Lösung und das am Zuckerteil acetylierte, amorphe Produkt **2** verseiften wir mittels alkoholischem Ammoniaks.

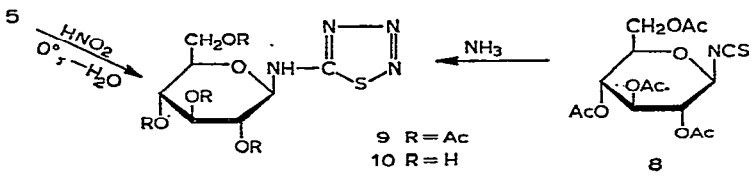


Mit guter Ausbeute erhielten wir mit dieser Methode das kristalline 4 Phenyl-2-(tetra-*O*-acetyl-D-glucopyranosylamino)-thiazol (**4**), indem **1** mit Phenacylbromid nach Beyer[12] kondensiert wurde. Die Glycosyl-Derivate des 2-Amino-4-methyl-thiazols konnten wir weder mit den von uns bekannten Methoden, noch mit derjenigen welche Panagopoulos *et al.*[13] mitteilten in entsprechend reiner, kristalliner Form erhalten.

Auf Grund des bekannten Aufbaues des 1,3,4-Thiadiazols synthetisierten wir das 2-(D-Glucopyranosylamino)-Derivat dieser heterocyclischen Verbindung. Durch Verseifung der mit der Kondensation[14,15] von 4-(Tetra-*O*-acetyl-D-glucopyranosyl)-thiosemicarbazid (**5**) und von Orthoameisensäure-äthylester erhaltenen Tetra-*O*-acetyl Verbindung (**6**) kamen wir zum 2-Glycosylamino-1,3,4-Thiadiazol (**7**).



Für die Synthese des 5-Amino-1,2,3,4-Thiatriazols sind zwei Verfahren bekannt[16,17]. Die Herstellung des entsprechenden *N*-Glykosyl-Abkömmlings gelang auf beiden Wegen. Einerseits liessen wir 4-(Tetra-*O*-acetyl-D-glucopyranosyl)-thiosemicarbazid (**5**) und salpetrige Säure bei 0° reagieren, andererseits wurde Tetra-*O*-acetyl-D-glucopyranosyl-isothiocyanat (**8**) mit Azoimid in alkoholischer Lösung zur Reaktion gebracht. In beiden Fällen sind wir zu demselben, kristallinen Reaktionsprodukt, 5-(Tetra-*O*-acetyl-D-glucopyranosylamino)-1,2,3,4-Thiatriazol (**9**) gekommen. Die Struktur der erhaltenen Verbindung wurde durch die beiden verschiedenen Synthesewege, sowie durch das IR-Spektrum bewiesen.

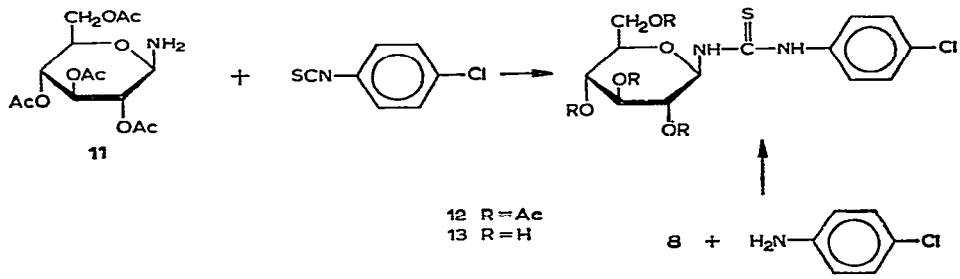


Für das IR-Spektrum der 5-Alkylamino-1,2,3,4-thiatriazole ist eine Absorptionsbande zwischen 1540–1590 cm^{-1} charakteristisch[18]. Ebenso kennzeichnend ist es, dass die Absorption der Azid-Gruppe um 2200 cm^{-1} fehlt[16b,18] im Spektrum, und das schliesst die eventuelle struktur-isomere Thiosäureazid-Struktur aus. Im Spektrum

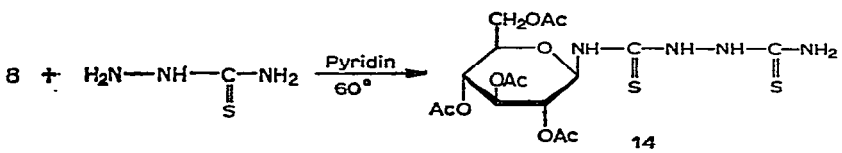
der Verbindung **9** erscheint eine scharfe Bande bei 1545 cm^{-1}, sie zeigt keine Absorption im Bereich um 2200 cm^{-1}.

Es ist bekannt, dass aus **8** und Ammoniak sich Tetra-*O*-acetyl-D-glucopyranosyl-thioharnstoff bildet[19]. Van de Kamp und Micheel[20] stellten aus **8** mit primären Aminen *N'*-substituierte-*N*-D-glucopyranosyl-thioharnstoffe her, andererseits synthetisierten Bognár und Somogyi[19] ebenfalls aus **8** mit Tetra-*O*-acetyl-D-glucopyranosylamin den symmetrischen Bis (tetra-*O*-acetyl-D-glucopyranosyl)-thioharnstoff.

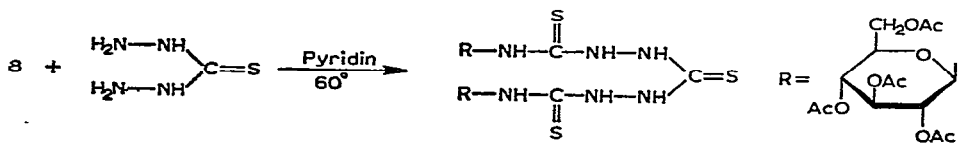
Wir bestätigten, dass es möglich ist, auf diesen beiden Wegen [also aus Tetra-*O*-acetyl-D-glucopyranosyl-isothiocyanat (**8**) mit primärem Amin bzw. aus Tetra-*O*-acetyl-D-glucopyranosylamin mit dem entsprechenden Isothiocyanat] zu demselben *N'*-D-glucosyl-*N*-substituierten Thioharnstoff zu kommen : die Reaktion von **8** und *p*-Chloranilin, sowie von **11** und *p*-Chlorphenylisothiocyanat ergab den *N*-(Tetra-*O*-acetyl-D-glucopyranosyl)-*N'*-*p*-chlorphenyl-thioharnstoff (**12**). Die Verseifung des Produktes nach Zemplén lieferte den *N*-glycosyl-*N'*-*p*-chlorphenyl-thioharnstoff (**13**).



Die Verbindung **8** wurde zur Einleitung der Reaktionen obigen Typs als sehr geeignet gefunden. Die Substanz **8** lieferte mit Thiosemicarbazid in Pyridin den *N*-[(Tetra-*O*-acetyl-D-glucopyranosyl)-thiocarbamoyl]-thiosemicarbazid [*N*-(Tetra-*O*-acetyl-D-glucopyranosyl)-dithio-hydrazodicarbonamid] (**14**).



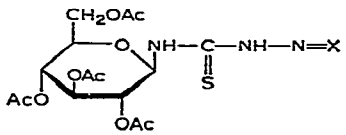
Zwei Moleküle von **8** ergaben mit einem Molekül Thiocarbohydrazid nach bekannter Methode[21] den 1,5-Bis[(tetra-*O*-acetyl-D-glucopyranosyl)-thiocarbamoyl]-thiocarbohydrazid (**15**).

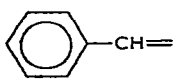
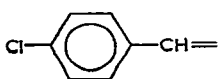
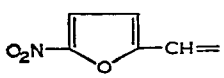
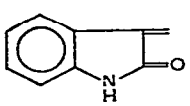
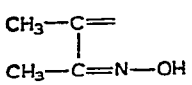


Zuletzt berichten wir darüber, dass wir nach den Angaben von van de Kamp und Micheel[20] durch Kondensation aus **5** und den entsprechenden Karbonylverbindungen mehrere, bisher unbekannte, 4-(Tetra-*O*-acetyl-D-glucopyranosyl)-thiosemicarbazone hergestellt haben, die nach Zemplénscher Verseifung die kristallinen 4-D-Glucopyranosyl-thiosemicarbazone lieferten. In Tabelle I und II stellten wir die wichtigsten Angaben über die Verbindungen zusammen.

TABELLE I

4-(TETRA-O-ACETYL-β-D-GLUCOPYRANOSYL)-THIOSEMICARBAZONE



X	*Schmp.*,°	$[\alpha]_D$,°	*Ausb.* (%)	*Analyse*	*C*	*H*	*N*	*Umkrist. aus:*
	180–181	− 74 (Chloroform)	96	Gef. Ber.	52.42 51.90	5.16 5.30	8.18 8.25	Äthanol–Wasser
	216–218	−89 (Chloroform)	92	Gef. Ber.	49.07 48.45	4.72 4.77	7.61 7.71	Methanol
	136–138	−69.4 (Aceton)	88	Gef. Ber.	43.10 42.72	4.41 4.43	10.03 9.98	Methanol–Wasser
	240	−163 (Pyridin)	95	Gef. Ber.	50.03 50.50	4.68 4.72	9.97 10.15	Äthylacetat
	204–205	−50.1 (Chloroform)	80	Gef. Ber.	45.50 45.40	5.42 5.55	11.87 11.90	Methanol–Wasser

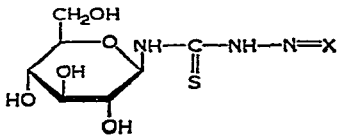
EXPERIMENTELLER TEIL

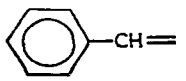
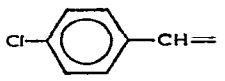
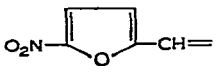
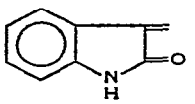
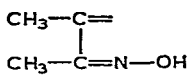
4-Methyl-2-(tetra-O-acetyl-D-glucopyranosylamino)-thiazol (**2**). — Die Substanz (**1**) (0.81 g, 2 mMol), 0.2 ml Chloraceton und 0.84 ml (2 mMol) Triäthylamin werden in 7.5 ml Äthylacetat 2 Stdn. gekocht. Das ausgefallene Triäthylamin HCl wird filtriert und der Rückstand i. Vak. zum Sirup eingedamft. Es gelang nicht, das Reaktionsprodukt zu kristallisieren.

2-D-Glucopyranosylamino-4-methyl-thiazol (**3**). — Der obige, dicke Sirup wird in 10 ml absol. Methanol gelöst und 1.2 ml, bei 0° gesättigter, alkoholischer Ammoniak-Lösung zugesetzt. Nach 20-stdn. Stehenlassen wird die hellgelbe Lösung ein-

TABELLE II

4-β-D-GLUCOPYRANOSYL-THIOSEMICARBAZONE



X	Schmp.,°	$[\alpha]_D$,°	Ausb. (%)	Analyse	C	H	N	Umkryst. aus:
	91–95	18.5[a]	90	Gef. Ber.	49.50 49.20	5.71 5.56	12.27 12.30	Methanol–Wasser
	166–169	34.7[a]	78	Gef. Ber.	43.70 44.70	4.29 4.78	11.00 11.17	Methanol–Wasser
	190–191	77[b]	92	Gef Ber.	38.80 38.20	4.35 4.25	14.16 14.90	Äthanol abs.
	240	11.4[a]	84	Gef. Ber.	46.45 47.15	4.80 4.71	14.03 14.65	Äthanol abs.
	186	52.9[b]	76	Gef. Ber.	39.40 39.20	5.95 5.93	17.00 16.63	Methanol

[a] *N,N*-Dimethylformamid–Wasser. [b] *N,N*-Dimethylformamid.

gedampft, dreimal mit Äthylacetat ausgekocht und der Rückstand aus absol. Äthanol umkristallisiert. Nach zweimaligem Umkristallisieren beträgt die Ausbeute an bräunlichen Kristallen 0.15 g (33.8%), Schmp. 167–171° (Kofler–Block), $[\alpha]_D^{26}$ −46.2° (*c* 1, Methanol); Lit.[13] 163–169°; $[\alpha]_D$ −52.9° (Methanol). Gef.: C, 42.36; H, 6.02; N, 10.13; S, 11.39. $C_{10}H_{16}N_2O_5S$ (276.3) Ber.: C, 43.46; H, 5.84; N, 10.14; S, 11.61%.

4-Phenyl-2-(tetra-O-acetyl-D-glucopyranosylamino)-thiazol (**4**). — Die Substanz **1** (2.03 g, 5 mMol), 0.2 g wasserfreies Natriumacetat und 1.0 g (5 mMol) Phenacylbromid werden in 16 ml Eisessig 1 Stunde auf dem Dampfbad gehalten. Das Reaktionsgemisch wird in 100 ml Eiswasser gegossen, das ausgeschiedene Produkt mehrmals mit Wasser gewaschen. Weisse, amorphe Substanz. 2.25 g (88.7%). Schmp. 70–73° (Kofler–Block). Der Schmelzpunkt ändert sich beim Umfällen aus Tetrachlorkohlenstoff–Petroläther nicht. $[\alpha]_D^{21}$ +21.9° (*c* 1, Tetrachlorkohlenstoff). Gef.: C, 54.76; H, 5.25; N, 5.27; S, 5.94; Acetyl, 33.99. $C_{23}H_{26}N_2O_9S$ (506.5) Ber.: C, 54.53; H, 5.17; N, 5.53; S, 6.33; Acetyl, 33.99%.

2-(Tetra-O-acetyl-D-glucopyranosylamino)-1,3,4-thiadiazol (**6**). — Die Substanz **5** (0.84 g, 2 mMol) wird in 1.8 ml Orthoameisensäure-äthylester auf dem Dampfbad 130 Min. erwärmt. Nach einer Stunde entstehen Kristalle. Das filtrierte Produkt (0.37 g, 42.7%) wird aus viel wässrigem Äthanol umkristallisiert. Feine Nadeln von Schmp. 209–213° (Zers.), $[\alpha]_D^{26}$ −32.9° (*c* 1, Pyridin). Gef.: C, 44.98; H, 4.91; N, 9.77; S, 7.42. $C_{16}H_{23}N_3O_9S$ (431.4) Ber.: C, 44.54; H, 4.91; N, 9.74; S, 7.43%.

2-D-Glucopyranosylamino-1,3,4-thiadiazol (**7**). — Eine Suspension von **6** (0.7 g) in 40 ml Methanol wird mit 0.4 ml N Natriummethylat-Lösung versetzt. Die Substanz löst sich schnell auf. Nach einem Tag wird die Lösung mit 50%-iger Essigsäure neutralisiert, i. Vak. eingedampft und der Rückstand mit absol. Alkohol verrieben. Es wird in minimalem heissem Wasser gelöst und absol. Alkohol zugegeben. Feine Nadeln. 0.24 g (56.6%). Schmp. 226° (Zers.). $[\alpha]_D^{23}$ −73.2° (*c* 1, Wasser). Gef.: C, 36.80; H, 6.02; N, 15.73; S, 12.14. $C_8H_{13}N_3O_5$ (263.3) Ber.: C, 37.34; H, 6.07; N, 16.33; S, 12.46%.

5-(Tetra-O-acetyl-D-glucopyranosylamino)-1,2,3,4-thiatriazol (**9**). — (*a*) Die Substanz **5** (4.21 g, 10 mMol) wird in 32 ml (30 mMol) N HCl gelöst und die Lösung wird unter Rühren mit einer Lösung von 0.69 g (10 mMol) $NaNO_2$ in 5 ml Wasser tropfenweise versetzt. Der ausgefallene weisse Niederschlag wird nach 10 Min. abgesaugt, mit Eiswasser gewaschen und i.Vak. über P_2O_5 getrocknet: 3.85 g (89%). Schmp. 117–118° (Zers.). Das Rohprodukt wird durch Auflösen in 58 ml Äthylacetat und Zugabe von 100 ml Petroläther (Sdp. 40–60°) in der Kälte umkristallisiert. Farblose Nadeln nach dreimaligem Umkristallisieren. Schmp. 126–127° (Zers.). $[\alpha]_D^{23}$ −36.2° (*c* 0.8, Chloroform). Gef.: C, 41.50; H, 4.53; N, 12.94; S, 7.43. $C_{15}H_{20}N_4O_9S$ (432.4) Ber.: C, 41.60; H, 4.63; N, 12.95; S, 7.40%.

(*b*) In eine Lösung von 2.10 g (5.4 mMol) **8** in 50 ml Äthanol wird aus 1.0 g (17 mMol) Natriumazid mit Schwefelsäure freigesetztem Azoimid in 5 ml Äther hineindestilliert und das Reaktionsgemisch verstopft bei Raumtemperatur stehengelassen. Kurz nach dem Auflösen von **8** fällt **9** in Form von farblosen Kristallen aus: 1.26 g. Schmp. 124–125° (Zers.). Aus der Mutterlauge können weitere 0.57 g (Gesamtausb.: 80%), Schmp. 123–124° gewonnen werden. Nach dreimaligem Umkristallisieren aus Äthylacetat–Petroläther schmilzt es bei 125–127° (Zers.); $[\alpha]_D^{23}$ −35.2° (*c* 0.8, Chloroform). Die Substanz erwies sich auf Grund von Misch-Schmp., Dünnschichtchromatographie (auf Kieselgel G Merck, Benzol–Aceton 8:2, entwickelt mit 50%-er Schwefelsäure bei 150°) und IR-Spektren mit der nach (*a*) hergestellten als identisch.

5-D-Glucopyranosylamino-1,2,3,4-thiatriazol (**10**). — Die Desacetylierung von 1.5 g **9** nach Zemplén ergibt 0.23 g (25%). Farblose Kristalle, Schmp. 140° (Zers.), $[\alpha]_D^{23}$ −37.2° (*c* 0.86, Äthanol). Die Substanz ist dünnschichtchromatographisch (Kieselgel G Merck, 1-Butanol–Aceton–Wasser 4:5:1) einheitlich. IR-Spektrum (KBr): 3300–3500 (OH und NH) und 1545 cm^{-1} (C=N und NH). Gef.: C, 32.20; H, 4.57 ; N, 20.97; S, 11.90. $C_7H_{12}N_4O_5S$ (264.27) Ber.: C, 31.80; H, 4.54; N, 21.20; S, 12.12%.

3-p-Chlorphenyl-1-(tetra-O-acetyl-D-glucopyranosyl)-thioharnstoff (**12**). — (*a*) Eine Lösung von **8** und 1.27 g (10 mMol) *p*-Chloranilin in 20 ml Äthanol wird 30 Min. bei 50° gehalten, nach dem Abkühlen mit Wasser bis zur beginnenden Trübung versetzt und über Nacht stehen gelassen. Das Rohprodukt: 4.12 g (80%), Schmp. 125–130°, wird zweimal aus wässrigem Methanol umkristallisiert. Viereckige Kristalle vom Schmp. 128–130°; $[\alpha]_D^{23}$ −12.3° (*c* 1.8, Chloroform). Gef.: C, 48.81; H, 4.76; N, 5.42; Cl, 6.75. $C_{21}H_{25}ClN_2O_9S$ (516.97) Ber.: C, 48.78; H, 4.83; N, 5.31; Cl, 6.87%.

(*b*) Eine Lösung von **11** (0.70 g, 2 mMol) und 0.34 g (2 mMol) *p*-Chlorphenylisothiocyanat in 3 ml Äthanol wird 30 Min. bei 50–60° gehalten und nach dem Abkühlen mit Wasser bis zur beginnenden Trübung versetzt. Nach einigen Wochen bei Raumtemperatur schieden 0.65 g (62%) grosse, tafelförmige Kristalle aus. Umkristallisiert wie oben. Schmp. 127–130°; $[\alpha]_D^{23}$ −13.6° (*c* 2, Chloroform). Die Substanz erwies sich auf Grund von Misch-Schmelzpunkt und IR-Spektren mit der nach (*a*) hergestellten als identisch.

3-p-Chlorphenyl-1-D-glucopyranosyl-thioharnstoff (**13**). — Eine Suspension von **12** (5 g) in 50 ml absol. Methanol wird mit 0.2N Natriummethylat-Lösung (5 ml) versetzt. Nach Stehen im Kühlschrank über Nacht wird das Gemisch mit Eisessig neutralisiert und i. Vak. zur Trockne eingedampft: 3.2 g (94%). Nach dreimaligem Umkristallisieren aus absol. Äthanol schmilzt es bei 150–152° (Zers., sintert ab 120°); $[\alpha]_D^{23}$ −50° (*c* 0.8, Wasser). Gef.: C, 45.03; H, 5.02; N, 7.80. $C_{13}H_{17}ClN_2O_5S$ (348.5) Ber.: C, 44.75; H, 4.87; N, 8.02%.

1-[(Tetra-O-acetyl-D-glucopyranosyl)-thiocarbamoyl]-thiosemicarbazid (**14**). — Eine Lösung von 3.89 g (10 mMol) **8** in 25 ml Pyridin wird mit 0.91 g (10 mMol) Thiosemicarbazid versetzt. Das Gemisch wird bis zum vollständigen Auflösen (100 Min.) bei 60° gehalten und über Nacht stehengelassen, dann i.Vak. zu Sirup eingeengt, mehrmals mit absol. Äthanol verrieben und i.Vak. zur Trockne verdampft. Der Rückstand wird aus wenig Äthanol umkristallisiert: 4.10 g (86%). Schmp. 152–153°; $[\alpha]_D^{23}$ −83.7° (*c* 1, Pyridin). Gef.: C, 39.52; H, 5.13; N, 11.33. $C_{16}H_{24}N_4O_9S_2$ (480.0) Ber.: C, 40.00; H, 5.00; N, 11.65%.

1,5-Bis-[(Tetra-O-acetyl-D-glucopyranosyl)-thiocarbamoyl]-thiocarbonohydrazid (**15**). — Aus 0.78 g (2mMol) **8** und 0.10 g (1 mMol) Thiocarbohydrazid erhält man bei **14** 3.27 g (74%) Rohprodukt. Nach dreimaligem Umkristallisieren aus Äthanol 2.82 g, Schmp. 174°; $[\alpha]_D^{23}$ −15.0° (*c* 0.7, Chlorofom). Gef.: C, 41.84; H, 4.90; N, 9.48. $C_{31}H_{44}N_6O_{18}S_3$ (884.0) Ber.: C, 42.15; H, 4.98; N, 9.50%.

Darstellung der 4-(Tetra-O-acetyl-D-glucopyranosyl)-thiosemicarbazone (s. Tabelle I). — 4-(Tetra-*O*-acetyl-D-glucopyranosyl)-thiosemicarbazide (**5**, 10 mMol) und die äquivalente Menge der Carbonyl-Verbindung werden in 50 ml Äthanol gelöst bzw. suspendiert und nach Zugabe von 2 ml Eisessig 3 Stdn. gekocht. Nach Abkühlen werden die ausgeschiedenen Kristalle abgesaugt und aus dem in der Tabelle I angegebenen Lösungsmittel umkristallisiert. Falls sich das Rohprodukt beim Abkühlen nicht ausscheidet, wird das Gemisch auf Eis gegossen und danach isoliert.

4-D-Glucopyranosyl-thiosemicarbazone (s. Tabelle II). — Die entsprechenden Acetate werden nach Zemplén verseift: 10 mMol der Acetylverbindung werden in 100 ml absol. Methanol gelöst bzw. suspendiert und nach Zugabe von 2.5–3 ml N Natriummethylat-Lösung wird das Gemisch 30 Min. bei Raumtemp. bald über Nacht im Kühlschrank aufbewahrt. Daraufhin neutralisiert man mit Eisessig gegen Phenolphtalein und dampft es i.Vak. zur Trockne ein. Der Rückstand wird aus dem angegebenen Lösungsmittel umkristallisiert.

DANK

Wir danken der analytischen Abteilung unseres Instituts (Leiterin: Frau Dr. É. Rákosi) für die Durchführung der Analysen, sowie der Ungarischen Akademie der Wissenschaften für die Unterstützung dieser Arbeit.

ZUSAMMENFASSUNG

Einige neue *N*-Glykosyl-Derivate werden aus einfacheren Glykosylamin-Abkömmlingen durch nachfolgenden Aufbau des Aglykons synthetisiert.

Die Kondensation von (Tetra-*O*-acetyl-D-glucopyranosyl)-thioharnstoff (**1**) mit Chloraceton ergibt 4-Methyl-2-(tetra-*O*-acetyl-D-glucopyranosylamino)-thiazol (**2**), mit Phenacylbromid das 4-Phenyl-Analogon (**4**). Die Reaktion von 4-(Tetra-*O*-acetyl-D-glucopyranosyl)-thiosemicarbazid (**5**) mit Orthoameisensäuretriäthylester liefert das 2-(Tetra-*O*-acetyl-D-glucopyranosylamino)-1,3,4-thiadiazol (**6**).
Das 5-(Tetra-*O*-acetyl-D-glucopyranosylamino)-1,2,3,4-thiatriazol (**9**) wird aus **5** mit salpetriger Säure bzw. aus (Tetra-*O*-acetyl-D-glucopyranosyl)-isothiocyanat (**8**) mit Azoimid synthetisiert. Die Addition von *p*-Chloranilin an **8** oder von Tetra-*O*-acetyl-D-glucopyranosylamin (**11**) an *p*-Chlorphenylisothiocyanat liefert den *N*-*p*-Chlorphenyl-*N'*-(tetra-*O*-acetyl-D-glucopyranosyl)-thioharnstoff (**12**). Die Acetate der neuen Glykosylamin-Abkömmlinge wurden desacetyliert. Das 1-[(Tetra-*O*-acetyl-D-glucopyranosyl)-thiocarbamoyl]-thiosemicarbazid (**14**) wird aus **8** mit Thiosemicarbazid, das 1,5-Bis[(tetra-*O*-acetyl-D-glucopyranosyl)-thiocarbamoyl]-thiocarbonohydrazid (**15**) aus **8** mit Thiocarbohydrazid synthetisiert. Anschliessend wird die Herstellung einiger neuer D-Glucopyranosyl-thiosemicarbazone bzw. ihrer *O*-Acetyl-Derivate beschrieben; die physikalischen Eigenschaften und IR-Daten dieser Verbindungen werden mitgeteilt.

SUMMARY

Some new *N*-D-glucosyl derivatives have been obtained from simpler D-glucosylamine derivatives by further substitution of the aglycon groups. Condensation of (tetra-*O*-acetyl-D-glucopyranosyl)thiourea (**1**) with 1-chloro-2-propanone gave 4 methyl-2-(tetra-*O*-acetyl-D-glucopyranosylamino)thiazole (**2**) and, with 2-bromoacetophenone, the corresponding 4-phenyl analogue (**4**). Treatment of 4-(tetra-*O*-

acetyl-D-glucopyranosyl)thiosemicarbazide (**5**) with triethyl orthoformate gave 2-(tetra-*O*-acetyl-D-glucopyranosylamino)-1,3,4-thiadiazole (**6**).5-(Tetra-*O*-acetyl-D-glucopyranosylamino)-1,2,3,4-thiatriazole (**9**) was prepared by the action of nitrous acid on **5** as well as by the action of azoimide on tetra-*O*-acetyl-D-glucopyranosyl isothiocyanate (**8**). The addition of *p*-chloroaniline to **8** or *p*-chlorophenyl isothiocyanate to tetra-*O*-acetyl-D-glucopyranosylamine (**11**) gave *N*-(*p*-chlorophenyl)*N'*-tetra-*O*-acetyl-D-glucopyranosyl)thiourea (**12**). The tetraacetates of the new D-glucosylamine derivatives were deacetylated. 1-[(Tetra-*O*-acetyl-D-glucopyranosyl)thiocarbamoyl]thiosemicarbazide, (**14**) was synthesised from **8** by treatment with thiosemicarbazide, and 1,5-bis[(tetra-*O*-acetyl-D-glucopyranosyl)thiocarbamoyl]thiocarbonohydrazide (**15**) was obtained from **8** and thiocarbohydrazide. Preparation of some new (*O*-acetyl-D-glucosyl)thiosemicarbazones is described, and physical and infrared data of these compounds are included.

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1-DEOXY-D-*erythro*-2,3-HEXODIULOSE, AN INTERMEDIATE IN THE FORMATION OF D-GLUCOSACCHARINIC ACID

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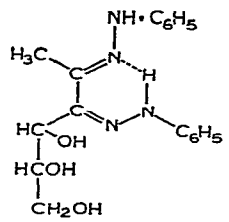
INTRODUCTION

According to the Nef–Isbell theory, the saccharinic acids are formed from reducing sugars *via* dicarbonyl intermediates, which are then rearranged to the saccharinic acids[1]. 3-Deoxy-D-*erythro*-hexosulose[2] and 4-deoxy-D-*glycero*-2,3-hexodiulose[3,4], the postulated intermediates in the formation of D-glucometasaccharinic and D-glucoisosaccharinic acids, respectively, have been isolated after alkaline treatment of suitably substituted sugars, and their facile conversions into the corresponding saccharinic acids on alkaline treatment have been demonstrated. From these intermediates, the two possible isomers ("α" and "β") of the saccharinic acids are formed in comparable amounts[5]. When D-fructose was treated with calcium hydroxide, however, only "α"-D-glucosaccharinic acid (2-*C*-methyl-D-ribonic acid) and no "β"-D-glucosaccharinic acid (2-*C*-methyl-D-arabinonic acid) was observed among the reaction products. Further, the formation of D-glucosaccharinic acid was not observed when D-fructose was treated with sodium hydroxide[6]. In the present paper, the synthesis of 1-deoxy-D-*erythro*-2,3-hexodiulose (**1**) and its reaction with alkali are reported.

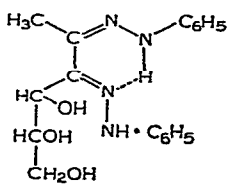
RESULTS AND DISCUSSION

D-Mannitol was converted into 1,5-anhydro-2,3,4-tri-*O*-benzoyl-6-deoxy-D-*lyxo*-hex-5-enitol, previously prepared by Zervas and Papadimitriou[7], but not characterised. This substance was deacylated to **2** and treated with aqueous acetic acid to yield 1-deoxy-D-fructose (**3**, $[\alpha]_D$ $-90°$ in water). The L-isomer, prepared according to Wolfrom *et al.*[8], showed $[\alpha]_D$ $+85°$, and the two substances were indistinguishable on paper chromatography and paper electrophoresis. The phenylosazone (**4**) of 1-deoxy-D-fructose showed $[\alpha]_{5780}$ $-28°$ (pyridine), which does not agree with the value ($[\alpha]_D$ $+52°$ in pyridine) reported for the same substance prepared from 1-deoxy-D-psicose[9]. The phenylosazone of 1-deoxy-L-fructose, prepared by us, showed $[\alpha]_{5780}$ $+29°$, and the two isomers had the same m.p. and n.m.r. spectrum, and were indistinguishable on t.l.c. They both gave two spots with tailing in between, indicating a slow equilibrium between two forms. When either spot was extracted and the extract chromatographed, the same picture was obtained. The elemental analysis was correct,

and the osazone consumed two moles of periodate per mole, with release of one mole of formaldehyde, in agreement with a non-cyclic structure (**4a** and **4b**). The n.m.r. spectrum showed signals corresponding to 3 methyl and 10 aromatic protons. The methyl protons appeared as a small peak at τ 7.84 and a larger one at τ 7.75, with relative areas of 1:4. These most probably correspond to the weaker (R_F 0.10) and the stronger (R_F 0.44) spots observed on t.l.c. In **4a** and **4b**, there is a *syn* and an *anti* relationship, respectively, between the methyl group and an $NH{\cdot}C_6H_5$ grouping. According to previous findings[10], the structure of an osazone is best represented by the *syn* form, the *anti* form being insignificant. The large peak at τ 7.75 might, by analogy, correspond to structure **4a**, and the smaller peak possibly to **4b**.

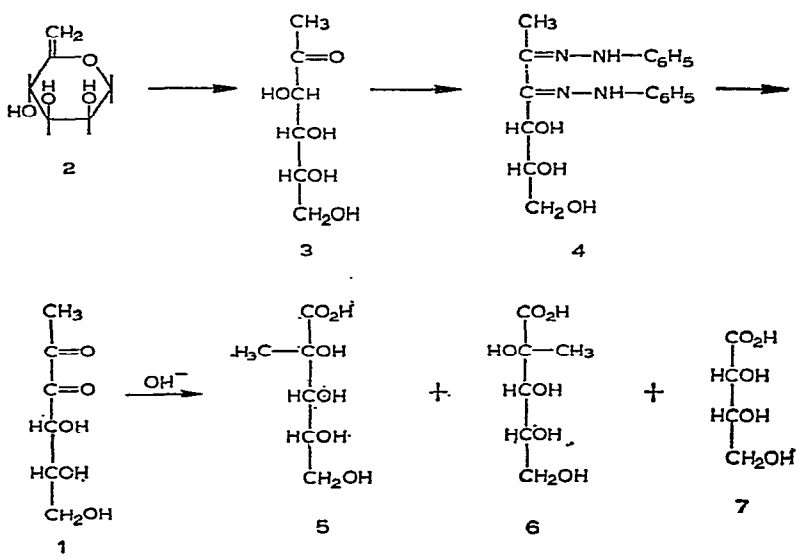


4a



4b

Treatment of the phenylosazone with benzaldehyde, a method often used to regenerate carbonyl compounds from their phenylhydrazine derivatives, produced only small proportions of hexodiulose. Treatment with nitrous acid, however, as devised by Ohle *et al.*[11], gave a better yield of carbonyl compound. This had a high electrophoretic mobility in bisulphite buffer, typical for dicarbonyl sugars[12]. The compound was stable only in acidic solution, and concentration of its solution, even by lyophilisation, caused decomposition, D-erythronic acid being the main product.



The same result was obtained on chromatography in non-acidic solvent systems. The substance was therefore kept in aqueous acetic acid. On reaction with phenylhydrazine, an osazone, indistinguishable from **4**, was formed. These results, together with those from the alkaline degradations reported below, show that the substance has the expected structure of 1-deoxy-D-*erythro*-2,3-hexodiulose (**1**).

Treatment of **1** with calcium hydroxide produced "α"-D-glucosaccharinic acid (**5**) and D-erythronic acid (**7**) in the relative proportions 10:3, as determined by g.l.c. of the trimethylsilyl derivatives of their lactones[5]. On treatment with sodium hydroxide, D-erythronic acid was the major product, the relative proportions between the two acids now being 2:10. The preponderant formation of saccharinic acid on treatment with calcium hydroxide and of cleavage on treatment with sodium hydroxide was also observed for another dicarbonyl intermediate, 4-deoxy-D-*glycero*-2,3-hexodiulose[4]. D-Erythronic acid, as the crystalline lactone, was also isolated from the product obtained when **1** was treated with sodium hydroxide.

The formation of "β"-D-glucosaccharinic acid (**6**), on treatment of **1** with calcium hydroxide or sodium hydroxide, was demonstrated by g.l.c., but, compared to the "α"-compound, this substance was formed in small proportions only. In agreement with earlier results[5], the rearrangement of **1** occurs with considerable stereoselectivity. In the formation of the other glucosaccharinic acids, a corresponding stereoselectivity is not observed. The obvious reason for these results is that, of the dicarbonyl intermediates rearranging into different glucosaccharinic acids, only **1** has an asymmetrically substituted carbon atom adjacent to the dicarbonyl grouping. A mechanistic interpretation of this result must await more-detailed studies of the reaction. It is interesting to note, however, that, on treatment of D-xylose with calcium hydroxide, the corresponding "α"- and "β"-saccharinic acids (2-*C*-methyl-D-erythronic and -D-threonic acid) were formed in comparable amounts. In the same investigation[6], no formation of "α"-D-glucosaccharinic acid and D-erythronic acid was observed on treatment of D-fructose with sodium hydroxide. These results, in conjunction with those of the present study, indicate that formation of **1** is insignificant when D-fructose is treated with sodium hydroxide.

EXPERIMENTAL

General methods. — Concentrations were carried out under diminished pressure below 45°, unless otherwise stated. Melting points are corrected.

The solvent systems used for paper chromatography (on Whatman No. 1 paper) and buffers used for paper electrophoresis (on Whatman 3 MM paper) were: *A*, ethyl acetate–acetic acid–water, 3:1:1; *B*, butanone, saturated with water; *C*, 0.1M borate, pH 10; *D*, 0.1M hydrogen sulphite, pH 4.7 (used at 50°).

The solvent system used for t.l.c., on "Kieselgel G nach Stahl", was ethyl acetate–light petroleum, 3:1.

G.l.c. of trimethylsilyl ethers[13] was carried out on a column of butanediol succinate at 160°, using a Perkin–Elmer 800 instrument.

N.m.r. spectra were recorded at 60 MHz on a Varian A-60 spectrometer. Deuterochloroform was used as solvent and tetramethylsilane as an internal reference.

Conventional spraying reagents for lactones, acids, alditols, and carbonyl compounds were used.

1,5-Anhydro-2,3,4-tri-O-benzoyl-6-deoxy-D-lyxo-hex-5-enitol. — A mixture of 1,5-anhydro-2,3,4-tri-*O*-benzoyl-6-deoxy-6-iodo-D-mannitol[7] (8.4 g) and silver fluoride (8.4 g) in pyridine (50 ml) was shaken for 3.5 h at room temperature. The product was extracted with ethyl ether (4 × 100 ml), the extract was concentrated, and a solution of the residue in ethyl ether was filtered and concentrated. Impurities were removed by chromatography, using a column of silicic acid with ethyl acetate as irrigant, to yield the pure substance (5.5 g), m.p. 119–120° (from ethanol), $[\alpha]_D^{24}$ −242° (*c* 1.4, chloroform), ν_{max} 1660 cm^{-1} (C=C) (Found: C, 71.7; H, 4.94. $C_{27}H_{22}O_7$ calc.: C, 70.8; H, 4.84%).

1,5-Anhydro-6-deoxy-D-lyxo-hex-5-enitol (**2**). — The above substance (1.0 g) in chloroform (2.5 ml) was added to a solution of sodium methoxide (from 75 mg of sodium) in methanol (2.5 ml), and the mixture was kept overnight at room temperature. Sodium hydrogen carbonate (0.5 g) and water (5 ml) were added, and the mixture was extracted with ethyl acetate (10 × 5 ml). The combined extracts were heated to 60°, flocculated salts were removed by filtration, and the solution was concentrated. The crystalline product (0.3 g) was separated from the oil (methyl benzoate) by decantation, washed with ether, and recrystallised from ethyl acetate, yielding the pure substance, m.p. 136–138°, $[\alpha]_D^{22}$ −181° (*c* 0.5, water), ν_{max} 1435 and 1650 cm^{-1} (C=C) (Found: C, 49.2; H, 7.08; O, 43.8. $C_6H_{10}O_4$ calc.: C, 49.3; H, 6.90; O, 43.8%).

1-Deoxy-D-fructose (**3**). — The substance **2** (0.85 g) in 10% aqueous acetic acid (8.5 ml) was kept for 24 h at room temperature and then concentrated. Acetic acid was removed from the residue by repeated codistillations with water, and water was removed by codistillation with ethanol–benzene. The resulting syrup (0.95 g), $[\alpha]_D^{20}$ −90° (*c* 1.5, water), gave a single spot on paper chromatography, which had the same mobility as 1-deoxy-L-fructose, prepared according to Wolfrom *et al.*[8]. It gave a light-brown colour with the anisidine hydrochloride reagent, and showed the same mobility as fructose on electrophoresis (buffer *C*). The i.r. spectrum was consistent with the assigned structure.

Phenylosazone of 1-deoxy-D-fructose (**4**).— A solution of **3** (0.64 g), sodium acetate trihydrate (3.8 g), and phenylhydrazine hydrochloride (2.4 g) in water (6 ml) was kept for 4 h at 100°. The brick-red precipitate was crushed, and washed with water. The crude product (1.1 g) gave three spots (R_F 0.74, 0.44, and 0.10) on t.l.c. Crystallisation from ethyl acetate–light petroleum removed the component having R_F 0.74. The product **4** showed m.p. 137–140°, $[\alpha]_{5780}^{21}$ −28° (*c* 1.3, pyridine). (Found: C, 63.1; H, 6.55; N, 16.3. $C_{18}H_{22}N_4O_3$ calc.: C, 63.3; H, 6.48; N, 16.3%).

On periodate oxidation, following the procedure of Hough *et al.*[14], the substance consumed 2.25 moles of periodate per mole and released 1.16 moles of formal-

dehyde. The oxidation was complete in 15 min, and precipitation of a cleavage product occurred during the reaction.

The phenylosazone from 1-deoxy-L-fructose was prepared analogously. It had m.p. 137–140°, $[\alpha]_{5780}^{21}$ +29° (*c* 0.65, pyridine), and was chromatographically indistinguishable from **4** (Found: C, 63.7; H, 6.54; N, 16.1%)

1-Deoxy-D-erythro-*2,3-hexodiulose* (**1**). — The substance **4** (200 mg) was dissolved in a mixture of water (0.3 ml), ethanol (2.2 ml), and 1.9M hydrochloric acid (0.95 ml). The solution was heated to 40°, and 2M aqueous sodium nitrite (0.7 ml) was added dropwise with stirring. After 1 h, the ethanol was distilled off at room temperature under diminished pressure, the resulting precipitate was removed by centrifugation, the supernatant solution extracted with chloroform, and the aqueous phase spotted onto prewashed Whatman 3-MM papers. These were then irrigated with solvent system *A*. The zone giving positive reactions with silver nitrate–sodium hydroxide and with 2,4-dinitrophenylhydrazine was cut out, while still wet, and extracted with water. The recovered compound, which was chromatographically pure, showed $M_{vanillin}$ 0.91 on electrophoresis in buffer *D*. The corresponding value for 1-deoxy-D-fructose was 0.0.

Part of the product was treated with phenylhydrazine to give an osazone, indistinguishable from **4** by t.l.c.

Alkaline treatment of **1**. — Aqueous solutions of **1** (pH 3) from 30 mg of **4** were added to 0.02M calcium hydroxide (90 ml) and 0.04M sodium hydroxide (90 ml), respectively. After 2 h at room temperature, when the reaction of **1** was complete, the solutions were treated with Dowex-50 (H^+), concentrated, dissolved in conc. hydrochloric acid (0.5 ml), and concentrated. The R_F values for "α"-D-glucosaccharino-, "β"-D-glucosaccharino-, and D-erythrono-lactone in solvent system *A* are 0.57, 0.54, and 0.50, respectively. A fraction, which should contain these substances, was isolated by paper chromatography in this solvent system and analysed by g.l.c.

D-Erythronolactone was isolated from the reaction of **1** with sodium hydroxide and was sublimed under diminished pressure to give crystals, m.p. 95–98°, indistinguishable from authentic D-erythronolactone (mixed m.p., i.r.).

ACKNOWLEDGMENT

The authors are indebted to Mr. K. I. Dahlqvist, Royal Institute of Technology, for determining the n.m.r. spectra.

SUMMARY

The postulated intermediate in the formation of D-glucosaccharinic acid, 1-deoxy-D-*erythro*-2,3-hexodiulose (**1**), has been synthesised. The substance, which was stable only in acidic solution, yielded "α"-D-glucosaccharinic acid and D-erythronic acid on treatment with base; with calcium hydroxide, the former acid was preponderant, and, with sodium hydroxide, the latter acid preponderated. "β"-D-Glucosaccharinic acid was formed in traces only.

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Notes

Virgilia oroboides gum
Part VI[1]. Further observations on the products of acid hydrolysis

Structural work on the polysaccharide gum exudate of *Virgilia oroboides* has included an investigation[2] of the products, both neutral and acidic, of graded hydrolysis. The monomeric acids identified as structural units are D-glucuronic acid and its 4-*O*-methyl derivative, the former in much the greater proportion. Recovery of two aldobiouronic acids, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (**1**) and 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose (**2**), establishes the modes of linkage of the D-glucuronic acid to D-galactose and D-mannose. A third aldobiouronic acid (**3**), recovered in amount equal to *ca.* 3% of the other two, appears, on the basis of the hydrolysis products from the acid and from its carboxyl-reduced derivative, to consist of 4-*O*-methylglucuronic acid linked glycosidically to galactose. Interest in the mode of linkage in this aldobiouronic acid has been stimulated by the isolation from *Encephalartos longifolius* gum of three such acids (reported[3] in an accompanying paper) whose identities have been shown to be **1**, **2**, and the 4-*O*-methyl derivative (on the acid moiety) of **1**.

Paper-chromatographic and ionophoretic mobilities of **3** and the 4-*O*-methyl-D-glucuronic acid analogue of **1** were identical, and the $[\alpha]_D$ value for the barium salt of **3** is similar to literature values given for the latter substance. Methylation of **3** gave a syrupy product, $[\alpha]_D$ −24° in chloroform, which ran concurrently on t.l.c. with the fully methylated derivative of **1**. Methanolysis then afforded the methyl glycosides (identified by their retention times on g.l.c. under standard conditions[4]) of 2,3,4-tri-*O*-methylglucuronic acid, 2,3,4-tri-*O*-methylgalactose, and 2,3,5-tri-*O*-methylgalactose (in trace amount); the molar ratio of acid to sugar was approximately 1:1.2. Acid hydrolysis of fully methylated **3** gave 2,3,4-tri-*O*-methylgalactose and methylated uronic acid, confirming the (1→6)-linkage from uronic acid to galactose.

It appears from the above evidence that the third aldobiouronic acid (**3**) isolated from *V. oroboides* gum is 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, *i.e.*, that the major aldobiouronic acid (**1**) residue present is accompanied by a small proportion of material that is *O*-methylated at C-4 of the D-glucuronic acid. This feature is being found frequently in current studies of plant polysaccharide gums[5].

EXPERIMENTAL

Experimental conditions used were as described in the accompanying paper[3].

The barium salt of aldobiouronic acid **3**, from acid hydrolysis of *V. oroboides* gum[2], showed identical behaviour on paper chromatography (in two solvents, *b* and *d*)

and paper ionophoresis (borate buffer) with specimens of 6-*O*-(barium 4-*O*-methyl-β-D-glucopyranosyluronate)-D-galactose derived from gum myrrh[6] and *Encephalartos longifolius* gum[3]. The barium salt, $[\alpha]_D$ *ca.* −7° (*c* 3.3), was methylated (Haworth and Purdie procedures) to give a syrup (15 mg) that was homogeneous on t.l.c. and similar in mobility to methylated 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and had $[\alpha]_D$ −24° (*c* 0.7, chloroform). Methanolysis and g.l.c. of a portion of this material gave methyl glycosides of methyl 2,3,4-tri-*O*-methylglucuronate (*T* 2.79, 3.75), 2,3,5-tri-*O*-methylgalactose (*T* 5.55), and 2,3,4-tri-*O*-methylgalactose (*T* 9.1). Hydrolysis (1.5N sulphuric acid for 18 h at 100°) of a further portion gave 2,3,4-tri-*O*-methylgalactose and methylated uronic acid, with a trace of 3,4,6-tri-*O*-methylmannose (originating from acid **2** present in the sample of acid **3**); identification of the sugars was by paper chromatography (in solvent *a* and in butanone–water azeotrope) and by t.l.c. Direct chromatographic comparison of the methanolysate and hydrolysate with those obtained from authentic, fully methylated 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose confirmed the identification.

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Acid stability of the *S*-oxides and *S,S*-dioxide of methyl 5-thio-β-D-xylopyranoside

Acid-catalyzed hydrolysis of methyl glycosides having sulfur as the ring heteroatom has been shown[1-3] to be much faster than for the ring-oxygen counterparts. This high rate of hydrolysis of the thio sugar derivatives has been explained[1] in terms of the inductive effect of the heterocyclic sulfur atom in releasing p electrons for stabilization of the anomeric carbonium ion. In the sulfoxide and sulfone structures, there is a net loss of electrons from the sulfur to the sulfoxide and sulfone oxygen atoms, decreasing the electron flow from the heterocyclic sulfur atom to the anomeric carbon atom, and hence greatly reducing the possibility of forming a carbonium–sulfonium ion.

The stability to acid of the methyl glycosides of the sugar sulfoxides and sulfone is shown by their resistance to hydrolysis by dilute mineral acid. Methyl 5-thio-β-D-xylopyranoside *S*-oxides ("α- and β-sulfoxides") and *S,S*-dioxide ("sulfone") (**1**, **2**, and **3**) are unaffected by treatment with 0.5N hydrochloric acid during 100 h at 75°; the experiment was conducted in a polarimeter tube, and no change in specific optical rotation was observed during this time. After 100 h, samples were applied to paper chromatograms, and only one spot (corresponding to starting material) was observed. Under the same conditions, the time for complete hydrolysis of methyl β-D-xylopyranoside in 0.5N hydrochloric acid is approximately 24 h at 75°, whereas, for methyl 5-thio-β-D-xylopyranoside, it is about 1 h.

Compounds **1**, **2**, and **3** were prepared by deacetylation of the corresponding 2,3,4-triacetates with methanolic sodium methoxide. Attempted deacetylation of 1,2,3,4-tetra-*O*-acetyl-5-thio-β-D-xylopyranose *S,S*-dioxide to the free sugar sulfone, however, gives rise to three water-soluble, decomposition products. It is well known that the sulfonyl group renders acidic a proton attached to an α-carbon atom. It is noteworthy that the deacetylated sulfone of the free sugar is very labile to base, whereas the deacetylated glycoside sulfone is not.

EXPERIMENTAL

Analytical Methods. — Purity of crystalline products was determined by t.l.c. on microscope slides[4] coated with Silica Gel G, and irrigated with (A) 3:1:1 (v/v) butyl alcohol–ethanol–water, or (B) 9:1 (v/v) chloroform–acetone. Location of components was by spraying with 5% sulfuric acid in ethanol and charring until permanent spots were visible. Paper chromatograms were run on Whatman No. 1 filter paper irrigated with (C) 40:11:19 (v/v) butyl alcohol–ethanol–water; permanganate–periodate spray[5] indicator was employed. Deacetylation reactions were performed with portions of a solution of powdered sodium methoxide (500 mg) in absolute methanol (100 ml).

Methyl 5-thio-β-D-xylopyranoside S-*oxide* ("α-sulfoxide") (**1**). — Methyl 2,3,4-tri-*O*-acetyl-5-thio-β-D-xylopyranoside "α-sulfoxide"[6] (1.5 g) was dissolved in 10 ml of

abs. methanol, and to this was added 20 ml of the sodium methoxide solution. The mixture was kept for 16 h at 4°; and then neutralized by passing the solution through a column of methanol-equilibrated Amberlite IR-120 (H^+) resin. The effluent was concentrated under diminished pressure to a solid residue; this was dissolved in a small volume of hot ethyl acetate, and the solution was allowed to crystallize at 4°. The yield was 0.61 g (67%), m.p. 192°, $[\alpha]_D^{20}$ +125° (*c* 1.9, water).

Kunz *O*-acetyl determination[7], and spraying a developed thin-layer chromatogram with ferric hydroxamate spray[8], showed no acetyl groups to be present. Reacetylation of **1** with acetic anhydride in pyridine gave the known tri-*O*-acetyl compound, m.p. 150°, $[\alpha]_D^{20}$ +27°.

*Methyl 5-thio-β-*D*-xylopyranoside* S-*oxide* ("*β-sulfoxide*") (**2**). — Methyl 2,3,4-tri-*O*-acetyl-5-thio-β-D-xylopyranoside "β-sulfoxide"[6] (1.5 g) was dissolved in 15 ml of abs. methanol, and 25 ml of the sodium methoxide solution was added. The reaction was processed as for **1**, to give 0.65 g (72%) of **2**, m.p. 204°, $[\alpha]_D^{20}$ −230° (*c* 0.44, water). The absence of acetyl groups was shown, and reacetylation gave the known triacetate, m.p. 206°, $[\alpha]_D^{20}$ −133°.

*Methyl 5-thio-β-*D*-xylopyranoside* S,S-*dioxide* ("*sulfone*") (**3**). — Methyl 2,3,4-tri-*O*-acetyl-5-thio-β-D-xylopyranoside "sulfone"[6] (1.5 g) was dissolved in 15 ml of abs. methanol, and 25 ml of the sodium methoxide solution was added. The reaction was processed as for **1**, to give 0.66 g (70%) of **3**, m.p. 197°, $[\alpha]_D^{20}$ −65.5° (*c* 1.7, water). Again, the absence of acetyl groups was shown, and reacetylation gave the known triacetate, m.p. 176°, $[\alpha]_D^{20}$ −69°.

TABLE I

PROPERTIES OF OXIDATION PRODUCTS

Compound	*2,3,4-Tri-O-acetyl*		*2,3,4-Trihydroxyl*		*Hydrolytic rate constant* ($sec^{-1} \times 10^5$)
	m.p., degrees	$[\alpha]_D^{20}$	*m.p., degrees*	$[\alpha]_D^{20}$	
Methyl β-D-xylopyranoside	115	− 60.8	157	− 65.5	1.08[a]
Methyl 5-thio-β-D-xylopyranoside	122	− 70.6	162	− 66.3	15.66[b]
Methyl 5-thio-β-D-xylopyranoside "α-sulfoxide" (**1**)	150	− 27.2	192	+125	
Methyl 5-thio-β-D-xylopyranoside "β-sulfoxide" (**2**)	206	−133	204	−230	
Methyl 5-thio-β-D-xylopyranoside "sulfone" (**3**)	176	− 69.3	197	− 65.5	

[a]H. S. ISBELL AND H. L. FRUSH, *J. Res. Natl. Bur. Std.*, 24 (1940) 125. [b]See ref. 1.

Attempted hydrolysis. — The crystalline glycoside (0.05 g) was dissolved in 2 ml of 0.5N hydrochloric acid. The optical rotation was read at 25°, and then water at 75° was circulated through the water-jacketed, polarimeter tube, and readings were taken every 5 h. No change in the specific optical rotation was observed at 75° during 100 h. At the end of this time period, samples were spotted on paper chromatograms,

and developed in irrigant C; the developed chromatogram showed that no change in the starting material had occurred during the treatment.

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A method for the analysis of sugars in plant cell-wall polysaccharides by gas–liquid chromatography

Numerous attempts have been made to determine the sugar composition of plant cell-wall polysaccharides[1–4] by laborious methods which are subject to large experimental errors. A promising approach to the analysis of monosaccharides involves the conversion of the sugars into their respective alditol acetates, which are then separated by gas–liquid chromatography[5,6]. This method has been used for analyzing the acid hydrolyzates of polysaccharides[7,8], but, although it has several advantages over earlier methods of polysaccharide analysis, it is unsuitable for analysis of a large number of samples. Modifications that permit rapid and reproducible analysis of the sugar constituents of polysaccharides of plant cells are here described.

Trifluoroacetic acid was used for hydrolyzing the polysaccharides, because (*a*) the yields of most monosaccharides from plant cell-wall preparations are at least equal to those obtained by hydrolysis with mineral acids, and (*b*) it may be readily removed by evaporation. Fig. 1 shows the yields of sugars obtained from cell walls of pinto-bean hypocotyls on hydrolysis with 2N trifluoroacetic acid in a sealed tube at 121° for various periods of time. In general, 2N trifluoroacetic acid (2 ml) was added to the wall material (20 mg); *myo*-inositol (1 mg) was added as an internal standard, as it is not present in cell walls and has a retention time quite different from those of the various sugars of

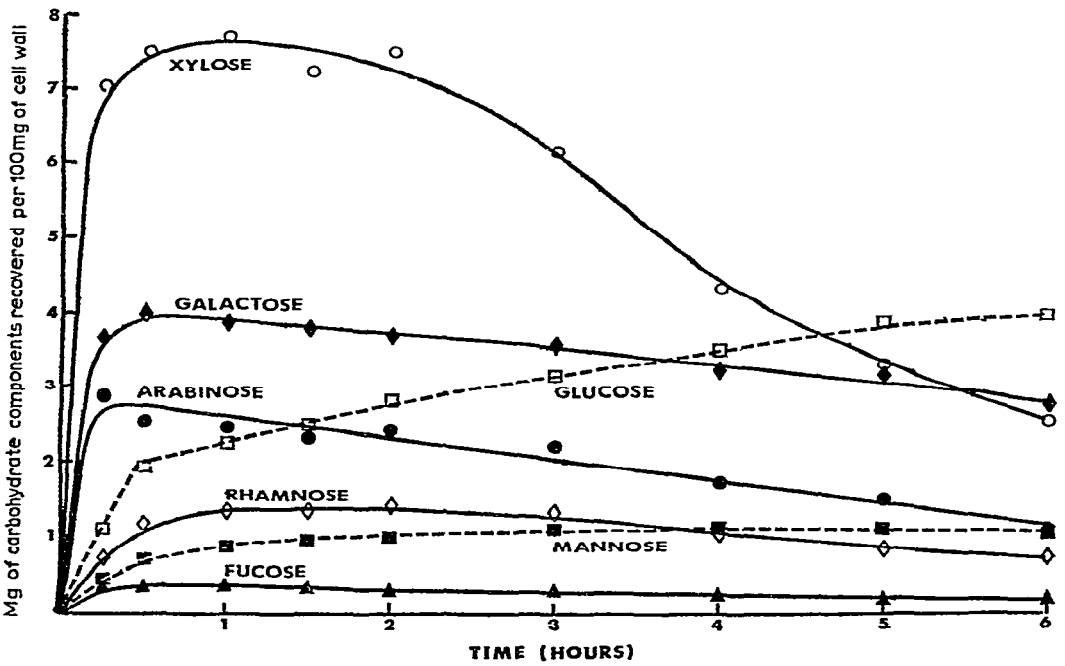

Fig. 1. Influence of the length of hydrolysis on the determination of the carbohydrate components of pinto-bean hypocotyl cell-walls. The cell walls were hydrolyzed with 2N trifluoroacetic acid at 121° for various periods of time.

the wall. After hydrolysis for 1 h (optimal for most of the sugars), the soluble portion was evaporated to dryness at 50° under a stream of filtered air.

The sugar mixtures obtained from hydrolyzed polysaccharides were reduced to their respective alditols with sodium borohydride (10 mg) in N ammonia (0.5 ml). The ammonia was added to ensure that those solutions that contained uronic acids and/or traces of trifluoroacetic acid were alkaline during reduction. After 1 h at room temperature (found sufficient for complete reduction of aldoses), the excess of borohydride was decomposed by dropwise addition of glacial acetic acid, until effervescence had ceased.

The borate produced on decomposition of the borohydride forms a complex with the alditols, and retards their acetylation. It was found that removal of boric acid improved the acetylation. A mixture of aldoses (rhamnose, xylose, and glucose) and alditols (arabinitol and mannitol) was subjected to the reduction. To aliquots (1 ml) of this mixture in test tubes, 1 to 8 additions of methanol (1 ml) were made, each followed by evaporation to dryness at 50° under a stream of filtered air. Acetic anhydride (1 ml) was then added and the tubes were sealed and heated for 1 h at 100°. It is evident from Fig. 2 that a single addition of methanol had little effect on the degree

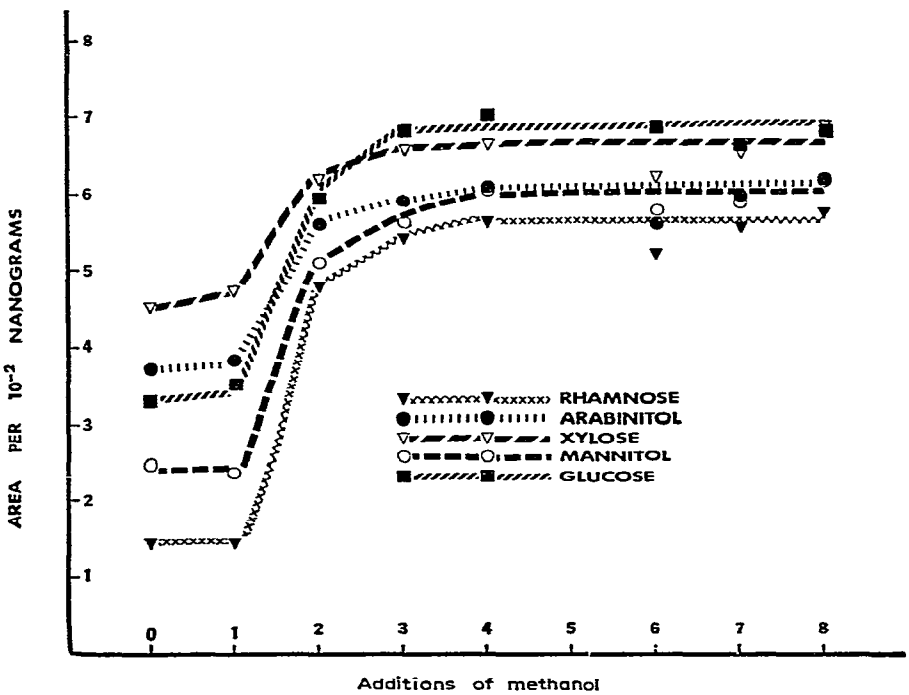

Fig. 2. Influence of the number of additions of methanol on the degree of acetylation. Aliquots (1 ml) were transferred from a mixture (10 ml) containing 1 mg of L-rhamnose, L-arabinitol, D-xylose, D-mannitol, and D-glucose per ml into each of 9 test tubes. These samples were reduced with N ammonia (0.5 ml) containing sodium borohydride (10 mg) and then subjected to various numbers of sequential additions of 1 ml of methanol with evaporation to dryness after each addition. The number of additions of methanol differed from tube to tube. The samples were acetylated, and assayed, as described in the text.

to which the alditols were acetylated (see below for details on chromatography). However, additional methanol evaporations, up to a total of 4, increased the degree of acetylation. Quantitative analysis of the boric acid remaining after each evaporation[9] showed that 5 additions and evaporations of methanol gave optimal results.

Acetylation of the alditols was performed in a sealed tube with acetic anhydride (1 ml) heated for 3 h at 121°. The sodium acetate remaining after the removal of boric acid (as its trimethyl ester) served as the basic catalyst for the acetylation reaction. Pyridine, used for this purpose by previous workers, had a deleterious effect on the column material and produced severe tailing of the peaks, especially of the solvent peak. The best g.l.c. separation was obtained with a liquid phase containing 0.2% of poly(ethylene glycol succinate), 0.2% of poly(ethylene glycol adipate) and 0.4% of silicone XF1150 coated on Gas-Chrom P (100–120 mesh) (Applied Science Labs., State College, Pa.). The dried material (*ca.* 1.1 g) was packed by vibration into a copper tube (4 ft × 1/8 in O.D.). A balancing column (5 ft long), containing about 1.4 g of the same material, was similarly packed. The columns were conditioned at 180° for 4 h. Separations with this column material were superior to those previously reported[6,8].

Fig. 3 illustrates the response of a 5.0-mV recorder to a 1-μl injection of an acetic anhydride solution containing 1 μg of each of the following: the pentaacetates

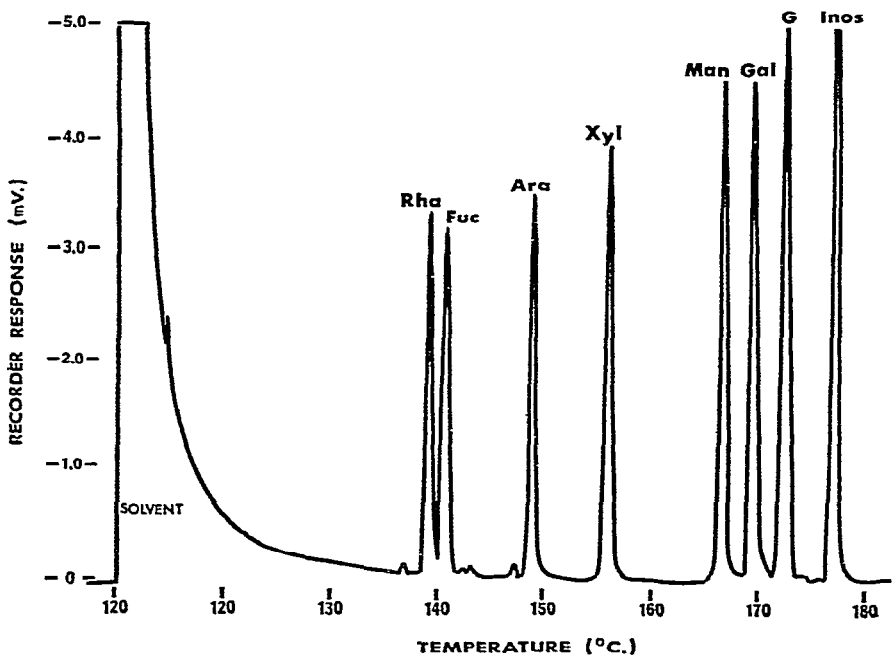

Fig. 3. Illustration of the recorder response for a 1-μl injection of acetic anhydride containing 1 μg each of L-rhamnose, D-fucose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucose, and *myo*-inositol after reduction and acetylation. The F & M gas chromatograph electrometer was set at range 10 and attenuation 1. Full-scale response on the strip-chart recorder was 5.0 mV. The injection-port temperature was 210°, and the detector temperature was 250°. The column temperature on injection was 120°; this was maintained for 10 min after injection, and then raised by 1° per min.

of arabinitol, fucitol, rhamnitol, and xylitol, and the hexaacetates of galactitol, glucitol, mannitol, and *myo*-inositol. The sensitivity achieved in this procedure (see Fig. 3) was obtained by setting the electrometer at range 10 and the attenuation at 1. The area under each peak was measured by electronic integration. The output of the gas chromatographs was recorded on instrumentation-quality magnetic tape (Scotch Brand #860, Minnesota Mining and Manufacturing Co.) with an Infotronics Model CRS-43R1D magnetic tape recorder, and was monitored by means of an Infotronics Model CRS-40TS playback system at 16 times the recording speed (7.5 in/sec). Integration of the response peaks was achieved with an Infotronics digital readout system Model CRS-11HS/42.

Results obtained in the analysis of cell-wall polysaccharides are given in Fig. 4. The cell walls analyzed in this experiment were obtained from sycamore (*Acer pseudoplatanus*) cells grown in suspension culture[10] on a defined (M1D) medium*. A sample of cell wall (16 mg) was hydrolyzed, and reduced, and then acetylated with acetic anhydride (1 ml). One μl was injected into the gas chromatograph to give the recorder responses shown in Fig. 4.

The accuracy and precision of this method of analysis were tested as follows (Table I): samples, each containing individually weighed amounts of the 7 sugars

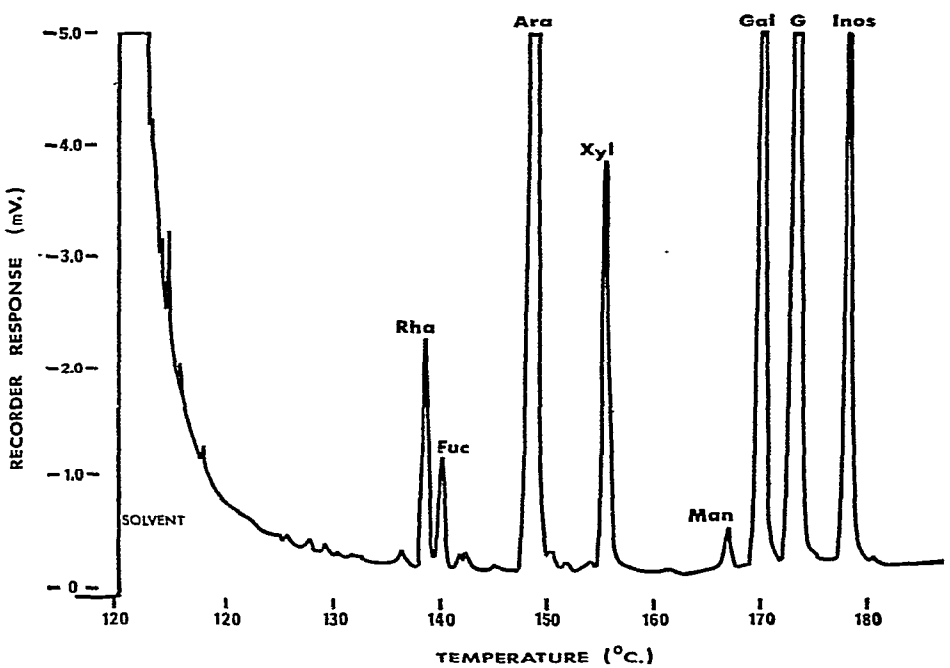

Fig. 4. Gas-chromatographic analysis of a 1-μl injection containing the material from sycamore (*Acer pseudoplatanus*) cell-walls. The cell walls were hydrolyzed, and the products reduced, acetylated, and analyzed as described in the text.

*Personal communication from Philip Filner, Michigan State University.

found in plant cell-walls and *myo*-inositol (1 mg/ml), were subjected to acid hydrolysis, reduction, acetylation, and assay by gas chromatography. L-Arabinose, D-fucose,

TABLE I

PRECISION AND ACCURACY OF THE METHOD

Injection No.	*Proportion of sugar components after repeated injection*[a]						
	L-Rhamnose	*D-Fucose*	*L-Arabinose*	*D-Xylose*	*D-Mannose*	*D-Galactose*	*D-Glucose*
1	0.439	0.351	0.444	0.550	0.644	0.563	0.789
2	0.462	0.367	0.444	0.546	0.655	0.580	0.760
3	0.458	0.377	0.439	0.575	0.660	0.579	0.802
4	0.428	0.353	0.422	0.534	0.631	0.555	0.808
5	0.452	0.369	0.445	0.564	0.661	0.578	0.807
6	0.453	0.367	0.441	0.559	0.668	0.587	0.815
Mean	0.449	0.364	0.439	0.555	0.653	0.574	0.797
Standard deviation	0.012	0.009	0.008	0.013	0.012	0.011	0.018
Standard value[b]	0.440	0.350	0.423	0.546	0.654	0.599	0.800

[a]Ratio of the area of 1 μg of sugar to the area of 1 μg of *myo*-inositol, obtained by repeated injection of 1-μl samples containing all sugars as their alditol acetates (see text). [b]The standard value represents the mean of 13 such samples injected at least 5 times each.

D-galactose, D-mannose, and D-xylose were purchased from Sigma Chemical Company, L-rhamnose from Pfanstiehl Laboratories, and D-glucose from J. T. Baker & Co.; these were found to contain less than 1% of monosaccharide contaminants. *myo*-Inositol (California Biochemical Corporation) was recrystallized 3 times from ethanol–water and contained no detectable contamination. The detector response was found to be a linear function of the amount of sugar injected over the range of 0.25 to 3 μg. Amounts of sugar above this range (as high as 12 μg) were also accurately determined. Each sample was injected repeatedly into 2 different FM gas chromatographs. The data are presented in Table I as the ratio of the area obtained for 1 μg of sugar to that for 1 μg of *myo*-inositol. The mean values obtained for the various sugars of this sample deviate only slightly from the standard values presented on the bottom line.

The major advantages of this procedure over the methods previously reported[6–8] are: (1) The trifluoroacetic acid used in the hydrolysis of polysaccharides is readily removed by evaporation. (2) The entire procedure may be performed in a single test-tube (a second test-tube is required when the hydrolyzed material contains an insoluble residue; this necessitates a single tranfer). (3) Acetylation of the alditols is catalyzed by the sodium acetate present, thus eliminating the need for pyridine. Hence, the acetylation mixture may be injected directly into the gas chromatograph. (4) Acetylation of the alditols is performed in a sealed tube, which eliminates the need for heating under reflux. (5) The column material described gives a better separation of the

derivatives of the sugars obtained from plant cell-walls than do commercial preparations currently available.

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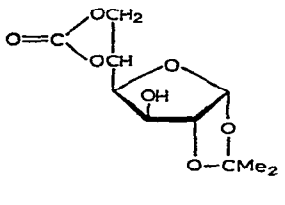
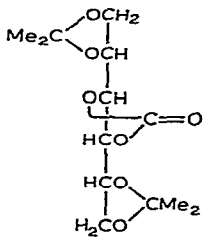
A novel method for preparation of some sugar carbonates

Sugar carbonates are most frequently prepared either by treating the sugars with an alkyl chloroformate or phosgene, in the presence of base, or by transesterifying with dialkyl, diaryl, or cyclic alkylene carbonates[1]. A novel method has been developed for the preparation of 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-carbonate (**1**) and 1,2:5,6-di-*O*-isopropylidene-D-mannitol 3,4-carbonate (**2**) by treatment of *O,O'*-



1

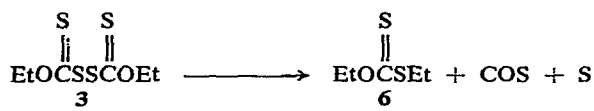


2

diethyl dithiobis(thioformate) (**3**) with 1,2-*O*-isopropylidene-α-D-glucofuranose (**4**) and 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**5**), respectively.

In an attempt to adapt a transesterification reaction for the preparation of thionocarbonate derivatives of sugars, **4** was treated in pyridine with an excess of **3** for a few days. If **3** undergoes exchange with the sugar molecule, the product should, as shown previously, undergo a subsequent rearrangement–fragmentation reaction, and 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate would be formed[2,3]. Although a reaction did occur (as evidenced by the disappearance of absorption maxima for **3** in the u.v. spectrum), no thionocarbonate could be identified in the resulting mixture. Instead, the carbonate **1** was isolated in about 30% yield. By using triethylamine as the base, and acetone or methyl sulfoxide–acetone as the solvent, the yield of **1** was increased to 55–85% in a reaction time of 7 min. Apparently, the corresponding thionocarbonate is not an intermediate in formation of the carbonate, as it was recovered almost quantitatively after it had been kept in methyl sulfoxide–acetone–triethylamine in the presence of **3**. A 33% yield of **1** was obtained when **4** was dissolved in ethanol and treated with *O,O'*-diisopropyl dithiobis(thioformate) in the presence of triethylamine.

By analogy with the behavior of some *O,O'*-dialkyl dithiobis(thioformates) in basic organic solvents[4], **3** was expected to undergo rearrangement–fragmentation as follows:



Indeed, *O,S*-diethyl dithiocarbonate (**6**), carbonyl sulfide, and elemental sulfur, together with a small proportion of carbon disulfide, were isolated and identified if **3**

was kept either in pyridine or triethylamine. Under the conditions used in the reaction leading to carbonate formation, neither **6** nor carbonyl sulfide reacted with **4** to give **1**; therefore, the intermediate product formed during the decomposition of **3** must have been the reactive species that gave **1**.

In other experiments (with triethylamine as the base and methyl sulfoxide–acetone as the solvent), the amounts of **1**, carbonyl sulfide, carbon disulfide, and **6** were measured and compared with those in a blank experiment that contained all of the reagents except **4**. For every mole of **1** formed, about 1 mole of carbonyl sulfide and 1.5 moles of **6** were lost, whereas the carbon disulfide content increased by about 2 moles; this increase was reached only after acidification of the reaction mixture. These results can be interpreted if it is assumed that two different reactions take place in the presence of **4**. In one, normal decomposition of **3** occurs to give **6**, carbonyl sulfide, and sulfur, as in the blank; in the other, 1 mole of **4** reacts with **3** to give 2 moles of *O*-ethyl dithiocarbonic acid (ethylxanthic acid) which, in the presence of triethylamine, forms the ethylxanthate salt. Indeed, a strong absorption at 303 nm, indicative of xanthate, was noted after the reaction was complete and before acidification.

When **5** was dissolved in ether and treated with **3** in the presence of triethylamine, **2** was isolated in about 50% yield.

EXPERIMENTAL

O,O′-*Diethyl dithiobis(thioformate)* (**3**) and O,O′-*diisopropyl dithiobis(thioformate)*. — Ethanol (100 ml) was mixed with pulverized sodium hydroxide (100 g) and the mixture was kept for 16 h at 25°. The crystalline mass that formed was dissolved in a small volume of water, and then carbon disulfide (150 ml) was added. After neutralization of excess alkali with aqueous acetic acid, and oxidation of the xanthate formed with iodine, the yellow solution was extracted with ether. Evaporation of the extract gave **3** as a yellow syrup which gave an acceptable analysis for sulfur.

By the same procedure, *O,O′*-diisopropyl dithiobis(thioformate) was obtained.

*1,2-*O*-Isopropylidene-α-*D*-glucofuranose 5,6-carbonate* (**1**). — (*a*) 1,2-*O*-Isopropylidene-α-D-glucofuranose (**4**, 300 mg) was mixed with methyl sulfoxide (0.5 ml), acetone (1 ml), triethylamine (1 ml), and **3** (2.5 g). The mixture was kept for 7 min in a water bath at 42°, during which time, bubbles of carbonyl sulfide were detected. The solvents were evaporated, and the residue was washed with carbon disulfide and water, and dried, to yield 285 mg of **1** as yellow crystals. When recrystallized from ethanol–water, the m.p., and mixed m.p. with an authentic sample, was 228–230°, and the i.r. spectrum was superimposable upon that of an authentic sample.

(*b*) To a solution of **4** (300 mg) in 1 ml of ethanol, *O,O′*-diisopropyl dithiobis (thioformate) (2.5 g) was added. The mixture was kept for 40 h at 25°, and then treated as in (*a*) to yield **1** (105 mg).

*1,2:5,6-Di-*O*-isopropylidene-*D*-mannitol 3,4-carbonate* (**2**). — 1,2:5,6-Di-*O*-isopropylidene-D-mannitol (**5**, 300 mg) was dissolved in ether (2 ml) and triethylamine

(1 ml). After addition of **3** (2.4 g), the mixture was kept for 20 h at 25°. Compound **2** (155 mg) was isolated by the method used for **1** (procedure *a*); m.p. 143–144°. Baker and Sachdev[5] reported m.p. 144–146° for **2**, whereas we observed m.p. 143–144° for **2** prepared by oxidation of 1,2:5,6-di-*O*-isopropylidene-D-mannitol 3,4-thionocarbonate with silver nitrate.

Determinations of carbonyl sulfide, carbon disulfide, O,S-*diethyl dithiocarbonate* (**6**), *and* **1**. — To a solution of **4** (500 mg) in a mixture of methyl sulfoxide (0.5 ml) and acetone (1 ml) were added triethylamine (1 ml) and **3** (1.0 g). This mixture was kept for 7 min at 42°, and the carbonyl sulfide and carbon disulfide were then collected in ethanol (500 ml) containing 4% of diethylamine by flushing the solution with nitrogen, acidifying it with dilute hydrochloric acid, and flushing it again with nitrogen. The concentrations of carbonyl sulfide and carbon disulfide in the ethanolic solution were calculated after dilution of a 5-ml sample to 125 ml with ethanol containing 0.4% of diethylamine. Calculations were based on $\lambda_{max}^{EtOH-0.4\%Et_2NH}$ 225 nm (ε 9,750) for carbonyl sulfide and 290 nm (ε 13,400) for carbon disulfide.

In another experiment under the same conditions, the reaction mixture, after 7 min at 42°, was diluted with ethanol (10 ml). A 1-ml sample of the solution was diluted with ethanol (2.5 liters) containing N hydrochloric acid (2 ml) and the concentration of **6** was calculated from the absorption at 280 nm. Pure **6** was prepared for reference by rearrangement of **3** in pyridine.

In a third experiment under the same conditions, **1** (285 mg) was isolated as described above.

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Formate contamination in borohydride reduction

Methods for the degradation of labeled carbohydrates by periodate oxidation often include borohydride reduction of intermediate aldehydes. Although the borohydrides are known to be essentially specific carbonyl reductants, an exception to this rule has been observed, to a small, but significant extent, in the reduction of CO_2 to formate. Since formate commonly appears as an end-product of the periodate oxidation of carbohydrates, the introduction of adventitious formic acid can seriously impair the validity of the results of degradation studies.

Interference from this reaction first became apparent in the chemical degradation of D-glucose-*5-C-d* synthesized in this laboratory. On periodate oxidation of a mixture of glycol and glycerol-*2-C-d* derived from methyl D-glucoside-*5-C-d* by periodate oxidation, followed by borohydride reduction, about three times as much formate-*d* was isolated as could be accounted for by the secondary alcoholic group of glycerol; deuterium abundance was correspondingly diminished, as compared to the expected value based on total glucose-deuterium content. After various fruitless attempts to explain this discrepancy in terms of contamination from reagents, overoxidation, etc., it was found that the excess formate results from the destruction of the excess of borohydride by saturation with CO_2, as described by Bevill *et al.*[1]. Since intermediates between methyl glucoside-*5-C-d* and its ultimate degradation products, formaldehyde and formate-*d*, were not isolated, extraneous formate, accumulated from two successive reductive steps in the degradation sequence, appeared as a diluent of formate-*d*. With ^{14}C instead of D, the same error would obviously have been observed.

The only known example of a reaction of this kind was reported by Wartik and Pearson[2] who observed the absorption of CO_2 by borohydride at 125° under anhydrous conditions and in ether at room temperature; in both circumstances, compounds were formed that produced formate on hydrolysis. Whether the reduction of CO_2 to formate can occur in aqueous solution had apparently never been tested until an explanation was sought for the discrepancy found in the glucose-*5-C-d* degradation. When CO_2 was passed through aqueous $NaBH_4$ under conditions identical to those used in the destruction of borohydride, small amounts of formate could be isolated from the reaction mixture; DCOONa was prepared similarly from $NaBD_4$.

Although there are alternative methods for destroying borohydride which do not lead to formate, there are other implications of this reaction to be considered in the use of this reagent. Frush and Isbell[3] recognized the reaction of Wartik and Pearson[2] as a potential source of formate in the borohydride reduction of carbohydrates in hydrogen carbonate buffer, a consideration validated by the results of the present investigation. Another possible mode of formate contamination incident to this reaction is exposure of borohydride to atmospheric CO_2. A recent method[4] for assay of formate arising from periodate oxidation of glycosides makes use of borohydride reduction for removal of interfering dialdehydes formed concomitantly; the accuracy of this method obviously depends on careful estimation of reagent blanks. In the use

of $NaBT_4$ for preparation of labeled sugars, unsuspected trace contamination by formate-*t* can be deleterious unless steps are taken to exclude it. Since borohydride is used in large excess, even small yields of formate can introduce substantial contamination into reduction products. It is therefore strongly recommended that, in general, products of borohydride reduction be isolated or appropriately treated to ensure removal of formate before further processing.

EXPERIMENTAL

Preparation of sodium formate. — An aqueous solution of $NaBH_4$ (600 mg in 25 ml) was saturated at room temperature with CO_2 for 30 min during which time it became to weakly basic. The solution was then treated with a small amount of IR-120 $(H)^+$ resin to liberate CO_2 and passed through a column of the same resin. After alkalinization with NH_4OH the effluent was evaporated to dryness in a rotary evaporator at 30°, and the residue was dried by addition and evaporation of methanol. The solid residue was sublimed *in vacuo* at 60° to give 24 mg (6% yield) of crystalline product, m.p. 115–117°, free from borate when examined with the flame test. The ammonium salt was converted into the sodium salt by treatment with conc. alcoholic NaOH; centrifugation and washing of the precipitate with cold ethanol gave, after drying, 20 mg of crystalline solid which softened at 253° and melted at 159–260°; the m.p. was unchanged after admixture with authentic sodium formate. Identity of the compound was confirmed by the preparation of *p*-bromophenacyl formate by the procedure of Gabriel[5]. The recrystallized ester migrated at the same speed as that of authentic formic ester on thin-layer silica gel plates in 95:5 benzene–ethyl acetate, as shown by u.v., H_2SO_4 charring, and (2,4-dinitrophenyl)hydrazine spray.

Preparation of sodium formate-d. — Repetition of this procedure with $NaBD_4$ (99% D) gave the same mass yield of DCOONa (99% D), assayed for deuterium by the procedure of Eisenberg[6] after appropriate dilution with carrier sodium formate.

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The degree-of-polymerization and polydispersity of cellulose in beechwood, and its radial variation across fibre cell-walls

Several workers have studied the fine structure of cellulose microfibrils, but few have considered structural changes across the walls of mature-plant cells. Hessler *et al.*[1] found that, for cotton, the degree-of-polymerization (DP) of cellulose is higher in the secondary wall than in the primary wall, and Frey-Wyssling[2] reached a similar conclusion when he compared cellulose in the primary wall of *Zea mays* with cellulose in the secondary wall of ramie fibres. More recently, Marx-Figini[3] has suggested that, in cotton, a template mechanism produces homogeneous, long-chain polymers in the secondary wall, whereas there is no such template in the primary wall where smaller molecules with a broad distribution of DPs are formed.

Woody tissue is more difficult to study than is cotton. In the only published paper[4] on changes of DP in cellulose across lignified cell-walls, it was concluded that there is a continual increase in the DP of cellulose from the middle lamella to the lumen in tracheids of *Pinus densiflora* and *Abies firma*, but some of the assumptions made are of very doubtful validity. Other work[5,6] has been done on the DP and polydispersity of cellulose from several species of wood but has been concerned with the material as a whole.

In order to study the variation in the DP and polydispersity of cellulose across the walls of beech fibres, the soft-rot fungus *Chaetomium globosum* was used to remove preferentially cellulose from the S_2 layer of beechwood-fibre walls. It has been shown[7] by optical and electron microscopy and chemical analysis that, in the early stages of cell-wall decomposition, the fungus grows within the S_2 layer in the

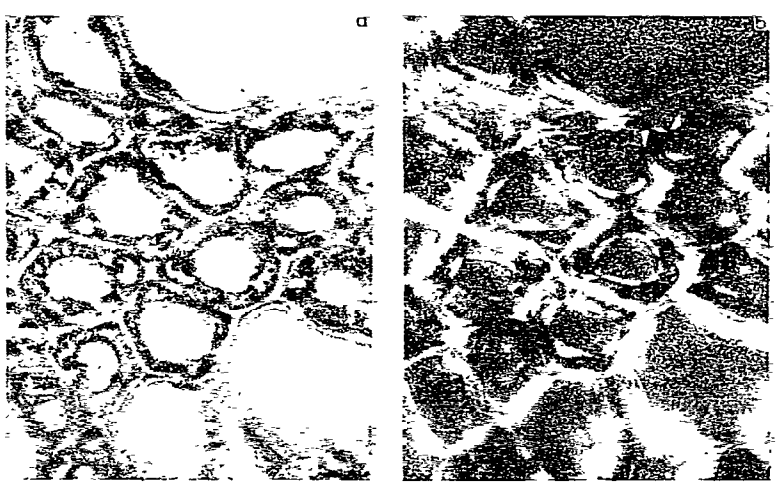

Fig. 1. Photomicrographs of a transverse section of beechwood cells showing cavities produced by the soft-rot fungus *Chaetomium globosum* in the S_2 layers of the cell walls, (*a*) in unpolarized light, (*b*) between crossed polarizers. Magnification × 700.

longitudinal direction of the microfibrils, and metabolises the carbohydrates in this layer. Dissolution is localised around the hyphae, and Fig. 1 shows typical cavities produced in this way. As decay proceeds, carbohydrates are removed throughout the wall, but the primary wall and the S_3 layer survive longest. Thus, by determining the DP of cellulose in wood decayed for different times, it is possible to draw conclusions about the DP of cellulose in the various layers.

Beech veneers were decayed with *Chaetomium globosum* for different periods[7], the cellulose was extracted by direct nitration[6], and its number-, weight-, and Z-average DP were calculated from light-scattering measurements made upon the cellulose nitrate in acetone[8], and by application of the theory due to Benoit *et al.*[9] for Gaussian chains.

Fig. 2 shows how the number-, weight-, and Z-average DP of cellulose in decayed wood vary with overall weight-loss. The significance of the viscosity-average DP has been discussed elsewhere[8].

The variation of each type of average with overall weight-loss may be explained in the following way. Except at very advanced stages of decay, the fungus has little effect upon the longer-chain molecules and removes only those of shorter or intermediate length. The chains are not removed in complete units, however, but are

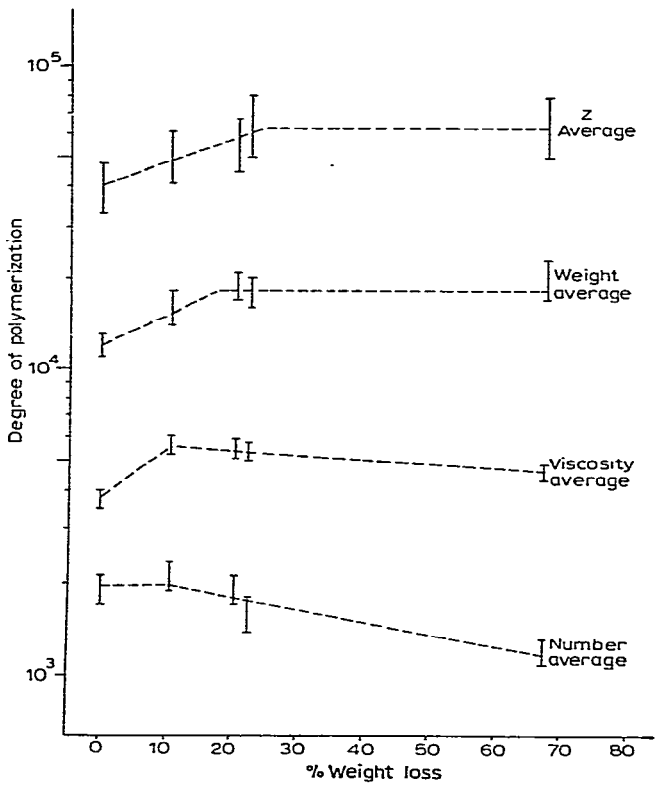

Fig. 2. Variation in the degree-of-polymerization of cellulose nitrate derived from the α-cellulose of beechwood veneers that had been subjected to various degrees of attack by *C. globosum*.

degraded. Thus, the residue consists of the longer chains, the unattacked chains of shorter or intermediate length, and degraded chains. As decay proceeds, the longer chains and the degraded chains account for a greater proportion of the residue. The system therefore becomes more polydisperse, and the ratio of weight-to number-average DP increases. Provided that the weight of degraded material present is sufficiently small, the residual, long-chain molecules will cause the weight-average DP to rise as decay proceeds. The number-average DP, however, depends upon the relative number of molecules present of each size. A small proportion by weight of highly degraded material will represent a relatively high proportion of the total number of molecules, and this causes the number-average DP to fall as decay proceeds. The Z-average DP varies in a similar manner to the weight average. This is to be expected as it depends even more upon the larger molecules. The fact that the weight- and Z-average DP cease to rise after about 20% weight-loss may be due to an increase in the proportion of degraded material or it may be due to a partial attack of the longer-chain molecules.

An essential requisite of the above explanation is that there is a region of long-chain molecules which is not affected until the final stages of decay. It has been shown by microscopy that the S_3 layer and the primary wall are most resistant to attack, and it is generally recognized that the primary-wall cellulose has a lower DP than secondary-wall cellulose[1-4]. It therefore follows that long-chain molecules are concentrated in the S_3 layer, in agreement with the findings of Shimizu *et al.*[4].

Apart from indicating the high degree of polydispersity of the material, Fig. 2 does not readily convey any pictorial impression of the distribution of DPs. It is not possible to obtain distribution curves unambiguously, given only number-, weight-, and Z-average DPs. If, however, one assumes arbitrarily a smooth distribution having only one peak, it is possible to select a suitable mathematical function having three constants and to determine these constants from the number-, weight-, and Z-average DP. The curves so obtained are not likely to vary very much with the mathematical function chosen, as three parameters are enough to fix the position and width of the peak, and the skewness. The commonly used Schulz–Zimm function[10] is not suitable for this purpose, as it has only two undetermined constants and predicts that $P_z - P_w = P_w - P_N$, where P is the DP. A modified version was therefore adopted *viz*:

$$F(P) = F_o P^a (1 + CP)\, \varepsilon^{-bP} \tag{1}$$

where $F(P)dP$ is the fraction by weight of material having DP between P and $P + dP$. F_o is the normalization function given by:

$$\frac{1}{F_o} = \frac{\Gamma(a+1)}{b^{a+1}}\left[1 + \frac{C(a+1)}{b}\right] \tag{2}$$

The number-, weight-, and Z-average DPs are then given by:

$$P_N = \frac{a}{b}\left[\frac{b + c(a+1)}{b + ca}\right]$$

$$P_w = \frac{a+1}{b}\left[\frac{b+c(a+2)}{b+c(a+1)}\right] \qquad (3)$$

$$P_z = \frac{a+2}{b}\left[\frac{b+c(a+3)}{b+c(a+2)}\right]$$

whence a, b, and c may be calculated. In view of the very broad distribution of DPs, it was decided to plot the logarithmic distribution function $G(P)$, where $G(P)\ d(\log P)$ is the fraction by weight of material whose logarithm of DP lies between $\log P$ and $\log P + d(\log P)$ and is given by:

$$G(P) = 2.3P\ F(P) \qquad (4)$$

Fig. 3 shows the curves obtained in this way for three different weight-losses. The normalization function has been adjusted so as to take into account the weight loss of α-cellulose (calculated from the overall weight-loss, using the data of Levi and Preston[7]). The curves therefore represent the actual amounts of material present at each DP, and clearly show that material of high molecular weight is not affected by the fungus until the advanced stages of decay. It would seem from these curves that the upper limit for the DP in the S_3 layer is about 100,000. At 20.5% weight-loss, only material

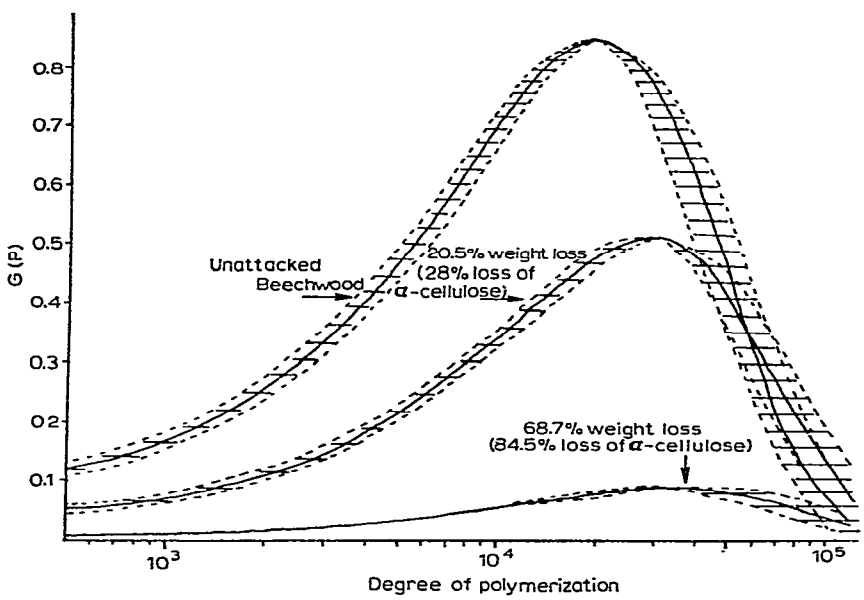

Fig. 3. Frequency-distribution curves for the α-cellulose of beechwood veneers that had been subjected to various degrees of attack by *C. globosum*. In each case, the area beneath the curve is proportional to the total weight of material present (see text). The shaded areas represent the errors estimated from the data of Fig. 2.

with DP below 50,000 has been removed. At higher weight-losses, it is possible that a partial attack on the S_3 has begun. The upper limit for the DP in the S_2 layer may therefore be as low as 50,000.

The distribution curve for unattacked wood is considerably broader than those obtained by Timell[5] who calculated his curves from viscometric measurements made upon a large number of fractions. This discrepancy may be due in part to a higher degree of degradation during the extraction procedure in the present work, although the extraction procedure used was that due to Timell. However, the validity of conventional methods for calculating distribution curves from measurements made upon several fractions has been severely questioned by several authors[11], especially where the fractions themselves are polydisperse. More-recent work on cellulose extracted from cotton, employing fractionation by gel-permeation chromatography[12], gives a distribution curve similar to that presented here.

In conclusion, it may be said that cellulose chains in the microfibrils of beech-fibre walls have a wide distribution of degrees-of-polymerization, and that the longer chains seem to be concentrated in the S_3 layer.

It must be emphasized that the fungus *Chaetomium globosum* was used in this work purely as a means of removing the S_2 layer of the cell wall. It must *not* be concluded at this stage that the S_3 layer is resistant to attack because it contains the longer-chain molecules, as there are other factors, such as the lignin content and degree of crystallinity, whose variation across the cell wall must be considered.

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The presence of sulphate in epithelial mucus

It has been shown by histochemical and autoradiographic studies in animals that sulphate is a constituent of epithelial mucus[1–4]. Similar findings have recently been made in man by histochemical[5] and autoradiographic techniques[6,7]. Acid hydrolysis of neuraminic acid-containing glycoproteins produces dark-coloured material that impedes colorimetric or turbidimetric analysis. Removal of the interfering matter by wet oxidation can oxidise non-sulphate sulphur to an appreciable extent, giving spuriously high analyses. Consequently, accurate determination of sulphate is difficult, and conflicting data have been reported on the estimation of sulphate in mucus by chemical procedures. Sulphate has been claimed to be absent from the glycoproteins of human nasal[8], tracheo-bronchial[8,9], cervical[8], gall-bladder[8], and bovine cervical[10] secretions. The sulphate present in gastric secretion[8,11] was originally thought to be due to the presence of mucoitin sulphate containing 2-acetamido-2-deoxy-D-glucose, D-glucuronic acid, and sulphate, but more-recent work[12–14] has shown that uronic acid is absent from human gastric secretion, and that the sulphate is linked to the glycoproteins. Sulphated glycoproteins have recently been isolated from ovine colonic mucus[15], canine submaxillary glands[16], and human bronchial mucus[17].

An improved method of determining sulphate in epithelial mucus was introduced by Gibbons[18], who used columns of Deacidite FF and hydrated zirconium oxide to remove the decomposition products of neuraminic acid prior to estimating the sulphate. This method has now been used to determine the sulphate content of a number of mucus secretions. The material under investigation was hydrolysed with N hydrochloric acid for 16 h at 100°, and the hydrolysate was lyophilised. The residue was dissolved in water and subjected to the column treatment[18], and the sulphate content was determined by the method of Gibbons and Wolfrom[19].

Sputum. — Samples collected from two adults suffering from chronic bronchitis and from two children with fibrocystic disease were dialysed for 4 days at 4° with 3 changes of distilled water. In order to solubilise the sputum, 15–25 mg of crystalline ficin was added to 100 ml of secretion, without adjusting the pH, and the solution was then incubated for 24 h at 37° and lyophilised. The sulphate content of these samples ranged from 0.68–1.72% of the dry weight. Bronchial mucosa from 2 of these 4 cases showed[7] an uptake of radioactive sulphate on tissue culture (the detailed results will be published later). Hydrolysis of sputum with 0.1N hydrochloric acid for 2 h at 80° removed the sialic acid present, as estimated by the procedure of Warren[20]. Dialysis of the hydrolysate for 5 days at 4° with 10 changes of distilled water did not decrease the sulphate content of the indiffusible material, indicating that the sulphate was not attached to a residue of sialic acid. Fractionation of an untreated sample of sputum (into a gel phase and a supernatant by centrifugation at 80,000 *g* for 75 min) revealed that most of the bound sulphate was associated with the gel phase.

Cervical mucus. — Mucus collected from cows at or near oestrus and from

pregnant animals was dialysed exhaustively at 0–4° against distilled water, and the indiffusible material was isolated by lyophilisation. These samples contain significant amounts of sulphate (oestrus mucus, 1.01%; pregnancy sample, 1.34%). Isolation[10] of the glycoproteins from oestrus and pregnancy mucus showed that these were the source of bound sulphate in mucus (glycoprotein from oestrus mucus, 2.09%; that from pregnancy mucus, 2.71%).

Ovarian-cyst fluid. — A glycoprotein with blood-group A activity was isolated from ovarian-cyst fluid by phenol fractionation[21]. The sulphate content (0.47%) of this material was much lower than that of the cervical glycoprotein.

Hog gastric glycoprotein. — A glycoprotein was isolated from commercial (B.D.H.) hog gastric-mucus by phenol extraction and further purified by fractionation with cetyltrimethylammonium bromide[22]. This contained only 0.51% of sulphate, which is much lower than the figures given by Häkkinen *et al.*[14] for human gastric glycoprotein, but this is probably due to removal of the more highly sulphated materials during the fractionation with cetyltrimethylammonium bromide.

Submaxillary-gland glycoproteins. — A glycoprotein isolated[23] from bovine submaxillary-gland contained only 0.41% of sulphate, whereas a glycoprotein from pig submaxillary[24] had an even lower content (0.23%) of sulphate.

These results provide chemical confirmation of the histochemical and autoradiographic data, showing that sulphate is an important constituent of epithelial mucus, the amount present varying with the source and the species.

Further, these results show that the sulphate in epithelial mucus is bound to the glycoprotein constituents and make it unnecessary to postulate the presence in mucus of such glucosaminoglycans at keratosulphate, unless these are isolated and definitely identified in epithelial secretions.

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A micro method for the determination of the configuration of glycosidic linkages

In the structural characterization of such complex polymers as teichoic acids[1], glycoproteins[2], and glycolipids[3], it is necessary to determine the configuration of the glycosidic linkages by which the carbohydrate residues are attached to the remainder of the polymer. Often, such determinations must be performed on micro-quantities of a sample, and as a result, are difficult to achieve by the usual chemical or enzymic procedures. We have utilized an enzymic micro-procedure for establishing the configuration of the glycosidic linkage in a hydrolytic fragment from the teichoic acid in the cell wall of *Streptococcus faecalis*. The method has been adequately tested with such known compounds as maltose, isomaltose, 1-*O*-(α-D-glucopyranosyl)glycerol, phenyl α-D-glucopyranoside, cellobiose, gentiobiose, and amygdalin. The procedure is equally useful for the determination of the configuration of the glycosidic linkage in *O*-(D-glucopyranosyl)serine, a possible hydrolytic fragment from glycoproteins[4]. Because the method should prove to be of general applicability in the structural analysis of many carbohydrate polymers, a description thereof is here presented.

The procedure involves application of a solution of the compound under study to a D-glucose oxidase test-strip. While the test strip is still moist, a solution of a specific D-glucosidase is placed directly on the compound on the test strip. Compounds having α-D-linked D-glucosyl residues will be hydrolyzed by an α-D-glucosidase, and compounds having a β-D-linked D-glucosyl residue will be hydrolyzed by a β-D-glucosidase. The D-glucose liberated in the reactions is oxidized by the D-glucose oxidase on the test strips, with a concurrent color change in the dye on the strips from red to blue. As the D-glucose oxidase strip is extremely sensitive for D-glucose[5] (10 nanograms of D-glucose is readily detected), a very small amount of the original compound is used, and the method is, indeed, an ultra-micro one. Many D-glucosidases (for example, the D-glucohydrolase of *Aspergillus niger*[6] and the β-D-glucosidase of almonds[7]) possess a broad substrate-specificity, but are extremely specific for the configuration of the D-glucosyl linkage; thus, the configuration of the D-glucosidic linkage in a large variety of polymeric fragments can be determined by this procedure. A sufficient number of blanks and controls must be used simultaneously with the test, in order to minimize possible error due to contamination of the enzyme solution or polymer fragments with free D-glucose. Should the compound under test be slowly hydrolyzed by the enzyme, pre-incubation of the enzyme with the compound, prior to the use of the D-glucose oxidase test-strips, is recommended.

Although D-glucose oxidase reacts very rapidly with D-glucose, other monosaccharides, for example, D-galactose and D-mannose, are slowly oxidized by the enzyme[8]. It is likely that the method can be adapted for determination of the configuration of glycosidic linkages in galactosyl and mannosyl compounds by use of specific galactosidases and mannosidases. In the latter cases, preincubation of the enzyme with the compound will be necessary, in order that sufficient amounts of the monosaccharides are produced to give a positive test.

EXPERIMENTAL

Materials. — Bacterial cell-walls from *Streptococcus faecalis* were prepared as described previously[9]. The teichoic acid was not isolated as such from the cell walls, but was hydrolyzed *in situ* by the use of 60% hydrofluoric acid[10]. A dried sample (5–10 mg) of cell-wall fragments was placed in a tube made of cellulose nitrate, 0.1 ml of 60% hydrofluoric acid was added to the sample, and the mixture was kept in a refrigerator for 15 min. During this time, the phosphoric diester bonds of the teichoic acid were hydrolyzed, but the glycosidic bonds by which some of the carbohydrate residues are attached to the glycerol or ribitol portion of the teichoic acids remained intact.

The hydrolytic fragments in the reaction mixture were separated by paper chromatography in a solvent system of 9:3:7 (v/v) butyl alcohol–pyridine–water. Fragments containing carbohydrate were located by staining marker strips from the paper with silver nitrate reagent. The hydrolytic fragments were extracted with water from the unstained portions of the chromatogram, and the extracts were concentrated to a small volume. These solutions were used for the tests.

A sample of *O*-(β-D-glucopyranosyl)serine was provided by Dr. S. Roseman, Johns Hopkins University, Baltimore, Maryland, and a sample of 1-*O*-(α-D-glucosyl)-glycerol was provided by Dr. E. J. Hehre, Albert Einstein College of Medicine, New York, N. Y. Samples of maltose, isomaltose, phenyl α-D-glucopyranoside, cellobiose, gentiobiose, and amygdalin were available in our laboratory.

A solution of the D-glucohydrolase of *Aspergillus niger*, having 800 units of activity per ml, was prepared in our laboratory[11]. The almond β-D-glucosidase was a product of the Nutritional Biochemicals Corporation, Cleveland Ohio. Because D-glucosidases possess broad optima of pH, aqueous solutions (1–5%) of the β-D-glucosidase were suitable for use in the experiments. D-Glucose oxidase test-strips were obtained from the Ames Laboratories, Division of Miles Laboratories Inc., Elkhart, Indiana.

Procedure. — Aqueous solutions (0.1—1%) of the compounds under study were suitable for the test. Aliquots of 1 μl of the solution were placed on several D-glucose oxidase strips by means of a micropipette or a microbiological loop. While the test strip was still moist, a 1-μl sample of the enzyme solution was placed directly on the compound on the various test strips. Two control test-strips, one moistened with the enzyme solution and the other with the compound, were also prepared. All of the test strips were examined continually for changes in color.

Appearance of a blue color on the test strip indicates the liberation, by the D-glucosidase, of D-glucose from the compound. D-Glucose oxidase converts D-glucose into D-gluconic acid and hydrogen peroxide, and the hydrogen peroxide reacts with the organic dye on the test strips, under catalysis by the peroxidase on the test strip, to give the observed change of color. (If a color change is obtained on the control test-strips, the sample is contaminated with D-glucose, and the test cannot be used.) As the D-glucohydrolase is specific for an α-D-glucosidic linkage, a positive

test with this enzyme shows that the compound under study contains a D-glucosyl residue linked by an α-D-linkage to an aglycon. Positive tests were obtained with maltose, isomaltose, 1-*O*-(α-D-glucosyl)glycerol, and phenyl α-D-glucopyranoside, and with hydrolytic fragments from the teichoic acids. With the D-glucohydrolase, negative results were obtained with compounds containing the β-D-glucosidic linkage, *e.g.*, cellobiose, gentiobiose, amygdalin, and *O*-(β-D-glucopyranosyl)serine. The latter compounds showed positive tests with β-D-glucosidase.

Compounds that are hydrolyzed at only low rates by the D-glucosidases required pre-incubation with the enzyme. Samples (10–20 μl) of the solutions of these compounds were mixed with an equal volume of the enzyme solutions. After suitable intervals of time (10–60 min), an aliquot of the reaction mixture was placed on the D-glucose oxidase test-strip, and the appearance or lack of appearance of color was noted. Adequate controls, consisting of enzyme and compound blanks, were prepared and analyzed in a similar manner.

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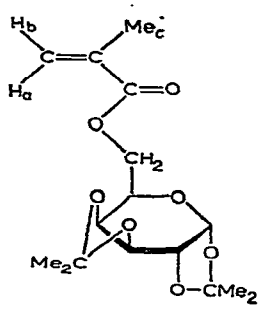
Polymerisable monomers of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose

The preparation and free-radical polymerisation of the crystalline monomer, 1,2:3,4-di-*O*-isopropylidene-6-*O*-methacryloyl-α-D-galactopyranose (**1**) have been described recently[1]. Deacetonation of the homopolymer with aqueous formic acid yielded the novel, water-soluble, synthetic "polysaccharide", poly(6-*O*-methacryloyl-D-galactose)[1]. Determination of the reactivity ratios[2] in the radical copolymerisation of acetal **1** with methyl methacrylate showed that this complex, carbohydrate monomer behaves as a simple alkyl methacrylate in radical polymerisation, and subsequent work[3] has shown it to be a valuable intermediate for the introduction of hydrophilic properties into hydrophobic polymers. We now report an improved and much cheaper synthesis of acetal **1**, together with the synthesis of a new monomer, 6-*O*-allyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**).



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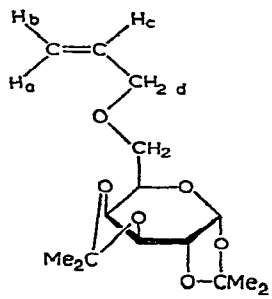

2

Acetal **1** was prepared previously[1] by treatment of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**3**) in pyridine with the expensive chemical, methacrylic anhydride. It has now been prepared in better yield (65.5%) by a simple transesterification between compound **3** and the readily available methyl methacrylate, in the presence of tetraisopropyl titanate as catalyst and *p*-methoxyphenol as polymerisation inhibitor[4]. Continuous removal by distillation of methanol formed in the reaction drove the equilibrium to the right, and reaction was essentially complete after 1 h.

6-*O*-Allyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**2**) was prepared by a modification of the allylation procedure of Bladon and Owen[5]. Two treatments of compound **3** in *p*-dioxane with allyl bromide, in the presence of an excess of powdered sodium hydroxide, were necessary for complete reaction. The pure compound was obtained in 91.8% yield as a colourless oil.

The molecular structures of compounds **1** and **2** were confirmed by p.m.r. spectroscopy. Cone and Hough[6] have analysed the p.m.r. spectra of a series of derivatives of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose, and assignments for com-

pounds **1** and **2** (Table I) were made by comparison with these spectra and with those of methyl methacrylate[7] and allyl alcohol[8].

Integration of all peaks in the p.m.r. spectrum of acetal **1** showed the expected 24 protons. The one-proton peak at τ 3.91 was identical to that of methyl methacrylate and was assigned to H_a. It was possible to distinguish the H-1 doublet ($J_{1,2}$ 5.0 Hz) in the pattern of peaks corresponding to two protons at τ *ca.* 4.5. The other proton was assigned to H_b. The α-methyl group (H_c) was readily detected at τ 8.07. The signal for H-3 at τ 5.44 was a quartet ($J_{3,2}$ 2.4 and $J_{3,4}$ 7.8 Hz). The remaining ring protons could not be detected, as the two H-6 protons had been moved downfield by the deshielding effect of the ester carbonyl group.

TABLE I

CHEMICAL SHIFTS (τ-VALUES)

Compound[a]	H_a	H_b	H_c	H_d	H-1	H-2	H-3	H-4	H-5	H-6	*Isopropylidene C-Me*		
1	3.91	*ca.* 4.5	8.07	—	4.50	[b]	5.44	[b]	[b]	[b]	8.57	8.59	8.71×2
2	4.75	4.87	*ca.* 4.0	[c]	4.55	[c]	5.44	[c]	[c]	6.44, 6.55	8.51	8.61	8.71×2

[a]**1**, 1,2:3,4-Di-*O*-isopropylidene-6-*O*-methacryloyl-α-D-galactopyranose; **2**, 6-*O*-allyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose. [b]Complex multiplet, 5.6–6.2. [c]Complex multiplet, 5.6–6.3.

The pattern of peaks in the p.m.r. spectrum of compound **2** centred at τ 4.0, 4.75, and 4.87 corresponded to three protons which were identical in shape and position to the olefinic protons of allyl alcohol and were assigned to H_c, H_a, and H_b, respectively. The H-1 doublet at τ 4.55 was distorted by a part of the signal from H_a. The signal for H-3, at τ 5.44, was a quartet ($J_{3,2}$ 2.4 and $J_{3,4}$ 7.8 Hz). The two H-6 protons were assigned to the signals at τ 6.44 and 6.55, by comparison with other derivatives of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose. The complex multiplet at τ 5.6–6.3, corresponding to five protons, contained the two allylic protons H_d, and H-2, H-4, and H-5. The total integral showed 24 protons.

EXPERIMENTAL

P.m.r. spectra were recorded in carbon tetrachloride solution on a Perkin–Elmer R10 (60 MHz) n.m.r. spectrophotometer, and chemical shifts (τ) were measured relative to tetramethylsilane. T.l.c. was performed on Silica Gel G with chloroform–ethanol (99:1); spray reagent, 90% sulphuric acid (10 min at 140°).

1,2:3,4-Di-O-isopropylidene-6-O-methacryloyl-α-D-galactopyranose (**1**).—1,2:3,4-Di-*O*-isopropylidene-α-D-galactose[9] (6.45 g) and *p*-methoxyphenol (1.3 g) were dissolved in methyl methacrylate (39 ml), and tetraisopropyl titanate (1.3 ml) was added. The flask was flushed with nitrogen for 10 min, and the temperature was raised to 110°. A methanol–methyl methacrylate azeotrope distilled at 82–85° and

ceased after *ca.* 30 min (final volume, 4 ml). The bath temperature was maintained at 110° for a further 30 min, the condenser was then fitted vertically, water (4 ml) and light petroleum (20 ml, b.p. 60–80°) were added, and the temperature was allowed to fall to 20° (18 h). The resulting orange precipitate was centrifuged, and washed with light petroleum, and the yellow supernatant and washings were evaporated under reduced pressure at 30°. A solution of the thin, syrupy residue in 50% aqueous ethanol (40 ml) deposited the crude methacrylate (6.20 g) as yellow crystals after 18 h at 0°. This material was purified with charcoal in the presence of tetrachloroquinol (60 mg) in ethanol (30 ml) and water (3 ml) for 15 min at 70°. The colourless filtrate was made up to 50% ethanol with water, and the pure methacrylate then crystallised, as before, as needles (5.33 g, 65.5%), m.p. 62.5–63°, $[\alpha]_D$ −49.3° (*c* 1.12, chloroform), and showed the same i.r. spectrum as the authentic material[1] (Found: C, 58.1; H, 7.21. $C_{16}H_{24}O_7$ calc.: C, 58.5; H, 7.36%). Polymerisation of the monomer in benzene solution, with azobisisobutyronitrile as catalyst, as previously described[1], gave polymer having an inherent viscosity of 3.2 dl/g (*c* 0.5, chloroform, 25°).

6-O-Allyl-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**2**). — 1,2:3,4-Di-*O*-isopropylidene-α-D-galactose[9] (19.9 g) was dissolved in anhydrous *p*-dioxane (100 ml), and powdered sodium hydroxide (8 g) was added. Allyl bromide (80 ml) was added over 20 min to the stirred mixture at 45°. Heating was increased to 50° for 30 min, and finally to 60° for 2 h. Water (100 ml) was added, and the mixture was extracted with chloroform (3 × 100 ml). The chloroform extract was washed with water (3 × 250 ml), dried over anhydrous sodium sulphate, and evaporated to a yellow oil (22.1 g). This material was re-allylated as before to give the crude allyl ether (22.08 g) which was distilled to give a colourless oil (21.08 g, 91.8%), b.p. 86°/0.03 mm. T.l.c. showed one component (R_F 0.89), and infrared analyses showed absorptions at 3080 (=CH.) and 1645 cm^{-1} (C=C); OH absorption was absent. The allyl ether had $[\alpha]_D$ −71.8° (*c* 1, chloroform) and n_D^{25} 1.4595 (Found: C, 60.2; H, 8.13. $C_{15}H_{24}O_6$ calc.: C, 60.0; H, 8.06%).

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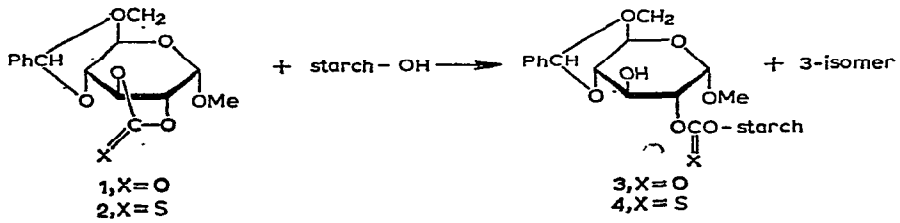
Preliminary communications

New polymers through reaction of *trans*-carbonates of glycosides with polysaccharides

In this report, it is shown that simple pyranoid sugars possessing vicinal *trans*-hydroxyl groups can be readily substituted onto starch. Such substitution occurs when the sugar is first converted into the *trans*-fused cyclic carbonate[1] or thionocarbonate[2] and then added to starch in the presence of a catalyst at 25°.

A high degree of reaction between methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate (**1**), or methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-thionocarbonate (**2**), and starch was realized under various conditions. The starch treated,



had either been dissolved in 90% methyl sulfoxide in water, or pregelatinized and suspended in water. With the methyl sulfoxide system, compound **1** (0.5 mole/mole of D-glucopyranosyl residue of the starch) was added to form a homogeneous mixture that was treated with triethylamine as catalyst. The reaction was followed by the disappearance of absorption for **1** in the 1809–1840 cm^{-1} region (cyclic carbonate[1]) and the appearance of absorption in the starch product (**3**) near 1750 cm^{-1} (acyclic carbonate). After 24 h, only absorption for acyclic carbonate was apparent. The product was isolated by adding the reaction mixture to several volumes of acetone, successively washing the resulting precipitate with acetone and ether, and then drying. Based on the weight of the starch derivative isolated, about 80% of **1** had been introduced. The product showed carbonyl absorption near 1750 cm^{-1} and phenyl absorption near 700 cm^{-1}. A similar yield was realized when starch was treated with **2** in the same way. The sulfur content of the product (**4**) revealed a degree of substitution near 0.4.

The reaction between starch and **1** was much faster when an aqueous suspension of pregelatinized starch was treated with the cyclic carbonate **1** in the presence of either triethylamine or sodium carbonate. A solution of **1** in acetone was added to the starch suspension; although **1** was then precipitated, it redissolved upon addition of triethylamine or aqueous sodium carbonate solution. Within several seconds, the

starch product began to be precipitated. After 2 min, the precipitate was collected, successively washed thoroughly with water and acetone, and dried. The yield and i.r. spectrum of the product were similar to those of the product obtained with **1** in the methyl sulfoxide system.

This investigation is being continued along a broad front to determine the generality of the reaction. Exploratory experiments have shown that starch and other polysaccharides react with various cyclic *trans*-carbonates of glycosides. The only apparent restriction for the sugar derivative is that it should possess the *trans*-fused structure. When the cyclic group is *cis*-fused, as with methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 2,3-carbonate, no reaction with starch occurs under the reaction conditions described.

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Carbohyd. Res., 5 (1967) 366–367

The synthesis of uridine 5-(6-deoxy-α-D-glucopyranosyl dihydrogen pyrophosphate) and its interaction with UDP-glucose dehydrogenase

Syntheses of analogues of uridine 5-(α-D-glucopyranosyl dihydrogen pyrophosphate) (UDPG) having a modified heterocyclic nucleus have been reported[1]. The behaviour of these analogues in chemical and enzymic reactions[2] is in good agreement with the hypothesis[3] that relates intramolecular interactions of glycosyl esters of nucleotides and their biological specificity.

We now report the synthesis and some biochemical properties of a UDPG analogue having the monosaccharide moiety modified at C-6, *viz.*, uridine 5-(6-deoxy-α-D-glucopyranosyl dihydrogen pyrophosphate) (UDP-quinovose, UDPQ). It is of interest to know whether UDPQ can participate in UDPG enzymic reactions, in order to obtain information on the significance of the hydroxyl group at C-6 of the monosaccharide residue in the biological activity of UDPG. Also, UDPQ is a potential inhibi-

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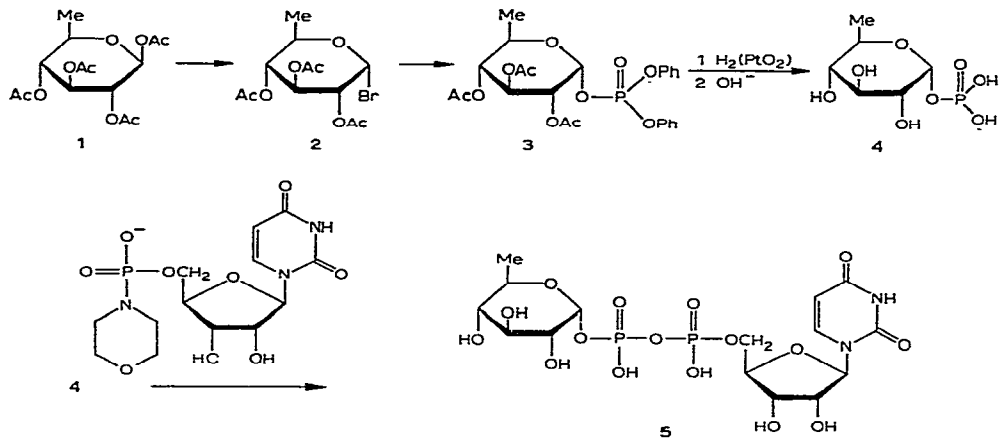
tor of enzymic reactions at C-6 of UDPG, for example, the conversion of UDPG into UDP-glucuronic acid, catalyzed by UDPG-dehydrogenase (EC 1,1,1,22)[4]. UDPQ may also be a precursor in the biosynthesis of plant glycosides, containing 6-deoxy-D-glucose.

The synthesis of UDPQ (5) was performed as follows:



Treatment of tetra-*O*-acetyl-6-deoxy-β-D-glucopyranose[5] (1) with a 40% solution of hydrogen bromide in glacial acetic acid gave tri-*O*-acetyl-6-deoxy-α-D-glucopyranosyl bromide (2, 89% yield), m.p. 125–127°, $[\alpha]_D^{20}$ +242° (*c* 0.43, chloroform) (lit.[6,7], m.p. 135–137°, $[\alpha]_D^{20}$ +226°; and m.p. 150–152°, $[\alpha]_D^{20}$ +247°). Treatment of a boiling solution of 2 in benzene with four equivalents of silver diphenyl phosphate for 4 h gave 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosyl diphenyl phosphate (3), isolated by t.l.c. on silica gel (chloroform–methanol, 20:1; R_F 0.60–0.65). Hydrogenation of 3 over platinum and deacetylation with ammonia gave 6-deoxy-α-D-glucopyranosyl phosphate (4) which was purified on Dowex-1 (HCO_3^-) and isolated as the triethylammonium salt. The product was homogeneous by paper chromatography and paper electrophoresis. The ratio (1:0.95) of deoxy sugar[8] to acid-labile phosphate[9], and an electrophoretic mobility equal to that of α-D-glucopyranosyl phosphate (Table I)

TABLE I

PAPER CHROMATOGRAPHIC AND ELECTROPHORETIC DATA[a]

Substance	*Paper chromatography*		*Paper electrophoresis*	
	A	*B*	*C*	*D*
α-D-Glucopyranosyl phosphate	—	1.14	1.25	—
6-Deoxy-α-D-glucopyranosyl phosphate	—	1.15	1.25	—
UDPG	1.09	—	0.80	1.50
UDPQ	1.12	—	0.80	1.50

[a]Mobilities relative to that of uridine 5-phosphate. [b]Solvent systems: *A*, ethanol–M ammonium acetate (5:2) (pH 7.5); *B*, isopropyl alcohol–ammonia–water (7:1:2); *C*, 0.05M triethylammonium hydrogen carbonate (pH 7.5); *D*, 0.05M triethylammonium acetate (pH 4.0).

prove **4** to be a sugar monophosphate. The α-D-configuration of the glycosidic bond in **4** is indicated by the large, positive, optical rotation ($[\alpha]_D^{20}$ +88.6° in water), and by the n.m.r. signal for H-1 (τ 4.70, quartet, $J_{1,2}$ 3Hz and $J_{1,P}$ 7Hz), which closely corresponds to the H-1 signal for α-D-glucopyranosyl phosphate.

For the synthesis of **5**, the triethylammonium salt of **4** was treated with triethylammonium uridine 5′-phosphomorpholidate[10] (3 equivalents) in anhydrous methyl sulfoxide for 3 h at 60°. UDPQ (**5**) was isolated by ion-exchange chromatography on Dowex-1X2 (Cl^-) and desalted by gel filtration[11] on Sephadex G-25. The resulting sodium salt of **5** was homogeneous by paper chromatography and paper electrophoresis, and its mobilities were closely similar to those of UDPG (Table I). The proportions of reducing sugar[12] after acid hydrolysis, to acid-labile phosphate, to total phosphate were 1:1.07:2.02 and correspond to the structure of UDPQ.

The interaction of UDPQ with UDPG-dehydrogenase was investigated with a purified[4,13] enzyme preparation. No reduction of NAD (nicotinamide adenine dinucleotide) under the influence of UDPG-dehydrogenase was obtained in the presence of UDPQ. When UDPQ was added to a mixture of UDPG, NAD, and UDPG-dehydrogenase, a characteristic change in the rate of NAD-reduction was seen. Initially, the rate was very low, but, after several minutes, it increased and became comparable with the rate of reaction in the absence of UDPQ (Fig. 1).

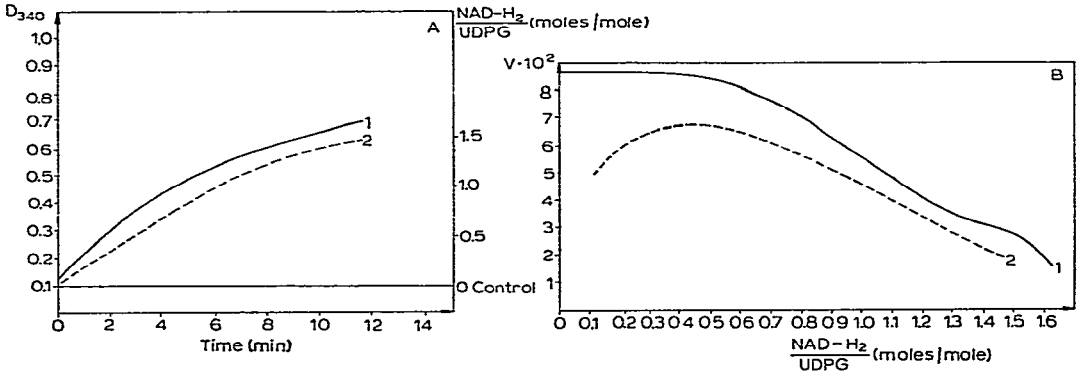


Fig. 1. Kinetics of the interaction of UDPG with UDPG-dehydrogenase in the presence of UDPQ. A. Change of absorbance at 340 nm (D_{340}) *versus* time. B. The rate of NAD-reduction (expressed as the change of D_{340} per min) *versus* the quantity of reduced NAD (calculated from D_{340}, assuming $\varepsilon_{340}=6.22\times10^3$). The curves in Fig. 1B were obtained by graphical differentiation of the curves in Fig. 1A.
Curve 1 (————). The incubation mixture contains enzyme (41 units[4]), glycine buffer (510 μmoles) pH 8.75, NAD (3 μmoles), and UDPG (0.17 μmoles) in a total volume of 3 ml. Curve 2 (— — — —). The incubation mixture contains UDPQ (0.56 μmoles) in addition to the above constituents. The reaction was begun by addition of the enzyme, and the scanning with a Hitachi ESP-2 recording spectrophotometer was begun within 20 sec. All solutions (except that of the enzyme) and the cell compartment of the spectrophotometer were kept at 30°.

Simonart *et al.*[14] have shown that the interaction of UDPG with NAD catalyzed by UDPG-dehydrogenase is a two-step reaction having a large difference in the

velocities of the stages:

$$\text{UDPG} + \text{NAD} \rightarrow \text{X-UDPG} + \text{NADH} + \text{H}^+ \quad (1)$$

$$\text{X-UDPG} + \text{NAD} \rightarrow \text{UDP-glucuronic acid} + \text{NADH} + \text{H}^+ \quad (2)$$

The initial rate of NAD-reduction is close to the rate of stage (1), but the rate of the final section of the kinetic curve is approximately that of the rate of stage (2). The two-step character of the kinetic curve in the reduction of UDPG may be clearly seen in Fig. 1 B.

The effect of UDPQ on the UDPG-dehydrogenase reaction may be interpreted as strong inhibition of stage (1) with weak or no inhibition of stage (2). Differential inhibition of stages (1) and (2) by the same inhibitor may be connected with catalysis of stages (1) and (2) of the UDPG-dehydrogenase reaction by different active sites of the enzyme or by different enzymes. A more-detailed study of enzyme reaction kinetics in the presence of UDPQ is now under investigation in this laboratory.

These results show the importance of UPDQ for the study of the mechanism of enzymic reactions of glycosyl esters of nucleotides.

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Acid hydrolysis of polysaccharides containing uronic acid residues

In a previous publication[1], we showed that, at pH values above 2, the polyuronide, alginic acid, was hydrolysed at a higher rate than neutral polysaccharides. Possible explanations of this high rate of hydrolysis are either an increased concentration of protons in the vicinity of the negatively charged polymer, compared with the bulk of the solution, or intramolecular catalysis by the undissociated carboxyl group. Experiments with cellobiose, cellobiouronic acid, and pseudocellobiouronic acid, reduced with borohydride, showed that the observed effect was most prominent for the reduced form of pseudocellobiouronic acid. Based on these results, we suggested that the carboxyl group in the sugar residue forming the reducing end of the disaccharide has a catalytic effect in the reaction[1]. As shown in Fig. 1, the steric configuration is in favour of an interaction between the carboxyl group and the glycosidic linkage entering C-4 of the carboxyl-carrying moiety.

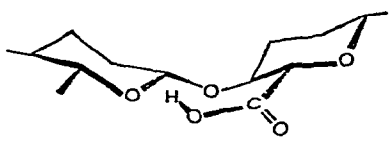

Fig. 1

Further evidence for intramolecular catalysis has been obtained by studying the rate of hydrolysis of alginate fragments. Using partial hydrolysis of alginic acid, it is possible to prepare three different fragments, all having a degree-of-polymerization of approximately 20 and consisting only of residues of D-mannuronic and L-guluronic acid[2]. The fragments differ in uronic acid composition, as shown in Table I. Preparation *A* has been shown to be characterized by a predominantly alternating structure.

TABLE I

RATE OF DEGRADATION OF ALGINATE FRAGMENTS AT pH 2.8

Preparation	D-*Mannuronic acid*, %	k (h^{-1})
A	65	0.05
B	92	0.08
C	13	0.02

The rate of hydrolysis at pH 2.8 in 0.05M citrate buffer was determined for all three preparations by following the increase in reducing power of the solutions. Preparation *C* was insoluble at this pH and had to be degraded at a higher pH value (3.6) before the start of the experiment. Straight lines were obtained for preparations *B* and *C* when the results were plotted according to a first-order reaction (60–80% of the bonds broken). For preparation *A*, curved lines were obtained, and the result

given below refers to the initial rate of the reaction. The rates, expressed as the slopes of the curves, are given in Table I.

When a polysaccharide consisting only of the monomer *M* is hydrolysed at a higher rate than a polymer consisting of the monomer *G*, it would be expected for intramolecular catalysis that the disaccharide *GM* would hydrolyse at a higher rate than the disaccharide *MG*. For neutral disaccharides, it is generally accepted that the rate of the proton-catalysed hydrolysis is primarily determined by the sugar residue forming the non-reducing end of the disaccharide[3]. Recent results, obtained by De and Timell[4] by hydrolysis in 0.5M sulfuric acid, demonstrate an effect also of the reducing end of an aldobiouronic acid, although the effect of the non-reducing moiety seems to be the dominating factor. It is therefore reasonable to assume that, for proton-catalysed hydrolysis, the disaccharide *MG* should hydrolyse at the higher rate. Partial hydrolysis of a polymer having alternating *G* and *M* units, with investigation of the disaccharides in the hydrolysate, should therefore yield information about the mechanism of hydrolysis.

Preparation *A* was hydrolysed at pH 2.8 until an average degree-of-polymerization of 2 was obtained. The products of hydrolysis were separated on a column of Sephadex G-25, as described previously[2]. The diuronide fraction was examined by paper electrophoresis[2] and found to consist of 80% of diuronides containing both monomers (*MG* and *GM*). The diuronide fraction was reduced with borohydride and hydrolysed, and the proportions of D-mannuronic and L-guluronic acid were determined. The hydrolysate was found to contain 90% of D-mannuronic acid, thus showing that the main diuronide in the mixture contained L-guluronic acid at the reducing end (*MG*). The results are thus in agreement with our assumption that intramolecular catalysis is the main mechanism responsible for the hydrolysis, at pH values above 2, of polysaccharides containing uronic acids.

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FORMATION OF PARTIALLY TRIMETHYLSILYLATED DERIVATIVES OF CARBOHYDRATES. THE TRIMETHYLSILYL TRIS-*O*-TRIMETHYLSILYL-D-GLUCOPYRANOSIDES AND TETRAKIS-*O*-TRIMETHYLSILYL-D-GLUCOSE

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INTRODUCTION

Simple, versatile methods have been described for the separation and estimation of carbohydrates and related polyhydroxy compounds by gas–liquid chromatography (g.l.c.). In our own work, we have used poly-(trimethylsilyl) ethers[1], which are readily prepared on a small scale and can be chromatographed with relative ease. The identification of an unknown compound can often be made by comparing the g.l.c. retention time of a derivative with those of authentic reference compounds under standardized conditions. Since a given partition coefficient is by no means exclusively characteristic of only one compound, such data must be interpreted with caution. It is usually expedient to compare the behavior of the unknown, on more than one type of liquid phase, with established retention data of standards.

Preparation of the trimethylsilyl ethers of sugars with a limited amount of reagent yields a mixture of partially substituted products, in addition to the usual completely substituted form. This observation led to a new type of qualitative g.l.c. analysis of carbohydrates in which the resolving power of the g.l.c. column was used to produce a complex array of peaks from a single sugar[2]. The collection of retention times, thus obtained, reduces to a minimum the possibility of a mistaken identification of an unknown substance. The value of this method of "partial derivative analysis" for identifications of carbohydrates is based on the premise that the number and relative proportions of partially substituted trimethylsilyl derivatives are dependent on the conformation and stereochemistry of the molecule. Although subtle structural differences may not be sufficiently great to allow any separation of the completely substituted trimethylsilyl derivatives of two compounds, it is expected that one or more of the partial derivatives will have a different retention time or will constitute different proportions of the whole mixture. This view was substantiated by the finding of qualitative and quantitative differences in the mixtures obtained after brief exposure of a number of simple sugars to hexamethyldisilazane[2]. Similar observations on the

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formation of partially substituted derivatives of bile acids were made some time ago by Sjövall[3] and more recently by Briggs and Lipsky[4].

The preparation and properties of partial trimethylsilyl derivatives of carbohydrates have received very little attention. The present work was, therefore, undertaken as part of a general study of the major partial trimethylsilyl derivatives obtained by the reaction of a carbohydrate with a limited amount of the silylation reagent. It was hoped that the work would provide some information on the relative reactivities of carbohydrate hydroxyl groups, and that ultimately it might be possible to propose a favored pathway for the sequence of substitution reactions which lead to the completely trimethylsilylated derivatives.

RESULTS AND DISCUSSION

In our early studies, partial trimethylsilyl derivatives were obtained by reaction of various carbohydrates with an excess of the standard silylating mixture for very short periods of time[2]. It was, however, later found more convenient to prepare the partial derivatives by using limited amounts of a trimethylsilyl group donor, as described by Birkofer *et al.*[5]. Under such conditions, g.l.c. revealed many more components in the reaction mixtures than had been detected previously. When the reaction was performed with β-D-glucose and hexamethyldisilazane, in the molar ratio 4.6:1 mixed with catalytic amounts of chlorotrimethylsilane and with pyridine as solvent

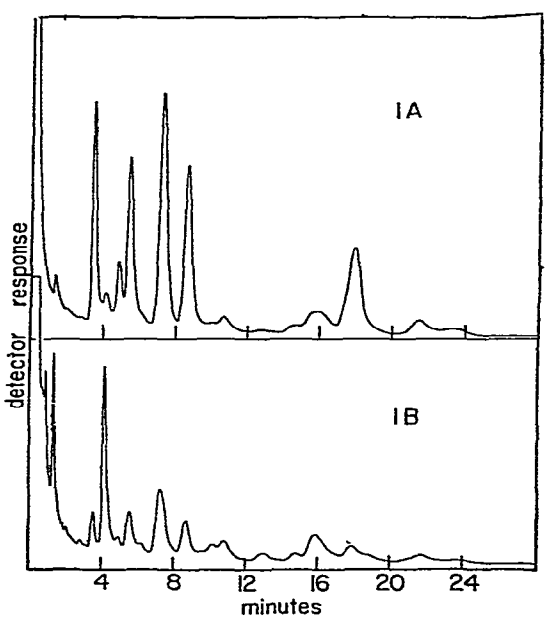


Fig. 1. Gas–liquid chromatogram of the mixture of partially trimethylsilylated derivatives obtained from 150 mg of β-D-glucose and 250 μl of silylation reagent under the conditions described in the text. The column was 3% XE-60 at 170°. In 1A, the chromatogram was obtained immediately after the end of the reaction period. In 1B, the chromatogram was obtained after allowing the mixture to stand for three days at room temperature.

the complex chromatogram shown in Fig. 1A was obtained. Analysis of the mixture was performed immediately after the 5-minute reaction period. It showed the presence of at least 18 components. Further reactions occurred in such mixtures when they were kept longer. For example, the chromatogram shown in Fig. 1B was obtained after the mixture was kept for 3 days at room temperature. Some new peaks are evident, and the amounts of other components have decreased. These changes are attributed to transsilylation reactions.

In order to simplify the identification problem, we have, in the first place, prepared partial derivatives under conditions where the number of products is limited. Secondly, we have studied the synthesis of partial derivatives having four trimethylsilyl groups. For such work, an obvious requirement was a protective group which could subsequently be removed by a method which would leave intact the trimethylsilyl ethers. The availability of benzyl α,β-D-glucopyranoside and of 2-, 3-, 4-, and 6-*O*-benzyl-D-glucose, and the possibility of cleavage of the benzyl groups by nonhydrolytic methods, suggested that 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-glucose (**1**) and the trimethylsilyl glycosides of the four possible tris-*O*-trimethylsilyl ethers might be prepared. When anomeric mixtures of benzyl D-glucoside and the four benzyl ethers were treated with an excess of hexamethyldisilazane, the expected benzyl 2, 3, 4, 6-tetrakis-*O*-trimethylsilyl-D-glucoside, and the trimethylsilyl *O*-benzyl-tris-*O*-trimethylsilyl-D-glucosides were obtained quantitatively. G.l.c. showed that the products contained, as expected, two major components, which are attributed to the presence of the α-D and β-D-pyranoside forms. In Table I are given the relative retention times of all of the mono-*O*-benzyl-tetrakis-*O*-trimethylsilyl-derivatives of α- and β-D-glucose. The reaction mixtures usually contained small amounts of other materials in addition

TABLE I

RELATIVE RETENTION TIMES OF BENZYL AND TRIMETHYLSILYL ETHERS OF BENZYL AND TRIMETHYLSILYL α,β-D-GLUCOPYRANOSIDES[a]

Compound	*Relative retention time*
Trimethylsilyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-β-D-glucoside	1.00
Benzyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-α-D-glucoside	2.93
Benzyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-β-D-glucoside	3.49
Trimethylsilyl 2-*O*-benzyl-3,4,6-tris-*O*-trimethylsilyl-α-D-glucoside	2.65
Trimethylsilyl 2-*O*-benzyl-3,4,6-tris-*O*-trimethylsilyl-β-D-glucoside	3.43
Trimethylsilyl 3-*O*-benzyl-2,4,6-tris-*O*-trimethylsilyl-α-D-glucoside	2.24
Trimethylsilyl 3-*O*-benzyl-2,4,6-tris-*O*-trimethylsilyl-β-D-glucoside	3.26
Trimethylsilyl 4-*O*-benzyl-2,3,6-tris-*O*-trimethylsilyl-α-D-glucoside	2.37
Trimethylsilyl 4-*O*-benzyl-2,3,6-tris-*O*-trimethylsilyl-β-D-glucoside	3.48
Trimethylsilyl 6-*O*-benzyl-2,3,4-tris-*O*-trimethylsilyl-α-D-glucoside	3.26
Trimethyisilyl 6-*O*-benzyl-2,3,4-tris-*O*-trimethylsilyl-β-D-glucoside	3.97

[a]The retention times were determined on a 6-foot column of 2.5% SE-30; temperature, 220°. The anomeric assignments are based on the assumption of a longer retention time for the β-D anomer in each case[1].

to the two major components. Small amounts of a third material, possibly possessing a furanose structure, were present even in trimethylsilyl 6-*O*-benzyl-2,3,4-tris-*O*-trimethylsilyl-D-glucoside (2) purified by distillation. Evidence that the reaction conditions did lead to the formation of the tetrakis substituted compounds, was obtained by determining the mass spectrum of trimethylsilyl 6-*O*-benzyl-2,3,4-tris-*O*-trimethylsilyl-*β*-D-glucopyranoside (3). As shown in Fig. 2, a peak for the molecular ion was

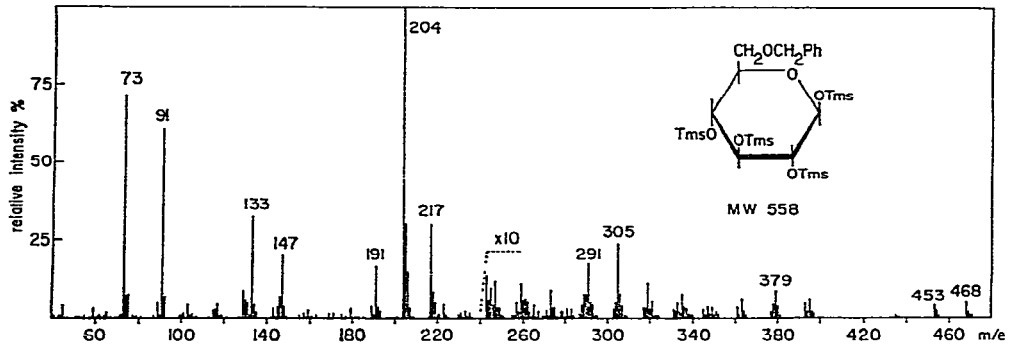


Fig. 2. Mass spectrum of benzyl 2,3,4,6-tetrakis-*O*-trimethylsilyl-*β*-D-glucopyranoside.

not obtained, but the molecular weight was clearly indicated by peaks at *m/e* 468 and 379 for $(M\text{-}90)^+$ and $(M\text{-}90\text{-}89)^+$. Peaks at *m/e* 91$(\text{-}CH_2C_6H_5)^+$ and *m/e* 147$(\text{-}CH{=}CHCH_2OCH_2C_6H_5)^+$ provided conclusive evidence for the presence of a benzyl group. It was not possible, however, to deduce the location of the benzyl group from inspection of the mass spectrum.

The anomeric mixtures of the monobenzyltetrakis-*O*-trimethylsilyl derivatives were subjected to hydrogenolysis with palladium on charcoal as the catalyst. The removal of the benzyl groups was followed by the appearance of new peaks on the gas chromatogram. In most cases*, two new major peaks appeared, which where assumed to be the two anomers of the trimethylsilyl tris-*O*-trimethylsilyl-D-glucoside. That this was the case was shown by isolation of the peaks by preparative gas chromatography, followed by further silylation of the liberated hydroxyl group to give the known trimethylsilyl tetrakis-*O*-trimethylsilyl-*α*- or *β*-D-glucoside (4 or 5, respectively). In this way, assignment of the anomeric configuration could be made for the tetrakis derivatives. The relative retention times for the trimethylsilyl *α*-D- and *β*-D- glycosides of 2,3,6- (6,7), 2,4,6- (8,9), 3,4,6- (10,11), and 2,3,4-tris-*O*-trimethylsilyl-D-glucose (12,13) are shown in Table II. The rate of hydrogenolysis was surprisingly low. After 48 h at room temperature and atmospheric pressure, the yields of the trimethylsilyl tris-*O*-trimethylsilylglucosides ranged from 40 to 70%.

The product of hydrogenolysis of benzyl tetrakis-*O*-trimethylsilyl-*α*, *β*-D-glucopyranoside (14) was not the expected 1. It consisted mainly of a mixture of trimethyl-

*When 3-*O*-benzyl-D-glucose was the starting material, only one new major peak appeared, consistent with the fact that the starting material was essentially the *β*-D anomer. When 3-*O*-benzyl-D-glucose was heated in pyridine prior to the silylation reaction, two new major peaks were observed (see Experimental section).

TABLE II

RELATIVE RETENTION TIMES AND YIELDS OF THE TETRAKIS AND TRIS-TRIMETHYLSILYL ETHERS OF TRIMETHYLSILYL D-GLUCOSIDE

Position of trimethylsilyl ether group	*Position of free OH group*	*Relative retention time*		*Yields from hexamethyldisilazane*[a] *α-D-glucose*[c] *and β-D-glucose*[d]				*Yields from N-(trimethylsilyl)acetamide*[b] *α-D-glucose*[c] *and β-D-glucose*[c]			
		XE-60	*SE-30*	*A* %	*B* %	%		*A* %	*B* %	*A* %	*B* %
Trimethylsilyl β-D-glucopyranoside											
2,3,4,6	None	1.00	1.00			5.9	(2.3)	0.8	8.6	3.8	1.2
2,3,6	4	1.35	0.61			31.3	(5.4)	12.6	11.5	11.5	24.7
2,4,6	3	0.88	0.43			23.1	(2.8)	15.3	12.7	12.6	15.3
3,4,6	2	1.24	0.61			10.1	(0.8)	6.0	4.3	2.7	4.0
2,3,4	6	1.85	0.76			10.2	(3.8)	5.3	1.9	6.2	5.9
Trimethylsilyl α-D-glucopyranoside											
2,3,4,6	None	0.58	0.61	38.9	29.0			5.2	14.0	8.4	1.3
2,3,6	4	0.95	0.46	32.0	36.4			32.1	22.4	11.8	26.1
2,4,6	3	0.72	0.37	14.0	18.8			9.1	12.0	17.8	12.4
3,4,6	2	0.90	0.49								
2,3,4	6	1.46	0.64					5.1	1.8	3.6	1.4
Unidentified				15.1	15.8	19.4	(6.0)	8.5	10.8	21.6	7.7

[a]The reactions with hexamethyldisilazane were performed with a molar ratio of 1.5:1 for glucose:hexamethyldisilazane, as described in the text. Freshly prepared reaction mixtures were used in each case. [b]The reactions with *N*-trimethylsilylacetamide were performed with a molar ratio of 4.4:1 for *N*-trimethylsilylacetamideglucose, as described in the text. [c]Duplicate runs, A and B, were carried out in these cases. [d]The values recorded are the means for four runs. The figures in parentheses are the standard deviations.

silyl tetrakis-*O*-trimethylsilyl-α- and β-D-glucoside (**4** and **5**). It is believed that the anomeric hydroxyl groups are too reactive to remain unsubstituted under the conditions used for hydrogenolysis, and that intermolecular transsilylation therefore occurs. This phenomenon was also observed to a minor extent during hydrogenolysis of trimethylsilyl 2-*O*-benzyl-tris-*O*-trimethylsilyl-D-glucoside (**15**), although the major product, as expected, was a mixture of the anomers of trimethylsilyl 3,4,6-tris-*O*-trimethylsilyl-D-glucoside (**10,11**).

Of the two liquid phases used for g.l.c., the polar cyanomethyl silicone (XE-60) gave more widely separated peaks than did the nonpolar methyl silicone (SE-30). For example, a mixture of **7**, **11**, and **4** was not resolved on SE-30 columns, but 3 well-separated peaks were obtained with an XE-60 column (see Figs. 3 and 4). On an XE-

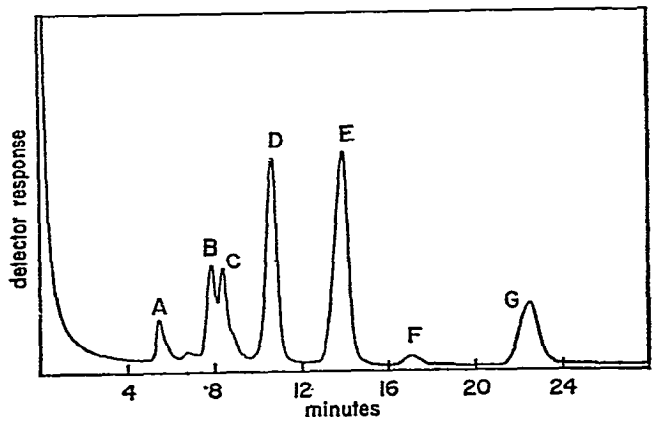


Fig. 3. Gas–liquid chromatogram of a mixture of partially trimethylsilylated derivatives obtained from 100 mg of β-D-glucose and 500 μl of silylation reagent under the conditions described in the text. The column was 2.5% SE-30 at 170°; (D **9**; (E) mixture of **11**, **7**, and **4**; (F) **13**; (G) **5**.

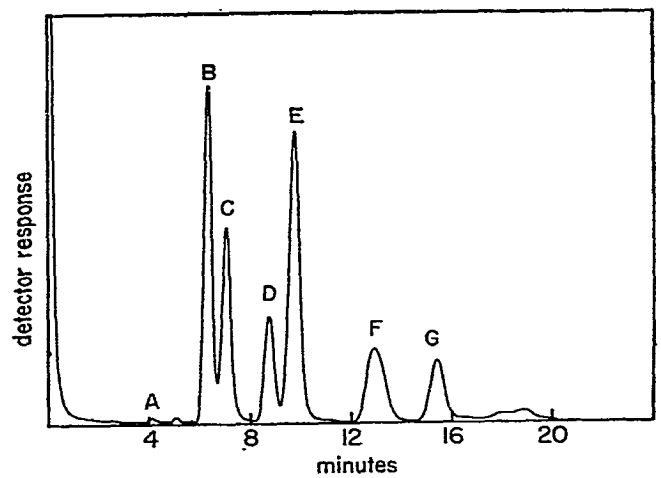
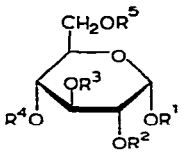
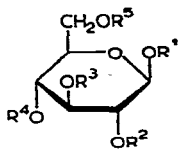


Fig. 4. Gas–liquid chromatogram of a mixture of partially trimethylsilylated derivatives prepared from β-D-glucose, on 3% XE-60 at 170°. The sample was prepared in the same way as that of Fig. 2; (A) **4**; (B, **9**; (C, **5**; (D) **11**; (E) **7**; (G) **13**.

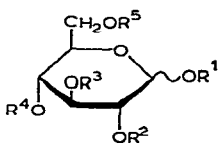
60 column, the trimethylsilyl tris-*O*-trimethylsilyl-α-D-glucopyranosides had retention times longer than that of **4**; similarly, with the exception of **9** the trimethylsilyl tris-*O*-trimethylsilyl-β-D-glucopyranosides had retention times longer than that of the fully trimethylsilylated glucoside **5**. The partial derivatives showed shorter retention times on SE-30 than those of the corresponding compounds **4** and **5**, with the exception of **12**. On XE-60, the retention times of the partial derivatives increased in the order, **8** < **10** < **6** < **12**, for the α-D series, and in the order **9** < **11** < **7** < **13**, for the β-D series. The order of retention times was essentially the same on SE-30 except for the fact that the pairs, **10** and **6**, and **11** and **7**, were scarcely separated.



	R^1	R^2	R^3	R^4	R^5
4	Tms	Tms	Tms	Tms	Tms
6	Tms	Tms	Tms	H	Tms
8	Tms	Tms	H	Tms	Tms
10	Tms	H	Tms	Tms	Tms
12	Tms	Tms	Tms	Tms	H



	R^1	R^2	R^3	R^4	R^5
3	Tms	Tms	Tms	Tms	CH_2Ph
5	Tms	Tms	Tms	Tms	Tms
7	Tms	Tms	Tms	H	Tms
9	Tms	Tms	H	Tms	Tms
11	Tms	H	Tms	Tms	Tms
13	Tms	Tms	Tms	Tms	H



	R^1	R^2	R^3	R^4	R^5
1	H	Tms	Tms	Tms	Tms
2	Tms	Tms	Tms	Tms	CH_2Ph
14	CH_2Ph	Tms	Tms	Tms	Tms
15	Tms	CH_2Ph	Tms	Tms	Tms

Tms=trimethylsilyl

Prior to our work, little attention had been directed to the identification of partially substituted trimethylsilyl derivatives of sugars. In 1956, Schwarz *et al.*[6] described the preparation of tris- and tetrakis-*O*-triethylsilyl derivatives of glucopyranose, but the positions of substitution were not determined. Birkofer *et al.*[5] reported the synthesis of trimethylsilyl 2,4,6-tris-*O*-trimethylsilyl-D-glucopyranoside (mixture of anomers?) from the 3-*O*-benzyl derivative by hydrogenolysis with palladium on charcoal. They also reported that trimethylsilyl 2,3,4-tris-*O*-trimethylsilyl-D-glucopyranoside was formed from glucose (1 mole) and *N*-trimethylsilylacetamide (4.4 moles) in pyridine. Our experiments, discussed below, indicate that complex mixtures are obtained under these conditions.

Other approaches were also considered in attempting to establish structures for the partial derivatives. One possibility was to convert individual products, isolated by preparative g.l.c., into known derivatives. In contrast to the properties of partially trimethylsilylated derivatives of methyl α-D-glucopyranoside[7], the derivatives of D-glucopyranose were found to be insufficiently stable to survive conditions for the conversion of free hydroxyl groups into acetyl, methyl, or benzyl derivatives. For example, additions of acetic anhydride and dry pyridine to any of the partially trimethylsilylated derivatives always resulted in the formation of penta-*O*-acetyl-D-glucose, exclusively. Methylations and benzylations were equally unsuitable. In attempts to prepare pure sodium salts of partially substituted derivatives, with freshly cut sodium and a solution of the substance in absolute diethyl ether, transsilylations occurred. Under these conditions, **9** was converted into **7** (53% of **9** at equilibrium).

It is important to note that isomerization of individual, partially trimethylsilylated derivatives during passage through the g.l.c. column (SE-30 or XE-60) did not occur to a significant extent. When single components, collected by preparative g.l.c., were rechromatographed, only one peak, having the same retention time as that originally found in the mixture, was observed. It is probable, therefore, that the number and relative proportions of partial derivatives, formed under limiting conditions, reflect differences in the rates of silylation of various hydroxyl groups, and that g.l.c. records of the mixture provide reasonably accurate data on the composition of these mixtures.

The availability of the reference compounds, reported here, allows some conclusions to be drawn concerning the composition of the partial mixtures obtained from trimethylsilylation of α- and β-D-glucose. Conditions were so selected that the composition was less complex than that of the mixture shown in Fig. 1A. With 0.66 mole of hexamethyldisilazane per mole of β-D-glucose, the products contained predominantly four and five trimethylsilyl groups, as shown in Figs. 3 and 4. The major compounds were identified as **9** and **7** (peaks B and E in Fig. 4) on the basis of their relative retention times. One substantial component (peak F in Fig. 4) and about three minor components remained unidentified. When α-D-glucose was used under the same conditions, the mixture contained a larger proportion of the fully trimethylsilylated glucoside, as shown by the results recorded in Table II. With α-D-glucose, mixtures corresponding roughly in proportion to those obtained with β-D-glucose were best obtained using a value of 1.85:1 for the molar ratio, glucose to hexamethyldisilazane. The reaction was not easy to control, and considerable variability in the relative proportions of the components was observed from run to run (see Table II).

An example of the mixture produced by reaction of *N*-(trimethylsilyl)acetamide and α-D-glucose under the conditions of Birkofer *et al.*[5] is shown in Fig. 5. In this case, the major compounds were again **8** and **6**. In this reaction, considerable anomerization had apparently taken place, since peaks with the retention times of **9**, **11**, and **7** were also present (peaks E, F, and G in Fig. 5). There were also four unidentified materials (peaks A, H, I, and J in Fig. 5). Quantitative data on the reaction of both α- and β-D-glucose with *N*-(trimethylsilyl)acetamide are given in Table II. In all cases,

there was considerable anomerization, and considerable variation in the proportions observed in duplicate reactions. The highest yield of the combined α- and β-D-glucosides having substitution at O-2, O-3, and O-4 was about 10%. It should be noted that this observation is contrary to that of Birkofer *et al.*[5]. Furthermore, trimethylsilyl 2,3,4-tris-*O*-trimethylsilyl-D-glucopyranoside was only a minor product in the reaction of β-D-glucose with hexamethyldisilazane (peak G in Fig. 4; see also Table II) and could not be observed at all in the same reaction with α-D-glucose (Table II).

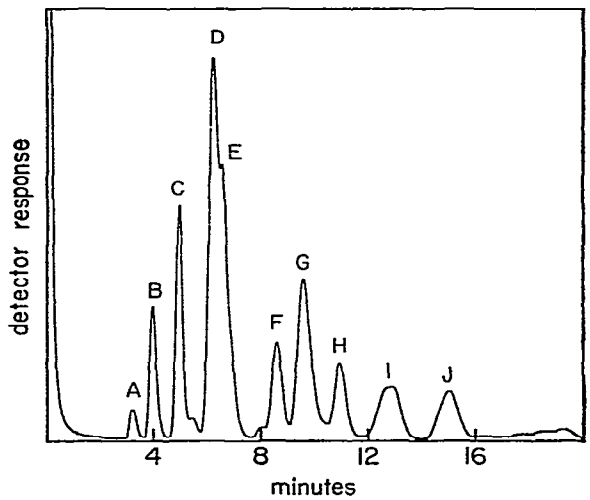


Fig. 5. Gas–liquid chromatogram of a mixture of partially trimethylsilylated derivatives of α-D-glucose, prepared with *N*-(trimethylsilyl)acetamide as described in the text. The column was XE-60 at 170°; (B) **4**; (C) **8**; (D) **6**; (E) **9**; (F) **11**; (G) **7**.

Our work provides some information on the relative reactivities of carbohydrate hydroxyl groups toward trimethylsilylation. Such reactivity differences with other reagents have been widely studied[8]. Thus, in some reactions (*e. g.* glycoside formation), only the anomeric hydroxyl group reacts. Other reactions exhibit a marked selectivity for the primary hydroxyl groups (*e. g.*, tosylation, tritylation). In reactions requiring alkaline conditions, the hydroxyl group at C-2 may be particularly reactive as a result of the electron-withdrawing effect of the anomeric center. Under the conditions described in this paper, the reaction of glucose with either hexamethyldisilazane or *N*-(trimethylsilyl)acetamide always yields, as the major tetrasubstituted products, trimethylsilyl glycosides substituted at positions 2,3,6 and 2,4,6. It is therefore clear that the hydroxyl groups at C-1, C-2, and C-6 are particularly reactive in this process.

Although insufficient information is available to distinguish the most reactive of these three positions, it seems likely that the early reactions occur primarily with hydroxyl groups at C-1 and C-2 of the glucose molecule; in later steps steric factors may control the nature of products formed. Assuming that trimethylsilylation gives first a trimethylsilyl glycoside substituted at O-2, the third substitution reaction could

occur at O-3, O-4, or O-6. This step could yield three different derivatives, by random substitution, or differences in rates or steric factors might limit the number of trisubstituted products. If either one of the hydroxyl groups at C-3 and C-4 has been substituted, it is presumed that, because of steric hindrance, the fourth trimethylsilyl group would be linked to O-6 rather than to the remaining position 3 or 4. This might explain why the trimethylsilyl 2,3,6- and 2,4,6-tris-*O*-trimethylsilylglucosides are the major products from α-D-glucose and β-D-glucose.

It is apparent that considerable work will be necessary to gain a complete understanding of the reaction sequence involved in the formation of a trimethylsilyl tetrakis-*O*-trimethylsilylhexoside. As one possible approach, we plan to obtain kinetic data on the relative rates of trimethylsilylation of compounds containing four trimethylsilyl groups.

EXPERIMENTAL

General. — Commercially available preparations of α-D-glucose and β-D-glucose were used. Hexamethyldisilazane and chlorotrimethylsilane were obtained from Applied Science Laboratories, Inc., State College, Pa. Pyridine, used as solvent in preparing trimethylsilyl derivatives, was of reagent grade, and was stored over KOH pellets. Palladium on charcoal (10%), used for hydrogenolysis, was purchased from Matheson, Coleman & Bell, East Rutherford, N. J. *N*-(Trimethylsilyl)acetamide was prepared by the method of Birkofer *et al.*[9].

For g.l.c., the instruments used were the F & M Models 400 and 402, each of which was equipped with flame ionization detectors. Two U-shaped glass columns (6 ft × 1/8 in i.d.) were employed; one was packed with 3% XE-60 on acid-washed, silanized Gas Chrom S (100 to 120 mesh), the other with 2.5% SE-30 on the same support. Column packings were prepared from toluene solutions of SE-30 or acetone solutions of XE-60, with the solution coating technique[10]. The liquid phases and packings were obtained from Applied Science Laboratories, Inc., State College, Pa. Both columns were conditioned for several h at 300° without gas flow, and then for an additional period of several h at 250° with normal gas flow. Analyses were performed at 170° with an injection port temperature of 210°.

Mass spectra were recorded with an LKB 9000 combined gas chromatograph-mass spectrometer, in which chromatographic effluents are conducted directly into the ion chamber of the mass spectrometer after removing helium preferentially with a jet-type molecular separator of the Becker–Ryhage type[11]. All spectra were recorded with an electron impact energy of 20 e.v., 3500 volts accelerating voltage, and ion source temperature of 250°.

Preparation of partially trimethylsilylated derivatives of D-*glucose.* — Several procedures were used in the synthesis of partially trimethylsilylated derivatives of D-glucose. In one series of experiments, amounts of glucose ranging from 5 to 150 mg were trimethylsilylated as follows. A portion (250 μl) of a mixture of hexamethyldisilazane, chlorotrimethylsilane, and pyridine (2:1:10, v/v) was diluted to 1.5 ml with

pyridine. The solution was warmed to 70°, D-glucose was added, and the mixture was shaken intermittently for 5 min. An appropriate aliquot was then analyzed directly by g.l.c. The complexity of the sample increased as the molar ratio, glucose to hexamethyldisilazane, was changed from 0.15:1 to 4.5:1. With the ratio, 0.15:1, only two peaks corresponding to **4** and **5** were obtained. Fig. 1A shows a chromatogram of the complex mixtures obtained when the ratio was 4.5:1.

To prepare less complex mixtures, in which it was hoped that compounds with four trimethylsilyl groups would predominate, the following conditions were used. To solid β-D-glucose (0.1 g) was added a mixture (0.5 ml) of hexamethyldisilazane, chlorotrimethylsilane, and pyridine (2:1:10) and a further portion of pyridine (4 ml). The molar ratio, glucose to hexamethyldisilazane, was 1.5:1. The mixture was refluxed for 5 min, after which pyridine and silane components were removed by distillation (10 mm pressure), and the residue was dissolved in abs. ethyl acetate (10 ml). The white precipitate, consisting of ammonium chloride and unreacted glucose, was removed by filtration, and aliquots of the ethyl acetate solution were used for g.l.c. analyses. Figs. 3 and 4 show typical chromatograms of this mixture on columns of XE-60 and SE-30. The identifications recorded in these figures were made on the basis of the retention times of known compounds, synthesized by the methods subsequently described. A similar mixture of partially substituted derivatives was prepared from α-D-glucose in the same way. In this case, however, the sugar (0.1 g) was mixed with the mixed silylation reagent (0.4 ml) to diminish the formation of **4**.

Mixtures prepared with *N*-trimethylsilylacetamide were also investigated. A chromatogram of the mixture obtained by the reaction of α-D-glucose and *N*-trimethylsilylacetamide in the molar ratio 0.22:1 according to Birkofer *et al.*[5], is shown in Fig. 5.

Benzyl α,β-D-glucopyranoside and benzyl ethers of D-*glucose.* — Benzyl α,β-D-glucopyranoside[12], 2-*O*-benzyl-[13], 3-*O*-benzyl-[14], and 4-*O*-benzyl-D-glucose[15] were prepared as described in the literature. When small samples of the benzyl derivatives were trimethylsilylated under our usual conditions[1], two peaks were obtained in all cases, indicating that the original samples were mixtures of the anomeric forms. With 3-*O*-benzyl-D-glucose, the second component was very small but it was increased considerably by first refluxing briefly a suspension of the solid in dry pyridine. The major component of the solid 3-*O*-benzyl-D-glucose was shown to be the β-D anomer by the method subsequently described (conversion into trimethylsilyl 3-*O*-benzyl-2,4,6-tris-*O*-trimethylsilyl-β-D-glucoside, and thence to the known **5**).

The compound, *N*-(3,4,6-tris-*O*-acetyl-D-glucopyranosyl)piperidine, which was required as an intermediate in the synthesis of 2-*O*-benzyl-D-glucose, was prepared as follows, with slight modification of the method described by Hodge and Rist[16]. Penta-*O*-acetyl-α-D-glucopyranose (39 g, 0.1 mole) and piperidine (25.8 g, 0.3 mole) were mixed first with manual, then later with mechanical stirring. After a short time, the mixture became fluid; stirring was continued until a thick crystalline mass formed in the flask (about 30 min). After addition of diethyl ether (150 ml), and stirring for an additional hour, the mixture was chilled in the refrigerator, and the crystalline product was removed by filtration. Recrystallization from abs. ethyl al-

cohol gave a material having m.p. 149° (42% yield). When the reaction was repeated under the same conditions with penta-*O*-acetyl-β-D-glucopyranose, the product had m.p. 130°. The two products showed identical retention times on g.l.c. analysis, and both were dextrorotatory, $[\alpha]_D^{23}$ +31.7° (*c* 2, chloroform). Since these m.ps. fell outside the range of 121–126° (dependent on the manner and rate of heating) reported previously[16], further evidence was sought for the correctness of the assigned structure. The product was treated with hexamethyldisilazane[1] to trimethylsilylate the remaining free hydroxyl group. Mass spectrometry of the product, *N*-(3,4,6-tri-*O*-acetyl-2-*O*-trimethylsilyl-D-glucopyranosyl)piperidine, confirmed the identity of the synthetic material. Peaks were observed at *m/e* 445 = molecular ion, 430 = M-15 (loss of CH_3), 402 = M-43 (loss of CH_3CO), 370 = M-75 (loss of CH_3 and CH_3COOH), and 84 $(C_5H_{10}N)^+$; the most intense peak was at *m/e* = 114 $(CH(OCOCH_3)=CH-CHO)^+$.

6-*O*-Benzyl-D-glucose was prepared by the following method: To 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-D-glucofuranose[17] (2.9 g) were added *N*,*N*-dimethylformamide (50 ml, dried over BaO and freshly redistilled) and benzyl bromide (15 ml). After the solution was cooled briefly, dried silver oxide (15 g) was added in small portions over a period of about 10 min. The flask was stoppered tightly, and the mixture was stirred overnight. The silver salt was removed, and the filtrate was concentrated *in vacuo* to a thick syrup. Methanol (50 ml) and 2N sulfuric acid (30 ml) were added, and the mixture was refluxed for 1 h. Methanol was removed by distillation, and the conc. solution was refluxed for 4 h. The water-insoluble portion which formed was removed in a separatory funnel, and the aqueous solution was neutralized with excess barium carbonate. The aqueous solution of 6-*O*-benzyl-D-glucose, free of sulfate ions, was concentrated *in vacuo* to a dry residue which was used without further manipulation, for preparation of the trimethylsilyl derivative.

Trimethylsilylation of the benzyl derivatives of D-*glucose.* — In most cases, 10 ml of the "2:1:10" silylation mixture was added to 500 mg of the required benzyl compound. After a reflux of 5 min, the volatile materials were removed by distillation, abs. ethyl acetate (30 ml) was added, and the precipitate was removed by filtration. This reaction gave a mixture of the trimethylsilyl mono-*O*-benzyl-tris-*O*-trimethylsilyl-α- and β-D-glucopyranosides in quantitative yield. For preparation of the 6-*O*-benzyl derivative, the dry residue of 6-*O*-benzyl-D-glucose, obtained as described earlier, was treated with 60 ml of the 2:1:10 silylation mixture. After a reflux of 5 min, the precipitate was filtered off, and the volatile materials were removed by distillation at atmospheric pressure. Trimethylsilyl 6-*O*-benzyl-2,3,4-tris-*O*-trimethylsilyl-D-glucoside (anomeric mixture, **2**) was recovered in 80% yield (3.5 g) by high-vacuum distillation: b.p. 156°/0.03 mm.

*Hydrogenolysis of benzyl tetrakis-*O*-trimethylsilyl-α-β-*D*-glucoside* (**14**) *and trimethylsilyl mono-*O*-benzyl-tris-*O*-trimethylsilyl-*D*-glucopyranosides.* — To the ethyl acetate solution of compound **14** or the mono-*O*-benzyl-tris-*O*-trimethylsilyl-D-glucosides (usually prepared from 500 mg of *O*-benzyl derivative) was added 10% palladium–charcoal catalyst (1 g) . Dry hydrogen was bubbled through the solution

with continuous stirring. After 48 h, the mixture was filtered, and aliquots of the ethyl acetate solution were analyzed by g.l.c. The various trimethylsilyl derivatives of D-glucose were recovered in yields ranging from 40 to 70%.

For the assignment of anomeric configuration, the two peaks obtained in each case were separately collected as they were eluted from a preparative SE-30 column (10%, 250°). Small samples of the pure anomeric forms were then converted by further trimethylsilylation[1] into the completely substituted derivatives (**4** or **5**). Configurations were assigned by comparison of the retention times of these derivatives with those of the known **4** or **5**.

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SUMMARY

When mixtures of the α and β anomers of benzyl D-glucopyranoside, and of 2-, 3-, 4-, and 6-*O*-benzyl-D-glucose were treated with hexamethyldisilazane, the tetrakis-*O*-trimethylsilyl derivatives of the benzyl glycosides and the tris-*O*-trimethylsilyl ethers of the trimethylsilyl glycosides were obtained (anomeric mixtures). On hydrogenolysis of the trimethylsilyl 2-, 3-, 4-, and 6-*O*-benzyl-tris-*O*-trimethylsilyl glycosides, the α- and β-anomers of the trimethylsilyl tris-*O*-trimethylsilyl-D-glucosides having the following substitution patterns were obtained, respectively: 3,4,6; 2,4,6; 2,3,6; and 2,3,4. The anomers were separated by gas–liquid chromatography. Hydrogenolysis of benzyl tetrakis-*O*-trimethylsilyl-α,β-D-glucopyranoside gave mainly a mixture of trimethylsilyl tetrakis-*O*-trimethylsilyl-α- and β-D-glucosides. Direct trimethylsilylation of α- and β-D-glucose with limiting amounts of trimethylsilyl donor [hexamethyldisilazane or *N*-(trimethylsilyl)acetamide] yielded complex mixtures of partially trimethylsilylated derivatives. The composition of such mixtures has been determined by g.l.c. The major components were the trimethylsilyl tetrakis-*O*-trimethylsilyl and the 2,3,6- and 2,4,6-tris-*O*-trimethylsilyl-D-glucosides.

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STRUCTURE OF THE CARBOHYDRATE MOIETY OF THE α_1-ACID GLYCOPROTEIN OF HUMAN PLASMA

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INTRODUCTION

Partial structures of the carbohydrate moiety of the α_1-acid glycoprotein (orosomucoid)[1,2] of human plasma have been determined mainly by Jeanloz and his co-workers[3–9], using the native α_1-acid glycoprotein, or its modification, and oligosaccharides isolated from the glycoprotein.

We have been able to isolate the carbohydrate units by cleaving the protein moiety from the glycoprotein by hydrazinolysis[10], and we now report on a structural study of the carbohydrate moiety, by periodate oxidation and Smith degradation[11–13], which has led to the postulation of an average structure.

EXPERIMENTAL

Materials. — The partially *N*-deacetylated polysaccharide (DeAc-Ps), obtained by hydrazinolysis of the α_1-acid glycoprotein, and its *N*-acetylated derivative (PS) were prepared as described previously[10]. Polysaccharide free from sialic acid (Asialo-PS) was prepared as follows: a solution of 100 mg of PS in 10 ml of 0.05N sulfuric acid was heated for 1 h at 80° and then passed through a column (1.5 × 15 cm) of Dowex-1X2 (acetate form), which was subsequently washed with water. The concentrated effluent was lyophilized, yielding 70 mg of Asialo-PS as a white powder.

Analytical procedures. — 2-Amino-2-deoxy-D-glucose (glucosamine) was determined[14] as 2,5-anhydro-D-mannose, using the indole–hydrochloric acid reagent[15]. After deamination of DeAc-PS with nitrous acid, most of the glucosamine residues could be determined directly, without prior acid hydrolysis. L-Fucose was determined with cysteine–sulfuric acid[16], and the acetyl content was determined colorimetrically by the method of Ludowieg and Dorfman[17]. *N*-Acetylneuraminic acid was determined by Warren's procedure[18] on the hydrolysate obtained with 0.05N sulfuric acid for 1 h at 80°. However, the neuraminyl linkage was resistant to mild, acid hydrolysis. Moreover, neuraminic acid was not oxidized by periodate at the amino and hydroxyl groups under the acidic conditions of Warren's method, but it was oxidized in neutral medium[19]. Therefore, the content of *N*-deacetylated sialic acid in DeAc-PS was determined as follows. An aqueous solution of DeAc-PS (2.1 mg in 3 ml) was adjusted to pH 7.8 with 4N sodium hydroxide, and kept overnight at room temperature. To this

solution was added 0.5 ml of 0.3M aqueous sodium metaperiodate, the pH was adjusted to 7.0, and the mixture was kept for 1 h at room temperature. A portion (0.1 ml) of this solution was mixed with 0.1 ml of 9M phosphoric acid and 1.0 ml of 10% aqueous sodium arsenite, and then treated with thiobarbituric acid, according to Warren's method. The content of galactose and mannose was calculated from the value of the total amount of hexose, and the ratio of galactose to mannose was determined by gas–liquid chromatography (g.l.c.). The total amount of hexose was determined by the thionalide–sulfuric acid reagent (method HR_{10})[20]. G.l.c. of the trimethylsilyl derivatives of the methyl glycosides was carried out by the method of Yamakawa and Ueta[21], using a 3-m column packed with 5% Ucon LB 500 on Gaschrom CLH at 205°. In the cases of DeAc-PS and its oxidation products, the galactose to mannose ratio was determined after *N*-acetylation, and prior to methanolysis and trimethylsilylation. Glycolaldehyde, erythritol, and threitol have the same retention time under these conditions, but, at 160°, glycolaldehyde was separable from erythritol and threitol. Glycerol was also determined at 160°.

The products of periodate oxidation of sialic acid were checked by paper chromatography at room temperature on Toyo filter paper No. 3 (15 × 50 cm), using (*a*) ethyl acetate–acetic acid–water (7:2:2) and (*b*) butyl acetate–acetic acid–water (3:2:1), by the ascending technique; Ehrlich's reagent was employed for staining. The oxidation product was hydrolyzed with 0.05N sulfuric acid for 1 h at 80°, and the hydrolysate was passed through a column (1.5 × 10 cm) of Amberlite IR-4B (carbonate form). The effluent was concentrated to a small volume and applied to the paper; *N*-acetylneuraminic acid was used as a reference to give R_{NANA} values.

Periodate oxidation — The amounts of sodium metaperiodate consumed, formic acid released, and formaldehyde formed during the oxidation were determined as follows : a solution of 150 mg of the sample (PS or Asialo-PS) in 15 ml of 0.1M acetate buffer (pH 4.5) was cooled to 4°, and 30 ml of chilled 0.1M sodium metaperiodate in the acetate buffer was added. The mixture was kept at 4° in the dark, and the consumption of periodate was determined on aliquots by the procedure of Fleury and Lange[22]. Formic acid was determined on 2-ml aliquots by titration with 0.01N sodium hydroxide. Formaldehyde was determined by the procedure of Smith and Montgomery[23], but on a smaller scale. Methyl α-D-glucopyranoside and galactitol were used in reference oxidations.

The unoxidized sugar components remaining in the above mixture (20 ml) after 72-h oxidation were analyzed as described below for the first product (*1-OR*) of the Smith degradation.

DeAc-PS was oxidized as follows. An aqueous solution of the sample (150 mg in 15 ml) was adjusted to pH 7.0 with 4N sodium hydroxide, 0.03M aqueous sodium metaperiodate (150 ml) was added, and the pH of the mixture was adjusted to 7.0. The oxidation was carried out in the dark for 72 h at 4°, and analyses were then effected as described above.

Smith degradation. — Portions (70 mg) of DeAc-PS, PS, and Asialo-PS were subjected to Smith degradation by a modification of the method of Montgomery

et al.[24]. DeAc-PS was oxidized under neutral conditions, and PS and Asialo-PS were oxidized in the buffer described above. After oxidation, each reaction mixture was treated with 3 ml of 50% aqueous ethylene glycol for 3 h at room temperature, and then passed through a column (3.1 × 60 cm) of Sephadex G-25, which was subsequently washed with water. The Molisch-positive effluent was concentrated and analyzed for sialic acid. The remaining solution was treated with 0.2 g of sodium borohydride and kept in a refrigerator overnight. Acetone (1 ml) was then added, and the solution was kept for 1 h at room temperature to destroy excess reductant. The reaction mixture was passed through a column (3.1 × 60 cm) of Sephadex G-25, which was subsequently washed with water. The Molisch-positive effluent (*1-OR*) was concentrated and analyzed for hexose, glucosamine, and L-fucose. This solution was mixed with an equal volume of 0.1N sulfuric acid and heated for 1 h at 80°. The hydrolysate was passed through a column (2.5 × 130 cm) of Sephadex G-10 to remove small fragments. The effluent was concentrated, and mixed with the same volume of 0.2M acetate buffer (pH 4.5). The buffered solutions obtained from all three above samples were treated by the procedure applied to PS and Asialo-PS, as described above, affording the twice-treated product (*2-OR*). Product *2-OR* was hydrolyzed with 0.1N hydrochloric acid overnight at room temperature, followed by the same treatment as above, to give product *3-OR*.

RESULTS

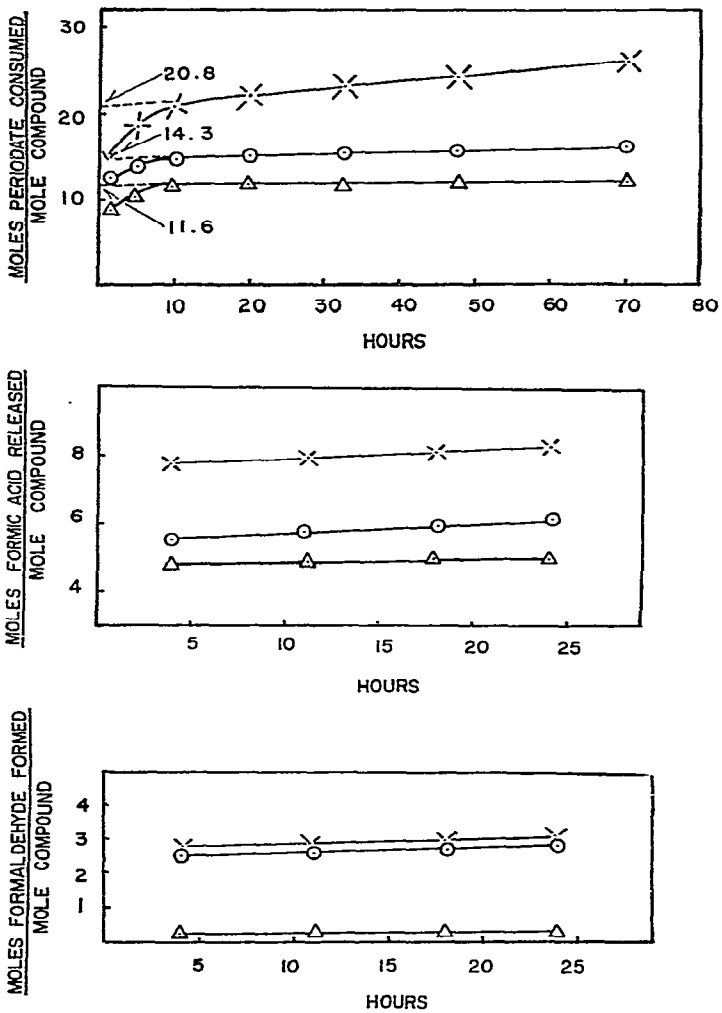
Periodate oxidation. — The consumption of sodium metaperiodate, and the amounts of the titratable acid released and of formaldehyde formed during periodate oxidation, are shown in Figs. 1–3.

As can be seen in the Figs., periodate reacted with DeAc-PS, PS, and Asialo-PS to the extent (by extrapolation) of 20.8, 14.3, and 11.6 moles/mole, respectively. After 24-h oxidation, 8.2, 6.1, and 5.0 moles/mole of formic acid, 3.1, 2.9, and 0.3 moles/mole of formaldehyde, respectively, were released.

Analytical data for sugar components of DeAc-PS, PS, and Asialo-PS, before and after periodate oxidation, are shown in Table I. Only 0.4 mole/mole of D-galactose was detected in Asialo-PS, whereas about 1 mole of D-galactose remained in DeAc-PS and PS. Moreover, another sample of Asialo-PS containing about 0.2 mole of L-fucose, which was obtained by a rather more-drastic treatment than that of the sample listed, contained less than 0.2 mole of D-galactose after oxidation. However, about 1.5 moles of D-galactose were found in DeAc-PS and PS at the early stage (1.5 h) of the oxidation. Approximately 1 mole of D-mannose of all samples was easily oxidized, even within 1.5 h, and no further oxidation was observed after 72 h. L-Fucose was not detected after the oxidation.

DeAc-PS was formed on hydrazinolysis of α_1-acid glycoprotein[10], and 79% of the glucosamine residues could be determined as 2,5-anhydro-D-mannose by the deamination procedure[14], without prior hydrolysis with acid. However, 70% of the glucosamine residues of DeAc-PS were oxidized with periodate under neutral con-

ditions. Approximately 38% of the glucosamine residues of the *N*-acetylated samples were destroyed by oxidation followed by reduction. The *N*-deacetylated sialic acid residues of DeAc-PS, amounting to 74% of the total, were oxidized completely between the amino and hydroxyl groups under neutral conditions.



Figs. 1–3. The amounts of oxidant consumed (Fig. 1), formic acid released (Fig. 2), and formaldehyde formed (Fig. 3) during the oxidation of DeAc-Ps (×), PS (○), and Asialo-PS (△) by sodium metaperiodate at 4°.

The *N*-acetylated sialic acid residues of DeAc-PS and PS were also oxidized. After mild hydrolysis (0.05N sulfuric acid, 80°, 1 h), the products formed gave color by Warren's method equivalent to 0.3 and 0.9 mole/mole of *N*-acetylneuraminic acid, respectively. The mild, acid hydrolysates of these products gave two or three blue spots with Ehrlich's reagent, having R_{NANA} 1.4 (x_1) and 2.0 (x_2, faint) [solvent (*a*)],

TABLE I

ANALYTICAL DATA FOR DEAC-PS, PS, AND ASIALO-PS[a], BEFORE AND AFTER OXIDATION WITH SODIUM METAPERIODATE FOR 72 H AT 4°

	Substance					
	Before oxidation			*After oxidation*		
	DeAc-PS	*PS*	*Asialo-PS*	*DeAc-PS*	*PS*	*Asialo-PS*
D-Galactose, %[b]	20.2	22.4	29.6	4.3	5.8	3.2
(moles/mole)	(4.0)	(3.9)	(3.8)	(0.9)	(1.0)	(0.4)
D-Mannose	14.3	16.3	22.0	9.2	11.3	15.0
	(2.9)	(2.8)	(2.8)	(1.8)	(1.9)	(1.9)
2-Amino-2-deoxy-D-glucose	26.4[c]	29.6	38.0	8.0	18.0	23.5
	(5.3)	(5.1)	(4.9)	(1.6)	(3.1)	(3.0)
L-Fucose	2.3	2.4	2.7	0	0	0
	(0.5)	(0.45)	(0.4)			
N-Acetylated sialic acid	6.3	24.9	0	3.1[g]	9.5[g]	—
	(0.7)	(2·4)		(0.3)	(0.9)	—
N-Deacetylated sialic acid	15.7	0	0			
	(2.0)					
Acetyl content, %	2.3	11.0	—			
(moles/mole)	(1.9)	(7.9)				
Molecular weight	3,600[d,e]	3,100[d]	2,300[f]			

[a]DeAc-PS: partially *N*-deacetylated polysaccharide fraction obtained from α_1-acid glycoprotein by hydrazinolysis; PS: polysaccharide units obtained by the *N*-acetylation of DeAc-PS; Asialo-PS: sialic acid-free PS. [b]Free sugar. [c]The corresponding amount of glucosamine that could be determined directly by the method of Yosizawa[14], without prior, acid hydrolysis, was 20.8 (4.2). [d]Molecular weight was determined by the sedimentation–equilibrium procedure[25]. [e]The analysis indicates that the carbohydrate content of DeAc-PS is less than 87.5% of the substance. [f]Calculated by subtraction of the amount of sialic acid from the molecular weight of PS. [g]The amount of the oxidized product, capable of color formation by Warren's method, expressed as *N*-acetylneuraminic acid.

and 1.4 (x_1), 1.9 (x_2), and 2.7 (x_3, faint) [solvent (*b*)], respectively. The methyl glycoside of *N*-acetylneuraminic acid, treated as described for the above samples, gave the same spots on the chromatograms. Of these products, x_1 was shown to be a C_7 compound by the ratio of the absorptions in the resorcinol reaction[26] at 580 and 630 nm. Also, the absorption maximum of the spectrum shifted from 590 (authentic sialic acid) to 630 nm, as reported for the C_7 compound[26].

Smith degradation. — Analytical data for the products of the Smith degradation are listed in Table II, and the small fragments detected by g.l.c. are shown in Table III.

Identical results were obtained for the first products (*1-OR*) of the Smith degradation and those of the periodate oxidation. Product *2-OR* from DeAc-PS contained 1 mole of mannose and 0.5 mole of glucosamine per mole, whereas *3-OR* contained only a small amount (0.3 mole) of mannose. On the other hand, approximately 2 moles each of mannose and glucosamine were found in *2-OR* from PS, and 1 mole each of these sugars in *3-OR* from this sample. Products *2-OR* and *3-OR* from Asialo-PS showed analytical data similar to those for the products from PS, except that *2-OR* from the former contained a lesser amount (0.6 mole) of glucosamine.

TABLE II

SMITH DEGRADATION OF DeAc-PS, PS, AND Asialo-PS

Product	*Sugar: %[a], moles/mole*									
	D-*Galactose*		D-*Mannose*		*2-Amino-2-deoxy-* D-*glucose*		*Sialic acid*		L-*Fucose*	
DeAc-PS, original	20.2, (100)	4.0	14.3, (100)	2.9	26.4, (100)	5.3	6.3[b],	0.7[b]	2.3,	0.5
1-OR[c]	4.1, (28)	0.8	8.9, (62)	1.8	7.9, (30)	1.6	3.0[d],	0.3[d]	0,	0
2-OR	0,	0	5.0, (35)	1.0	2.6 (10)	0.5				
3-OR			1.4, (10)	0.3	0,	0				
PS, original	22.4, (100)	3.9	16.3, (100)	2.8	29.6, (100)	5.1	24.9[b],	2.4[b]	2.4,	0.5
1-OR	5.6, (25)	1.0	11.1, (68)	1.8	17.5, (59)	3.0	9.5[d],	0.9[d]	0,	0
2-OR	1.0, (4)	0.2	10.6, (65)	1.8	11.8, (40)	2.0	0,	0		
3-OR	0,	0	6.3, (39)	1.1	5.6, (19)	1.0				
Asialo-PS, original	29.6, (100)	3.8	22.0, (100)	2.8	38.0, (100)	4.9	2.7,	0.4		
1-OR	3.0, (10)	0.4	14.9 (68)	1.9	23.0, (61)	3.0	0,	0		
2-OR	0,	0	13.7, (62)	1.8	10.6, (28)	1.4				
3-OR			8.8, (40)	1.1	6.8, (18)	0.9				

[a]Free sugar; the value in parenthesis shows the percentage of the remaining monosaccharide. [b]The amount of *N*-acetylated sialic acid. [c]Periodate oxidation was carried out under neutral conditions. [d]The amount of the oxidized product, capable of color formation by Warren's method, expressed as *N*-acetylneuraminic acid.

By hydrolysis with 0.1N hydrochloric acid overnight at room temperature, the degraded sialic acid (still capable of color formation by Warren's method) obtained by oxidation of DeAc-PS and PS was not removed from the fraction of higher molecular weight even by treatment with Dowex-1 (acetate form). It was, however, separated after hydrolysis with 0.05N sulfuric acid for 1 h at 80°. About 0.4 mole of galactose was found in the fractions of higher and lower molecular weight, separated by Sephadex G-10, from the mild, acid hydrolysate of *1-OR* derived from DeAc-PS. However, no noticeable amount of hexose was detected in the fraction of lower molecular weight obtained from *1-OR* derived from PS.

Product *3-OR* from DeAc-PS was shown to contain glycerol, glycolaldehyde, and a small proportion of mannose, by g.l.c., as well as by paper chromatography [butyl alcohol–pyridine–water (5:3:2); staining reagent, alkaline silver nitrate]. Product *1-OR* from PS and *N*-acetylated *1-OR* from DeAc-PS were hydrolyzed with N sulfuric

TABLE III

THE AMOUNTS[a] OF GLYCOLALDEHYDE, GLYCEROL, ERYTHRITOL, AND/OR THREITOL DETECTED IN THE SMITH-DEGRADATION PRODUCTS OF DeAc-PS, PS, AND Asialo-PS

Product	*Glycolaldehyde*	*Glycerol*	*Erythritol (E) and/or threitol (T)*
DeAc-PS[b]			
1-OR	+++	+++	+++(E+T)
2-OR	+	++	−
3-OR	+	++	−
PS			
1-OR	+++	+++	+(T)
2-OR	+	++	−
3-OR	+	++	−
Asialo-PS			
1-OR	+++	+++	−
2-OR	+	++	−
3-OR	+	+	−

[a]The amounts of the small fragments detected by g.l.c. are expressed as follows: −, <0.1; +, 0.25−1.5; ++, 1.6–2.5; +++, >2.5 moles/mole. [b]Since DeAc-PS was degraded into oligosaccharides of small molecular weight, even after the first Smith-degradation, it was difficult to determine the small fragments quantitatively.

acid for 6 h at 100°. Sulfuric acid was removed from the hydrolysates with Amberlite IR-4B (OH^-), and the tetritol of each hydrolysate was isolated by paper chromatography and then examined by paper electrophoresis[27] (20 volts/cm, 30 milliamperes, 1 h); staining was effected with alkaline silver nitrate. Rates of migration of erythritol and threitol, compared with that of galactitol, were 0.83 and 0.54, respectively. The results showed that *1-OR* from DeAc-PS contained a moderate proportion of erythritol and a small proportion of threitol, whereas that from PS contained a small proportion of threitol.

DISCUSSION

In the previous paper[10], 2-acetamido-2-deoxy-D-glucose was shown to be at the reducing end of PS. This reducing sugar of Asialo-PS was gradually oxidized with periodate, affording a small proportion of formaldehyde (Fig. 3). Most of the formaldehyde released from PS must, however, be accounted for by oxidation of sialic acid. Further oxidation of glucosamine at the reducing end of DeAc-PS might release an additional fraction of the formaldehyde. In the course of Smith degradation, the glucosamine residue at the reducing end of the molecule must be modified. The fragment (*e.g.*, 2-substituted glycerol) thus produced could be retained until the adjacent sugar was oxidized. The amounts of formaldehyde and formic acid liberated during the

periodate oxidation of these compounds suggest that the reducing glucosamine is linked to its adjacent sugar through position 4.

Approximately 1 mole of the D-mannose residues of all of the samples was oxidized within 1.5 h, suggesting substitution at position 6. It is less probable that the D-mannose was present at the non-reducing end of the molecule. The absence of tetritol in *1-OR* derived from Asialo-PS (Table III) excludes the presence of D-mannose substituted only at position 4. On the other hand, approximately 3 out of the 4 moles of the D-galactose residues of DeAc-PS and PS were degraded by the first oxidation, and, since 2 out of the 3 residues were oxidized rapidly, they must be at the non-reducing end or substituted at position 6 or 2. The slowly oxidized residue should be substituted at position 4, and is the precursor of the threitol obtained from *1-OR* derived from PS. Further decrease of the D-galactose content (about 0.5 mole) in the product of oxidation of Asialo-PS indicated that this D-galactose residue was substituted at position 3 with sialic acid in PS. The unoxidized D-galactose residue of *1-OR* from DeAc-PS was found to be in approximately equivalent molar proportions in the fractions of higher and lower molecular weight obtained after mild, acid hydrolysis. No hexose was found in the fraction of lower molecular weight obtained from *1-OR* of PS. Therefore, approximately half of the unoxidized mole of D-galactose residues was linked to an oxidizable adjacent sugar, probably at position 4 of the glucosamine residue of DeAc-PS, whereas the remaining half was linked at position 3 of an unoxidized glucosamine residue.

L-Fucose was readily oxidized, indicating its location at the non-reducing end of the molecule. Another sample of Asialo-PS, which contained a lesser proportion of L-fucose, underwent extensive oxidation of the D-galactose residues. These findings suggest that D-galactose is linked through position 3 to L-fucose.

After the first oxidation followed by reduction (*1-OR*), DeAc-PS, PS, and Asialo-PS lost 70, 38, and 38%, respectively, of their glucosamine residues. The tetritol found in *1-OR* from DeAc-PS was a mixture of threitol and erythritol. Only threitol was detected in the corresponding *1-OR* from PS, whereas *1-OR* obtained from Asialo-PS contained neither tetritol. We interpret these facts as meaning that the erythritol is formed by oxidation of glucosamine residues substituted at position 4, by cleavage between position 2 and 3. Approximately 80% of the glucosamine residues of DeAc-PS could be detected directly as 2,5-anhydro-D-mannose after deaminative cleavage of the glucosaminidic linkages (Table I). Nevertheless, 30% of the glucosamine residues were resistant to oxidation, indicating the presence of 3-substituted glucosamine residues.

One of the two moles of glucosamine residues destroyed by the first sequential oxidation and reduction of PS and Asialo-PS is apparently linked at position 6, whilst the second is at the reducing end of the molecule.

The *N*-deacetylated residues of sialic acid of DeAc-PS were completely oxidized between the amino and hydroxyl groups under neutral conditions. After periodate oxidation, the *N*-acetylated sialic acid of DeAc-PS and PS gave color, by Warren's method, equivalent to 0.3 and 0.9 mole/mole, respectively, of *N*-acetylneuraminic acid. The products appeared to be mainly a C_7 compound[26]. The degraded sialic acid was

not removed from the fraction of higher molecular weight after mild, acid hydrolysis of the oxidation products from DeAc-PS or PS, even when followed by treatment with Dowex-1 (acetate form). When the hydrolysis condition was changed from 0.1N hydrochloric acid, at room temperature overnight, to 0.05N sulfuric acid for 1 h at 80°, the degraded sialic acid was cleaved. The amount of this substance retained in the fraction of higher molecular weight from both samples was approximately 0.3 mole/mole. Therefore, most of the color-producing oxidation product of the *N*-acetylated sialic acid of DeAc-PS appeared to be attached through position 3 of D-galactose to the unoxidized portion of this sample. However, the remaining, degraded sialic acid in *1-OR* from PS should be linked to the oxidizable galactose.

The inner core of DeAc-PS should contain approximately 1.5 moles of the oxidizable glucosamine residues as indicated by the first periodate oxidation. These glucosamine residues were not oxidised when they were *N*-acetylated, suggesting 4-, or 4- and 6-substitution. Moreover, the removal of these glucosamine residues results in the oxidation of approximately one mole of the D-mannose residues by the second, Smith degradation. The D-mannose residues therefore appear to be substituted at position 3. In addition, the isolation of *O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-α-D-mannopyranosyl-(1→2)-glycerol[28] from an acid hydrolysate of *1-OR* from DeAc-PS showed the presence of another 3-substituted D-mannose residue. The remainder of the oxidizable glucosamine residues may be linked at positions 2, 4, or 6 of one of the unoxidized D-mannose residues, making a branch-point. There remain 1.6 glucosamine residues that have not been accounted for in *1-OR* derived from DeAc-PS, and, as mentioned above, these should be linked at positions 3, or 3 and 4 (or 6), by the D-mannose or D-galactose residues.

As can be seen in Table II, when the sample was free from sialic acid, an additional 0.6 mole of the galactose residues was oxidized by the first periodate oxidation. The removal of this galactose residue resulted in subsequent oxidation of 0.6 mole of glucosamine in *2-OR* derived from Asialo-PS. However, *3-OR* preparations from PS and Asialo-PS showed identical analytical data, each containing *ca.* one mole of D-mannose and glucosamine. Furthermore, as mentioned above, *1-OR* and *2-OR* from DeAc-PS contained 1.6 and 0.5 moles of glucosamine residues, respectively. These observations suggest the presence of oligosaccharide branches that might consist of *ca.* 0.5 mole of each of the component sugars. These branches may be attached to a single D-mannose residue adjacent to the reducing end of the molecule.

Based on these observations, a possible, average structure of the polysaccharide units (PS) of the α_1-acid glycoprotein is shown in Fig. 4. Anomeric configurations are taken from previous reports[5,6,8,29–31].

As shown already[7,9,32–35], the carbohydrate moiety of the α_1-acid glycoprotein is composed of several polysaccharide units. Each unit has a different chain length, composition, and structure. Hence, it is difficult to clarify the exact structure of each unit by using a pooled α_1-acid glycoprotein or modifications thereof, or a mixture of the polysaccharide units. Therefore, the results of the present study allow postulation only of a possible, average structure of these units.

Many investigators[4,5,29,33,36–39] have reported that sialic acid is located at the non-reducing end and is linked to D-galactose as in Fig. 4.

Most of the structural work reported so far on the carbohydrate moiety of the α_1-acid glycoprotein has been carried out by Jeanloz and his co-workers[3–9]. The results of their enzymic studies, involving neuraminidase, β-D-galactosidase, and 2-acetamido-2-deoxy-β-D-glucopyranosidase from *Diplococcus pneumoniae*, are incorporated in Fig. 4.

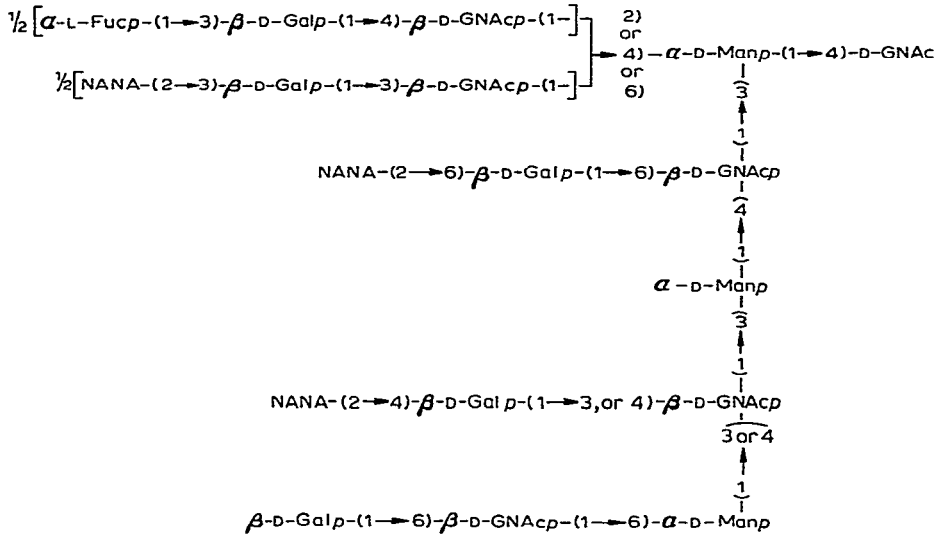


Fig. 4. A possible, average structure for the polysaccharide units of α_1-acid glycoprotein. Abbreviation: NANA, *N*-acetylneuraminic acid.

Eylar and Jeanloz[3] isolated "*N*-acetyllactosamine", a tetrasaccharide, and an octasaccharide from the hydrolysate of the α_1-acid glycoprotein. The octasaccharide contained the sequence, *O*-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-(1→4 or →2)-*O*-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-(1→2 or →3)-*O*-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose, having a branch at position 4 of the D-mannose residue adjacent to the reducing sugar of the molecule. The branch had a sequence, *O*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose. These workers recently reported[9] that the α_1-acid glycoprotein contains a carbohydrate moiety consisting of a total of 16 chains. Each chain possesses an *N*-acetylneuraminic acid residue as the non-reducing, terminal group, with the exception of two chains where it is replaced by L-fucose. Some of the chains were terminated by the sequence *O*-(*N*-acetylneuraminyl)-(2→4)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose. In order to obtain information on the chemical structure of the inner core of the polysaccharide moiety, the sialic acid-free glycoprotein and a derivative thereof, obtained by treatment with neuraminidase and β-D-galactosidase from *Diplococcus pneumoniae*, were subjected to sequential periodate oxidation. The results indicate that each molecule of glycoprotein has 8–9

chains containing *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2 or →6)-D-mannopyranosyl residues, and five chains containing *O*-β-D-galactopyranosyl-(1→6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2 or →6)-D-mannosyl residues, with 30 to 40% of the chains possessing branch points at C-3 or C-4 of the D-mannose residues, and also contains *O*-(2-acetamido-2-deoxy-D-glucosyl)-2-acetamido-2-deoxy-D-glucose residues.

A discrepancy between our results and those of Jeanloz's group[9] involves the proportion of D-mannose residues remaining in the oxidation products of the sialic acid-free and sialic acid-containing samples. Our products contained a higher percentage of D-mannose residues. It was reported[9] also that sialic acid-containing α_1-acid glycoprotein retained a considerable proportion of carbohydrate including D-galactose and D-mannose, as well as 2-acetamido-2-deoxy-D-glucose, even after five, sequential oxidations with periodate. Our work gave comparable results for the carbohydrate samples containing and free from sialic acid (PS and Asialo-PS), when more drastic conditions of hydrolysis (0.05N sulfuric acid for 1 h at 80°) were used to remove the oxidized sialic acid in the first step of the Smith degradation. On the other hand, Hughes and Jeanloz[9] reported incompatible values for the D-mannose content of the products derived from sialic acid-free α_1-acid glycoprotein before and after treatment with β-D-galactosidase.

A partial structure, similar to that described above, was proposed by Willard[39] for the carbohydrate chain of urinary orosomucoid. He indicated that 2-acetamido-2-deoxy-D-glucose must be linked at position 3 or 4, or be involved in a branch point. The production of large proportions of glycerol and formic acid on Smith degradation favoured a 1→6 linkage for some of the D-galactose and/or D-mannose residues. The absence of erythritol proved that no D-mannose was linked at position 1 and 4 alone, whereas the presence of a trace of threitol showed that only a small proportion of D-galactose was linked at 1 and 4. Some of the D-mannose (and D-galactose) residues must be substituted at positions 1 and 3 or be involved in a branch point in order to account for the resistance to oxidation. In addition, the anomeric configurations (except for that of the D-mannosyl linkages) of the units in the carbohydrate chain of urinary orosomucoid, as determined by Barker *et al.*[29] using enzymes obtained from *Klebsiella aerogenes*, have been incorporated into Fig. 4. The α-D-mannosyl configuration of α_1-acid glycoprotein was proposed by Li[30] and by Wagh *et al.*[31].

SUMMARY

Periodate oxidation and Smith degradation of the carbohydrate moiety obtained by hydrazinolysis of the α_1-acid glycoprotein of human plasma have been studied, and an average structure for the polysaccharide has thereby been obtained. The inner core of the polysaccharide appears to be *O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-(1→3)-*O*-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-(1→3)-*O*-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose, to which several oligosaccharides are attached. To the residue of D-mannose that is linked to the reducing 2-acetamido-2-deoxy-D-glucose residue, equimolar amounts of *O*-sialyl-(2→3)-

O-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)- and *O*-L-fucopyranosyl-(1→3)-*O*-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-chains are attached at positions 2, and/or 4, and/or 6. An *O*-sialyl-(2→6)-D-galactopyranose residue is attached at position 6 of the second residue of 2-acetamido-2-deoxy-D-glucose. An *O*-sialyl-(2→4)-D-galactose residue is attached at positions 3 or 4 of the third residue of 2-acetamido-2-deoxy-D-glucose, and an *O*-D-galactopyranosyl-(1→6)-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-(1→6)-*O*-D-mannopyranosyl chain is attached to the same residue of 2-acetamido-2-deoxy-D-glucose at position 4 or 3.

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A NEW APPROACH TO THE SYNTHESIS OF HIGHER 3-DEOXYGLYCULOSONIC ACIDS

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INTRODUCTION

3-Deoxyglyculosonic acids are currently of special interest. Higher 3-deoxyglyculosonic acids, containing 7–9 carbon atoms, play an important role in some biosynthetic processes[1] and in the formation of specific biopolymers[2–4]. The biological significance of sialic acids, which also belong to this group, is well known[5,6].

The only general method of synthesis of higher 3-deoxyglyculosonic acids involves condensation of monosaccharides with oxalacetic acid[7] or di-*tert*-butyl oxalacetate[8]. However, this method gives mixtures of several isomeric acids that are difficult to separate, and, in consequence, synthetic 3-deoxyglyculosonic acids are rather inaccessible.

We now wish to describe a new approach to the synthesis of higher 3-deoxyglyculosonic acids, including the conversion of monosaccharides (*e.g.* D-galactose) by the Wittig reaction into 3,4-dideoxyglyculos-3-enonic acids.

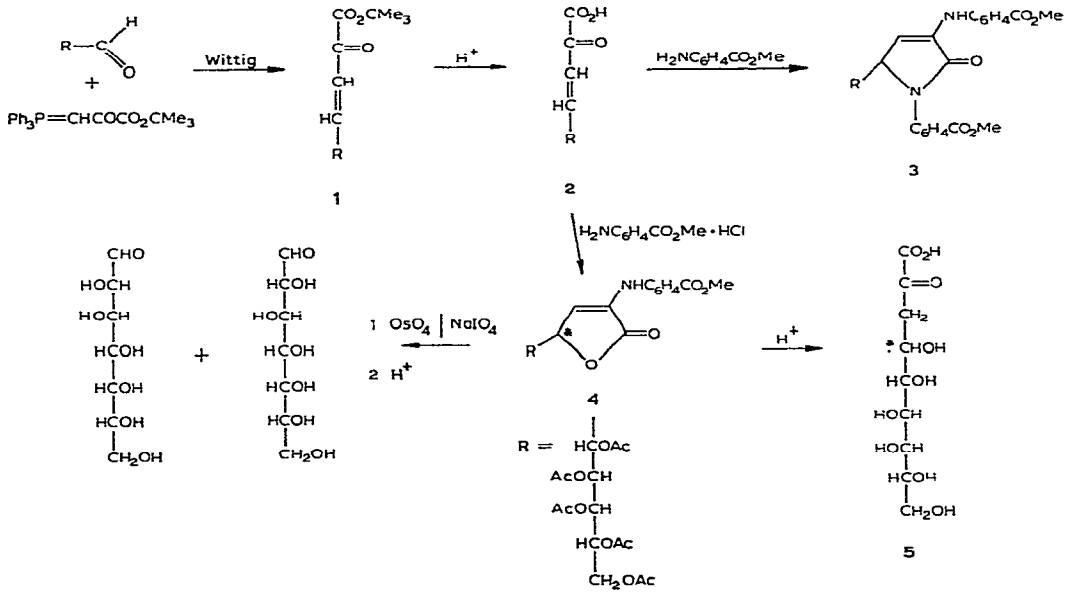
RESULTS AND DISCUSSION

The unsaturated glyculosonic acids, which are the key intermediates in the new syntheses, were obtained by the condensation of *aldehydo*-forms of monosaccharides with (alkoxyoxalyl)methylenetriphenylphosphorane, the latter being synthesized from an alkyl bromopyruvate[9]. This reaction results in elongation of the original carbon chain by three carbon atoms, with simultaneous introduction of the required functional groupings.

The reaction of penta-*O*-acetyl-*aldehydo*-D-galactose with (*tert*-butoxyoxalyl)-methylenetriphenylphosphorane proceeds smoothly in boiling benzene to give a high yield of *tert*-butyl 5,6,7,8,9-penta-*O*-acetyl-3,4-dideoxy-D-*galacto*-nonulos-*trans*-3-enonate (**1**). The use of (*tert*-butoxyoxalyl)methylenetriphenylphosphorane is of especial convenience, since the transformation of the *tert*-butyl ester **1** into the corresponding unsaturated acid **2** could be accomplished in quantitative yield with trifluoroacetic acid under mild conditions.

The conversion of the unsaturated acid **2** into a 3-deoxyglyculosonic acid might be achieved either by hydration of the double bond or by cyclisation, the latter process giving the corresponding lactone. The hydration and/or lactonisation of β,γ-enoic

acids proceeds in the presence of concentrated mineral acids under drastic conditions that cannot be applied to the carbohydrate derivatives. Arylidenepyruvic acids have been converted into *N*-aryl-enaminolactones by the action of aromatic amines[10]. However, under these conditions, the unsaturated acid **2** gave mainly the enaminolactam instead of the expected enaminolactone. Treatment of acid **2** with methyl



p-aminobenzoate resulted in formation of the *N,N'*-di[(*p*-methoxycarbonyl)phenyl]-enaminolactam **3** as the major product, the *N*-[(*p*-methoxycarbonyl)phenyl]enaminolactone **4** being isolated in much lower yield. Structure **3** follows from the n.m.r. spectrum (Fig. 1), which showed peaks at δ *ca.* 2 (five *O*-acetyl groups), 3.88 and 3.91 (two methoxy-groups), 4.97–5.40 (complex multiplet, C-4–C-8 protons), 6.15 (singlet, olefinic proton), and 6.98 (NH proton). Two doublets centered at δ 7.00 and 7.93 (J 8 Hz) correspond to protons of the aromatic nucleus linked with the nitrogen atom of the enamino group, and the protons of the aromatic nucleus linked to the nitrogen atom of the lactam ring gave two doublets centered at δ 7.60 and 8.67 (J 9 Hz).

Formation of the enaminolactam **3** results from nucleophilic addition of the amine to the conjugated double bond, followed by cyclisation of the γ-amino acid. In the case of arylidenepyruvic acids, no addition to the double bond occurs, and the reaction appears to proceed *via* anil formation. In order to obtain the enaminolactone **4** from the unsaturated acid **2**, nucleophilic attack must be prevented, *e.g.*, by use of the amine salt instead of the free base. Indeed, the condensation of the unsaturated acid **2** with methyl *p*-aminobenzoate hydrochloride in glacial acetic acid at 70° resulted in formation of the enaminolactone **4**, isolated in *ca.* 45% yield after chromatography. The n.m.r. spectrum of **4** (Fig. 2) supports the suggested structure and contains two doublets centered at δ 6.98 and 7.97 (J 8.0 Hz, 4 aromatic protons), peaks corresponding to five *O*-acetyl groups and one methoxyl group (δ 3.84), a

complex multiplet (δ 5.07–5.66, H-4–H-8), and an AB octet of an ABX system (δ 3.67–4.47, C-9 methylene protons). The singlet at δ 6.18 is assigned to the olefinic

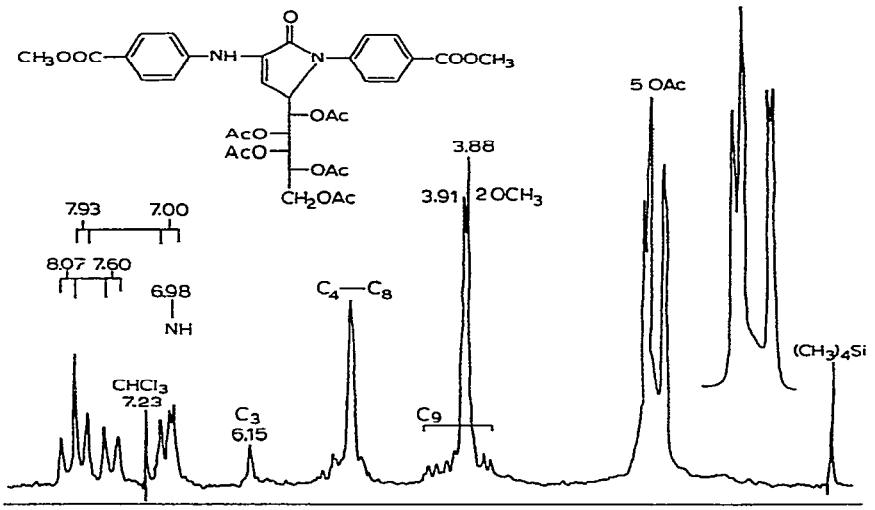


Fig. 1. N.m.r.-spectrum of *N,N'*-di[(*p*-methoxycarbonyl)phenyl]-2-enamino-4-(D-*galacto*-pentaacetoxypentyl)-4-butanelactam (**3**). (The right-hand, upper portion shows the signals for the *O*-acetyl groups recorded at smaller sweep-width).

proton at C-3, whilst that at 6.91 corresponds to the NH-group at C-2, since the latter signal showed an upfield shift on dilution.

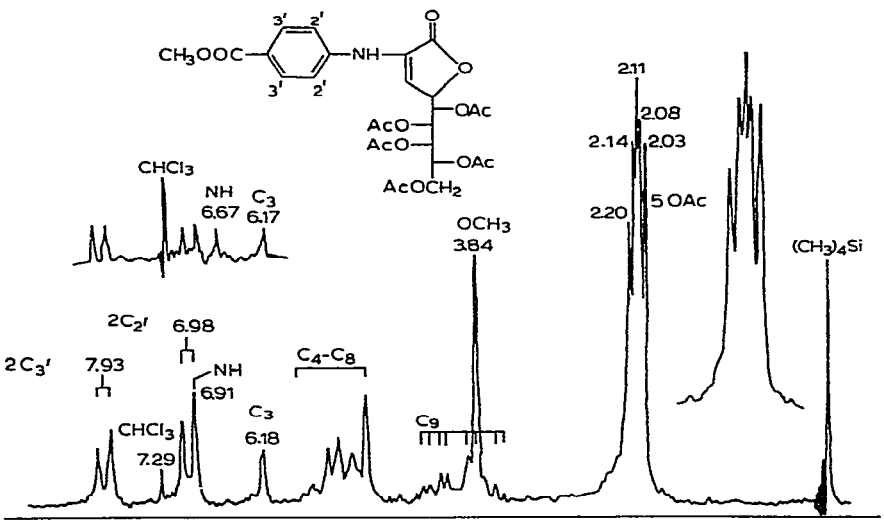


Fig. 2. N.m.r.-spectrum of 2-[*N*-(*p*-methoxycarbonyl)phenyl]enamino-4-(D-*galacto*-pentaacetoxypentyl)-4-butanolide (**4**) (the left-hand upper portion corresponds to the diluted solution, the right-hand one to the *O*-acetyl signals recorded at smaller sweep-width).

The presence of the enamino group in the lactone **4** was proved by identification of methyl *p*-aminobenzoate formed on mild acid hydrolysis. The condensation of the acid **2** with methyl *p*-aminobenzoate hydrochloride may give rise to two enaminolactones isomeric at C-4. Indeed, the product obtained, in spite of its apparent homogeneity on t.l.c., was shown to be a mixture of epimers by the formation of two heptoses on scission of the double bond in lactone **4** by treatment with sodium metaperiodate–osmium tetroxide in aqueous *N,N*-dimethylformamide. Methanolysis and hydrolysis of the product gave a mixture (identified by paper chromatography[11]) of D-*glycero*-L-*manno*-heptose, D-*glycero*-L-*gluco*-heptose, and D-galactose. The above heptoses were synthesized from D-galactose by Sowden's method[12]. The appearance of D-galactose in the degradation mixture is apparently due to hydrolytic elimination of *p*-(methoxycarbonyl)oxanilic acid with subsequent periodate oxidation of the sugar moiety.

The final step in the synthesis, involving conversion of enaminolactone **4** into free acid **5**, was accomplished by the action of 0.2N methanolic hydrogen chloride at 75°, followed by hydrolysis. The acids **5**, isolated as the barium salts, could be separated by paper chromatography (see Experimental) and gave positive periodate–thiobarbituric acid[13] (λ_{max} 550 nm) and *o*-phenylenediamine tests[14] (λ_{max} 335 nm), thus proving them to be 3-deoxyglyculosonic acids. The isolation of individual acids is now in progress.

EXPERIMENTAL

General. — Paper chromatograms were run on the Leningrad factory No. 2 paper "M" by the ascending technique with the following systems (v/v): (*A*) butyl alcohol–acetic acid–water (4:1:1), and (*B*) butyl alcohol–pyridine–water (5:3:2), and by the descending technique in (*C*) butyl alcohol–pyridine–water (6:4:3), (*D*) propyl alcohol–benzyl alcohol–85% formic acid–water (5:7:2:2), and (*E*) butyl alcohol–pyridine–0.1N hydrochloric acid (5:3:2). Paper electrophoresis was performed in 0.01M pyridine-acetate buffer, pH 4.5, at 20 volts/cm for 0.5–1 h. Substances were detected on paper by the following spray reagents: periodate–benzidine, alkaline silver nitrate, sodium periodate–thiobarbituric acid (Warren's reagent[15]), *p*-anisidine phosphate, and a specific reagent[16] for heptoses. Thin-layer chromatography (t.l.c.) was performed on silica gel KSK in chloroform–methanol (19:1), and detection was effected with conc. sulfuric acid, aqueous potassium permanganate, or iodine vapour. Evaporations were carried out under diminished pressure at temperatures not exceeding 40°. Melting points were determined on a Kofler micro-heating stage. N.m.r. spectra were measured on an RS-60 NMR spectrometer on solutions in deuteriochloroform with tetramethylsilane as the internal standard.

tert-*Butyl 5,6,7,8,9-penta*-O-*acetyl-3,4-dideoxy*-D-galacto-*nonulos*-trans-3-*enonate* (**1**)[9]. — A solution of penta-*O*-acetyl-*aldehydo*-D-galactose (0.39 g) and (*tert*-butoxy oxalyl)methylenetriphenylphosphorane[9] (0.5 g, 25% excess) in dry benzene (50 ml) was refluxed for 20 h. The cooled mixture was concentrated and placed on a column

of silicic acid (50 g). The column was washed with gradients of increasing concentration of ether in benzene from 0 to 50%. Fractions were monitored by t.l.c., and those containing pure substance (R_F 0.80) were combined and evaporated to dryness. Crystallization of the residue from ethyl acetate–hexane gave the ester **1** (425 mg, 80%), m.p. 102–104°, $[\alpha]_D^{20}$ +27.2° (*c* 4.18, benzene).

Anal. Calc. for $C_{23}H_{32}O_{13}$: C, 53.48; H, 6.25. Found: C, 53.89; H, 6.61.

*5,6,7,8,9-Penta-O-acetyl-3,4-dideoxy-*D-galacto-*nonulose-*trans-*3-enonic acid* (**2**). — Ester **1** (100 mg) was dissolved in trifluoroacetic acid (1 ml), the clear solution was kept for 40 min at room temperature and evaporated to dryness, and benzene (5 ml) was distilled from the residue. After threefold evaporation with benzene, the residue was dried *in vacuo* at 40°, and the acid **2** was obtained in quantitative yield as a white, amorphous powder. The analytical sample was obtained as follows: a solution of the dry residue in saturated, aqueous sodium hydrogen carbonate (5 ml) was washed with chloroform (2 × 5 ml), acidified to pH 3 with 2N hydrochloric acid, and extracted with chloroform. The extract was dried, evaporated, and crystallized from ethyl acetate–hexane to give **2** (40 mg), m.p. 98–104°, $[\alpha]_D^{20}$ +26.9° (*c* 3.22, chloroform).

Anal. Calc. for $C_{19}H_{24}O_{13}$: C, 49.56; H, 5.25. Found: C, 49.53; H, 5.63.

N,N′-*Di-*[(p-*methoxycarbonyl)phenyl*]-*2-enamino-4-*(D-galacto-*pentaacetoxypentyl*)-*4-butanelactam* (**3**). — To a solution of acid **2** (from 256 mg of ester **1**) in glacial acetic acid (2.5 ml) was added methyl *p*-aminobenzoate (75.5 mg), and the solution was kept for 5 days at room temperature. The reaction mixture was evaporated to dryness, the residue was dissolved in chloroform (10 ml), and the solution was washed successively with 2N hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried with magnesium sulfate, and evaporated. The residue was dissolved in a few ml of benzene and chromatographed on a column of silica gel (20 g), using benzene–ether mixtures with increasing proportions of ether (0 to 50%, v/v). Fractions were monitored by t.l.c., and evaporation of appropriate fractions gave 200 mg of crystalline residue, which, on recrystallization from 4 ml of methanol, gave lactam **3** (140 mg) as needles, m.p. 207–209°, $[\alpha]_D^{20}$ −3.4° (*c* 4.14, chloroform).

Anal. Calc. for $C_{35}H_{38}N_2O_{15}$: C, 57.85; H, 5.27; N, 3.86. Found: C, 57.91; H, 5.45; N, 3.71.

Evaporation of the mother liquor and recrystallization from methanol (2 ml) gave lactone **4** (40 mg) as rosettes, m.p. 180°.

2-[N-(p-*Methoxycarbonyl)phenyl*]*enamino-4-*(D-galacto-*pentaacetoxypentyl*)-*4-butanolide* (**4**). — To a solution of acid **2** (from 520 mg of ester **1**) in glacial acetic acid (5 ml) was added methyl *p*-aminobenzoate hydrochloride (190 mg), and the solution was kept for 6 h at 70°. The darkened mixture was evaporated to dryness, and a solution of the residue in chloroform (10 ml) was washed with water, saturated aqueous sodium hydrogen carbonate (4 × 25 ml), and water, dried with magnesium sulfate, and evaporated. The residue was chromatographed on silica gel (30 g), using benzene–ether mixtures, to yield 270 mg (45%) of lactone **4**. It was twice recrystallized from methanol, and 250 mg of analytically pure lactone **4** were obtained as rosettes, m.p. 181° (sinters at 168–170°), $[\alpha]_D^{23}$ +57.0° (*c* 3.38, chloroform).

Anal. Calc. for $C_{27}H_{31}NO_{14}$: C, 54.63; H, 5.26; N, 2.36. Found: C, 54.45; H, 5.24; N, 2.52.

Conversion of enaminolactone **4** *into heptoses.* — To a stirred solution of enaminolactone **4** (10 mg) in 80% aqueous *N*,*N*-dimethylformamide (1 ml) was added one crystal of osmium tetroxide. After 15 min, small portions of sodium metaperiodate (total, 50 mg) were added to the darkened mixture, which was stirred for 2 h. The colourless solution was filtered, diluted with chloroform (5 ml), and washed with 5% aqueous sodium thiosulfate and water, dried with magnesium sulfate, and evaporated to dryness. A solution of the syrupy residue in 0.2N methanolic hydrogen chloride (1 ml) was boiled under reflux for 2 h and then evaporated to dryness. The residue was heated with 2N hydrochloric acid (1 ml) for 3 h at 100°. The cooled solution was diluted with water (10 ml) and passed through columns of Amberlite IRA-400 (AcO^-, 3 ml) and KU-2 (H^+, 2 ml), and the eluate was concentrated to 1 ml. This hydrolyzate contained D-galactose, D-*glycero*-L-*manno*-heptose, and D-*glycero*-L-*gluco*-heptose, indistinguishable from authentic sugars on paper chromatograms in systems *B*, *C*, and *D*.

3-Deoxy-D-glycero-L-manno- *and* -L-gluco-*nonulosonic acids* (**5**). — A solution of enaminolactone **4** (0.2 g) in 0.2N methanolic hydrogen chloride (100 ml) was boiled under reflux for 2 h, water (20 ml) was added, and the mixture was heated for 1 h at 80°. The colourless solution was cooled, the methanol was evaporated *in vacuo* with addition of water, and the solution was neutralized with sodium hydrogen carbonate and extracted with chloroform (3 × 25 ml). The chloroform extract contained (t.l.c. and electrophoresis) methyl *p*-aminobenzoate. The aqueous solution was treated (to pH 3) batchwise with cation-exchanger KU-2 (H^+), filtered, stirred with excess of silver carbonate, filtered, and passed through a column of cation-exchanger KU-2 (H^+). The eluate was concentrated *in vacuo* to a small volume and then freeze-dried. The resulting product contained the free acids **5** and their lactones (R_F 0.10 and 0.20 in system *A*; two spots, one moving towards the anode and the second being immobile, were detected on a paper electrophoretogram). The product was dissolved in water (5 ml), 0.1N barium hydroxide was added with stirring to pH 7.5, and the stirring was continued for 20 min. The mixture was neutralized with carbon dioxide, filtered, concentrated *in vacuo*, and filtered, and the clear solution was freeze-dried. The resulting white solid (94.3 mg) was the trihydrate, $[\alpha]_D^{20}$ −21.0° (*c* 4.27, water), of the barium salt of the title compound.

Anal. Calc. for $(C_9H_{15}O_9)_2Ba.3H_2O$: C, 29.78; H, 5.00; Ba, 18.93. Found: C, 29.77; H, 4.85; Ba, 19.59.

The product migrated as a single spot (R_F 0.07) on chromatography in system *A*, and on electrophoresis, but could be separated in system *E* (Whatman No. 2 paper, 3 days) into two spots, with mobilities of 14.7 and 19.3 cm, in the ratio of *ca.* 10:1.

SUMMARY

A novel approach to the synthesis of 3-deoxyglyculosonic acids is reported. The key step in this synthesis involves the transformation of a monosaccharide by the

Wittig reaction into a 3,4-dideoxyglyculos-3-enonic acid, and subsequent lactonisation.

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NATURE OF THE STRUCTURAL CHANGE DURING THE MUTAROTATION OF SUGAR OSAZONES

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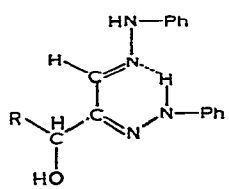
INTRODUCTION

The structure of sugar osazones was for long controversial, and despite many investigations, the exact nature of the structural change during mutarotation was not understood.

When Emil Fischer first prepared the sugar osazones, he attributed to them an open-chain structure[1,2]. The mutarotation of sugar osazones was first observed in 1909 by Levene and Jacobs[3], but they did not attempt to explain the phenomenon. Besides Fischer's open-chain structure, tautomeric azohydrazone structures[4] and ring structures[5,6] were proposed for the osazones, and their mutarotation was considered to be the result of an equilibrium between the different forms. In 1935, Engel[7] endeavored to show that this mutarotation is due to hydrolysis, with liberation of phenylhydrazine.

On theoretical grounds, Fieser and Fieser[8] proposed, in 1944, two alternative chelate structures for the osazones, these being stabilized by resonance. In 1955, Mester[9] supplied the first chemical evidence in favor of structure (A), one of the two chelate structures advanced by Fieser and Fieser. Supplementary arguments were adduced later by chemical and physical studies[10–14]. Further confirmation of this structure* came recently from n.m.r. data published almost simultaneously by three independent laboratories[15–18].

In 1957, Mester and Major[11] decided that mutarotation is a consequence of a reversible change in the structure of the osazones through the action of a basic solvent;



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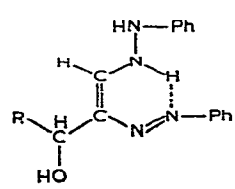

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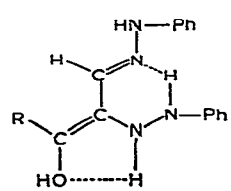

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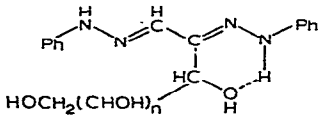
* For reviews, see L. Mester, *Angew. Chem. Intern. Ed. Engl.*, 4 (1965) 574, and H. El Khadem, *Advan. Carbohydrate Chem.*, 20 (1965) 157.

and a progressing equilibrium between the initial structure (A) and the *N*-chelated structure (B) or the *O*-chelated structure (C) has been advanced[19].

Based essentially on u.v. spectrophotometric investigations, Henseke and Köhler[20] attributed the *O*-chelated structure (D) to the osazones, and suggested the opening of this structure through formation of Fischer's open-chain structure during mutarotation. Since then, the chelate structure (A) of the sugar osazones has been repeatedly confirmed, and for this reason, no mechanism starting with the *O*-chelated structure (D) proposed by Henseke and Köhler can be accepted.



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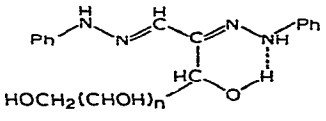

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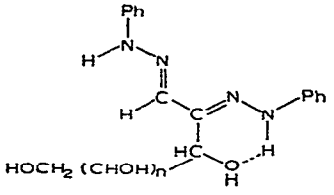

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From the changes of the proton signals in the n.m.r. spectra of the osazones during mutarotation, Chapman and co-workers[16] accepted the chelate structure A proposed by Mester and Major[11] as the initial structure, but they advanced for the final form of the osazones in mutarotation, structure D, which had earlier been proposed by Henseke and Köhler for the initial form. (Erroneously, Chapman and co-workers attributed to Henseke the O-H···N chelated structure E, instead of the O···H-N chelated structure D.) The evidence advanced by Chapman and co-workers for the *O*-chelated structure D for the product formed in the mutarotation of sugar osazones is not conclusive for the following reasons: (i) the position of the two new N-H signals appearing in the region of the solvent-bonded N-H signals supports a nonchelated, Fischer type of structure, rather than an *O*-chelated one; (ii) not only the formation of the *O*-chelated structure D but many other structural changes may provoke similar displacement in the position of the C-3 hydroxyl proton signal; and (iii) the proposed structure conflicts with data obtained through formazan formation. Therefore, we have re-examined the changes by measurements of the n.m.r. spectra, u.v. spectra, and formazan formation.

RESULTS AND DISCUSSION

N.m.r. spectra. — N.m.r. spectroscopy showed the initial and the final forms of the osazones to be in progressing equilibrium during mutarotation. Calculated from the intensity of the proton signals[21], the equilibrated solution of hydroxypyruvaldehyde bis(phenylhydrazone) (glycerosazone) (**1**) contains 50% of the final form, and that of D-*threo*-pentulose phenylosazone (D-xylosazone) (**2**) contains about 10% of the final form. A similar equilibrium was found for 6-deoxy-L-*arabino*-hexulose phenylosazone (L-rhamnosazone) (**3**), for D-*arabino*-hexulose phenylosazone (D-glu-

cosazone) (4), and for 1,2-pentanedione bis(phenylhydrazone) (propylglyoxal osazone) (6). No isomerization was observed for 3-methyl-1,2-butanedione bis(phenylhydrazone) (isopropylglyoxal osazone) (5).

Proton signals of the osazones during mutarotation in deuteriopyridine and *p*-dioxane are given in Table I. The initial form of the osazones **1–5**, and the final form of **6**, show a strongly chelated and a solvent-bonded N-H proton. In the initial form of **6** and the final form of osazones **1–4**, two N-H signals appear in the solvent-bonded region. To ascertain the chelated or nonchelated nature of the N-H protons, we have made use of the following method.

In solution in *p*-dioxane, first recommended by Mester and Major for the study of the mutarotation of sugar osazones, the solvent-bonded N-H protons show a lowered rate of proton exchange. Addition of water to the *p*-dioxane solution manifests itself in a downfield shift of the nonchelated N-H proton signals by about one p.p.m., due certainly to solvation of the N-H group by water. Imino protons that are *N*- or *O*-chelated do not show this phenomenon. This method established the presence of a chelated imino group, besides a nonchelated one, in the final form of sugar osa-

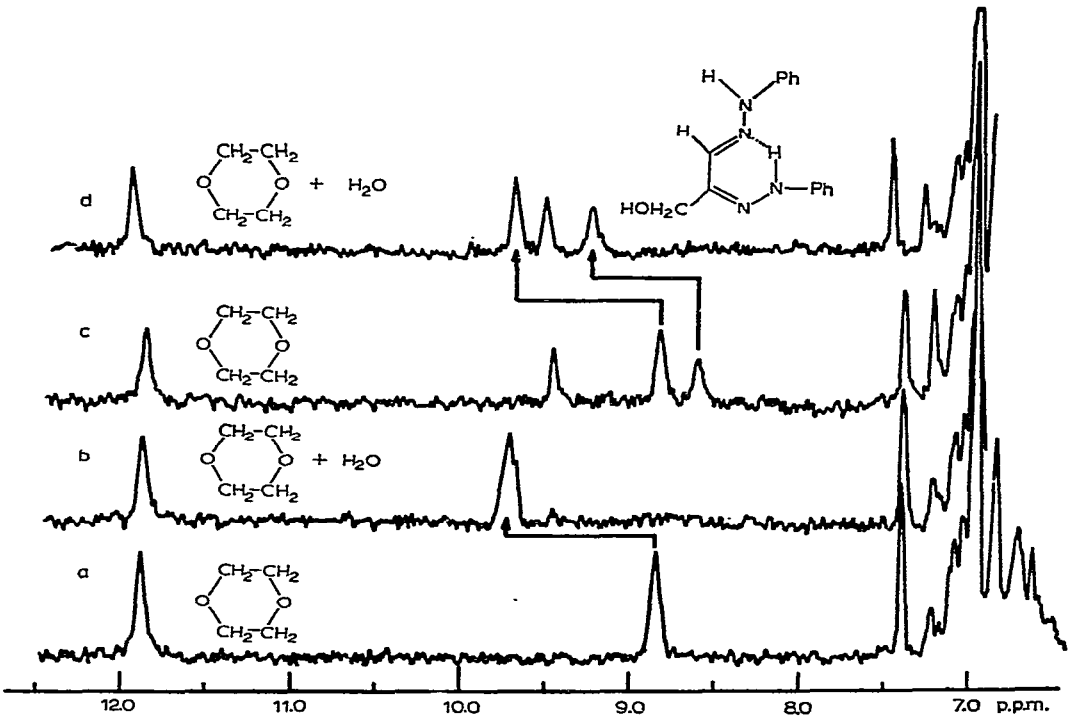

Fig. 1. Proton signals of hydroxypyruvaldehyde bis(phenylhydrazone) (glycerosazone) during reversible isomerization in *p*-dioxane: (a) initial spectrum; (b) initial spectrum after addition of water; (c) final spectrum (24 h); (d) final spectrum (24 h) after addition of water.

TABLE I

NUCLEAR MAGNETIC RESONANCE SPECTRAL DATA (P.P.M.) OF OSAZONES DURING REVERSIBLE ISOMERIZATION

No.	*Compound*	*In deuteriopyridine*						*In* p-*dioxane*					
		Initial form			*Final form*			*Initial form*			*Final form*		
		N-H	N-H	H-1	N-H	N-H	H-1	N-H	N-H	H-1	N-H	N-H	H-1
1	D-Glycerosazone	12.71	11.36	8.22	11.36	10.97	7.99		11.88	8.84	7.40	9.46	8.62	7.21
								$+H_2O$:	11.94	9.69	7.46	9.50	9.23	7.28
2	D-Xylosazone								11.89	8.90	7.40	9.95	8.53	*
								$+H_2O$:	11.97	9.68	7.40	9.98	9.15	*
3	L-Rhamnosazone	12.74	11.18	8.69	11.44	10.73	8.04							
4	D-Glucosazone	12.53	11.40	8.36	11.40	10.57	7.77							
5	Isopropylglyoxal osazone	12.50	11.17	7.70	unchanged				11.65	8.64	7.13	unchanged		
								$+H_2O$:	11.65	9.45	7.26			
6	Propylglyoxal osazone								8.45	7.95	7.09	11.52	8.55	*
								$+H_2O$:	9.03	8.36	7.14	11.52	9.38	*

*Signal included with benzene peaks.

zones (**1–4**) (see Fig. 1). The position of *N*-chelated N-H proton signals being shifted much farther downfield, the chelated imino proton in the final form of sugar osazones **1–4** must be *O*-, rather than *N*-, chelated.

By the same method, we ascertained the presence of two nonchelated imino protons in the initial form of **6**, whereas the final form of the same osazone, and the initial form of **5**, showed the presence of a chelated and of a nonchelated N-H proton.

U.v. spectra. — The absence of *N*-chelation in the final form of sugar osazones in mutarotation was also confirmed by u.v. spectroscopy.

Mester and Major[11] reported the displacement of the maxima at 396 nm in the spectra of sugar osazones towards shorter wavelengths during mutarotation. The initial and final forms of the osazones being in progressing equilibrium during mutarotation, the simultaneously registered shift of the u.v. maximum is the result of the superposition of the u.v. spectra of the two forms in equilibrium.

To obtain the u.v. spectrum of the final form, we recorded the u.v. spectrum of each sugar osazone after complete mutarotation (24 h) in *p*-dioxane, against a freshly prepared solution of the same osazone in *p*-dioxane, a solution which contained only the nonisomerized (initial) form of the osazone. The concentration of the osazone in the freshly prepared solution was so selected as to be equal to the concentration of the initial form in the equilibrium mixture after 24 h. The concentration of the initial form in the equilibrated solution was estimated from the intensity of the signals corresponding to the two forms in the n.m.r. spectrum[21], and was also established by

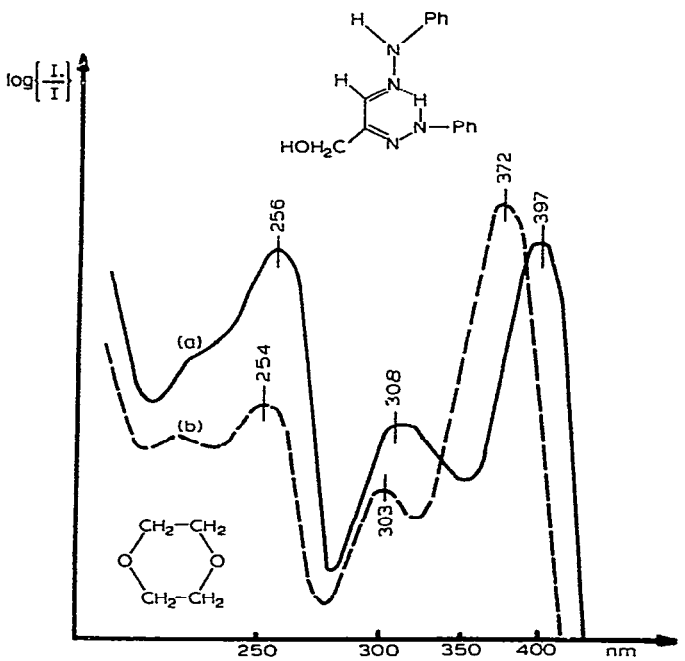

Fig. 2. U.v. spectra, in *p*-dioxane, of hydroxypyruvaldehyde bis(phenylhydrazone) (glycerosazone) during reversible isomerisation; (a) initial form; (b) final form.

following the changes in the overlapping[22] u.v. signals by subtraction of the u.v. spectra of freshly prepared solutions of the initial form at different concentrations.

The u.v. spectra of the initial and final forms of hydroxypyruvaldehyde bis(phenylhydrazone) (**1**) are shown Fig. 2. Similar spectra were obtained for the initial and final forms of D-*threo*-pentulose phenylosazone (**2**).

The u.v. spectra of the initial forms of sugar osazones (max. at 394–396 nm) correspond with the spectrum of the chelated 3-methyl-1,2-butanedione bis(phenylhydrazone) (**5**) (max. at 395 nm); and the spectra of the final forms of the sugar osazones obtained by mutarotation in *p*-dioxane (max. at 368 nm for **1** and at 372 nm for **2**) are identical with the spectrum of the nonchelated 1,2-pentanedione bis(phenylhydrazone) (**6**) (max. at 366 nm) (see Fig. 3). Thus, the u.v. spectral evidence also supports the rupture of the N-H···N chelation and the formation of a bis(phenylhydrazone) or similar structure during mutarotation.

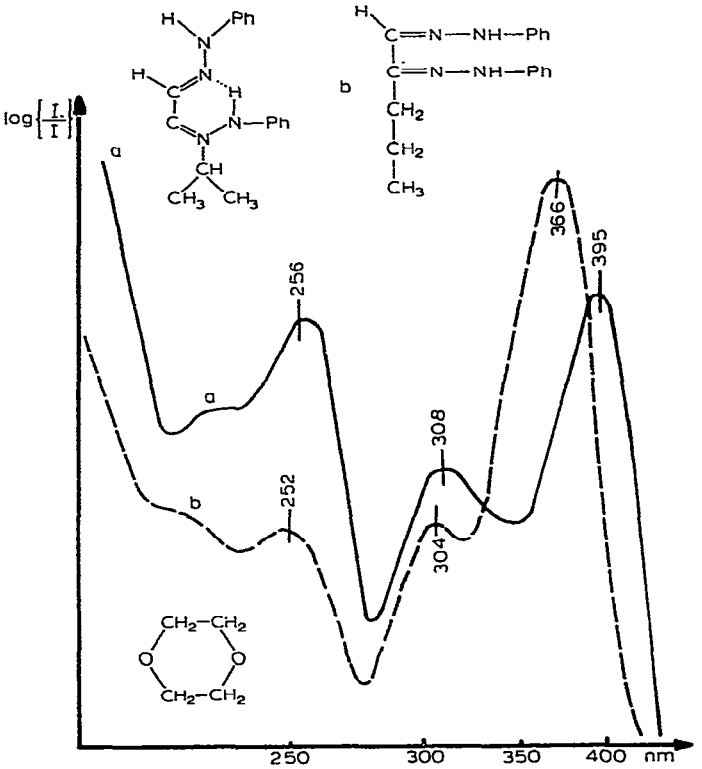

Fig. 3. U.v. spectra in *p*-dioxane: (a) 3-methyl-1,2-butanedione bis(phenylhydrazone) (isopropylglyoxal osazone); (b) 1,2-pentanedione bis(phenylhydrazone) (propylglyoxal osazone).

Long-range spin–spin coupling. — A long-range, spin–spin coupling was reported by Mester, Moczar, and Parello[10,11] between the C-1 proton and the nonchelated N-H proton in the n.m.r. spectra of the initial form of sugar osazones. A similar, long-range coupling, characteristic of the *syn* (*i.e.*, *cis* C-1–H and NH-Ph) geometric

isomers of *aldehydo* phenylhydrazones[15], has now been observed for the final form in the n.m.r. spectra of the sugar osazones in mutarotation (see Fig. 4.).

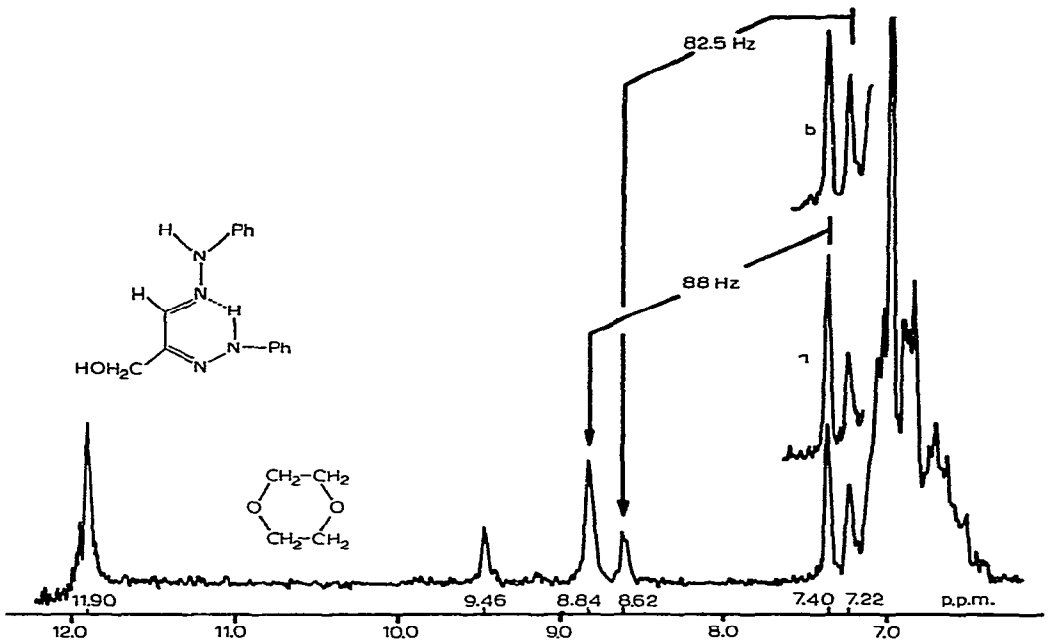

Fig. 4. N.m.r. spectrum of hydroxypyruvaldehyde bis(phenylhydrazone) (glycerosazone) during reversible isomerization in *p*-dioxane: (a) decoupling figure of the H-1 proton (at 7.40 p.p.m.) with the nonchelated N-H proton (at 8.84 p.p.m.) in the initial form; (b) decoupling figure of the H-1 proton (at 7.22 p.p.m.) with the nonchelated N-H proton (at 8.62 p.p.m.) in the final form.

This evidence indicates that the geometry of the final form of sugar osazones in mutarotation is very similar to that of the initial form (A) and, along with arguments previously adduced, has led us to consider structure F. This structure is a restricted-rotation isomer of structure D about the C-1–C-2 bond. Examination of structure D by means of atomic models shows the phenylimino group of the C-1-phenylhydrazone to be hindered by the sugar chain, so that the formation of structure D is highly improbable. Of the other possible rotational isomers, structure F is the most stable, since it is the only one which is coplanar.

Formazan formation. — The formation of structure F during mutarotation has also been confirmed by measuring the yield of radioactive formazan obtained through reaction of diazotized aniline-^{14}C with samples taken from the respective solutions of hydroxypyruvaldehyde bis(phenylhydrazone) (**1**), of D-*arabino*-hexulose phenylosazone (**4**), and of D-*lyxo*-hexulose phenylosazone (D-galactosazone) (**7**) during mutarotation in pyridine.

Data presented in Table II show a decrease in formation of formazan from **4** and **7**. In contrast, **1** shows a distinct increase in ability to form formazan during progressing isomerization. The transition of the initial structure A to the structure F

TABLE II

FORMATION OF OSAZONE-FORMAZANS THROUGH REACTION OF DIAZOTIZED ANILINE-^{14}C WITH OSAZONES IN MUTAROTATION

No.	*Obtained from*	*Yields (%) in osazone-formazan-^{14}C*	
		before mutarotation	*after mutarotation*
1	Hydroxypyruvaldehyde bis(phenylhydrazone) (Glycerosazone)	48.5	54.5
4	D-*arabino*-Hexulose phenylosazone (D-Glucosazone)	31.0	25.0
7	D-*lyxo*-Hexulose phenylosazone (D-Galactosazone)	26.5	23.0

is a satisfactory explanation of these data. In form F of the sugar osazones **4** and **7**, formazan formation is rendered more difficult, because attack of the diazonium cation on the C-1 proton is hindered by the sugar chain, and this results in diminished formation of formazan. The same difficulty is not found with hydroxypyruvaldehyde bis(phenylhydrazone) (**1**), because of the absence of a sugar chain; and, in this case, the yield of formazan increased during progressing isomerization, as expected.

Thus, formazan formation and n.m.r. and u.v. spectral data support the existence of the restricted-rotation isomer F of the *syn* (*i.e.*, *cis* C-1–H, NH-Ph) isomerized, N-H···O chelated, bis(phenylhydrazone) structure as the final form of these sugar osazones in mutarotation.

EXPERIMENTAL

N.m.r. spectra. — The n.m.r. spectra of the compounds reported in Table I were determined at 60 MHz, with tetramethylsilane as the internal reference, by use of an A-60 Varian Associates spectrometer (Palo Alto, California).

To demonstrate the presence of chelated N-H protons, the n.m.r. spectra were recorded in *p*-dioxane solution (20 mg of osazone/0.4 ml of solvent), and 100 µl of distilled water was added to bring about displacement of the nonchelated N-H protons (see Fig. 1).

Long-range, spin–spin coupling. — Decoupling of the C-1 protons in the initial and final forms of **1** and **2** was obtained by irradiation of the nonchelated N-H protons in *p*-dioxane solution (see Fig. 4), by use of the double-resonance method, with a Varian spin-decoupler.

U.v. spectra. — U.v. spectra were determined with a UNICAM SP-700 spectrophotometer. A 0.4 mM solution of **1** in *p*-dioxane was used for studying the reversible isomerization. After equilibration for 24 h (50% of final form), the spectrum was recorded against a *p*-dioxane solution containing only half the amount of **1**. In this way, the spectrum of the final form was obtained by subtraction, and is compared in Fig. 2 with the spectrum of a freshly prepared 0.2 mM solution of **1** in *p*-dioxane (initial form).

Similar spectra were obtained for **2**. After equilibration for 24 h (10% of final form), the spectrum was recorded against *p*-dioxane solution containing 90% of the total amount of **2**.

The spectra of **5** and **6** were determined in a *p*-dioxane solution (0.02 mM) (see Fig. 3).

Formazan formation. — Two 1.13-mmole (299-mg) samples of hydroxypyruvaldehyde bis(phenylhydrazone) (**1**) were each dissolved in 3 ml of pyridine. Ethanolic potassium hydroxide (9 ml of 2N) was added to one of the solutions, which was then allowed to react at −5° with 1.18 mmole (124 mg) of diazotized aniline-^{14}C (297,700 ipm/mmole) without delay. The second solution was kept for 24 h at room temperature to permit complete isomerization. Ethanolic potassium hydroxide and diazotized aniline-^{14}C solution were then added as above. Five minutes after treatment with the diazotized aniline, each sample was poured onto ice, and the black precipitate was collected, and washed with ice-cold water. After being dried *in vacuo* over phosphorus pentaoxide, the precipitates were separately dissolved in 1:9 *p*-dioxane–ethanol, and the yields of osazone-formazan were calculated from the radioactive counts (measured by a Tracerlab Type No. 1 counter), after evaporation of the solutions on plates.

Values presented in Table II for D-*arabino*-hexulose phenylosazone (**4**) and for D-*lyxo*-hexulose phenylosazone (**7**), before and after mutarotation, were obtained similarly, except that 1 mmole (358 mg) of osazone was dissolved in 10 ml of 1:1 pyridine–ethanol, and 10 ml of 2M ethanolic potassium hydroxide was added before reaction with the diazotized aniline-^{14}C solution. The osazone-formazans formed were isolated from the solution, and were identified by comparison with authentic samples.

Materials. — All of the compounds used had melting points in accordance with those reported in the literature.

1-Phenylhydrazone of 3-methyl-1,2-butanedione (1-phenylhydrazone of isopropylglyoxal). — Ethyl isobutyrylacetate (7.9 g), ethanol (60 ml), sodium hydroxide (3 g), and water (5 ml) were mixed and cooled to 4°, and 4.5 g of diazotized aniline, as a 10% aqueous solution, was added with stirring. After 1 h, the separated oil was dissolved in a solution of potassium hydroxide (8 g) in 60 ml of ethanol and 25 ml of water, the solution was refluxed during 30 min, 750 ml of water was added, and 3.4 g of crude product was obtained by filtration. Repeated recrystallization from a mixture of heptane and ethyl acetate afforded the hydrazone, m.p. 113°.

Anal. Calc. for $C_{11}H_{14}N_2O$: C, 69.44; H, 7.42; N, 14.73. Found: C, 69.82; H, 7.53; N, 14.45.

1-Phenylhydrazone of 1,2-pentanedione (1-phenylhydrazone of propylglyoxal). — The phenylhydrazone was prepared similarly, starting with ethyl butyrylacetate.

Anal. Calc. for $C_{11}H_{14}N_2O$: C, 69.44; H, 7.42; N, 14.73. Found: C, 69.61; H, 7.81; N, 15.03.

Bis(phenylhydrazone) of 3-methyl-1,2-butanedione (isopropylglyoxal osazone) (**5**). — The phenylhydrazone (0.8 g) was dissolved in 15 ml of ethanol, and 0.6 g of

phenylhydrazine, 2 ml of water, and 0.2 g of sodium hydroxide were added; the mixture was refluxed for 1 h. Next day, the precipitate was collected (1.1 g); repeated recrystallization from heptane yielded the osazone, m.p. 116°.

Anal. Calc. for $C_{17}H_{20}N_4$: C, 72.82; H, 7.19; N, 19.99. Found: C, 72.52; H, 6.95; N, 19.88.

Bis(phenylhydrazone) of 1,2-pentanedione (propylglyoxal osazone) (**6**). — The osazone was similarly obtained from the corresponding phenylhydrazone; m.p. 123°.

Anal. Calc. for $C_{17}H_{20}N_4$: C, 72.82; H, 7.19; N, 19.99. Found: C, 72.95; H, 7.28; N, 19.77.

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SUMMARY

Mutarotation of sugar osazones is due to an equilibration, through the action of a basic solvent, of the initial N-H···N chelated structure A with an N-H···O chelated isomer. Formazan formation and u.v. and n.m.r. spectral data support the restricted-rotation isomer F of the *syn* (*i.e.*, *cis* C-1–H, NH-Ph) isomerized, N-H···O chelated, bis(phenylhydrazone) structure as the final form of sugar osazones in mutarotation.

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PARTITION OF SUGARS BY THIN-LAYER CHROMATOGRAPHY AT LOW TEMPERATURES*

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INTRODUCTION

The separation of sugars by thin-layer chromatography (t.l.c.) is a rapid and sensitive procedure in carbohydrate analysis[1–8]. We have noticed that t.l.c. of pure sugars on cellulose layers at low temperatures may give, in certain cases, elongated or double spots, in contrast to the appearance of a single, well-defined spot at 20–30°. We now report a systematic investigation of this phenomenon as occurring at −18°.

METHODS

Chromatography. — T.l.c. was performed on Cellulose D (Camag AG, Muttenz, Switzerland) on glass plates with propyl alcohol–ethyl acetate–water (6:3:3). The *o*-aminobiphenyl[9] and the urea–phosphoric acid[10] reagents were used for the detection of aldoses and ketoses. Cellulose plates, which were dried before use for 2 h at 105°, were slowly cooled to room temperature in a dry chamber. When used for separations in the cold, the plates were precooled at −20°, and the sugar samples (0.5–1.0 μl) were quickly applied at room temperature on the cold plates (at 0 to 5°) and dried immediately with a stream of cold air. The plates were not pre-equilibrated with the developing solvent.

Preparation of radioactive substrates. — ^{14}C-Labelled sugars were purchased from the Radiochemical Centre, Amersham, Bucks.

[^{14}C]-Levanbiose (6-*O*-β-D-fructofuranosyl-D-fructose) was isolated from a partial, acid hydrolysate of [^{14}C]-levan synthesized by levansucrase from [^{14}C]-sucrose[11]. For this purpose, a 4% levan solution (0.1 μcurie per μmole of anhydrofructose unit) was hydrolyzed in sulphuric acid at pH 2.1 for 80 min at 60°. The solution, after neutralization with Dowex-1 (CO_3^{2-}), was reduced in volume, and levanbiose as well as other oligofructosides were separated and isolated by paper chromatography[11,12].

[^{14}C]-Sucrose, labelled exclusively in the D-fructose residue, was synthesized enzymatically as follows. A reaction system contained 1.0 μmole of UDPG [uridine 5-(D-glucopyranosyl pyrophosphate)]; 0.8 μmole of [^{14}C]-D-fructose (9 μcuries);

*A preliminary report of this work has been published: *Israel J. Chem.*, 4 (1966) p. 96.

20 μmoles of phosphate buffer (pH 7.5); 0.03 units of purified, sugar-beet UDPG; fructose transglucosylase[13]; and 0.05 units of alkaline phosphatase[14] from *Escherichia coli* ML 308 in a total volume of 0.32 ml. After 2 h at 30°, 3 ml of water and 100 mg of Amberlite MB-3 (mixed-bed resin) were added. The suspension was filtered, concentrated *in vacuo*, and applied to Whatman No. 3MM paper for chromatography[11]. The labelled sucrose was eluted with water to yield 0.5 μmole of disaccharide (5.6 μcuries).

[^{14}C]-β-D-Fructose (30 μcuries per mmole) was recrystallized from ethanol. [^{12}C]- or [^{14}C]-α- and β-D-mannose (11 μcuries per mmole) were obtained by crystallization from ethanol or from acetic acid, respectively[15].

Source of other reagents. — Most of the sugars used were commercial products (Fluka, AG, Buchs, Switzerland) of high purity. Rare hexoses were kindly given by Drs. H. S. Isbell and N. K. Richtmyer. Isomaltulose was obtained from Dr. R. Weidenhagen. Inulobiose was isolated from a partial, acid hydrolysate of inulin[16]. Other ketose oligosaccharides were obtained as described elsewhere[17]. Invertase (N. B. Co., Cleveland, Ohio) was a melibiase-free preparation. UDPG was purchased from the Sigma Chemical Co., St. Louis, Mo.

Physical determinations. — To measure ^{14}C-labelled sugars, the spots revealed by the spray reagents on the t.l.c. plates were scraped off into vials containing scintillation liquid[18]. Counting was performed in a Packard Instrument Co. Model 3003 Tri-Carb scintillation spectrometer, with 80% efficiency as calibrated with an internal, ^{14}C standard. G.l.c., for comparative analysis of equilibrium solutions of pure monosaccharides, was performed[19] on a Packard Instrument Co. Series 7800 gas chromatograph. Optical rotations were measured with a Bellingham and Stanley Model A Polarimeter.

RESULTS

Qualitative observations. — When an equilibrated, aqueous solution of D-fructose was analyzed by t.l.c. at low temperatures, two components were frequently detected (Fig. 1). At room temperature, only one spot was obtained on the chromatogram. Sucrose, on the other hand, always gave one spot, irrespective of the temperature employed.

In some of the earlier experiments, D-fructose and sucrose yielded, in addition to the spots separated as described above, trace amounts of one or two spots moving close to the solvent front. This was due to the use of very dry (oven-heated) cellulose plates. When the dry plates were pre-cooled prior to the application of samples at room temperature, the moisture absorbed by the cellulose layer was sufficient to prevent the appearance of the very fast components. It was also observed that the appearance of these minor spots did not occur on plates that were not oven heated, but dried slowly and kept at room temperature.

Examination of other sugars showed that they differ in their tendencies to separate into two components during t.l.c. at low temperatures (Fig. 2; Table I), but

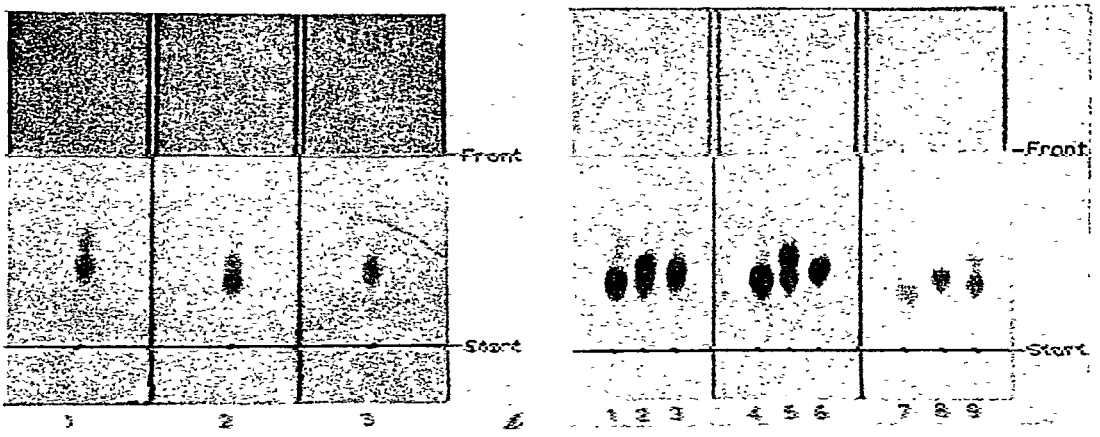

Fig. 1. T.l.c. on cellulose of an equilibrated, aqueous solution of D-fructose for (1) 45 min at −18°; (2) 30 min at 0°; (3) 20 min at 30°. The solvent front advanced 5 cm.

Fig. 2. T.l.c. on cellulose at −15° of equilibrated, aqueous solutions of 1, arabinose; 2, lyxose; 3, ribose; 4, galactose; 5, mannose; 6, glucose; 7, isomaltulose; 8, sorbose; 9, fructose. The slower, faint spot observed in the galactose preparation is a lactose contaminant.

TABLE I

SEPARATION OF VARIOUS SUGARS BY T.L.C.[a] AT −18°

Sugar	R_F		*Sugar*	R_F	
	Slow component	*Fast component*		*Slow component*	*Fast component*
D-Glucose	0.27	—	Turanose	0.23	0.33
D-Galactose	0.23	0.38	Maltulose	0.21	0.33
D-Mannose	0.25	0.35	Lactulose	0.24	0.35[c]
D-Talose	0.30	0.44	Leucrose	0.19	—
D-Altrose	0.38	0.41[b]	Isomaltulose	0.27	—
D-Fructose	0.27	0.39	Levanbiose	0.34	—
D-Sorbose	0.31	—	Inulobiose	0.28	0.35[c]
D-Xylose	0.38	0.40[b]	1-*O*-β-D-Glucosyl-		
D-Lyxose	0.33	0.43	D-fructose	0.19	0.30
D-Arabinose	0.31	0.50	Sucrose	0.31	—
D-Ribose	0.41	—	Lactose	0.17	—
D-*glycero*-D-*gulo*-			Maltose	0.21	—
Heptose	0.38	—	Cellobiose	0.19	—
2-Deoxy-D-*arabino*-			Melibiose	0.17	—
hexose	0.57	—	Raffinose	0.15	—
2-Deoxy-D-*erythro*-			2-Amino-2-deoxy-D-		
pentose	0.64	—	glucose	0.30	—
L-Rhamnose	0.37	0.51			
D-Fucose	0.47	—			

[a]Samples (1 μl containing 20 μg) were taken from equilibrated, aqueous solutions. Conditions of separation were as described in Methods; the solvent front moved 8.8 cm. [b]Poor separation of spots. [c]Fast component was present in barely detectable amounts.

TABLE II

PERCENTAGE OF ANOMERIC AND RING FORMS OF HEXOSES AT EQUILIBRIUM IN AQUEOUS SOLUTION, AS EVALUATED BY DIFFERENT METHODS

	T.l.c.[a]		*G.l.c.*[b]			*N.m.r.*[f]			*Mutarotation and oxidation*[i]		
	Fast component	*Slow component*	*α-D-Pyranose*	*β-D-Pyranose*	*γ*	*α-D-Pyranose*	*β-D-Pyranose*	*Furanose*	*α-D-Pyranose*	*β-D-Pyranose*	*Furanose*
D-Fructose	25	75		[c]						70–80[j]	20[k]; 31[l]
D-Galactose	10	90	32	63	5	27	60	trace[g]	30	69	12[m]
			33	53	14[d]	35	65	trace[h]			
D-Mannose	66	34	72	28	—[e]	67	33[g]	—	69	31	—
						64	36[h]				
D-Glucose	one spot		40	60	—[e]	36	64[g]	—	36	64	—

[a]Samples (0.5 μl) from an equilibrated [^{14}C]-hexose solution in water (5%; 10 mμcuries/μmole) at room temperature were analyzed as described in Methods. [b]Ref. 19. [c]Sweeley *et al.*[19] reported one peak for fructose. Employing the same separation method, but using a column of 15% Apiezon-L on Gas-Chrom P (Applied Science Labs., State College, Pa.) at 202°, we have noticed one major and two or three smaller peaks for fructose. The relative amounts of these components differed in different runs. [d]Ref. 20. [e]In pyridine, values of 24 and 3% were obtained for D-mannose and D-glucose, respectively[19]. [f]Refs. 21–25. [g]In methyl sulfoxide[24], values were 15 for D-galactofuranose, 14 for β-D-mannopyranose, and 62 for β-D-glucopyranose. [h]Ref. 25. [i]Refs. 26–28. [j]It is accepted that D-fructose in solution exists predominantly as the more stable β-pyranoid form[21,29]. [k]Ref. 30. [l]Ref. 31; 80% furanose was found in *N,N*-dimethylformamide[32]. [m]Refs. 33, 34.

a clear-cut correlation with anomeric or ring forms cannot be formulated. For example, D-glucose yields one spot, although both the α- and β-D pyranoid forms are present in an equilibrated solution (Table II). D-Ribose, which, at equilibrium in solution, exists to a large extent as a pentofuranose[21–24], yields one spot. D-Mannose or D-xylose, which, in aqueous solution at equilibrium, are present to a negligible extent in the furanoid form[21–24] (Table II), yield two distinct spots. Disaccharides containing a reducing fructose residue separate into two spots only when substitution in the fructose permits formation of both furanoid and pyranoid rings.

Quantitative observations. — For several sugars, the relative amounts of the material found in the two separable spots could be measured by using ^{14}C-labelled monosaccharides (Table II). The comparison of these values with those found in the literature for the amount of the various sugar forms in aqueous solution suggests that the two spots correspond (*a*) in the case of fructose, to the furanoid and pyranoid forms; (*b*) in the case of D-mannose, to the α- and β-hexopyranose anomers; and (*c*) in the case of D-galactose, to the hexo-furanoid and -pyranoid forms. The results of the following experiments give substantial support to these conclusions.

Separation of D-fructose in β-D-fructofuranoside hydrolysates. — An instantaneous hydrolysis of [^{14}C]-sucrose (labelled only in the fructosyl moiety) and of [^{14}C]-levanbiose was achieved with a large excess of yeast invertase at 0°. T.l.c. of these reaction systems showed (Fig. 3) that the relative quantity of the slow-moving D-fructose

component increased up to the amount of the pyranoid form expected to exist in an equilibrated solution of D-fructose (Table II).

Comparison between mutarotation and appearance of separable spots in t.l.c. — The rate of appearance of the two components separable by t.l.c. was compared with the mutarotation of D-fructose (Fig. 4) and D-mannose (Fig. 5). It was clear that, under similar conditions, the pattern of appearance of the fast-moving component in a freshly prepared solution of β-D-fructose corresponded to the appearance of β-D-fructofuranose, as seen by the course of mutarotation. In the case of D-mannose, formation of the fast-moving component followed the appearance of α-D-mannopyranose in a freshly prepared solution of β-D-mannopyranose. On the other hand, the appearance of the slow-moving spot corresponded to the formation of β-D-mannopyranose in a freshly prepared solution of α-D-mannopyranose.

Effect of temperature on the relative amounts of the two spots separated by t.l.c. — Aqueous solutions of D-fructose and D-mannose were equilibrated at different temperatures, and samples were analyzed by t.l.c. at low temperature (Fig. 6). In the

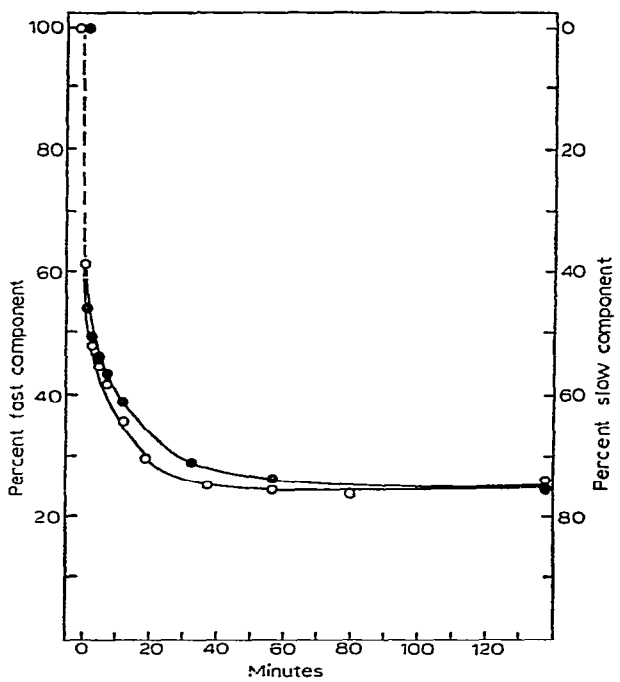

Fig. 3. Appearance of a fast-moving D-fructose component after hydrolysis of β-D-fructofuranosides; 150 μl of a 1% invertase solution at 0° were added to 50 μl of a solution containing 20 μmoles of [^{14}C]-D-fructose-labelled sucrose (0.5 μcurie per μmole) and 5 μmoles of phosphate–citrate buffer (pH 6.1) at 0°. In a preliminary experiment, it was found that, under these conditions, total hydrolysis of sucrose was effected in less than one min. Samples (1 μl) were withdrawn at suitable time intervals for t.l.c. at −18°. The two, separated, [^{14}C]-D-fructose spots were eluted and measured as described in Methods. In a second experiment, 7.5 μmoles of [^{14}C]-levanbiose (0.03 μcurie per μmole) were used as the substrate in a similar reaction system. Duplication of these experiments, carried out in the absence of buffer, yielded equivalent results to those obtained with buffer: ●, sucrose; ○, levanbiose.

case of D-fructose, a significant increase in the relative amount of the fast component occurred with increase in temperature. This picture has a marked resemblance to that associated with the change of optical rotation of equilibrated solutions of D-fructose caused by elevation of temperature[35]. This phenomenon was attributed to an increase in the relative amount of β-D-fructofuranose[15,26,36]. Temperature-dependent changes in the rotation of equilibrated solutions is not significant in such sugars as D-mannose and D-glucose that exist mostly in one ring form in solution[15,26,34,36]. The lack of any change in the ratio of the two mannose spots at different temperatures therefore supports the conclusion that they represent the two anomers of the manno-pyranoid form.

Two-dimensional t.l.c. — D-Fructose and D-mannose were separated in one direction at −18°. The plates were then kept wet for 6 h at room temperature to allow the sugar to re-equilibrate. When rechromatographed in the second dimension at 25°, only two spots could be revealed, whereas, when the second separation was carried out at −18°, four spots of sugar were detected.

DISCUSSION

The mobilities of sugars on thin-layer chromatograms are similar to those for paper chromatography[37–39]. Partition between α- and β-D anomers, or the two ring

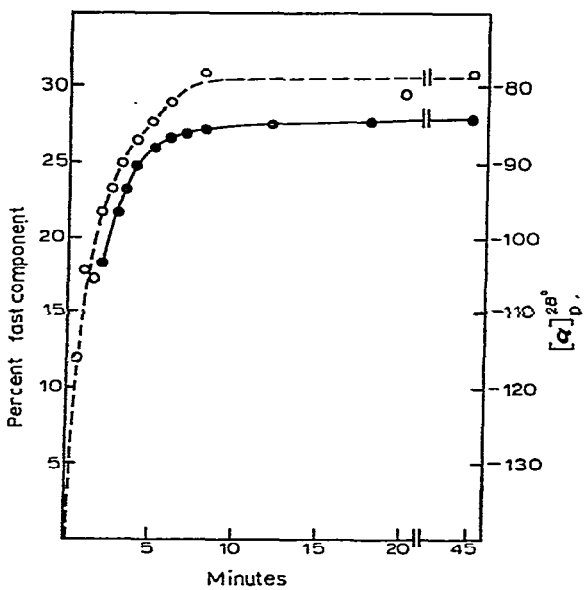

Fig. 4. Mutarotation of D-fructose in comparison with appearance of the fast-moving component in t.l.c. The change in optical rotation of a 4.5% aqueous solution of freshly dissolved β-D-fructose was followed at 28°. A small volume of 5% [^{14}C]-β-D-fructose solution (3 mμcuries/μmole) was similarly prepared. Samples (0.5 μl) were taken for analysis by t.l.c. at −18°, as described in Methods: ●, optical rotation; O, amount of fast-moving component separated chromatographically.

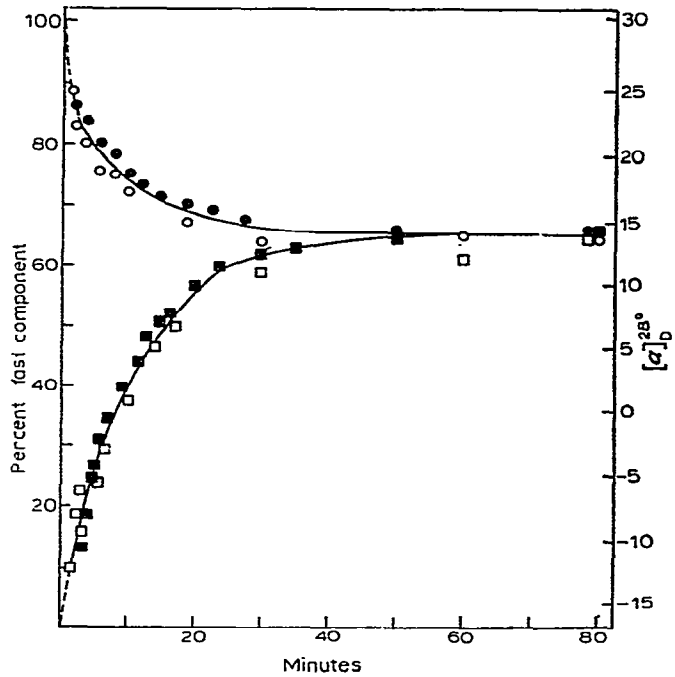

Fig. 5. Mutarotation of α- and β-D-mannose in comparison with the relative amounts of D-mannose components separated by t.l.c. The change in optical rotation of a 4.3% aqueous solution of freshly dissolved α-D-(●) or β-D-mannose (■) was followed at 28°. Small volumes of 5% solutions of α- and of β-[^{14}C]-D-mannose (5.5 mμcuries per μmole) were similarly prepared. Samples (0.5 μl) were taken for analysis by t.l.c. at −15°: O, α-[^{14}C]-D-mannose; □, β-[^{14}C]-D-mannose.

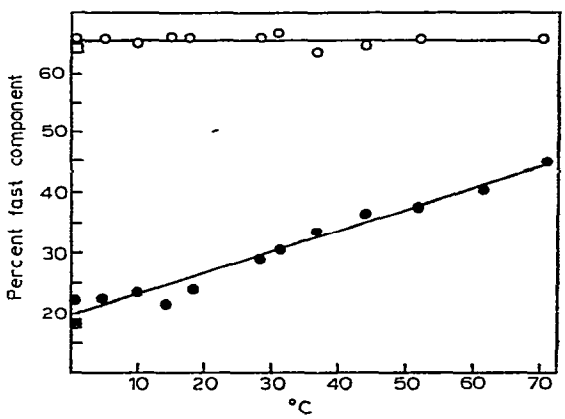

Fig. 6. Effect of temperature on the relative amount of the fast-moving component in D-mannose and D-fructose. Samples (1.0 μl) of [^{14}C]-sugars (10 mμc per μmole) from 3% aqueous solutions, pre-incubated for 3 h at various temperatures, were analysed by t.l.c. at −15°. The separated spots were eluted and measured as described under Methods: O, D-mannose; ●, D-fructose; □, equilibrated D-mannose and ■, D-fructose solutions that were kept in the deep freeze, and then thawed at 1° and immediately applied to the chromatoplate.

forms in which a particular reducing sugar might be present in solution, is not observed when chromatography is performed at ambient temperatures. A rapid equilibrium exists between all these forms during the slow movement of the sugars with the developing solvent, and the final result is that only one spot is apparent. It is probable that when chromatography is carried at very low temperatures in presence of an organic solvent, the rate of equilibration between the different sugar forms pre-existing in the solution is drastically inhibited. Under such circumstances, forms having different mobilities in a particular solvent would be separated[40]. The speed of development is most probably an important factor contributing to the sharpness of the resolution. An increase in developing time to more than 1 h (when the solvent front moved 5–6 cm) usually brought about tailing and coalescence of the two components.

In the present study, the fractionation at low temperatures of D-mannose into its α and β anomers and of D-fructose into the furanoid and pyranoid forms has been clearly demonstrated. Several other sugars were shown to give two components, but only a further, quantitative study, using radioactive substrates, would establish which forms appear as distinct spots in each individual case.

As it is well known, the relative amount of the various sugar forms in solution may change according to the nature of solvent in which the sugar is dissolved and equilibrated. The procedure described in this work could be helpful in the determination of these ratios for specific solvents.

The results of the present study indicate that, in some cases, t.l.c. at low temperature could furnish a useful, simple, analytical tool for the identification of minute amounts of reducing sugars. In particular, it can be applied to mono- or di-saccharides which, at room temperature, have closely similar chromatographic mobilities, but which may give more than one spot at low temperatures. Chromatography at very low temperatures could also prove helpful in detecting and measuring transient forms in a rapidly equilibrating, chemical system. Attention should be paid also to the dangers inherent in interpretations when analysis of sugars by t.l.c. is unintentionally performed at low temperatures.

SUMMARY

Several monosaccharides give two components when analysed by thin-layer chromatography on cellulose at $-18°$. For D-fructose, the two spots corresponded to the furanoid and pyranoid forms. For D-mannose, the two spots could be correlated with the α and β anomers of the pyranoid form, and for D-galactose, the minor component is probably the hexofuranose. Other sugars, including D-glucose, gave a single spot under these conditions. It is suggested that chromatography at low temperatures is of potential value as a useful analytical tool for the identification of minute amounts of certain reducing sugars.

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PREPARATIVE, THIN-LAYER CHROMATOGRAPHY IN CARBOHYDRATE CHEMISTRY*

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Preparative, thin-layer chromatography (t.l.c.) of colorless substances that do not fluoresce under ultraviolet light necessitates the use of a detecting reagent for locating the zones. Detection is usually accomplished[1] by shielding most of the plate, except for guide strips, and then streaking or spraying the exposed guide strips with the reagent. A disadvantage of this procedure is that a considerable proportion of the material separated on the plate is lost in the guide strips. A second disadvantage is that use of sulfuric acid, and other detecting reagents[2] commonly employed, may require that the whole plate be heated to reveal the zones on the guide strips. This procedure can be undesirable if the separated components are volatile, heat-labile, or extremely sensitive to the fine mist of the detecting reagent that may come into contact with the protected portion of the plate after the guide strips have been sprayed. These problems are particularly evident when separations by preparative t.l.c. are attempted with carbohydrates and their derivatives.

A number of procedures have been proposed for overcoming some of these difficulties, especially in t.l.c. on silica gel. These include incorporation of various substances, such as zinc silicate–zinc sulfide[2] or a fluorescent organic compound[3], into the adsorbent layer. Iodine[2,4] and water[2,5] have been used as nondestructive detecting agents. Each of these procedures has certain limitations.

This paper describes a simple technique for locating components on preparative chromatoplates, with negligible loss of material, and without the necessity of heating the plate or using the detecting reagent in the vicinity of the plate. The technique is applicable with chromatoplates coated with silica gel, alumina, or microcrystalline cellulose. The procedure involves removal from the surface of the stationary phase, by means of adhesive tape or a greased glass plate, of a very thin layer for use as a guide strip. The method is described as part of a general separation procedure for resolution of product mixtures from synthetic organic reactions, and is illustrated with an example from the carbohydrate field. On a one-gram scale, separation is achieved with four standard (20×20 cm) chromatoplates, and final purification is effected, where possible, by vacuum microdistillation.

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PROCEDURE

Preparation of chromatoplates. — *Silica gel.* Silica Gel G (E. Merck, Darmstadt, Germany) (100 g) is mixed with water (200 ml) and applied to five clean glass plates (20 × 20 × 0.3 cm) at a thickness of 1.0–1.5 mm by means of a variable-thickness applicator (Desaga, Brinkmann Instruments, Great Neck, New York). After the slurry has set (~2 h) the plates are transferred to a storage rack (Desaga), and are kept for about 18 h at room temperature to dry by spontaneous evaporation. The rack is then kept for 3 h in an oven at 130°; the plates are cooled, and are kept in a desiccator or a dry box.

Alumina. Chromatographic grade alumina (Woelm, activity grade 1, neutral, acidic, or basic; M. Woelm-Eschwege, Germany) is applied directly to clean glass plates (20 × 20 × 0.3 cm), either by the "dry" method of Mottier and Potterat[6], or as a slurry in water, with subsequent reactivation, as described by Albano, Horton, and Tsuchiya[7]. In neither case is a binder used.

Microcrystalline cellulose. "Avicel"–Technical Grade (formerly termed "Avirin"), obtained from the Avicel Sales Division of American Viscose Division, FMC Corporation, Marcus Hook, Pennsylvania, is blended with water and applied at 1 mm thickness to clean glass plates (20 × 20 × 0.3 cm) by the method of Wolfrom, Patin, and de Lederkremer[8]. The plates are allowed to dry at room temperature for at least 24 h, and are not heated.

Application of the sample. — The mixture to be separated is applied, either as a neat liquid or as a concentrated solution in a volatile solvent, along a line parallel to, and 3.0 cm from, one edge of the plate. A multiple-spot[9] or syringe type[10,11] of applicator may be used, but manual application of a row of contiguous spots 0.4 cm in diameter by means of a fine-tipped capillary tube is satisfactory. The resultant band should not be more than 0.5 cm wide. For most separations, up to 250 mg of sample per plate may be applied to plates coated with silica gel or alumina, and up to 50 mg on plates coated with microcrystalline cellulose. The total amount applied to each plate should be lessened if the mixture contains substances whose mobilities differ by less than 0.1 R_F unit.

Development of the chromatoplates. — The developing solvent is selected on the basis of preliminary tests with microscope slides (2.5 × 7.5 × 0.1 cm) that have been coated with a 250-μm layer of the appropriate stationary phase. A solvent is chosen that gives the desired separation, and it is usually desirable that the polarity of the developer be such that the R_F value of the fastest-moving component is 0.8–0.9. When the developer contains ether or other highly volatile solvent, the developing jar is surrounded by ice and water. The plates are developed in pre-equilibrated glass jars by the usual ascending method. A single ascent of the solvent is usually satisfactory for adsorption chromatograms on alumina or silica gel. Multiple development frequently gives better resolution for separation on microcrystalline cellulose; again, the polarity of the developer is so selected that the R_F value of the fastest-moving component, after multiple development, is 0.8–0.9. For plates coated with alumina

by the "dry" method, the developing procedure of Mottier and Potterat[6] is used. After development, the plates are removed and allowed to dry.

Detection and excision of the zones. — Adhesive-tape method. Either masking tape or transparent adhesive tape is used. Suitable commercial tapes are Scotch Masking Tape and Scotch Magic Tape (Minnesota Mining and Manufacturing Co., St. Paul, Minnesota). The developed plate is placed flat, face upward, on a table. One, two, or more strips of ½-inch wide adhesive tape, long enough to overhang the sides of the plate, are applied lightly, adhesive side down, along the direction of solvent flow and perpendicular to the base line (see Fig. 1, A). It is desirable to mark the point of the base line on the tape. A second glass plate, having the same size as the chromatoplate, is then placed over the chromatoplate. The ends of the tape are brought over the top plate, adhesive side up, and are secured to the top plate by means of short lengths of adhesive tape (see Fig. 1, B). The top plate is now pressed uniformly

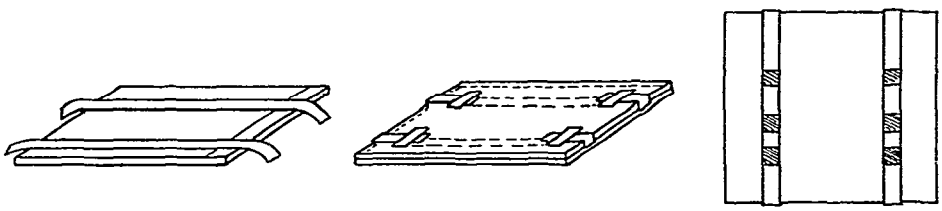

Fig. 1. Detection of zones on preparative chromatoplates by the adhesive-tape method. A. Application of the tape. B. Attachment of the tape to a carrier plate for the guide strips. C. Carrier plate and guide strips after spraying.

against the chromatoplate by hand pressure. Moderate pressure is required when masking tape is used, but transparent tape requires only very light pressure. The top (carrier) plate is then lifted off. The strips of adhesive tape carry with them a very thin film of the stationary phase from the point of contact with the chromatoplate, but most of the coating at the point of contact remains on the chromatoplate. The strips of tape, still attached to the carrier plate, are sprayed with the detecting reagent to indicate the zones (see Fig. 1, C). These guide strips are then lined up with the chromatoplate and the appropriate zones are removed by excision with a spatula or by use of a suction apparatus[11].

When sulfuric acid is used as the indicator, certain substances give black bands on the guide strips in the cold. Many other substances give black bands after gentle heating with this reagent. Prolonged heating (over 15 min at 105°) should be avoided with masking tape, because general blackening of the tape then makes observation of the zones difficult. Higher temperatures can be used for detection when transparent tape is employed, and there is good contrast between the background and the indicated zones. When strong heating is required for visualization, it is better to detect the zones by the greased-plate method.

Greased-plate method. Silicone high-vacuum grease (Dow-Corning Corp., Midland, Michigan) (1 part by wt.) is mixed thoroughly in a mortar with Silica Gel G

(0.45 parts by wt.), and the resultant paste is smeared thinly and uniformly on one side of glass strips that are to be used as guide strips. Microscope slides may be used, but glass strips that are 20 cm long and 1–2 cm wide are more convenient. It is not necessary to grease all of the area of the strips. The strips are placed, greased side down, on the developed chromatoplate, in the direction of solvent flow, and a glass plate, having the same size as the chromatoplate, is placed over the strips. Moderate pressure is applied to the top plate, which is then removed. The strips are then lifted off carefully, to remove a thin film of the stationary phase from the surface of the chromatoplate. The zones are detected on the guide strips by heating after spraying with sulfuric acid, or by some other suitable technique, and the bands are located on the chromatoplate in the usual way.

The use of silicone grease, unmixed with silica gel, is satisfactory when alumina is used as the stationary phase, and is to be preferred when the greased-plate technique is used with microcrystalline cellulose. Silicone grease alone, used with chromatoplates coated with silica gel, sometimes causes separation of the whole layer at the point of contact.

RESULTS

The following separation, that of a mixture of products formed by acid-catalyzed alcoholysis of an unsaturated sugar (D-glucal)[12], provides a typical example of the use of the foregoing procedure. The principal products are somewhat volatile, and are readily decomposed by traces of sulfuric acid.

Treatment of D-glucal (1.0 g) with 0.025M ethanolic sulfuric acid (55 ml) for 2 h at 70° gave a mixture of products (1.03 g). An exploratory, analytical chromatogram on Silica Gel G, with 2:1 benzene–ether as the developer and sulfuric acid as the indicator, showed (see Fig. 2) major components having R_F 0.81, 0.50, and 0.40, together with several minor components. The major components have been identified[12,13] as 2-(1,2-diethoxyethyl)furan (**1**), 2,6-diethoxy-4-oxohexanal diethyl acetal (**2**), and 2-(1-ethoxy-2-hydroxyethyl)furan (**3**), respectively. The mixture was applied to four preparative chromatoplates coated with silica gel, and the plates were developed with 2:1 benzene–ether at 0°. After location of the zones with sulfuric acid by the masking-tape procedure, and elution of the excised adsorbent with ether, the solutions were clarified by passage through small columns (1 × 3 cm) of silica gel (Silica Gel Davison, grade 950, 60–200 mesh, Davison Division of the W. R. Grace Co., Chattanooga, Tennessee), and evaporated. The recoveries of the separated components were **1**, 234 mg; **2**, 254 mg; and **3**, 155 mg. The products were homogeneous when examined in the same t.l.c. system.

Substances **1**, **2**, and **3** were further purified by vacuum microdistillation. A modified design of a simple microdistillation apparatus[14] is shown in Fig. 3. Samples (10–100 mg) of **1**, **2**, and **3**, isolated by the foregoing procedure, were introduced into the bulb (not containing glass wool) as solutions in ether (0.2 ml). Distillation was effected by the procedure described[14], and recoveries of distilled product were in the range of 65–90%. Purified in this way, substances **1**, **2**, and **3** gave correct elemental

analyses, and gave very sharply resolved n.m.r. spectra that were fully concordant with the structures and that showed no impurity peaks. Substances **1** and **3** were homogeneous by gas–liquid chromatography (g.l.c.); substance **2** underwent some

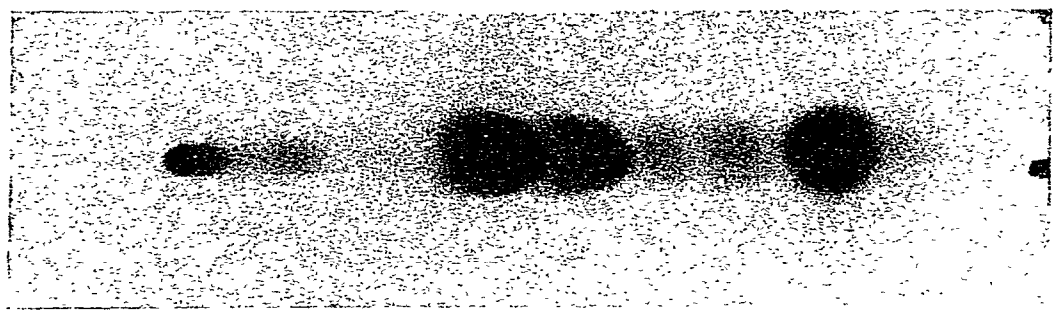

Fig. 2. Analytical, thin-layer chromatogram of the mixture of products formed by acid-catalyzed ethanolysis of D-glucal.

decomposition on attempted g.l.c. A Wilkens Model 705 Autoprep gas chromatograph, equipped with a flame-ionization detector and a column of 30% SE-30 on Chromosorb W (Analabs, Inc., Hamden, Connecticut) was used, at a column temperature of 140°, with programming to 240°.

DISCUSSION

The procedures described are in routine use in this laboratory for the separation of mixtures of products from exploratory organic reactions, especially in the carbohydrate field. For work involving compounds that may be heat-labile and of low volatility, the method is much superior to gas–liquid chromatography, because the introduction of artifacts by thermal degradation or during derivatization is avoided. Even in those cases where the products can be distilled, the method provides a safeguard in that analytical t.l.c. before and after distillation provides direct indication of thermal stability or lability, and the conditions for vacuum microdistillation are not so severe as those normally used in gas–liquid chromatography.

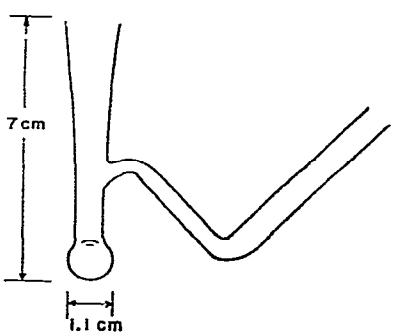

Fig. 3. Apparatus for vacuum microdistillation.

The procedure used for preparing plates coated with silica gel gives uniform layers of adsorbent that are free from the cracks that frequently result when accelerated drying is attempted. Commercial, pre-coated, preparative t.l.c. plates may be used, but are less economical.

The zone-detection procedure that employs adhesive masking tape is the method of choice wherever it can be used, because this tape is easier to manipulate than transparent tape. The zone-detection techniques described can undoubtedly be applied with stationary phases other than the three described herein. Adhesive tape has been used[15] for monitoring preparative paper-chromatograms, and also for removal of already detected zones from the surface of thin-layer chromatoplates[16].

In the example given, the major components of a complex mixture are separated, and obtained in high purity. The volatility of some of the products may have led to some losses, but overall recoveries are good. A purification step that appears to be generally useful involves passing the separated products, in ether solution, through a small bed of activated silica gel having no binder. The purified products **1** and **3** were homogeneous by g.l.c., indicating that they did not undergo thermal degradation on the column in the pure state. Substance **2** suffered some decomposition on the column. It is noteworthy that the original mixture, when subjected to gas–liquid chromatography under the same conditions, gave a very complex mixture that gave evidence of extensive decomposition of some of the principal components during passage through the column. This observation illustrates the advantages of preparative t.l.c. separations, and emphasizes the need for caution in interpreting the results of preparative gas–liquid chromatography applied to complex mixtures.

SUMMARY

Procedures are described for the location of zones on preparative, thin-layer chromatograms, by removal of a very thin film from the surface of the developed plate by means of adhesive tape or a greased glass plate, followed by conventional detection of zones on the removed film. The method permits detection of zones with negligible loss of material, and has been employed with adsorption chromatograms on silica gel and alumina, and partition chromatograms on microcrystalline cellulose. The method is described as part of a general scheme for preparative, thin-layer, chromatographic separations of mixtures of carbohydrate derivatives or other organic compounds on a one-gram scale.

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MASS SPECTROMETRY OF PARTIALLY METHYLATED ALDITOL ACETATES

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INTRODUCTION

Gas–liquid chromatography has become an important method for the separation and tentative identification of the methylated sugars obtained on methylation analysis of polysaccharides. One advantage of the method is that only small amounts of material are required. The full use of this advantage, however, cannot be realised as long as the unambiguous identification of the methylated sugars requires the preparation of crystalline derivatives for comparison with authentic samples. Attempts to circumvent this by characterising the methylated sugars by mass spectrometry have been made. In these studies, the mass spectra of methyl glycosides of methylated sugars are determined after permethylation with deuteriomethyl iodide[1], acetylation[2], or trimethylsilylation[3]. In the last two methods, the mixture can be separated by g.l.c. and the components directly introduced into the ionization chamber of the mass spectrometer; in the first method, they must be separated and permethylated before the mass spectra are determined. A drawback of these methods is that each methylated sugar generally gives rise to several methyl glycosides, thus rendering the g.l.c. separation more difficult and often incomplete.

RESULTS

In a recent publication[4], we reported that partially methylated alditol acetates, obtained from methylated sugars by borohydride reduction followed by acetylation, could be separated by g.l.c. on an ECNSS-M column. By this method, each methylated sugar is transformed into a single derivative, and, as generally good separations are obtained, this method offers definite advantages. We also found that some components, not separated on this column, may be separated on a polyphenyl ether column[5]. The present paper reports studies on the mass spectra of partially methylated alditol acetates. In almost all of the recorded spectra, the base peak was *m/e* 43 (CH_3CO^+). Table I gives the prominent peaks in the mass spectra of different, partially methylated alditol acetates, derived from methylated pentoses, hexoses, and 6-deoxyhexoses. Only peaks having an intensity of more than 10% of the base peak are included.

Some results are immediately evident on inspection of Table I. Firstly, mass spectra of isomeric alditols having the same substitution pattern are not sufficiently

TABLE I

PROMINENT PEAKS (*m/e*) IN THE MASS SPECTRA OF ACETATES OF METHYLATED ALDITOLS

Parent sugar	*m/e*																						
	43	45	59	71	85	87	89	99	101	113	115	117	127	129	131	139	143	145	161	175	189	203	205	233	261
3-Me-Ara	+					+								+							+				
2,3-Me$_2$-Ara	+	+				+			+			+		+							+				
2,4-Me$_2$-Ara	+	+										+													
2,5-Me$_2$-Ara	+	+								+		+											+		
2,3,5-Me$_3$-Ara	+	+		+		+			+			+		+					+						
2-Me-Xyl	+											+													+
2,3-Me$_2$-Xyl	+					+			+			+		+							+				
2,4-Me$_2$-Xyl	+											+													
3,5-Me$_2$-Xyl	+	+		+		+			+					+					+		+				
2,3,4-Me$_3$-Xyl	+	+							+			+							+						
2-Me-Fuc	+											+													
3-Me-Fuc	+	+				+			+					+			+				+				
2,3,4-Me$_3$-Fuc	+	+							+			+			+					+					
2,3,5-Me$_3$-Fuc	+	+	+						+			+													
2-Me-G	+	+										+				+									
6-Me-G	+	+				+					+			+											
2,3-Me$_2$-G	+	+							+			+													+
2,4-Me$_2$-G	+	+				+						+		+							+				
2,6-Me$_2$-G	+	+				+						+		+											
3,6-Me$_2$-G	+	+				+		+		+				+							+		+		
4,6-Me$_2$-G	+	+				+			+					+					+						+

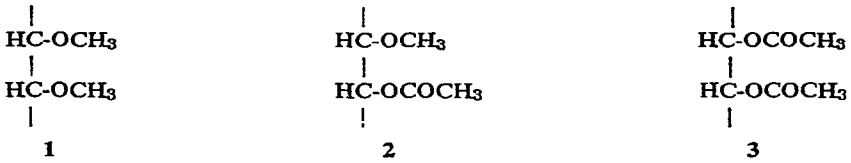
Parent sugar	m/e																							
	43	45	59	71	85	87	89	99	101	113	115	117	127	129	131	139	143	145	161	175	189	203	205	233	261
2,3,4-Me_3-G	+					+		+	+			+		+					+		+				
2,3,6-Me_3-G	+	+				+		+	+	+		+												+	
2,4,6-Me_3-G	+	+				+			+			+		+					+						
2,3,4,6-Me_4-G	+	+		+		+			+			+		+				+	+				+		
3-Me-Gal	+				+	+		+					+	+							+			+	
2,4-Me_2-Gal	+					+						+		+							+				
2,6-Me_2-Gal	+	+				+						+		+											
3,4-Me_2-Gal	+					+		+						+							+				
2,3,4-Me_3-Gal	+					+		+	+			+		+					+		+				
2,3,5-Me_3-Gal	+								+			+													
2,3,6-Me_3-Gal	+	+				+		+	+	+		+												+	
2,4,6-Me_3-Gal	+	+				+			+			+		+					+						
2,3,4,6-Me_4-Gal	+	+		+		+			+			+		+				+	+				+		
2,3,5,6-Me_4-Gal	+	+	+				+		+			+											+		
2,4-Me_2-Man	+					+						+		+							+				
3,4-Me_2-Man	+					+		+						+							+				
2,3,4-Me_3-Man	+					+		+	+			+		+					+		+				
2,4,6-Me_3-Man	+	+				+			+			+		+					+						
3,4,6-Me_3-Man	+	+				+		+	+					+					+		+				
2,3,4,6-Me_4-Man	+	+		+		+			+			+		+				+	+				+		

different to allow a distinction between them. No significant differences could be detected even when comparing peaks down to 0.1% of the base peak. The same is true for the mass spectra of isomeric, methylated glycosides[1–3]. This means that information will sometimes be lost when converting methylated sugars into alditols. The alditols derived from 2,3- and 3,4-di-*O*-methylpentoses will, for example, give almost identical mass spectra, as will those derived from 2,4- and 3,5-di-*O*-methylhexoses. By reducing with deuterioborohydride, this restriction could be eliminated. Secondly, it is seen that, for each substitution pattern, a unique mass spectrum is obtained. This means that, in methylation analysis of polysaccharides, the mass spectrum of a partially methylated alditol acetate obtained from a methylated sugar, in combination with the sugar composition of the original polysaccharide and T-values[4], will, in most cases, give sufficient evidence for an unambiguous characterisation of the methylated sugar. A requirement is that the mass spectrum of a methylated alditol acetate having the same substitution pattern should be known. For heteropolysaccharides, T-values of different, alternative derivatives should also be known and be sufficiently different from each other. The mass spectra of most substitution patterns for pentitols and hexitols are given in Table I, but some, derived from furanosidic residues, are lacking. Only part of the combinations deriving from 6-deoxyhexose residues are represented and none deriving from heptose, other deoxy sugar, or aminodeoxy sugar residues. An analysis of the fragmentation pattern of the substances studied has, however, revealed principles, which can be applied for characterisation of substances having substitution patterns for which the mass spectra have not been previously recorded.

DISCUSSION

In their study of mass spectra of alditol acetates, Chizhov *et al.*[6] distinguish between primary and secondary fragments. The former arise by fission between two carbon atoms in the alditol carbon chain; either of the two fragments formed could carry the positive charge. The latter are formed from the primary fragments by elimination of acetic acid (60) and/or ketene (42). The intensity of the primary fragments increased with decreasing mass number. As the intensities of peaks may vary with changes in the experimental conditions, the determination of mass spectra of some fully acetylated alditols was repeated. The results, which are in good agreement with those of Chizhov *et al.*[6], show that the intensities of the primary fragments given by the alditol acetates are much lower than the intensities of the corresponding peaks from the partially methylated alditol acetates. However, it was found that mono-*O*-methylhexitols also give prominent fragments corresponding to those obtained from fully acetylated hexitols. This indicates that fission takes place between carbon atoms in the partial structures **1** and **2** in preference to structure **3**, and that the positive ion is stabilised by a methoxyl grouping. An inspection of Table II, in which the prominent, primary peaks of different partially methylated alditol acetates are summarized, supports this assumption. Further, primary fragments of high intensity,

containing two vicinal methoxyl groups, are not observed, except in one case, unless they can be formed from the alditol by fission between two other vicinal methoxyl groups, indicating that structure **1** is cleaved in preference to structure **2**.



The secondary fragments observed may be derived from the primary fragments by single or consecutive eliminations of acetic acid (60), ketene (42), methanol (32), or formaldehyde (30).

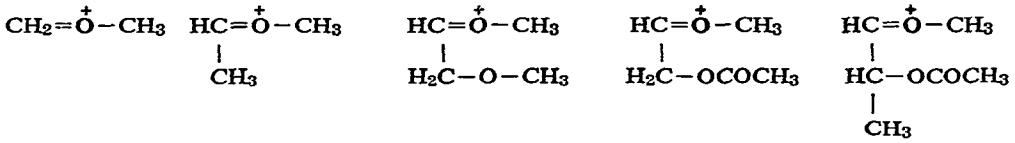
TABLE II

PRIMARY FRAGMENTS IN THE MASS SPECTRA OF ACETATES OF METHYLATED ALDITOLS

	m/e											
	45	59	89	117	131	161	175	189	203	205	233	261
Pentitol[a]												
2				+								+
3								+				
1,3	+					+		+				
1,4	+			+							+	
2,3				+				+				
2,4				+								
1,3,4	+			+		+						
2,3,4				+		+						
Hexitol[a]												
1	+											
2				+								
3								+				+
1,3	+					+						+
1,4	+							+			+	
1,5	+			+								
2,3				+								+
2,4				+				+				
3,4								+				
1,3,4	+					+		+				
1,3,5	+			+		+						
1,4,5	+			+							+	
2,3,4				+		+		+				
2,3,5				+								
1,2,4,5	+		+	+						+		
1,3,4,5	+			+		+				+		
6-Deoxyhexitol[a]												
2				+								
3								+	+			
2,3,4				+	+		+					
2,3,5		+		+								

[a]Figures refer to positions of methylation.

The lowest primary fragment, *m/e* 45 (*A*), is given by substances having a methoxyl group at C-1, but not by any other substance. A peak (*m/e* 45) of low intensity (<5%) may be seen in mass spectra of other substances, and is probably a secondary fragment. A corresponding fragment, *m/e* 59 (*B*), is obtained from 6-deoxyhexitols when the 5-position is methylated. A secondary fragment having the same mass number may sometimes be obtained, as discussed below.

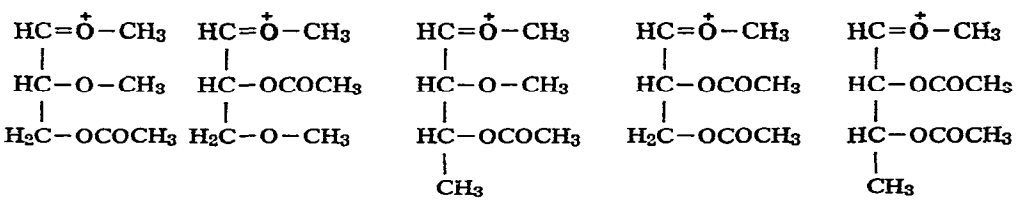


A *B* *C* *D* *E*

The primary fragment *C*, *m/e* 89, is obtained when positions 1 and 2 in the alditol are methylated. This is the exception to the principle, referred to above, that an ion of high intensity, containing a pair of vicinal methoxyl groups, is formed only when fission can occur between another pair of vicinal methoxyl groups. The intensities of the primary fragments generally increase with decreasing mass number, and this may counterbalance the less favorable structural disposition for the formation of this ion. A secondary fragment, identical with *B*, is obtained from *C* by loss of formaldehyde.

The primary fragment *D*, *m/e* 117, is obtained when C-1 is acetylated and C-2 methylated. An analogous, primary fragment, *m/e* 131 (*E*), is obtained from a 6-deoxyhexitol, methylated in the 4- but not in the 5-position.

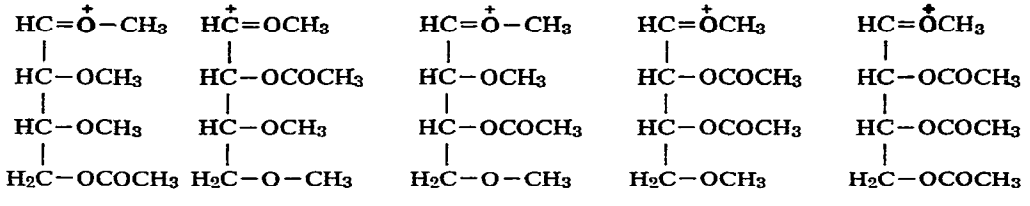
Two primary fragments having *m/e* 161, F_1 and F_2, are expected. Of these, F_1 is obtained in low intensities from alditols methylated at positions 2 and 3, unless position 4 is also methylated when it becomes a prominent fragment. The primary fragment *m/e* 175 (*G*) is obtained from a 6-deoxyhexitol methylated at positions 2, 3, and 4. Fragment F_2, obtained from alditols methylated at positions 1 and 3, is always of high intensity. The secondary fragments having *m/e* 129 and *m/e* 101 are obtained from F_1 and F_2 by loss of methanol and acetic acid, respectively. Further loss of ketene from *m/e* 129 gives *m/e* 87, and loss of formaldehyde from *m/e* 101 might give *m/e* 71.



F_1 F_2 *G* *H* *I*

The primary fragment *m/e* 189 (*H*) is given by alditols methylated at position 3 but not at positions 1 and 2. The secondary fragment *m/e* 129 may be formed by elimination of acetic acid, and the fragments *m/e* 99 and *m/e* 87 by further loss of formaldehyde and ketene, respectively. The analogous, primary fragment *m/e* 203 (*I*) is obtained from a 6-deoxyhexitol, methylated at position 3 but not at positions 4 or 5.

Three primary fragments of *m/e* 205, K_1, K_2, and K_3 may be expected. The first, expected from alditols methylated at positions 2, 3, and 4, is not observed in high intensities, but might be if position 5 was also methylated. Fragment K_2 is observed from alditols methylated at positions 1, 2, 4 and 5, and K_3 from alditols methylated at positions 1, 3, 4, and 5. If, in either case, position 5 is not methylated, the corresponding peak appears with only low intensity. Several secondary peaks may be obtained from these fragments, and *m/e* 145, obtained from K_3 by loss of acetic acid, appears to be especially important.



Three primary fragments of *m/e* 233 may be expected. One of these (*L*) is obtained from alditols methylated at positions 1 and 4. A prominent secondary peak, *m/e* 113, is most probably derived from *L* by loss of two molecules of acetic acid.

The primary peak having the highest mass number, *m/e* 261 (*M*), and a relatively high intensity, is given by alditols methylated at position 3 and acetylated at positions 4, 5, and 6.

EXPERIMENTAL

The partially methylated, acetylated alditols were prepared from the parent sugars by reduction with sodium borohydride and subsequent acetylation with acetic anhydride–pyridine[4]. The compounds, dissolved in chloroform, were injected into an ECNSS-M column fitted in a LKB combined gas chromatograph–mass spectrometer at 180°. The mass spectra were recorded at an inlet temperature of 250°, ionizing potential of 70 e.v., ionizing current of 60 μamp., and a temperature of the ion source of 290°.

SUMMARY

The mass spectra of a number of partially methylated alditol acetates, derived

from methyl ethers of pentoses, hexoses, and 6-deoxyhexoses by reduction and acetylation, have been determined. Isomeric alditols having identical substitution patterns gave similar mass spectra that unambiguously determine the substitution pattern. Mass spectra should also allow determination of substitution patterns of partially methylated alditol acetates that have not previously been investigated. The method will be useful, in combination with gas–liquid chromatography, for the characterisation of different methylated sugars obtained on methylation analysis of polysaccharides.

ACKNOWLEDGMENTS

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THE REDUCTION OF AZIDES WITH SODIUM BOROHYDRIDE: A CONVENIENT SYNTHESIS OF METHYL 2-ACETAMIDO-4,6-*O*-BENZYLIDENE-2-DEOXY-α-D-ALLOPYRANOSIDE

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(Received July 11th, 1967)

INTRODUCTION

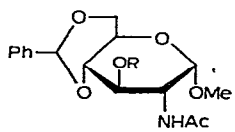
Carbohydrate azides, prepared by replacement of sulphonyloxy groups by azide, are valuable intermediates in the synthesis of amino sugars. The azide group is usually reduced to the amino function by catalytic reduction (using Raney nickel, palladium, etc) or by treatment with lithium aluminium hydride. We have found that the yields of the amine obtained by catalytic hydrogenation are, in some cases, low or variable, and the products are difficult to purify[1]. Moreover, the use of lithium aluminium hydride is often precluded because of the presence of other groups sensitive to this reagent. The results of Boyer and Ellzey[2] suggested that sodium borohydride was not a satisfactory reductant for azides, whereas Stevens *et al.*[3] used this reagent in 2-propanol, without comment, for the reduction of ethyl 4-azido-2,3,4,6-tetradeoxy-α-D-*threo*-hexopyranoside and obtained the corresponding amine in 85% yield. In the course of our studies on the synthesis of amino sugars, we have found sodium borohydride to be an excellent reagent for the reduction of azido derivatives, and our results are described below.

RESULTS AND DISCUSSION

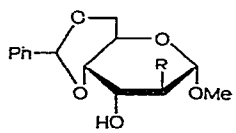
In pursuit of a synthesis of methyl 2,3,4,6-tetra-acetamido-2,3,4,6-tetradeoxy-α-D-galactopyranoside[4], methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside (**10**) was required as starting material. We found that the method[5] involving solvolysis of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-mesyl-α-D-glucopyranoside (**3**) was inconvenient for large-scale work. We considered that the method of Baker and Buss[6], in which the configuration at C-3 of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**1**) was inverted by sequential oxidation with the Pfitzner–Moffatt reagent and reduction of the resulting ketone with sodium borohydride, was more promising, particularly if methyl sulphoxide–acetic anhydride[7] could be used. However, it was subsequently found that oxidation of compound **1** with the latter reagent gave a mixture of two products, suspected of being the required ketone and the corresponding 3-acetate (**2**) formed by acetylation.

We considered that competing acetylation, which effectively blocks oxidation, would be less likely for an axial hydroxyl group. Baker and Buss[6] have shown that,

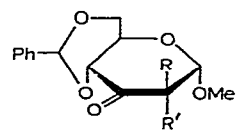
during the oxidation of a hydroxyl group with the Pfitzner–Moffatt reagent, epimerisation of the axial substituent on an adjacent position may occur. Thus, methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (**4**) afforded methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hex-3-ulopyranoside (**6**), identical with that obtained from the *gluco*-isomer **1**. We have therefore studied the oxidation of methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (**5**) with methyl sulphoxide–acetic anhydride. The 2-azide (**5**) was readily prepared in 85% yield by ring-opening of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside by azide[8]. This epoxide is normally prepared from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside *via* the ditoluene-*p*-sulphonate[9], but we have found it more convenient, and less time-consuming, to go by way of the dimethanesulphonate (readily obtained in near quantitative yield), which yields the epoxide in 70% yield when heated with ethanolic sodium ethoxide. The formation of the epoxide by this route was first described by Honeyman and Morgan[10], but little emphasis was placed on it, and its usefulness has not been widely appreciated.



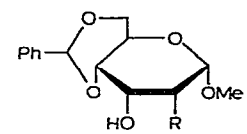
1 R=H
2 R=Ac
3 R=Ms



4 R=NHAc
5 R=N_3



6 R=H, R'=NHAc
7 R=H, R'=N_3
8 R=D, R'=N_3

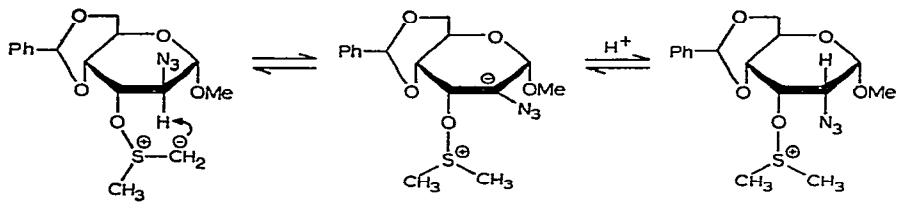


9 R=N_3
10 R=NHAc

Oxidation of the 2-azide (**5**) with methyl sulphoxide–acetic anhydride gave a ketone in 65% yield. Reduction of this ketone with a large excess of borohydride gave a basic product, the infrared spectrum of which indicated the absence of an azide group. The product was best isolated, in an overall yield of 86%, as its *N*-acetyl derivative (formed by treatment with acetic anhydride in ethanol), which was shown to be methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside (**10**) by comparison with a sample prepared by the method of Jeanloz[5]. Hence, not only had borohydride reduced the ketone stereospecifically by equatorial attack, but reduction of the azide substituent and inversion of configuration at C-2 had also occurred. It was found that the ketone could be specifically reduced, to give a mono-azide (**9**), if a smaller proportion of borohydride was used. Since further reduction of this azide, followed by *N*-acetylation, afforded the 2-acetamido-alloside (**10**), and oxidation with methyl sulphoxide–acetic anhydride gave the same ketone (**7**) from which it was derived, all three products must have the same configuration at C-2. Hence, epimerisation must have occurred during the oxidation step and not under the mildly basic conditions of the borohydride reduction.

The exact mechanism by which this epimerisation takes place is worthy of discussion. Epimerisation at asymmetric centres adjacent to a carbonyl function are more generally observed under basic conditions, than under the mildly acidic con-

ditions used for the oxidation. We therefore considered initially that epimerisation may occur during the transition-state phase of the oxidation reaction. It is probable that the sulphonium "ylid" **11** is a key intermediate in the oxidation[11,12], and we



11 **12** **13**

speculated initially that this could function as a sufficiently powerful base to remove H-2 to give carbanion **12**, which would re-combine with a proton from the solvent to give the thermodynamically more-favoured, equatorial epimer **13**. If epimerisation took place by such a mechanism, since the reaction is irreversible, no further exchange of H-2 would occur subsequent to the collapse of "ylid" **11** to the ketone. However, if epimerisation is caused by an acid-catalysed enolisation, exchange of H-2 in the resulting ketone (**7**) should be observed. To test these two possible mechanisms, we allowed the azido-ketone **7** to stand in a mixture of methyl sulphoxide, acetic anhydride, and deuterium oxide, in order to simulate the post-oxidation conditions experienced by the ketone in the oxidation reaction mixture. We then re-isolated the ketone and found that, in agreement with the acid-catalysed enolisation mechanism, complete exchange of H-2 for deuterium had taken place to give the 2-deutero derivative **8**. The completeness of the exchange was convincingly demonstrated by n.m.r. spectroscopy. Before exchange, the H-1 doublet of **7** was the only ring-proton readily recognised (τ 4.84, $J_{1,2} \sim 4$ Hz), but, after exchange, this resonance collapsed to a singlet, and a doublet previously observed at τ 6.22 disappeared completely, and was consequently assigned to H-2 (Fig. 1). No other changes in the spectrum were observed, indicating that no exchange took place at C-4, which is a bridgehead carbon atom. The ketone **7** should be a valuable intermediate for the synthesis of amino sugars specifically tritiated or deuterated at C-2, and, if tritiated or deuterated borohydride is used, at C-2 and C-3. The 2-acetamido-alloside **10** should be readily converted into the *gluco*-isomer **1** by treatment of its 3-methanesulphonate with sodium benzoate in *N*,*N*-dimethylformamide (*cf.* ref. 4), hence providing a convenient route to specifically tritiated and deuterated derivatives of 2-amino-2-deoxy-D-glucose. Horton and Jewell[13] have previously utilised hexulopyranose derivatives for the preparation of specifically deuterated carbohydrates by base-catalysed exchange.

Application of this oxidation–reduction procedure to methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (**14**) was only partially successful. The oxidation product was obtained as an oil that contained at least three components. Reduction of the crude product, followed by *N*-acetylation, gave methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-glucopyranoside (**16**; 19%). The formation of the

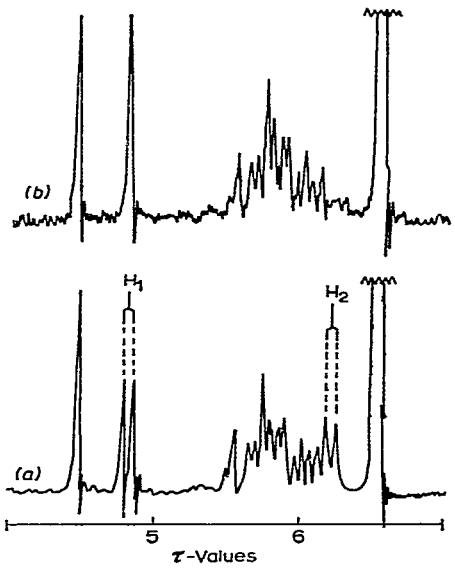
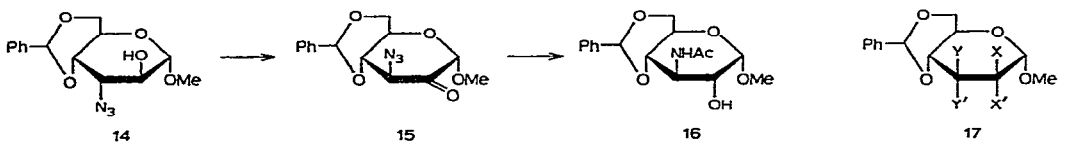


Fig. 1. N.m.r. spectrum of (*a*) methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hex-3-ulopyranoside and (*b*) its 2-deutero derivative.

glucoside, rather than the mannoside, by reduction of α-D-*arabino*-hexulopyranosides (*e.g.*, **15**) with borohydride has been observed by Baker and Buss[6]. It is probable that equatorial approach by borohydride is hindered by the axial methoxyl group, whereas axial approach suffers no such hindrance.



The reduction of azide groups to amino functions in these compounds prompted us to look more closely at this reagent to see whether it could be utilised generally for effecting this type of transformation. In addition to the azides referred to above, seven azide and diazide derivatives of the general type **17** have been reduced to the corresponding amines in 60–80% yields. In most cases, the amines have been characterised as *N*-acetyl derivatives. With methyl 3-azido-4,6-*O*-benzylidene-2-*O*-methanesulphonyl-α-D-altropyranoside, the amine was isolated after 1 h, but, on one occasion, when the reaction mixture was kept overnight, a 60% yield of the epimine, methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino-α-D-allopyranoside, isolated as the *N*-acetyl derivative, was obtained.

EXPERIMENTAL

Melting points were determined on a Kofler microscope hot-stage and are

uncorrected. Unless otherwise stated, optical rotations were determined in chloroform to an accuracy better than ±0.002° with a Bendix-N.P.L. automatic polarimeter, using a 2-cm cell at 21 ±2°. Thin-layer chromatography was carried out on microscope slides coated with silica gel (Whatman SG41), using chloroform–methanol (9:1) or ether; detection was effected with a 10% v/v solution of sulphuric acid in ethanol followed by heating. Unless otherwise stated, all named products gave single spots on chromatograms, and their infrared spectra were in accord with their structures.

Methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside. — Methanesulphonyl chloride (100 ml) was added slowly to a chilled solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (100 g) in dry pyridine (250 ml), and the resulting solution was kept overnight at *ca.* 5°. Decomposition of the reaction mixture with crushed-ice afforded the crude, solid disulphonate (150 g, 97%). This material was suitable for the next stage but could be recrystallised from chloroform to give needles, m.p. 187–188°, $[\alpha]_D$ +48.5° (*c* 0.5). Lit.[10], m.p. 188–189°, $[\alpha]_D$ +49°.

The disulphonate was then added to a solution prepared by adding sodium (16.25 g) to ethanol (1500 ml) (*i.e.*, 0.5N sodium ethoxide), and the resulting mixture was heated under reflux for 1 h with stirring. Addition of water to the cooled reaction mixture yielded the epoxide, which was washed well with water and ethanol and finally dried to give the product (66 g, 70% overall yield). Recrystallisation from chloroform–ether gave needles, m.p. 200°, $[\alpha]_D$ +140° (*c* 1). Lit.[14], m.p. 200°, $[\alpha]_D$ +140°. The infrared spectrum was identical with that of an authentic specimen of the epoxide[9].

In some preparations, the product was contaminated with some of the corresponding *manno*-2,3-epoxide, and we believe that this arises because of incomplete esterification during the first stage of the preparation. This can usually be overcome by the use of a larger proportion of the acid chloride or longer reaction times for the sulphonylation.

Methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-altropyranoside (5). — A suspension of the *allo*-epoxide (40 g) in hot ethanol (500 ml) was treated with a solution of sodium azide (40 g) and ammonium chloride (40 g) in water (120 ml), and the mixture was heated under reflux for 22 h with stirring. After cooling, the reaction mixture was diluted with water (*ca.* 800 ml), and the solid was filtered off, washed well with water, and dried to give the azide (40 g, 85%). Recrystallisation from ether–light petroleum gave the product as plates, m.p. 77–80°, $[\alpha]_D$ +64.2° (*c* 1.0). Lit.[8], m.p. 79–80°, $[\alpha]_D$ +65°.

Methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-ribo-hex-3-ulopyranoside (7). — (*a*) The 2-azido-altroside (5; 30 g) was dissolved in methyl sulphoxide (300 ml), and acetic anhydride (150 ml) was added. The resulting solution was kept at room temperature overnight. Addition of water to the reaction mixture initially precipitated an oil that soon crystallised. The product was then filtered off, washed well with water, and, without drying, recrystallised from ethanol to give ketone 7 (19 g, 64%), m.p. 153–155°, $[\alpha]_D$ +550° (*c* 0.44) (Found: C, 54.7; H, 5.5; N, 13.55. $C_{14}H_{15}N_2O_5$ calc.: C, 55.0; H, 5.6; N, 13.7%).

When the ketone (100 mg) was allowed to stand in a mixture of methyl sulphoxide (1 ml), acetic anhydride (0.5 ml), and deuterium oxide (0.32 ml) for 48 h and then re-isolated by addition of water to the reaction mixture, the n.m.r. spectrum of the product (75 mg) indicated (Fig. 1) that complete exchange of H-2 for deuterium had taken place.

(*b*) The 2-azido-alloside **9** (500 mg) was oxidised in a manner exactly similar to the above to give the azido-ketone (**7**) (60%), m.p. 151–152°, mixed m.p. with the product obtained from the altroside, 152–154°.

Methyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-allopyranoside (**9**). — To a stirred solution of ketone **7** (5 g) in *N,N*-dimethylformamide (10 ml) was added methanol (100 ml) followed by the portionwise addition of sodium borohydride (1 g). After 30 min, the mixture was diluted with water and extracted with chloroform (3 × 75 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to a crystalline solid. Recrystallisation from ethanol gave the 2-azido-alloside (**9**) (4 g), m.p. 117.5–118.5°, $[\alpha]_D$ +139.2° (*c* 0.53) (Found: C, 54.9; H, 5.0; N, 13.8. $C_{14}H_{17}N_3O_5$ calc.: C, 54.7; H, 5.5; N, 13.7%).

Reduction of azides with sodium borohydride. — (*a*) Compound **7** (12.5 g) was dissolved in *N,N*-dimethylformamide (20 ml), and the solution was diluted with methanol (150 ml). Sodium borohydride (10 g) was then added portionwise to the stirred solution. After 45 min, the reaction mixture was diluted with water and extracted with chloroform (3 × 100 ml). In some cases, the reaction mixture was briefly boiled prior to dilution with water. The combined extracts were washed with water, dried ($MgSO_4$), and taken to dryness. The resulting, crystalline solid was dissolved in ethanol (50 ml), and acetic anhydride (7 ml) was added, causing immediate crystallisation of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside (**10**) (10 g, 86%), which, after recrystallisation from ethanol, had m.p. 210.5–211.5°, $[\alpha]_D$ +64.7° (*c* 1.0). Lit.[5], m.p. 214–215°, $[\alpha]_D$ +64°.

(*b*) Compound **9** was reduced, in a manner exactly analogous to that described above, to give compound **10** (88%), m.p. 210–212°, $[\alpha]_D$ +63.8° (*c* 1.0), identical with the product of (*a*) above.

(*c*) Compound **5** was reduced as above to give methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside[15] (**17**; X = NH_2, Y′ = OH, X′ = Y = H) in 80% yield, m.p. 165–166°, $[\alpha]_D$ +105°. With benzoic anhydride in ethanol, the amine gave the *N*-benzoyl derivative, m.p. 161–163°, $[\alpha]_D$ −1° (*c* 1.0). Lit.[16], m.p. 162–164°, $[\alpha]_D$ −1°.

(*d*) Methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (**17**; Y′ = N_3, X = OH, X′ = Y = H) was reduced, as above, to give the 3-amine (**17**; Y′ = NH_2, X = OH, X′ = Y = H) in 60% yield. After recrystallisation from ethanol–light petroleum, it had m.p. 186–188°, $[\alpha]_D$ +91° (*c* 0.55). Lit.[15], m.p. 188°, $[\alpha]_D$ +89°.

(*e*) Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methanesulphonyl-α-D-altropyranoside (**17**; X = N_3, Y′ = OMs, X′ = Y = H) upon reduction, as above, with borohydride, gave the corresponding amine (**17**; X = NH_2, Y′ = OMs, X′ = Y = H) in 60% yield. The product, recrystallised from ethanol, had m.p. 128–129°, $[\alpha]_D$ +95° (*c* 0.7) (Found: C, 50.2; H, 5.75; N, 4.2. $C_{15}H_{21}NO_7S$ calc.: C, 50.1; H, 5.85; N, 3.9%).

With acetic anhydride in ethanol, the amine gave the *N*-acetyl derivative, m.p. 143–145°, $[\alpha]_D$ +74.5° (*c* 0.51). Lit.[16], m.p. 142–145°, $[\alpha]_D$ +64°.

(*f*) Methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-2-*O*-methanesulphonyl-α-D-altropyranoside (**17**; Y′=N_3, X=OMs, X′=Y=H) afforded a syrup upon reduction as described above, which, with acetic anhydride in ethanol, gave the corresponding *N*-acetyl derivative (**17**; Y′=NHAc, X=OMs, X′=Y=H) in 60% yield, m.p. 168–170°, $[\alpha]_D$ +40.8° (*c* 0.71). Lit.[17], m.p. 161–162°, $[\alpha]_D$ +30°.

When the reduction reaction mixture was kept overnight, the product (60%), after *N*-acetylation, was shown to be methyl 2,3-acetylepimino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-allopyranoside, by comparison with an authentic sample[16].

(*g*) Methyl 3-azido-4,6-*O*- benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (**17**; Y=N_3, X=X′=Y′=H) was reduced as above, and the product was treated with acetic anhydride in ethanol to give the corresponding, sparingly soluble *N*-acetyl derivative (**16**; Y=NHAc, X=X′=Y′=H) in 78% yield, m.p. 278–280° (subl.), $[\alpha]_D$ +62.4° (*c* 0.94) (Found: C, 62.1; H, 7.0; N, 4.3. $C_{16}H_{21}NO_5$ calc.: C, 62.6; H, 6.8; N, 4.5%). Lit.[18], m.p. 272–274° (subl.), $[\alpha]_D$ +65°.

(*h*) Methyl 2,3-diazido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-mannopyranoside (**17**; X=Y=N_3, X′=Y′=H) was reduced as above, and the syrupy diamine was treated with acetic anhydride in ethanol to give the corresponding di-*N*-acetyl derivative (**17**; X=Y=NHAc; X′=Y′=H) in 65% yield, m.p. 305–306° (subl.), $[\alpha]_D$ −36° (*c* 0.66, *N*,*N*-dimethylformamide) (Found: C, 58.9; H, 6.8; N, 7.4. $C_{18}H_{24}N_2O_6$ calc.: C, 59.3; H, 6.6; N, 7.7%). Lit.[19], m.p. 310–311° (subl.), $[\alpha]_D$ −36.2°.

(*i*) Methyl 2-acetamido-3-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucopyranoside[4] (**17**; Y=N_3, X′=NHAc, Y′=X=H) was reduced, as described above, to give methyl 2-acetamido-3-amino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucopyranoside (**17**; Y=NH_2, X′=NHAc, Y′=X=H), m.p. 261–262°, $[\alpha]_D$ +45.2° (*c* 0.62), in 75% yield (Found: C, 59.4; H, 6.9; N, 8.5. $C_{16}H_{22}N_2O_5$ calc.: C, 59.6; H, 6.6; N, 8.7%). With acetic anhydride in ethanol, the product gave the sparingly soluble di-*N*-acetyl derivative (93%), m.p. 324–330° (subl.), $[\alpha]_D^{50}$ +44.3° (*c* 0.56, *N*,*N*-dimethylformamide) (Found C, 58.9; H, 6.7; H, 7.6. $C_{18}H_{24}N_2O_6$ calc.: C, 59.3;H, 6.6; N, 7.7%).

Sequential oxidation and reduction of methyl 3-azido-4,6-O-benzylidene-3-deoxy-α-D-altropyranoside (**17**; Y′=N_3, X=OH, Y=X′=H). — The 3-azide (0.5 g) was oxidised with methyl sulphoxide–acetic anhydride, as described above for the 2-azide. The product was isolated as an impure syrup containing three components (t.l.c.). Reduction of this product, as described above, gave a syrup that was dissolved in ethanol (2 ml). Addition of acetic anhydride (0.2 ml) caused crystallisation of methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-glucopyranoside (100 mg, 19%), m.p. 292–295° (subl.), $[\alpha]_D$ +96.8° (*c* 0.46, *N*,*N*-dimethylformamide), identical by its infrared spectrum with an authentic sample. Lit.[20], m.p. 286–288° or[6] m.p. 311–313°.

ACKNOWLEDGMENT

The authors are indebted to Eli Lilly and Company for financial support.

SUMMARY

Sodium borohydride has been shown to be a valuable reagent for the reduction of azides to amines in yields of 60–85%. Oxidation of methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside with methyl sulphoxide–acetic anhydride gave, *via* epimerisation at C-2, methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hex-3-ulopyranoside. It has been demonstrated that epimerisation occurs subsequent to oxidation, *via* an acid-catalysed enolisation. The ketone, upon reduction with borohydride and *N*-acetylation, gave an 86% yield of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside, thus providing a convenient synthesis of this compound.

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NITROGEN-CONTAINING CARBOHYDRATE DERIVATIVES

PART XVII.* METHYL 4,6-*O*-BENZYLIDENE-2,3-DIDEOXY-2,3-DIPHENYLAZO-α-D-MANNOSIDE

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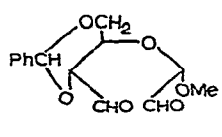
INTRODUCTION

The major product from the reaction[1] of periodate-oxidised methyl 4,6-*O*-benzylidene-α-D-glucoside (**1**) and aqueous phenylhydrazine has been shown[2] to be methyl 4,6-*O*-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside (**2**). This paper describes the structural investigation of a minor product (*A*) of this reaction.



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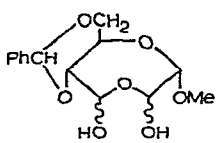

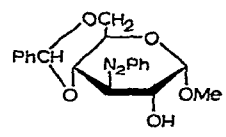

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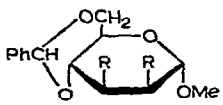
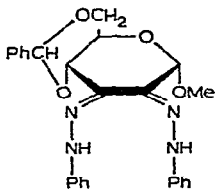


3 R = NH_2
4 R = NHAc
5 R = N_2Ph



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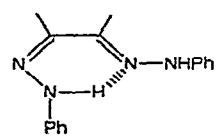

7

RESULTS AND DISCUSSION

From the mother liquors of a large number of preparations of compound **2**, we have isolated a second, yellow product (*A*) in 2–3% yield. The same product could be obtained in 63% yield by treatment of compound **1** with a four-fold excess of phenylhydrazine in *N,N*-dimethylformamide.

Ultraviolet and visible spectra, elemental analysis, and mass spectra suggested that compound *A* was a methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-diphenylazo-α-D-glycoside. Hydrogenation in the presence of Raney nickel gave the known[3] methyl

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2,3-diamino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-mannoside (3), further characterised as its di-*N*-acetyl derivative (4). It has been shown[2,4] that phenylazo groups are reduced under the above conditions to give the amino compound having the same configuration. Since the *ax-eq* arrangement of the substituents at C-2 and C-3 of compound *A* (*i.e.*, 5) was unexpected, the work[3] establishing the structure of compound 3 was repeated and was confirmed. It was further substantiated by comparison of the *manno* di-*N*-acetyl derivative with the authentic *gluco* compound: they were different.

The *manno* configuration of compound *A* was further established from the unsplit signal given by H-1 at τ 5.41 in the p.m.r. spectrum (C_6D_6); this is in accord[5] with an *eq-eq* arrangement of H-1 and H-2.

The *ax-eq* arrangement of the two phenylazo groups in *A* is surprising, since an *eq-eq* arrangement would be expected on steric grounds; the only other cyclisation of this type recorded in the literature[6] yields a diequatorial product. The mechanism is probably of the latter type[6], that is, formation of a bis(phenylhydrazone), cyclisation to a phenylazo-phenylhydrazine derivative, followed by oxidation.

There is, of course, no hope of following the formation of compound 5 in aqueous solution, where it is only a minor by-product. However, in the *N*,*N*-dimethylformamide reaction, the mixture is poured into brine to give a very pale-cream solid, which, if filtered and dried rapidly, shows a band at 280 nm with a shoulder at 300 nm in its u.v. spectrum; this spectrum is consistent with the bis(phenylhydrazone) structure 6. If this pale-coloured solid is dissolved in methanol, the yellow diphenylazomannoside 5 is deposited during several days. It appears therefore that no ring closure occurs in *N*,*N*-dimethylformamide.

Treatment of the phenylazo compound 2 with three moles of phenylhydrazine in *N*,*N*-dimethylformamide, with processing as above, gave only unchanged starting compound; compound 2 is therefore not an intermediate in the formation of compound 5.

Treatment of the diphenylazo compound 5 with methanolic sodium methoxide (*cf.* refs. 6–8) gave the bis(phenylhydrazone) 6, previously obtained[7] by another route that was not reproducible. From its p.m.r. spectrum (signals at τ −2.80 and 0.15 ascribable[9] to chelated and non-chelated NH protons), compound 6 has a structure such as 7. Attempts to obtain the parent diulose by treatment[7] of compound 6 with benzaldehyde gave an unstable, cream solid that, from spectral data, may have been the required product.

The reduction of compound 5 appears to be the best route at present available to derivatives of 2,3-diamino-2,3-dideoxy-D-mannose, starting from D-glucose.

EXPERIMENTAL

Compounds were identified where necessary by mixed m.p. and infrared spectrometry. Thin-layer chromatography was performed on silica gel (Kieselgel G nach Stahl) usually with included u.v. indicator; where u.v. indicator was not used, detection was by concentrated sulphuric acid or iodine vapour. All evaporations were

performed in a rotatory evaporator at <50°. All optical rotations are for chloroform solutions.

Methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-diphenylazo-α-D-mannoside (**5**). — A solution of dioxepan **1** (10 g) in *N,N*-dimethylformamide (50 ml) was added to water (3.5 l) at 80°. The solution was cooled to 60° with stirring, and a solution of phenylhydrazine hydrochloride (5.2 g) and sodium acetate (fused, 16.6 g) in water (100 ml) was added. A yellow solid separated, and the solution was cooled to *ca.* 35° and finally kept for 4 h at room temperature. Crystallisation of the resulting yellow solid from propan-2-ol yielded methyl-4,6-*O*-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside (**2**) (40%), m.p. 182–183° (lit.[1], 182–183°).

Concentration of the propan-2-ol mother liquors gave a further crop of the phenylazoglucoside. Further concentration to *ca.* half volume gave a third crop of yellow crystals. Repeated extraction with hot methanol gave a bright-yellow solid that was crystallised from a large volume of methanol to yield bright-yellow needles of compound **5** (2.5% based on **1**), m.p. 187–189°, $[\alpha]_D^{27}$ −423° (*c* 0.8), $\lambda_{max}^{CHCl_3}$ 272.5 and 413 nm (ε 18, 730 and 339.5) [Found: C, 68.0; H, 5.8; N, 12.1; mol. wt. 451 (by osmometry). $C_{26}H_{26}N_4O_4$ calc.: C, 68.1; H, 5.7; N, 12.2%; mol. wt. 458]. Mass spectral data: *m/e* 458 (M^+); *m/e* 105 (base peak; PhN_2^+, and possibly $PhC^+=O$).

Reduction of methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-diphenylazo-α-D-mannoside (**5**). — Compound **5** (1 g) in a 1:1 mixture of absolute ethanol and cyclohexane (80 ml) was treated with Raney nickel (1 g) and hydrogenated at 85 atmos. and 85° for 15 h. The nickel was removed by filtration, and the solution was concentrated, water being added repeatedly to remove aniline by steam distillation. Finally, the residue was boiled with water and filtered hot, and the solution was evaporated to a pale syrup that was dried by azeotropy with ethanol to yield methyl 4,6-*O*-benzylidene-2,3-diamino-2,3-dideoxy-α-D-mannopyranoside (**3**) (0.51 g, 84%). Periodate oxidation of this product was performed as described[3] for the authentic material.

Acetylation, using pyridine and acetic anhydride, gave methyl 4,6-*O*-benzylidene-2,3-diacetamido-2,3-dideoxy-α-D-mannoside (**4**), m.p. 304° (decomp.). Recrystallisation from water raised the m.p. to 310° (decomp.), $[\alpha]_D^{26}$ −35.9° (*c* 0.78); lit.[3], m.p. 310–311°, $[\alpha]_D^{20}$ −36.2°.

Preparation of methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-diphenylazo-α-D-mannopyranoside (**5**) *in* N,N-*dimethylformamide.* — The dioxepan **1** (2 g) in *N,N*-dimethylformamide (40 ml) was treated with redistilled phenylhydrazine (2.7 g). The solution was kept for 60 h and then added to water (400 ml); a white precipitate formed. Sodium chloride (2–3 g) was added to promote flocculation, and the suspension was kept for 1 day. The pale-yellow precipitate was collected (see text for u.v. spectrum), and dissolved in methanol (200 ml), and the solution was kept for 8 days to give yellow needles of compound **5**. Recrystallisation from methanol gave the diphenylazo sugar (1.4 g, 63%), m.p. 185–187°.

Treatment of compound **5** *with sodium methoxide.* — The diphenylazo sugar (**5**) (0.46 g) was added to N sodium methoxide in methanol under reflux; heating was continued for 10 min. Cooling and filtration gave the crude product (0.46 g), which,

on recrystallisation from methanol–water, gave methyl 4,6-*O*-benzylidene-α-D-*erythro*-2,3-hexodiulose bis(phenylhydrazone) (**6**, 87%), m.p. 210° (decomp.), $[\alpha]_D^{26}$ −557° (*c* 1.07); lit.[7], m.p. 210° (decomp.); it is regretted that an incorrect $[\alpha]_D$ value was given previously[7] for this compound.

Attempted preparation of the parent diulose from methyl 4,6-O-benzylidene-α-D-erythro-2,3-hexodiulose bis(phenylhydrazone) (**6**). — The bishydrazone (0.3 g) in 70% aqueous ethanol (50 ml) was treated with benzaldehyde (0.9 ml) and glacial acetic acid (0.09 ml). The reaction mixture was heated under reflux for 5 h, during which time the bishydrazone dissolved. Cooling and allowing to stand overnight gave no crystals of the unchanged bishydrazone. The mixture was concentrated *in vacuo* to give two layers consisting of a benzaldehyde solution and an aqueous layer. The two layers were separated, and the aqueous layer was concentrated *in vacuo*. Cream-coloured crystals separated (0.04 g). The infrared spectrum showed maxima at 1740 and 1750 cm^{-1}. Crystallisation from methanol–ether gave a white jelly that decomposed on drying.

Treatment of methyl 4,6-O-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside **2** *with phenylhydrazine.* — The phenylazoglucoside (1 g) in *N*,*N*-dimethylformamide (17 ml) was treated with phenylhydrazine (0.9 ml). The solution was kept for 8 h and then poured into water (170 ml). The yellow precipitate was collected, and recrystallised from methanol to give methyl 4,6-*O*-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside (95%), m.p. 186–189°.

ACKNOWLEDGMENTS

We thank I.C.I. (Pharmaceuticals Division) for financial support, Mrs. A. G. Pike for technical assistance, and Dr. A. C. Richardson for a sample of methyl 2,3-diacetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucoside.

SUMMARY

Methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-diphenylazo-α-D-mannoside (**5**) has been isolated in 2–3% yield from the reaction of periodate-oxidised methyl 4,6-*O*-benzylidene-α-D-glucoside with aqueous phenylhydrazine, and in 63% yield when phenylhydrazine in *N*,*N*-dimethylformamide was used. Compound **5** has been rearranged with base to the corresponding bis(phenylhydrazone).)

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THE ACID HYDROLYSIS OF GLYCOSIDES

V. EFFECT OF THE NATURE OF THE AGLYCON ON THE HYDROLYSIS OF GLYCOSIDURONIC ACIDS (GLYCURONIDES)

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INTRODUCTION

A carboxyl group at C-5 in the glycose moiety of a glycoside has long been known to stabilize the glycosidic linkage towards acids. Recently[1], it was observed that D-glucopyranosiduronic acids ("D-glucopyranuronides") containing an aglycon having a low electron-affinity are hydrolyzed more rapidly by acids than are the corresponding D-glucopyranosides. It was the object of the present investigation to adduce more evidence on this point. Several β-D-glucopyranosiduronic acids were accordingly synthesized, and their kinetic characteristics were established and compared with those of the corresponding β-D-glucopyranosides.

RESULTS

Fourteen β-D-glucopyranosiduronic acids were synthesized, most of them for the first time, by conventional methods. All intermediate peracetates, and all but three of the final β-D-glucopyranuronides, were obtained crystalline. Four β-D-glucopyranosides were also prepared. Hydrolysis was conducted as described earlier[1–3] in aqueous sulfuric acid at 60, 70, 80, and 90°; pseudo-first-order rate coefficients, and energies and entropies of activation, were determined from polarimetric measurements[1,2].

It is known that glycopyranosiduronic acids suffer decarboxylation when they are heated in acidic, aqueous solutions[4,5], a fact that renders measurements of the present type subject to considerable doubt. Easty[6] studied the acid-catalyzed hydrolysis of methyl α-D-glucopyranoside and methyl α-D-glucopyranosiduronic acid by determining the methanol produced on hydrolysis of these compounds. In the present series of investigations, similar measurements were made polarimetrically under the same conditions[1,2]. Comparison between the two sets of data revealed that all of the kinetic data were practically identical, indicating the accuracy of the polarimetric method, provided that measurements are made at the initial stage of the hydrolysis. Use of the Guggenheim[7] and Swinbourne[8] procedures, which do not require knowledge of the final optical rotation of the solution, also gave results in agreement with those afforded by the conventional method.

The kinetic data are presented in Table I, which also includes the ratio between

the rate coefficients at 60° of the glucuronides and corresponding glucosides[1,2]. New data for four glucopyranosides are summarized in Table II. Standard deviations for the rate coefficients were, with a few exceptions, in the range of 1 to 4%; energies of activation were accurate within ±0.2 kcal. mole^{-1}.

TABLE I

RATE COEFFICIENTS AND KINETIC PARAMETERS FOR THE HYDROLYSIS OF β-D-GLUCOPYRANOSIDURONIC ACIDS IN 0.5M SULFURIC ACID

Aglycon	$k \times 10^6$, *sec*$^{-1}$			*E, kcal mole*$^{-1}$	$\Delta S^{\ddagger}$ *at 60° cal. deg.*$^{-1}$ *mole*$^{-1}$	*Ratio*[a]
	60°	*70°*	*80°*			
Ethyl	3.91	13.8	45.9	28.8	+1.5	2.54
Propyl	4.54	16.4	54.3	29.0	+2.5	2.50
Isopropyl	18.8	63.2	205.0	27.9	+2.1	7.10
Butyl	4.61	16.3	53.5	28.7	+1.5	2.84
Hexyl	4.80	16.9	54.0	28.3	+0.4	—
(2-Hydroxyethyl)	0.614	2.35	8.34	30.5	+3.0	0.355
(2-Methoxyethyl)	0.65	2.28	7.56	28.7	−2.3	0.355
(2-Chloroethyl)	0.183	0.716	2.62	31.1	+2.5	0.089
(3-Chloropropyl)	0.791	2.84	9.94	29.6	+0.8	—
(Carboxymethyl)	0.317	1.19	4.92	32.1	+6.4	0.084
(2-Carboxyethyl)	—	1.41	—	—	—	0.310[b]
Cyclopentyl	16.8	57.5	179.6	27.7	+1.1	5.42
Cyclohexyl	17.3	59.8	183.0	27.6	+0.8	5.32
Phenyl	0.57	2.37	9.23	32.6	+9.0	—

[a] $k_{glucuronide}/k_{glucoside}$ at 60°. [b] At 70°.

It had previously been found[1] that methyl α- and β-D-glucopyranosiduronic acid are hydrolyzed at rates that are only slightly lower than those of methyl α- and β-D-glucopyranoside. In the present series, ethyl, propyl, butyl, and hexyl glucuronides were all hydrolyzed at approximately the same rate as the isobutyl and neopentyl compounds studied previously[1], and 2 to 3 times faster than their glycoside analogs. The isopropyl, cyclopentyl, and cyclohexyl D-glucuronides exhibited rates that were four

TABLE II

RATE COEFFICIENTS AND KINETIC PARAMETERS FOR THE HYDROLYSIS OF FOUR D-GLUCOPYRANOSIDES IN 0.5M SULFURIC ACID

Aglycon	$k \times 10^6$, *sec*$^{-1}$			*E, kcal mole*$^{-1}$	$\Delta S^{\ddagger}$ *at 60°, cal. deg.*$^{-1}$ *mole*$^{-1}$
	60°	*70°*	*80°*		
(2-Chloroethyl)	2.06	8.61	33.7	32.7	+11.9
(Carboxymethyl)	—	14.2	53.1	31.7	+10.1
(Carboxyethyl)	—	4.58	—	—	—
Cyclopentyl[a]	—	10.6	39.7	33.1	+13.5

[a] k=152.6 at 90°.

times those of the above glucuronides, and that were considerably greater than those of the corresponding glucosides. The (2-hydroxyethyl), (2-methoxyethyl), (3-chloropropyl), and phenyl glucuronides were hydrolyzed much more slowly. The (carboxymethyl), (2-carboxyethyl), and, especially, the (2-chloroethyl) glucuronides were even more stable towards acid.

The energies of activation were all within the range of 28 to 32 kcal. $mole^{-1}$, and were lower for the more labile and higher for the more stable glucuronides. Most of the entropies of activation lay between +0.5 and +2.5 cal.$deg.^{-1}mole^{-1}$ at 60°.

DISCUSSION

The unusual stability of many glucopyranosiduronic acids and of all aldobiouronic acids towards acid has been attributed by several investigators[9-14] to an inductive effect of the (electron-attracting) carboxyl group, whereas others[1,6,15] have objected to this interpretation, without, however, suggesting any alternative explanation. It is clear from the data in Table I that the polarity of the aglycon influences the rate of hydrolysis of glucopyranosiduronic acids. If ln (k/k_0) is plotted against σ^*, where k is the rate coefficient of a glucuronide, k_0 is the rate coefficient of methyl β-D-glucopyranosiduronic acid, and σ^* is Taft's[16] polar substituent constant, the relationships shown in Fig. 1 are obtained. Evidently, the glucopyranosiduronic acids are hydrolyzed at rates that are inversely proportional to the electron affinity of the aglycon. A corresponding plot for glucopyranosides (see Fig. 2) shows that the polarity of the aglycon does not influence the rate of hydrolysis of these compounds.

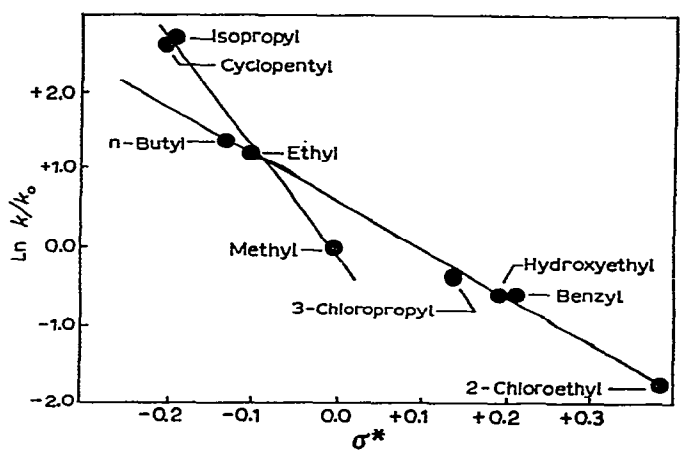

Fig. 1. Relation between the electron affinity of the aglycon and the rate of hydrolysis of β-D-glucopyranosiduronic acids.

The carboxymethyl group has a polar substituent constant of[16] +1.05. Inspection of Fig. 1 reveals that the expected rate of hydrolysis of the (carboxymethyl) β-D-glucopyranosiduronic acid should be approximately one hundredth of that actually observed. Capon[17] has shown that (*o*-carboxyphenyl) β-D-glucopyranoside

is hydrolyzed at about 10^4 times the rate for the *p*-analog at a pH of 3.5, and attributed this finding to intramolecular catalysis by the *o*-carboxyl group. Similar arguments may be invoked in the present case to explain the high rate of hydrolysis of the (carboxymethyl) β-D-glucuronide, although the carboxyl group in this compound is probably less favorably situated. In the strongly acidic solution used here, the interaction would probably involve general acid-catalysis *via* the undissociated carboxyl group.

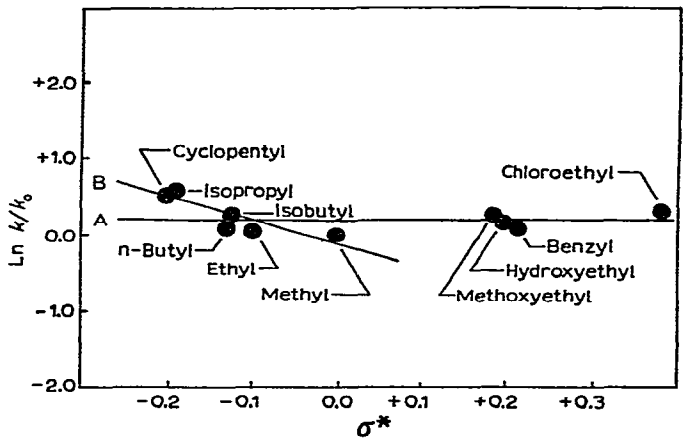

Fig. 2. Relation between the electron affinity of the aglycon and the rate of hydrolysis of β-D-glucopyranosides.

Another factor, besides the inductive effect of the aglycon, is apparent from Figs. 1 and 2, where a linear relationship is indicated between σ^* and ln (k/k_0) for the methyl, ethyl, and isopropyl glucuronides and glycosides. Evidently, the hydrolysis is facilitated by the substitution, in the aglycon, of alkyl groups for hydrogen atoms on the carbon atom adjacent to the glycosidic oxygen atom, probably because of repulsive interaction with the glycose residue. Substitution of all three hydrogen atoms would result in alkyl–oxygen fission and a much enhanced rate of hydrolysis[2,18]. Increase in bulk at other positions of the aglycon has little effect on the rate of hydrolysis[2].

Comparison of the data in Table I with those in Table II, and with those reported previously for other glucopyranosides[1,2,19], shows that energies of activation of the β-D-glucopyranosiduronic acids are 4 to 5 kcal.mole^{-1} lower than those of the corresponding β-D-glucopyranosides. Similarly, as observed by several investigators[1,6,20], albeit not by all[13], entropies of activation are consistently lower for the glucuronides, suggesting that the latter are more ordered in the transition state than are the glucosides.

The electron-attracting nature of the carboxyl group at C-5 undoubtedly lowers the electron density at the ring-oxygen atom, resulting in a destabilization of the intermediate carbonium ion, and hence a decrease in the rate of hydrolysis. Any conformational effect of the bulky carboxyl group would act in the same direction. As emphasized by Semke, Thompson, and Williams[13], the inductive effect of the carb-

oxyl group will depend on the electron density prevailing at the actual site of reaction. Substitution, in the ring, of sulfur for (the more electronegative) oxygen results in an enhanced rate of hydrolysis[21,22]. This effect has been attributed by Whistler and Van Es[21] to the greater ability of the sulfur to release electrons to the glycosidic oxygen atom, thus increasing the extent of protonation.

It is possible that the heterolysis step in the hydrolysis of glycopyranosiduronic acids having aglycons of low electron-affinity is facilitated by interaction between the carboxyl group on C-5 and the glycosidic oxygen atom, resulting in an intramolecular, general-acid catalysis of the type suggested by Capon[17]. An electron-attracting aglycon would lower the electron density at the glycosidic oxygen atom, thus impeding protonation, and, perhaps, would also cause the carboxyl group to interact with the ring-oxygen atom, which would then become less effective in stabilizing the intermediate, carbonium-oxonium ion. These factors could be sufficiently significant to overcome the increased ease of heterolysis brought about by the flow of electrons towards the aglycon, resulting in a lessened overall rate of hydrolysis.

As may be seen from Table I, the effect of the nature of the aglycon is pronounced. At 60°, isopropyl β-D-glucopyranosiduronic acid, for example, is hydrolyzed 100 times faster than the (2-chloroethyl) analog. The stability of cyclohexyl β-D-glucopyranosiduronic acid is almost one thirtieth of that of the benzyl[1] and phenyl analogs.

EXPERIMENTAL

All melting points are corrected. The purity of the three noncrystalline compounds was checked by paper, and by gas–liquid, chromatography.

Synthesis of methyl (2-chloroethyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate. — Drierite (30 g), freshly prepared[23] silver carbonate (60 g), 2-chloroethanol (100 g), dry benzene (200 g), and a small amount of iodine were stirred for one hour at room temperature with exclusion of moisture. Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[24], m.p. 105–106°, $[\alpha]_D^{25}$ +198° (chloroform), (40 g) was added, and stirring was continued for 36 h at room temperature. After the suspension had been filtered through Celite, and the solid had been washed with benzene, the filtrate and washings were combined, washed with water (to remove residual 2-chloroethanol), dried, filtered, and concentrated to a syrup that was dissolved in warm isopropyl alcohol (450 ml). The solution was cooled, to give crystals, 22.7 g (58%), m.p. 141–142°, $[\alpha]_D^{25}$ −23.9° (*c* 2.0, chloroform).

All other *O*-acetylated glycuronides were obtained crystalline in the same way. The yields, melting points, specific rotations, and analytical data are summarized in Table III.

Synthesis of 2-chloroethyl β-D-glucopyranosiduronic acid. — The above acetate (15 g) was suspended in anhydrous methanol (250 ml), and 0.1M methanolic sodium methoxide (250 ml) was added. The solution was kept at room temperature for 2 h, most of the methanol was removed, and water (250 ml) was added to saponify the methyl ester. Aqueous sodium hydroxide (30 ml) was added, and the solution was kept for 15 h at 4°. Sodium ions were removed with a cation-exchange resin, and the

TABLE III

EXPERIMENTAL AND ANALYTICAL DATA FOR THE METHYL (2,3,4-TRI-*O*-ACETYL-β-D-GLUCOPYRANOSID)-URONATES

Aglycon	*Yield, %*	*M.p., degrees*	$[\alpha]_D$, *degrees*	*Carbon, %*		*Hydrogen, %*	
				Calc.	*Found*	*Calc.*	*Found*
Ethyl	82	102–103	−21.0				
Propyl	82	108–109	−30.0	51.60	51.09	6.42	6.41
Isopropyl[25]	77	141–143	−36.7	51.06	50.44	6.42	6.38
Butyl[1]	77	89–90	−27.6	52.30	51.09	6.71	6.70
Hexyl	71	84–85	−31.1	54.53	53.86	7.23	7.33
(2-Hydroxyethyl)	86	162.5–163.5	−22.3	47.62	47.33	5.86	5.84
(2-Methoxyethyl)	81	105.5–106.5	−29.2	48.98	48.28	6.16	6.20
(2-Chloroethyl)[a],[26]	58	141–142	−23.9	45.40	44.95	5.43	5.45
(3-Chloropropyl)[b]	54	128–129	−11.0	46.78	46.16	5.64	5.80
(Carboxymethyl)[c]	25	100–102	−49.3	47.29	47.01	5.46	5.47
(Carboxyethyl)[c]	61	86.5–87.5	−22.1	48.57	48.64	5.76	5.92
Cyclopentyl	80	132–132.5	−46.5				
Cyclohexyl[1],[25]	83	140–141	−36.2				
Phenyl[13]	12	125–126.5	−29.6				

[a]Cl, Calc.: 8.94. Found: 9.16. [b]Cl, Calc.: 8.63. Found: 8.53. [c]Methyl ester.

solution was filtered, and concentrated to a syrup which was dissolved in ethyl acetate containing 10% of methanol. Crystals (5 g) formed at room temperature; m.p. 153–154°, $[\alpha]_D^{25}$ −47.5° (*c* 2.0, water).

The other β-D-glucopyranosiduronic acids were prepared in the same way; most of them were crystallized from ethyl acetate containing a small proportion of

TABLE IV

EXPERIMENTAL AND ANALYTICAL DATA FOR THE β-D-GLUCOPYRANOSIDURONIC ACIDS

Aglycon	*M.p., degrees*	$[\alpha]_D$, *degrees*	*Carbon, %*		*Hydrogen, %*	
			Calc.	*Found*	*Calc.*	*Found*
Ethyl	160–161	−64.4				
Propyl	125–127	−64.9	45.76	45.43	6.83	6.86
Isopropyl[1]	134–135	−66.6	45.76	45.00	6.83	6.85
Butyl[1]	102–104	−60.3	47.99	47.16	7.25	7.22
Hexyl	110–111	−55.3	51.79	51.02	7.97	8.05
(2-Hydroxyethyl)	87–89	−50.5	40.34	39.80	5.92	6.68
(2-Methoxyethyl)		−50.4				
(2-Chloroethyl)[a]	153–154	−47.5	37.44	37.51	5.10	5.34
(3-Chloropropyl)[b]	108–109	−54.1	39.93	40.72	5.59	5.71
(Carboxymethyl)		−65.9	38.00	38.97	4.80	5.39
(2-Carboxyethyl)		−31.0				
Cyclopentyl	124–126	−68.2				
Cyclohexyl[1],[25]	164–165	−62.2				
Phenyl[13]	162–163	−88.1				

[a]Cl, Calc.: 13.82. Found: 13.70. [b]Cl, Calc.: 13.10. Found: 12.56.

ethanol or isopropyl alcohol. Melting points, specific rotations, and analytical data are listed in Table IV.

Synthesis of β-D-glucopyranosides. — The three β-D-glucopyranosides were prepared as described elsewhere[1,2]. Experimental and analytical data are presented in Table V. None of the glucosides were obtained crystalline.

TABLE V

EXPERIMENTAL AND ANALYTICAL DATA FOR THE 2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSIDES AND THE β-D-GLUCOPYRANOSIDES

Aglycon	*Peracetate*			*Glucoside*
	Yield, %	*M.p., degrees*	$[\alpha]_D$, *degrees*	$[\alpha]_D$, *degrees*
(2-Chloroethyl)[2,27]	68	116–117	−10.6	−26.3
(Carboxymethyl)[2,28]	42	74–76[a]	−29.4[a]	−33.6
(2-Carboxyethyl)[2]	61	90–95[a]	−10.1[a]	−29.0

[a]Methyl ester.

Kinetic measurements. — Hydrolyses were conducted in 0.05M aqueous sulfuric acid at 60, 70, 80, or 90°, either in sealed ampoules or in a polarimeter tube; the concentration of sugar was 0.05M. Experiments with various concentrations of glycosides gave identical rate-coefficients, showing that the reaction was pseudo-first-order in the presence of an excess of acid. The extent of hydrolysis was determined, usually within the first 10% of conversion, with a Perkin–Elmer Model 141 photoelectric polarimeter. Owing to decomposition of the sugars, all data obtained beyond a conversion of 70% had to be discarded. Rate coefficients, energies and entropies of activation, and standard deviations were calculated with an IBM 1620 computer.

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SUMMARY

Rate coefficients and kinetic parameters have been determined for the acid-catalyzed hydrolysis of fourteen β-D-glucopyranosiduronic acids containing aglycons of different electron-affinity. Energies and entropies of activation of all of these compounds were lower than those of the corresponding β-D-glucopyranosides. The glucopyranosiduronic acids were hydrolyzed at rates that were inversely proportional to the electron affinity of the aglycon, whereas the rates of hydrolysis of the glucopyranosides were independent of the polarity of the aglycon. (Carboxymethyl) β-D-glucopyranosiduronic acid was hydrolyzed at one hundredth of the rate expected.

The phenomena are discussed, assuming a catalytic interaction between the carboxyl groups and the glycosidic oxygen atom. Substitution, in the aglycon, of alkyl groups for hydrogen atoms at the carbon atom adjacent to the glycosidic oxygen atom increased the rate of hydrolysis both of the glucopyranosiduronic acids and of the glucopyranosides.

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STUDIES ON URONIC ACID MATERIALS

PART XXII*. THE COMPOSITION OF THE GUM FROM *Acacia drepanolobium* HARMS EX SJÖSTED

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INTRODUCTION

A preliminary study[2] showed that *Acacia drepanolobium* is a further example (*cf. A. nilotica*[3], *A. nubica*[4], and *A. arabica*[1]) of an *Acacia* gum having a positive specific rotation and a low content of L-rhamnose that does not bear a unit molar correspondence to the uronic acid content. It was also noted that *A. drepanolobium* gum does not dissolve completely in cold water, giving about 20% of a water-insoluble gel that can be dispersed in cold, dilute, alkaline solution.

The solubility difficulty associated with some commercial specimens of gum arabic (*A. senegal* syn. *verek*) has received comment recently[5]. The reason for the occasional formation of mucilaginous gels by some *Acacia* species is not known. We have examined many specimens of *A. senegal* gum, but they have not given sufficient quantities of the insoluble gel to facilitate its study. In contrast, *A. drepanolobium* conveniently provides considerable proportions of water-insoluble material, and we now present the results of an analytical study of the differences in composition between the water-soluble gum, a fraction soluble in dilute sodium chloride solution, and the insoluble gel.

EXPERIMENTAL

Origin of specimens. — Nodules of the gum from *Acacia drepanolobium* Harms ex Sjösted were collected by Mr. W. M. C. Bagshawe, Provincial Forest Officer, at Tabora, Western Province, Tanganyika, in July, 1961.

Analytical methods. — The standard methods involved have been described previously[6].

Fractionation procedure. — Two portions of crushed, bark-free, *A. drepanolobium* gum (*ca.* 20 g) were each stirred with distilled water (500 ml) for 12 h; the soluble fraction (*A*) was collected by filtration (muslin, and then paper), dialysed against tap water, and then electrodialysed. The residue was stirred with M sodium chloride solution (500 ml) for 12 h; the salt-soluble fraction (*B*) was collected by filtration, dialysed against tap water, and then electrodialysed. The residual insoluble gel

*For Part XXI, see Ref. 1.

(Fraction *C*) was dialysed against tap water. The three fractions were freeze-dried from aqueous solutions or dispersions. The percentage yields of the three fractions are shown in Table I.

Viscosity measurements. — Determinations on Fractions *A* and *B* were made in M sodium chloride solution in an Ubbelohde dilution viscometer at 25° (solvent flow-time, 193.6 sec). Fraction *C* was studied in M sodium hydroxide solution at 25° (solvent flow-time, 215.2 sec).

Specific rotations. — Fractions *A* and *B* were studied in distilled water and in M sodium hydroxide; Fraction *C* was studied in M sodium hydroxide. Specific rotations of sugars were measured in aqueous solutions. All measurements were made at $19 \pm 1°$.

Periodate oxidations. — These were performed at room temperature in darkness by mixing equal volumes of 4% polysaccharide solutions and 0.25M sodium metaperiodate. The formic acid released was titrated (Methyl Red) with standard sodium hydroxide. The reduction of periodate was determined with standard arsenite solution. Periodate oxidation of oligosaccharides was performed at room temperature in darkness, using excess of sodium metaperiodate. Formaldehyde was estimated colorimetrically with chromotropic acid[7]. Periodate oxidations of *O*-methyl sugars were carried out by the method of Lemieux and Bauer[8].

Paper chromatography. — Whatman No. 1 and 3MM papers were used with the following solvent systems (v/v):(*a*) benzene–butyl alcohol–pyridine–water (1:5:3:3, upper layer); (*b*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (*c*) ethyl acetate–acetic acid–formic acid–water (18:8:3:9); (*d*) butyl alcohol–ethanol–water (4:1:5, upper layer); (*e*) ethyl acetate–pyridine–water (10:4:3); (*f*) butanone–acetic acid–water (9:1:1, saturated with boric acid); (*g*) butanone–water–ammonia (sp. gr. 0.880) (200:17:1). Chromatograms were developed as described previously[6], except that D-glucose was detected by the following enzymic method. Chromatograms were sprayed, in succession, with 0.5M phosphate buffer (pH 7.0), 0.05% peroxidase, 0.05% glucose oxidase, and 0.1% *o*-dianisidine in 90% aqueous ethanol. Glucose oxidase specifically oxidises D-glucose, giving hydrogen peroxide, which is coupled with *o*-dianisidine by peroxidase, giving a purple spot. R_{Gal}, R_G, and R_{Rha} values refer to distances moved relative to D-galactose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and L-rhamnose, respectively.

Gas–liquid chromatography. — G.l.c. of mixtures of *O*-methyl sugars was performed on a chromatograph (Type S3A, Gas Chromatography Ltd.) fitted with flame-ionisation detectors, at nitrogen flow-rates of *ca.* 100 ml/min on columns of (*i*) 15% by weight of poly(butane-1,4-diol succinate) on Celite (120×0.5 cm) at 175°, (*ii*) 15% by weight of poly(ethylene glycol adipate) on Celite (75×0.5 cm) at 160°. Retention times (T) are quoted relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Conversion of 4-O-methyl-D-glucuronic acid into 4-O-methyl-D-glucose. — 4-*O*-Methyl-D-glucuronic acid (29 mg) was converted into the methyl ester methyl glycoside by treatment for 8 h with boiling, 5% methanolic hydrogen chloride. The resulting solution was neutralised with silver carbonate and filtered, and the residue was washed

TABLE I

ANALYTICAL DATA[a] FOR *Acacia drepanolobium*, FRACTIONS *A*, *B*, AND *C*

	First series of extractions			*Second series of extractions*		
	A	B	C	A	B	C
Recovery, %	79	8	13	80	8	12
Ash, %	0.00	0.00	2.78	0.00	0.00	2.56
Nitrogen, %	1.11	1.21	0.58	1.18	1.24	0.61
$[\eta]$, cm^3g^{-1}	17.8	19.5	22.8	15.0	17.2	20.2
Methoxyl, %	0.43	0.43	0.41	0.42	0.42	0.42
$[\alpha]_D$ (*c* 1.0, water)[b]	+77.8°	+77.2°	—	+82.2°	+82.6	—
$[\alpha]_D$ (*c* 1.0, N sodium hydroxide)[b]	+77.2°	+78.2°	+78.0°	+81.5°	+81.9°	+80.0°
Equivalent weight [b,c]	1976	1997	—	1985	1980	—
Uronic anhydride, %[b,d]	8.9	8.8	—	8.8	8.8	—
Uronic anhydride, %[b,e]	9.6	9.9	9.7	9.5	9.8	9.8
Formic acid released on periodate oxidation (moles/g × 10^3)[b]	1.07	1.05	1.02	1.06	1.08	1.05
Periodate reduced (moles/g × 10^3)[b]	3.96	3.97	3.93	3.95	3.98	3.96
L-Arabinose, %[b]	52	51	49	53	53	51
D-Galactose, %[b]	38	39	41	37	37	39
L-Rhamnose, %[b,f]	1	1	1	1	1	1
D-Glucuronic acid, %[b,g]	7	7	7	7	7	7
4-*O*-Methyl-D-glucuronic acid, %[b,g]	2	2	2	2	2	2

[a]All data corrected to a dry-weight basis; [b]data corrected for all non-carbohydrate material; [c]direct titration (potentiometric) with 0.02N sodium hydroxide; [d]assuming that all acidity arises from uronic acid groups; [e]by acid decarboxylation (Ref. 14); [f]by acetaldehyde released on periodate oxidation (Ref. 15); [g]assuming that all the methoxyl content arises from 4-*O*-methyl-D-glucuronic acid.

with warm methanol. The extract was evaporated to dryness, and dissolved in distilled water (10 ml). This solution was mixed with sodium borohydride (0.25 g in 25 ml of water) and kept overnight. Excess of borohydride was destroyed by the addition of Amberlite IR-120 (H^+) resin. The resin and the solvent were removed, and the borate was removed as methyl borate. The product (4-*O*-methyl-D-glucose) was purified on sheets of filter paper with solvent (*a*).

Borohydride reduction of aldobiouronic acids. — Sodium borohydride (25 mg in 5 ml of water) was added dropwise to the aldobiouronic acid (5–10 mg), and the mixture was kept overnight. Excess of borohydride was destroyed by the addition of Amberlite IR-120 (H^+). The resin and the solvent were removed, and the borate was volatilised as methyl borate. The product was boiled with 5% methanolic hydrogen chloride for 4 h, the solution was neutralised (silver carbonate) and filtered, and the residue was washed with warm methanol. The extract was evaporated to dryness, the residue was dissolved in distilled water (10 ml), and sodium borohydride (25 mg in 5 ml of water) was added. After storage overnight, excess of borohydride was destroyed, and borate was removed as before; the reduced product, (glucopyranosylgalactitol and/or 4-*O*-methylglucopyranosylgalactitol) was isolated as a syrup.

Methylation of aldobiouronic acids. — The micro Kuhn method developed by Perila and Bishop[9] was used. The aldobiouronic acid (10 mg) was shaken with methyl iodide (1 ml), *N*,*N*-dimethylformamide (1 ml), and silver oxide (1 g) at room temperature, in darkness, for 18 h. The mixture was filtered, the residue washed with chloroform, and the filtrate evaporated to dryness. The product was boiled with 5% methanolic hydrogen chloride for 8 h. The resulting methyl glycosides were analysed by g.l.c. A portion of the methanolysate was evaporated to dryness, and reduced with sodium borohydride (25 mg in 5 ml of water) for 12 h. The methyl glycosides were hydrolysed with N sulphuric acid for 4 h at 100°, and the methylated reducing sugars were examined by paper chromatography in solvents (*d*) and (*g*).

RESULTS

Comparative study of Fractions A, B, *and* C. — Hydrolysis (N sulphuric acid, 7 h) of each fraction, followed by paper-chromatographic examination, showed the presence of galactose, arabinose, and acidic disaccharides. Paper chromatograms, examined in ultraviolet light after spraying with aniline hydrogen oxalate, revealed that a trace of rhamnose was present in each fraction. After more drastic hydrolysis (2N sulphuric acid, 7 h), examination in solvent (*b*) showed that D-glucuronic acid, D-glucurono-6,3-lactone, and 4-*O*-methyl-D-glucuronic acid were present in each fraction.

Analytical data for the fractions are shown in Table I. Methylated sugars were not detected in the gum hydrolysates; if the methoxyl content of the gum is assumed to be located solely in residues of 4-*O*-methyl-D-glucuronic acid, calculation shows that this acid is present in each fraction to the extent of 2%.

Periodate oxidation of each fraction was followed by simultaneous determination

(2-ml aliquots) of formic acid and sodium metaperiodate. Oxidation of all fractions was complete after 48 h. For Fraction *C*, the oxidation mixtures did not become homogeneous until after 18 h.

The intrinsic viscosities shown in Table I were measured within a few days after isolation of the fractions. When the measurements were repeated after 2 months, it was found that the intrinsic viscosity of each fraction had increased, and a further increase was apparent after storage for 6 months in the free-acid form. This phenomenon is under investigation.

Study of Fraction A. — *A. drepanolobium* gum (160 g) was extracted with cold water, as described above, to yield Fraction *A* (130 g), which contained 10% moisture and 7% protein.

The neutral sugars of Fraction A. — The polysaccharide (0.75 g) was hydrolysed (N sulphuric acid, 7 h). The hydrolysate was fractionated on Whatman 3MM sheets for 36 h with solvent (*a*); crystalline D-galactose and L-arabinose were isolated.

D-Galactose (114 mg) had $[\alpha]_D^{19}$ +81° (equil., *c* 1.0) and, after recrystallisation from ethanol, m.p. and mixed m.p. 168°. The X-ray diffractogram was identical with that of an authentic specimen.

L-Arabinose (180 mg) had $[\alpha]_D^{19}$ +105° (equil., *c* 1.0) and, after recrystallisation from aqueous ethanol, m.p. and mixed m.p. 159°. The X-ray diffractogram was identical with that of an authentic specimen. After recrystallisation from water, the phenylosazone had m.p. and mixed m.p. 165°.

Fraction *A* (3 g) was hydrolysed (N sulphuric acid, 7 h). The hydrolysate was fractionated on Whatman 3MM sheets for 16 h with solvent (*a*). The position of rhamnose was located by spraying side-strips on which L-rhamnose had been run as a marker. Elution of the appropriate zone with water gave L-rhamnose (21 mg) having $[\alpha]_D^{19}$ +6° (*c* 0.21). After recrystallisation from aqueous ethanol, the L-rhamnose had m.p. and mixed m.p. 93–94°.

The acidic sugars of Fraction A. — The polysaccharide (10 g) was hydrolysed for 7 h with N sulphuric acid. Paper chromatography of the hydrolysate in solvent (*b*) indicated the presence of galactose, arabinose, and two acidic fractions (I) and (II) having R_{Gal} values of 0.26 and 0.59: these acidic fractions were isolated as syrups from filter sheets developed in solvent (*b*).

Fraction I (147 mg) was examined by paper chromatography in solvent (*b*) for 72 h; two acidic components were observed, having R_{Gal} 0.23 and 0.29, respectively. Similarly, paper chromatography in solvent (*c*) for 24 h gave two acidic components (aldobiouronic acids *D* and *E*) having R_{Gal} 0.56 and 0.61. Fraction I was hydrolysed (2N sulphuric acid, 7 h); paper chromatography of the hydrolysate in solvent (*b*) showed *ca.* equal amounts of galactose and glucurono-6,3-lactone, accompanied by traces of glucuronic acid and unhydrolysed aldobiouronic acids *D* and *E*. Crystalline D-galactose and D-glucurono-6,3-lactone were isolated from Whatman 3MM sheets developed in solvent (*b*). D-Galactose (50 mg) had $[\alpha]_D^{19}$ +80° (equil., *c* 1.0) and m.p. and mixed m.p. 167°, after recrystallisation from ethanol. D-Glucurono-6,3-lactone (45 mg) had $[\alpha]_D^{19}$ +19° (equil., *c* 0.9); after recrystallisation from

aqueous ethanol, it had m.p. and mixed m.p. 176°, with an X-ray diffractogram identical with that of authentic material.

Fraction II (78 mg) was examined by paper chromatography in solvent (*b*) for 72 h; two acidic components were observed, having R_{Gal} 0.56 and 0.66. Similarly, paper chromatography in solvent (*c*) for 24 h gave two acidic components (aldobiouronic acids *F* and *G*) having R_{Gal} 0.90 and 0.94. Fraction II was hydrolysed (2N sulphuric acid, 7 h); paper chromatography of the hydrolysate in solvent (*b*) showed *ca.* equal amounts of galactose and 4-*O*-methylglucuronic acid, accompanied by traces of unhydrolysed aldobiouronic acids *F* and *G*. Crystalline D-galactose and pure 4-*O*-methylglucuronic acid were isolated from 3MM sheets developed in solvent (*b*). D-Galactose (25 mg) had $[\alpha]_D^{19}$ +80° (equil., *c* 0.5) and m.p. and mixed m.p. 168°. 4-*O*-Methylglucuronic acid (31 mg) had $[\alpha]_D^{18}$ +36° (*c* 0.62); borohydride reduction of the methyl ester gave 4-*O*-methylglucose, which was purified on filter sheets with solvent (*a*) and isolated as a syrup. 4-*O*-Methylglucose had R_{Gal} 1.92 and R_{Rha} 0.90 in solvent (*a*), R_{Gal} 2.19 and R_{Rha} 0.84 in solvent (*b*), and R_{Gal} 1.74 and R_{Rha} 0.93 in solvent (*d*). After recrystallisation from aqueous acetone, the phenylosazone had m.p. 156–158° (lit[10], 158–159°).

The aldobiouronic acids of Fraction A. — The hydrolysate from Fraction *A* was fractionated [solvent (*b*), 96 h] on 3MM filter sheets; four aldobiouronic acids *D*,*E*,*F*, and *G* were isolated as syrups, and identified as follows.

Acid D (102 mg) had R_{Gal} 0.23 [solvent (*b*)] and R_{Gal} 0.56 [solvent (*c*)], and was chromatographically identical with 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose in these solvents. The syrup had $[\alpha]_D^{19}$ −3° (*c* 1.02), and this suggests the presence of a β-D-linkage. The syrup (5 mg) was hydrolysed (2N sulphuric acid), and paper chromatography [solvent (*b*)] revealed *ca.* equal amounts of D-galactose and D-glucurono-6,3-lactone. Acid *D* (10 mg) was reduced, using borohydride, as described above. The resulting hydrolysate was examined chromatographically [solvent (*f*)]; *ca.* equal amounts of glucose and galactitol were detected. The oxidation of known weights (3–5 mg) of acid *D* with excess of sodium metaperiodate did not give formaldehyde. Acid *D* (10 mg) was methylated by the Kuhn method, and the methyl glycosides produced on methanolysis of the product were examined by g.l.c.; the products were 2,3,4-tri-*O*-methyl-D-glucuronic acid [column (*i*), *T* 2.33, 2.96; column (*ii*), *T* 2.08, 2.70]; 2,3,4-tri-*O*-methyl-D-galactose [column (*i*), *T* 6.59; column (*ii*) *T* 5.25]; and a trace of 2,3,5-tri-*O*-methyl-D-galactose [column (*i*), *T* 4.09, 4,50; column (*ii*), *T* 3.44, 4.14]. A portion of the methanolysate was reduced with sodium borohydride; after removal of methyl glycosides by mild hydrolysis with acid, the products were examined chromatographically in solvent (*d*). This revealed 2,3,4-tri-*O*-methyl-D-galactose (red spot, R_G 0.72) and a red–black spot (R_G 0.84) corresponding to 2,3,4-tri-*O*-methyl-D-glucose and 2,3,5-tri-*O*-methyl-D-galactose. Double development (solvent (*g*)] separated the 2,3,4-tri-*O*-methyl-D-glucose (red spot) from 2,3,5-tri-*O*-methyl-D-galactose (black spot). These experiments led to the identification of acid *D* as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose.

Acid E (91 mg) had R_{Gal} 0.29 [solvent (*b*)] and R_{Gal} 0.61 [solvent (*c*)], and

was chromatographically identical with 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose. The syrup had $[\alpha]_D^{20}$ +106° (*c* 0.91) and this suggests an α-D linkage. The syrup (5 mg) was hydrolyzed (2N sulphuric acid), and paper chromatography [solvent (*b*)] showed *ca.* equal amounts of D-galactose and glucurono-6,3-lactone. Acid *E* (5–10 mg) was reduced, using borohydride, as described above. The resulting glucosylgalactitol was hydrolysed (N sulphuric acid), and the hydrolysate was examined chromatographically [solvent (*f*)]; *ca.* equal amounts of glucose and galactitol were detected. The oxidation of known weights (3–5 mg) of acid *B* with excess of sodium metaperiodate gave 1.01 moles of formaldehyde per mole of acid *E*. Acid *E* (10 mg) was methylated by the Kuhn method, and the methyl glycosides produced on methanolysis of the product were examined by g.l.c.; the products were 2,3,4-tri-*O*-methyl-D-glucuronic acid [column (*i*), *T* 2.40, (2.99); column (*ii*), *T* 2.04, 2.70] and 2,3,6-tri-*O*-methyl-D-galactose [column (*i*), *T* (2.99), 3.88, and 4.23; column (*ii*), *T* 2.42, 3.07, and 3.41]. A portion of the methanolysate was reduced with sodium borohydride; after removal of methyl glycosides by mild hydrolysis with acid, the products were examined chromatographically in solvent (*d*). This revealed 2,3,6-tri-*O*-methyl-D-galactose (R_G 0.76) and 2,3,4-tri-*O*-methyl-D-glucose (R_G 0.84). Double development [solvent (*g*)] revealed the same *O*-methyl sugars. These experiments led to the identification of acid *E* as 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose.

Acid F (42 mg) had R_{Gal} 0.56 [solvent (*b*)] and R_{Gal} 0.90 [solvent (*c*)], and was chromatographically identical with 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose. The syrup had $[\alpha]_D^{19}$ +3° (*c* 0.42) (for lit. values, see Ref. 5); this suggests a β-D linkage. The syrup (5 mg) was hydrolysed; paper chromatography [solvent (*b*)] showed *ca.* equal amounts of D-galactose and 4-*O*-methylglucuronic acid. Acid *F* (5–10 mg) was reduced with borohydride to give a 4-*O*-methylglucosylgalactitol, and the hydrolysate was examined chromatographically [solvents (*a*) and (*f*)]; *ca.* equal amounts of galactitol and 4-*O*-methylglucose were detected. The remainder of the hydrolysate was oxidised by the method of Lemieux and Bauer[8], and the products were examined in solvent (*a*). Spraying with aniline hydrogen phthalate revealed 2-*O*-methylerythrose, a characteristic product of 4-*O*-methylglucose, as a yellow-green spot (R_F 0.55). Known weights of acid *F* (3–5 mg) did not give formaldehyde when oxidised with excess of metaperiodate. On methylation, acid *F* gave the same methylated sugars as acid *D*. These experiments led to the identification of acid *F* as 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

Acid G (31 mg) had R_{Gal} 0.66 [solvent (*b*)] and R_{Gal} 0.94 [solvent (*c*)], and was chromatographically identical with 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose. The syrup (5 mg) was hydrolysed; paper chromatography [solvent (*b*)] showed *ca.* equal amounts of D-galactose and 4-*O*-methyl-D-glucuronic acid. Acid *G* (5–10 mg) was reduced with borohydride to a 4-*O*-methylglucosylgalactitol; the hydrolysate in solvents (*a*) and (*f*) showed *ca.* equal amounts of 4-*O*-methylglucose and galactitol. The hydrolysate was periodate oxidised[8], and the aniline hydrogen phthalate spray revealed the presence of a 2-*O*-methylerythrose. Periodate oxidation led to the production of 0.96 moles of formaldehyde per mole of acid *G*. On methylation,

acid *G* gave the same methylated sugars as acid *E*. These experiments led to the identification of acid *G* as 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

The aldobiouronic acids of fractions B *and* C. — After hydrolysis under the same conditions as for Fraction *A*, fractions *B* and *C* were examined chromatographically and were found to contain similar proportions of the four aldobiouronic acids identified in Fraction *A*.

DISCUSSION

Acacia drepanolobium gum has been shown to contain the same sugars, and the same four aldobiouronic acids, as other gums of the genus *Acacia* having high, positive rotations (*cf.*, *A. nilotica*[3], *A. arabica*[1], *A. nubica*[4], and *A. seyal*[4]).

A study of the analytical data presented in Table I shows that there are no striking differences in the composition of the gum fractions *A* (water-soluble), *B* (salt-soluble) and *C* (water- and salt-insoluble, but soluble to the extent of 1% in molar sodium hydroxide). Fractions *A* and *B* have an arabinose–galactose ratio of $52\pm1:38\pm1$, and this must be compared with the ratio of $50\pm1:40\pm1$ for Fraction *C*; although this may not be analytically significant, some small differences may be sufficient to account for the solubility differences exhibited by Fractions *A*, *B*, and *C*. It is possible that the differences may involve some variation in fine structure or degree of branching, as in the case with soluble and insoluble laminaran[11]. Although the sugar composition and periodate oxidation data for Fractions *A*, *B*, and *C* are in good agreement, structural differences between these fractions cannot be discounted until a full structural study has been made.

The most significant analytical differences between Fractions *A*, *B*, and *C* lie in the ash and protein contents of the purified fractions. The high ash content of Fraction *C* may reflect the difficulty encountered in purifying an aqueous dispersion, as opposed to a true solution. It is possible, however, that the gel formation shown by Fraction *C* is a consequence of crosslinking effects arising from the presence of polyvalent cations, although no foundation for this theory was found in experiments with *A. senegal* gum[12].

It is of interest that the nitrogen content of Fraction *C* is only *ca.* 50% of that present in Fractions *A* and *B*. Protein–carbohydrate interaction has been offered in the past as a possible explanation of gel formation; the jelly isolated from *Acacia pycnantha*[13] contained three times as much nitrogen as the water-soluble gum.

A further explanation of the physical differences between Fractions *A*, *B*, and *C* may involve the molecular size and/or shape of the polysaccharides involved: Table I shows that the intrinsic viscosity of Fractions *A*, *B*, and *C* increases in the same order as the increase in insolubility. Physico-chemical studies of Fractions *A*, *B*, and *C* will be carried out in due course.

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SUMMARY

The gum polysaccharide from *Acacia drepanolobium* is not completely water-soluble, and has been subjected to graded extractions giving Fractions *A* (cold-water soluble, 80%), *B* (salt-soluble, 8%), and *C* (a gel soluble in dilute alkali, 12%). Fraction *A* contains L-arabinose (52%), D-galactose (38%), L-rhamnose (1%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%). Fractions *A*, *B*, and *C* each contain four aldobiouronic acids, *viz.*, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose; 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose; 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose; and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

The analytical differences between fractions *A*, *B*, and *C* have been investigated, and the factors that may account for their physical differences are discussed.

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THE INTERACTION OF STARCH WITH BROMINE IN ACID SOLUTION

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INTRODUCTION

The objects of the present study are the two interactions of starch with molecular bromine occurring in acid, aqueous solutions, namely adsorption and oxidation.

The similarity of the adsorption of gaseous bromine and iodine on dry starch has been shown by infrared spectroscopy[1] and X-ray study[2]; it is therefore reasonable to expect the existence of a starch–bromine complex in aqueous solution. Potentiometry has been applied to the thermodynamic and stochiometric study of the starch–iodine complex[3]. In the present work, an attempt was made to apply the same technique to the starch–bromine complex, and to study the oxidation of starch by bromine as a function of the pH. Drastic oxidation of amylopectin by bromine has been studied by Doane and Whistler[4]. The present paper is concerned with the kinetics of mild oxidation and is thus a continuation of previous investigations of the interaction of starch and hypohalites in the alkaline pH range[5,6]. As before, the study was carried out on granular starch.

EXPERIMENTAL

Materials. — The samples of wheat starch, waxy-maize starch, whole potato-starch, and the amylose fraction of potato starch were those characterized previously[5–7].

Precipitation phenomena. — Amylose was dissolved in boiling water, potassium bromide was then added, and the pH of the solution was adjusted to 3 by phosphate buffer. Bromine was then added to the mixture, causing the formation of a brown precipitate. After storage for 15 min at room temperature, the solution was filtered through glass paper. The brown precipitate turned blue upon addition of potassium iodide, whereas the filtrate turned dark brown when iodide was added. This shows that amylose is quantitatively precipitated by bromine. The precipitate forms only in the presence of bromide ions (which alone seem to have no effect on the solubility of amylose). Hence it is the tribromide ion which precipitates amylose. Also, the addition

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of iodine to amylose solutions causes the formation of the blue color; a precipitate is formed only when iodide ions are present. The same effect was observed in aqueous solutions of the synthetic polymers poly(vinylpyrrolidone) and poly(ethylene glycol)[8]. Chlorine does not precipitate amylose, presumably because the trichloride ion is not formed.

No precipitate is formed upon the addition of bromine to solutions of amylopectin (waxy-maize starch). When whole potato-starch was dispersed in boiling water containing potassium bromide, at pH 3, and bromine was added, a precipitate formed on cooling. This was filtered off, washed with alcohol (to remove the absorbed bromine), and dried *in vacuo*. The iodine affinity of the material was found to be close to that of pure amylose. Upon addition of alcohol to the filtrate, a precipitate, having extremely low iodine-affinity, was formed. Thus, in principle, the fractionation of starch by bromine is possible. It might be useful to solubilize starch by oxidation with bromine at elevated pH and to isolate the amylose by acidifying the reaction mixture, using the residual oxidant as precipitant.

The adsorption of bromine by granular starch. — A suspension of granular wheat-starch [34 AGU/l (anhydro D-glucose units per litre)] in an aqueous solution of bromine (2.2mM) and potassium bromide (0.1M), adjusted to pH 3 by phosphate buffer, was agitated for 2 h at 30°. A sample of the agitated suspension was then withdrawn, and its bromine content was determined volumetrically and found to be equal to the initial bromine content. The agitation was then stopped, and the starch was allowed to settle. The clear supernatant was found to contain less oxidant than the suspension. When agitation was renewed, the bromine concentration of the whole suspension was again found to be equal to that observed initially. The difference in concentration between the whole suspension and the supernatant is most probably due to the adsorption of bromine on the starch and corresponds to the adsorption of 1 molecule of bromine per 95 AGU. When the same suspension was equilibrated under the same conditions with 13mM bromine, the ratio of adsorbed bromine to AGU was found to be 1:40. In a suspension of waxy-maize starch (pure amylopectin) (34 AGU/l) equilibrated with 2.2mM bromine, the above ratio was found to be 1:300, indicating that the adsorption by amylopectin is less than by amylose (which constitutes 25% of the wheat starch).

Potentiometry. — Preliminary experiments revealed that, at pH 3 and 30°, amylose is not attacked by bromine even after 20 days. Therefore, the adsorption of bromine on amylose under these conditions could be studied by potentiometry. The potentiometric equipment consisted of a glass beaker equipped with a metal jacket, through which water thermostatted at 30° was circulated, and a perforated Teflon lid to carry the electrodes (this arrangement ensures both tightness of the system and complete darkness of the solution). The potential of a platinum electrode immersed in the bromine solution was read on a Cambridge Bench Type pH Meter against a saturated calomel electrode. In view of the volatility of bromine, no actual titrations were performed, but each point on both the calibration and titration curve was measured from a freshly prepared solution. The calibrating solutions contained 0.1M

potassium bromide and 0.1M phosphate buffer (pH 3) in addition to the desired amount of bromine. A linear dependence of the potential on log [Br_2] was found between 1 and 30mM bromine, the slope being 31 mvolts/log [Br_2]. Since the readings on the pH meter are ± 0.5 mvolts, the concentration of the bromine can be determined within $\pm 3\%$.

The amylose was dissolved in N potassium hydroxide in the cold by agitating for *ca.* 1 h, and the pH of the solution was then adjusted to 3 by addition of hydrobromic and phosphoric acids. The final solution contained amylose (15.5 AGU/l; the highest amylose concentration that could be prepared by this method), 0.1M potassium bromide, and 0.1M phosphate buffer, in addition to the volumetrically measured concentration of bromine. The potential of the electrodes immersed is these solutions was found to be constant for more than 1 h. Within a certain range, the concentration of bromine in the amylose solutions determined potentiometrically was found to be less than that determined volumetrically. This difference is ascribed to the adsorption of bromine by amylose. The results of the measurements, expressed as the ratio of bromine molecules adsorbed per AGU (c_b/m) *versus* the concentration of free bromine n equilibrium (c_f), are summarised in Fig. 1.

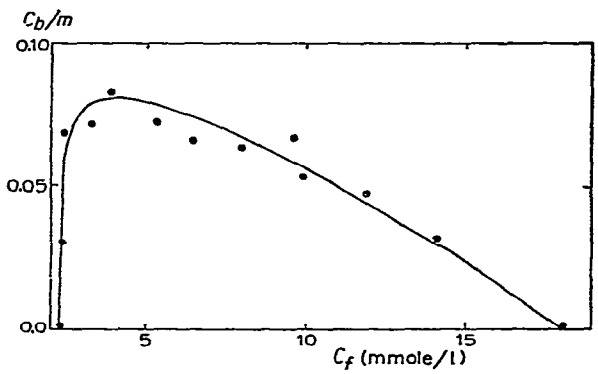

Fig. 1. The dependence of the ratio c_b/m (bound Br_2: AGU of amylose) on the concentration of the free bromine (c_f) at 30° and pH 3; concentration of amylose, 16 AGU/l.

In can be seen from Fig. 1 that measurable adsorption occurs when the concentration of free bromine is 2–15mM, and that the maximum in adsorption is about 0.08 molecule of bromine per AGU. Since the concentrations of free and bound bromine could be ascertained to within ± 3 and «3%, respectively, the uncertainty in the values shown in Fig. 1 is *ca.* $\pm 10\%$. At amylose concentrations of 7.8 and 3.9 AGU/l, bromine was adsorbed on amylose in the same ratio and concentration range of free bromine, but the uncertainty in these measurements is even larger due to the smaller difference in concentrations between free and bound bromine.

Volume contraction accompanying adsorption. — The complexing of bromine by starch was accompanied by a volume contraction. Thus, the density of a 0.1M potassium

bromide solution containing 5mM bromine at 30.0° is 1.0165, and that of 0.1M potassium bromide containing 0.25% of amylose is 1.0135. The density of a 3:2 mixture of the two is 1.0175.

Oxidation kinetics. — The oxidation of granular wheat and waxy-maize starch by bromine at pH 5.0, 3.0, and 1.7 was investigated at 30°, the initial bromine concentration being 2.8mM and that of the suspended starch 34 AGU/l. All solutions were 0.1M with respect to potassium bromide.

The kinetics of oxidation at pH 5.0 were measured with the apparatus used previously for the study of oxidation by chlorine at high pH[5]. The reacting solution was kept in darkness. Loss of oxidant through evaporation was evaluated by blank experiments. Parallel volumetric determination of the bromine concentration by the thiosulphate and the arsenite–iodine methods showed that no bromate is formed under the prevailing conditions, in agreement with previous results[4].

Because of the slow rate of oxidation in acid medium, the kinetics at pH 3.0 and 1.7 were studied with a slightly modified technique in order to cut down losses of bromine by evaporation. The reaction solutions (which were identical to that used at pH 5 except for the addition of 0.1M phosphate buffer adjusted to the pH under investigation), contained in dark-colored Erlenmeyer bottles provided with glass stoppers, were thermostatted externally and magnetically stirred. Blank experiments were also run for pH 3.0 and 1.7. The loss at pH 3.0 was found to be almost double that at pH 1.7, suggesting that, at these pH values, decomposition of hypobromous acid rather than evaporation of bromine, is the main cause of loss of oxidant. No bromate was found at either pH.

In the kinetic measurements, aliquots of the stirred starch suspension were withdrawn, and analyzed without filtering off the starch. Thus, the unconsumed oxidant includes that adsorbed on the starch. Just as in the study[5] of oxidation by chlorine at high pH values, the data could be fitted into the equation

$$1/t \ln(c_0/c) = k,$$

where c_0 and c are the respective bromine concentrations initially and after t minutes, and k is a constant. All of the measurements were duplicated. The values of k (min^{-1}), computed by the method of least squares, are given in Table I, together with the 95% confidence interval as an indication of accuracy.

The oxidation of amylose at pH 3 was also studied by the same technique. Amylose was found to be unoxidized by bromine even after contact for 20 days. This suggests that, in acid solution, bromine is a specific oxidant of amylopectin. Indeed, the iodine affinity of granular wheat-starch is slightly increased by subjection to mild oxidation by an acid solution of bromine[6]. This increase, however, may be due to the destruction of "native" aggregates and thus be the effect of exposure of the starch to an acid medium rather than the action of bromine.

There is the possibility that, during the contact with an acid solution of bromine, some hydrolysis occurs in the starch. In order to evaluate the possible influence of hydrolysis, both wheat and waxy-maize starch were suspended for 10 days

in potassium bromide solutions buffered at pH 3.0 or 1.7. Bromine was then added, and its consumption after 30 min was measured. This "initial" oxidant consumption of the pretreated starch was found to be identical with that of the untreated material, indicating that hydrolysis has no noticeable influence on the oxidation kinetics.

DISCUSSION

(*a*) *The amylose–bromine complex.* — The results of the present work, especially the potentiometric study, leave little doubt that a complex between molecular bromine and amylose is formed in aqueous solution. A better insight into the results is gained by assuming that the properties of this complex are analogous to that formed by iodine with amylose, and the precipitating action of bromine is also analogous to that of iodine; indeed, iodine has been proposed[9] as a fractionating agent for amylose.

It is true that the addition of bromine to amylose does not cause such a pronounced change in color as does iodine, which is ascribed to the passage of iodine from the polar, aqueous medium into the "paraffinic" interior of the amylose helix[10]. Whereas the colors of iodine dissolved in polar and non-polar media are markedly different, the color of dissolved bromine is not appreciably affected by the polarity of the medium[11]. It is therefore understandable that the complexing of bromine by amylose is not accompanied by a change in color. Moreover, the disappearance of the blue color of the iodine–starch complex[12] upon addition of bromine is readily understood as a displacement of iodine by bromine inside the helix. It was observed in the present work that this discoloration is reversible; upon addition of an amount of thiosulfate sufficient to decrease the excess of bromine, the blue color is restored.

It is well known that starch must be dispersed molecularly in order to attain its full adsorbing capacity towards iodine. Prior to potentiometric estimation of its iodine affinity, the starch sample is dispersed in potassium hydroxide. It is therefore not surprising that granular starch adsorbs less bromine than does the dispersed material.

In considering Fig. 1, it should be remembered that bromine has a dual function as ligand and precipitant. According to the mass law, binding should increase linearly with bromine concentration, but even small proportions of bromine added to water make it a poor solvent for starch (just as in the case of iodine or octanol), which causes the starch to pass into the crystalline state where it does not adsorb bromine. The balance of the two effects is such that, under the experimental conditions, adsorption is maximal at a concentration of 3mM. From the analogy with iodine, a larger binding of bromine per AGU (and possibly a linear increase of adsorption with concentration of the free bromine) is to be expected when smaller amylose concentrations are used[13]. This, however, requires extremely sensitive potentiometric measurements that were not attempted in this work. It is nevertheless interesting to point out that the binding of bromine to amylose is of the same order of magnitude (1 molecule of bromine per 12 AGU) as that of iodine.

(*b*) *Kinetic measurements.* — It is evident from Table I that the rate of oxidation of both species of starch increases rapidly with the pH of the solution. This is in full agreement with the results obtained by Doane and Whistler[4], who studied the oxi-

dation of dissolved amylopectin by excess of bromine. A complex series of reactions occurs in the complete oxidation of starch, whereas, in "mild" oxidation, the initial phase of that sequence is dominant. It is assumed[14] that the first step of the action of bromine on starch is the oxidation of a hydroxyl group on C-2, C-3, or C-6; it is reasonable to assume that this reaction is the main one occurring under conditions of "mild" oxidation, which might therefore resemble the oxidation of ethanol by bromine.

TABLE I

THE pH DEPENDENCE OF THE RATE OF OXIDATION OF GRANULAR STARCH BY BROMINE AT 30°. STARCH CONCENTRATION, 34 AGU/l; BROMINE CONCENTRATION, 2.8mM

pH	$k(min^{-1})$ *Wheat starch*	*Waxy-maize starch*
1.7	$(4.0 \pm 1.4) \times 10^{-6}$	$(1.5 \pm 0.2) \times 10^{-5}$
3.0	$(2.5 \pm 0.4) \times 10^{-5}$	$(2.0 \pm 0.5) \times 10^{-5}$
5.0	$(2.0 \pm 0.3) \times 10^{-3}$	$(5.6 \pm 1.8) \times 10^{-4}$

The influence of the pH on the rate of oxidation of ethanol by bromine was studied by Perlmutter-Hayman and Weissmann[15], who found that the oxidation is a first-order reaction with respect to bromine. The logarithm of the rate constant depends linearly on the pH above 5 and becomes almost independent of the acidity below pH 3. The first step[15] in the hydroxyl oxidation is the abstraction of a proton by molecular bromine. Two reactions occur simultaneously, namely, the comparatively slow proton abstraction from an ethanol molecule and the much more rapid abstraction from an ethanolate anion. The rate of the first reaction is independent of the acidity of the medium, whereas that of the second is proportional to the pH, since the anion concentration is low. Hence, at high acidity, the ethanol molecule is the main reacting species, whereas, at moderate acidity, the reaction with the anion is dominant.

The dependence of the logarithm of the rate of starch oxidation on the pH is shown in Fig. 2, and the results of the present work are summarized in Table I (the known[6] value at pH 7.0 is also given). In the case of pure amylopectin (waxy-maize

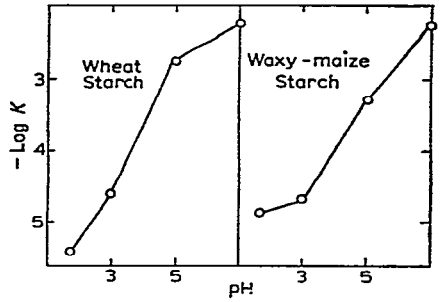

Fig. 2. The pH dependence of the logarithm of the rate of oxidation of granular starch by bromine at 30° (c_0 = 2.8mM; starch, 34 AGU). See text for definition of k.

starch), the curve resembles that of ethanol, in agreement with the postulate that the main reaction of "mild" oxidation is the attack on hydroxyl groups of the starch. This reaction also determines the pH dependence in the case of wheat starch; however, the dependence is complicated due to the complexing of bromine by the amylose fraction of this starch species. By analogy with the iodine reaction, it may be assumed that the complexed bromine is in the paraffinic interior of the amylose helix and thus unavailable for oxidation. It is also likely that the addition of bromine leads to dehydration and shrinkage of the swollen wheat-starch granule (by analogy with the precipitation of dissolved amylose); this would be reflected in the activity of starch towards oxidation. Yet another factor influencing the activity of starch is the destruction of "native aggregates" and retrogradation in acid solution[7]. The present state of knowledge of the various factors controlling the oxidation of wheat starch in acid medium does not enable us to make a more quantitative evaluation of the results.

SUMMARY

Amylose is precipitated from aqueous solution by the tribromide ion and can thus be separated from amylopectin. The adsorption of bromine by dissolved amylose was demonstrated potentiometrically. The rate of oxidation of starch by bromine at pH 1.7, 3.0, and 5.0 is a first-order reaction with respect to bromine, and increases rapidly with the pH.

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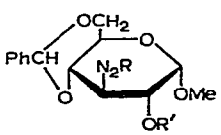
Notes

Nitrogen-containing carbohydrate derivatives
Part XVIII*. Methyl 4,6-*O*-benzylidene-3-deoxy-3-methylazo-α-D-glucoside

The formation of an arylazo sugar on treatment of a periodate-oxidised sugar derivative with an arylhydrazine is well known[1-3], and the reaction has recently been extended into the steroid field[4]. It has now been shown that methylhydrazine reacts with periodate-oxidised methyl 4,6-*O*-benzylidene-α-D-glucoside, in an analogous manner to arylhydrazines[1,2], to give the title compound (**1**). Thin-layer chromatography showed that several other products were formed, none of which has been investigated. The configuration of compound **1** was established by reduction to the known[1] methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-glucoside, and also by comparison of the p.m.r. spectrum of the 2-*O*-acetyl derivative (**2**) with that of methyl 4,6-*O*-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside 2-acetate (**3**). Assignments for the latter compound (in benzene-d_6 solution at 100 MHz) have been rigorously established by double resonance and deuterium substitution (to be published). The spectrum of compounds **2** and **3** are sufficiently similar to establish a common configuration about C-1, C-2, and C-3. Protons attached to these atoms exhibit nearly first-order multiplets, being displaced to low field from the other skeletal protons. The quartets for H-2 (τ 4.32 in **2**; 4.18 in **3**) have a repeated spacing ($J_{1,2}$ ~3.7 Hz, typical of *ax-eq* interaction) in common with that of the doublets for H-1 (τ 4.97 in **2**; 5.02 in **3**), and another ($J_{2,3}$ ~10.2 Hz, typical of *ax-ax* interaction) in common with that of the triplets for H-3 (1:2:1, τ 5.55 in **2**; 5.27 in **3**). The last signals indicate *ax-ax* coupling ($J_{3,4}$ ~9.5 Hz) also to H-4 in both compounds. The benzylidene-proton peak is unsplit (τ 4.70 in **2**; 4.73 in **3**). Aromatic protons in compound **3** give three distinct bands corresponding to 2:2:6 protons at τ 2.27, 2.62, and 3.0, that at lowest field being due to the phenylazo group, since it is absent from the spectrum of compound **2** (2:3 protons at τ 2.52, 2.9).



1 R=Me, R'=H
2 R=Me, R'=Ac
3 R=Ph, R'=Ac

EXPERIMENTAL

N.m.r. spectra were recorded for solutions in benzene-d_6 on a Varian HA-100

*Part XVII: B. E. DAVISON, R. D. GUTHRIE, AND D. MURPHY, *Carbohyd. Res.*, 5 (1967) 449.

spectrometer, and o.r.d. and c.d. curves were obtained on Polarmatic 62 and Jasco CD/ORD/UV 5 instruments, respectively.

Methyl 4,6-O-benzylidene-3-deoxy-3-methylazo-α-D-glucoside. — Periodate-oxidised methyl 4,6-*O*-benzylidene-α-D-glucoside (10 g) was dissolved in *N*,*N*-dimethylformamide (100 ml), and the resulting solution was added to water (3 l) at 70–80°. To this was added a solution of fused sodium acetate (10 g) and methylhydrazine sulphate (5 g) in water (100 ml). The resulting solution was cooled quickly to room temperature by the addition of ice, kept for 5–6 h, and then extracted with chloroform (6×250 ml). The combined extracts were evaporated to give a syrup, from which tetrachloroethylene was twice distilled. The solid residue (9.7 g) was recrystallised twice from ethanol–light petroleum to give the crude product (2.2 g), m.p. 154–157°. T.l.c. of the mother liquors showed a number of spots.

The methylazoglucoside was very difficult to obtain pure by recrystallisation. The pure product, homogeneous by t.l.c. (silica gel, ether), was obtained only by preparative t.l.c. The sample thus obtained had m.p. 155°, $[\alpha]_D$ +51° (*c* 1.0, chloroform); λ_{max}^{MeOH} 251 (ε 251), 257 (ε 303), 260.5 (ε 257), 257 (ε 184), and 352 nm (ε 50). The band at 352 nm was associated with a negative Cotton effect in the c.d. and o.r.d. curves in a number of solvents {c.d., (*c* 0.237, methanol), $[\theta]_{300}$ 0; $[\theta]_{355}$ −1480; $[\theta]_{408}$ 0} (Found: C, 58.6; H, 6.6; N, 8.85. $C_{15}H_{20}N_2O_5$ calc.: C, 58.4; H, 6.5; N, 9.1%).

Acetylation of the recrystallised methylazoglucoside with acetic anhydride in pyridine overnight gave methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-methylazo-α-D-glucoside, m.p. 125–127° (from ethanol–light petroleum), $[\alpha]_D$ +51° (*c* 1.5, chloroform) (Found: C, 58.2; H, 6.2; N, 7.9. $C_{17}H_{22}N_2O_6$ calc.: C, 58.2; H, 6.3; N, 8.0%). On standing, the product decomposed to form an additional component (t.l.c.) that was not further investigated. The recrystallised methylazoglucoside was reduced in the presence of Raney nickel in alcohol at 70° and 70 atmos. pressure for 6 h. The catalyst was removed, and the filtrate was evaporated to yield a solid that was acetylated with acetic anhydride–pyridine for 5 min to give methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-glucoside (25% overall, after one recrystallization), m.p. 283–286° (decomp.); lit.[1], m.p. 286–288° (decomp.). The product was identified by comparison of its infrared spectrum with that of an authentic sample.

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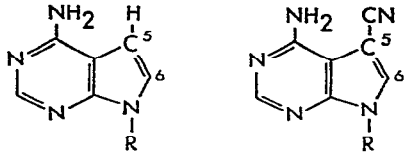
The synthesis of D-ribose-*1-t*

Suhadolnik and Uematsu[1] and Smulson and Suhadolnik[2] recently reported that D-ribose-*1-*^{14}C and D-ribose-$^{14}C_5$ are incorporated into the pyrrolo ring of the 7-deazaadenine D-ribonucleosides, tubercidin (I) and toyocamycin (II). The data obtained indicate that C-1 and C-2 of D-ribose become C-6 and C-5 of tubercidin, and

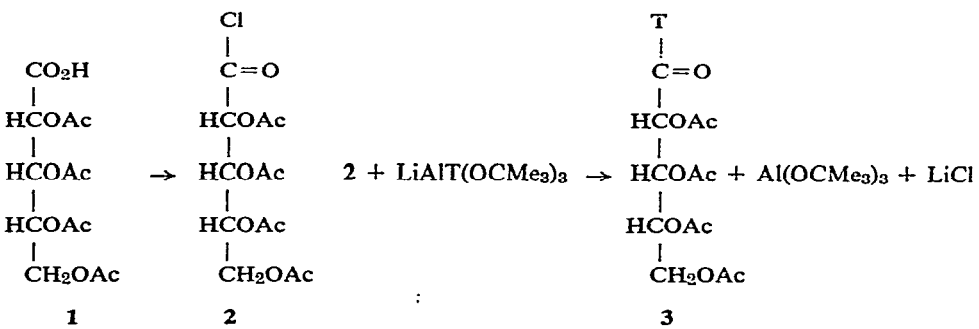


I II

R=D-Ribose

that C-1, C-2, and C-3 of D-ribose become C-6, C-5, and the cyano carbon atom of toyocamycin, respectively. The evidence obtained on the biosynthesis of toyocamycin from D-ribose-*1-*^{14}C indicates that the radioactivity resides at C-6 of the 7-deazaadenine. If D-ribose is incorporated as a three-carbon unit into toyocamycin and if C-1 of D-ribose becomes C-6 of the base portion of toyocamycin, then the tritium from D-ribose-*1-t* should be situated at C-6. However, if the incorporation of D-ribose, as a three-carbon unit, proceeds in such a way that C-1 of D-ribose becomes the cyano group of the 7-deazaadenine base of toyocamycin, there should be no tritium in the aglycon moiety. The experimental proof of such a mechanism requires the synthesis of D-ribose labeled with tritium or deuterium at C-1.

This Note describes the synthesis of D-ribose-*1-t* from tetra-*O*-acetyl-D-ribonic acid (**1**). Compound **1** was converted into tetra-*O*-acetyl-D-ribonyl chloride (**2**) with



thionyl chloride. Compound **2** was reduced with lithium tri-*tert*-butoxyaluminohydride-*t* according to the method of Brown and Subba Rao[3], affording tetra-*O*-acetyl-*aldehydo*-D-ribose-*1-t* (**3**).

The acetyl groups were removed with barium methoxide in methanol to yield D-ribose-*1-t* (**4**), which was further purified by paper chromatography. An aliquot of **4**, eluted

from the paper chromatogram, was converted into crystalline tetra-*O*-acetyl-D-ribopyranose-*1-t*, and recrystallized to a constant specific activity. In addition, the loss of the tritium by oxidation of the D-ribose-*1-t* to D-ribonolactone (with bromine water) showed that all of the tritium was situated at C-1 of the tritiated D-ribose.

EXPERIMENTAL

Lithium aluminum hydride-*t* was purchased from the New England Nuclear Corporation. Anhydrous ethyl ether was distilled from lithium aluminum hydride. Diglyme (diethylene glycol dimethyl ether) and *tert*-butyl alcohol were stored over Linde molecular sieve (No. 4A) and distilled. Lithium tri-*tert*-butoxyaluminohydride-*t* was prepared according to the method of Brown and McFarlin[4]. Four mmoles of *tert*-butyl alcohol (in 1.25 ml of ether) was added during 15 min to a solution of lithium aluminum hydride-*t* (48 mg; 1.27 mmoles; 3.6 mCi; specific activity, 2.85 mCi/mmole) in 3.5 ml of ether. The resulting white precipitate (lithium tri-*tert*-butoxyaluminohydride-*t*) was removed by filtration, washed with ether, dried *in vacuo* for 2 h, and used immediately; yield 170 mg (54%). Tetra-*O*-acetyl-D-ribonic acid was obtained from Dr. C. C. Christman, Merck and Co., Inc., Elkton, Virginia. Radioactivity was measured in a Packard Model 314 EX Tri-Carb Liquid Scintillation Spectrometer. The samples were counted in the scintillation solvent of Bray[5]. Melting points were determined with a Thomas–Hoover silicone-bath apparatus and are uncorrected.

Tetra-O-*acetyl*-D-*ribonyl chloride* (**2**). — A solution of **1** (3 g; 9.2 mmoles) and freshly distilled thionyl chloride (3 g; 25 mmoles) was heated for 1 h at 50°, and then kept for 30 min at room temperature. The excess of thionyl chloride was removed by evaporation *in vacuo*. The resulting syrup was crystallized from ether–hexane; yield 3 g (91%), m.p. 73–75° (lit.[6], 74–76°).

Tetra-O-*acetyl*-aldehydo-D-*ribose*-1-t (**3**). — A solution of **2** (225 mg; 0.64 mmole) in 5 ml of Diglyme was kept at −35 to −50° under nitrogen, and a solution of lithium tri-*tert*-butoxyaluminohydride-*t* (167 mg; 0.66 mmole) in 5 ml of Diglyme was added dropwise during 30 min, with continuous stirring. The mixture was allowed to warm to room temperature during 30 min, and poured over crushed ice (50 g). The suspension was filtered, the insoluble material was washed with two 25-ml portions of ethanol, and the filtrate and washings were combined and concentrated *in vacuo* to a yellow syrup, which was used without further purification. The amount of radioactivity was 225 μCi; yield 50%.

D-*Ribose*-1-t (**4**). — Tetra-*O*-acetyl-*aldehydo*-D-ribose-*1-t* (**3**) (64 μCi) was dissolved in 100 ml of absolute methanol, barium oxide (140 mg; 0.92 mmole) was added, and the solution was kept for 6 h at 0°. The barium was precipitated as barium carbonate by addition of Dry Ice, the suspension was filtered, and the filtrate was concentrated *in vacuo* to about 0.5 ml. The solution was applied to the origin of two paper chromatograms (Whatman 3 MM), and developed with 4:1:5 butyl alcohol–acetic acid–water. The D-ribose was detected on the paper chromatogram with 2-biphenylamine

spray reagent[7]. Hydrolysis of **3** was complete, as determined by the hydroxamate spray reagent[8]. All of the radioactivity on the paper chromatogram was coincidental with the D-ribose area. The D-ribose was eluted from the chromatograms with water, and was determined quantitatively by the method of Fernell and King[9]; yield 33.7 μCi (53%); 7.2 mg; the specific activity was 702 μCi/mmole. The overall yield from **2** to **4** was 26%.

Proof of the incorporation of tritium into the D-ribose was made, and the location of tritium at C-1 was determined as follows: carrier D-ribose was added to an aliquot of the D-ribose-*1-t*, and the mixture was converted into crystalline tetra-*O*-acetyl-D-ribopyranose-*1-t* by the method of Levene and Tipson[10]; m.p. 108–109° (lit.[10], 110°). The specific activity did not change on recrystallization. The D-ribonolactone obtained by the oxidation of D-ribose-*1-t* with bromine water[11] was not radioactive.

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Oxidative–reductive depolymerization: a note on the comparison of degradation rates of different polymers by viscosity measurements

Several authors[1–6] have reported that polymers of different types undergo depolymerization in the presence of autoxidants and molecular oxygen. The name oxidative–reductive depolymerization (ORD) has been proposed for the reaction[5], which is thought to be caused by the formation of peroxides by autoxidation, followed by the formation of radicals by reaction between the peroxides and the autoxidant[6]. The reaction is of considerable practical importance, since it takes place under physiological conditions where polysaccharides and other natural polymers usually are regarded as being completely stable.

Recently, Herp *et al.*[7] investigated the specificity of this reaction for a number of neutral and acidic polysaccharides and synthetic polymers, using ascorbic acid as the autoxidant. The degradation of the polymers was followed by viscosity measurements, and characterized by the decrease of the specific viscosity after a given time of degradation. No viscosity decrease was found for solutions of starch, glycogen, and dextrin. A relatively small decrease occurred for dextran solutions, whereas solutions of alginate, propylene glycol alginate, pectin, methylcellulose, polyacrylic and polymethacrylic acid, and a copolymer of methyl vinyl ether and maleic anhydride all rapidly decreased in viscosity in the presence of ascorbic acid. It was concluded[7] that the presence of carboxyl groups or ester groups in the molecule generally appears to increase the ease of degradation of polysaccharides, with the exception of methylcellulose.

Let P_{no} and P_{nt} be the number-average degree-of-polymerization at the start of the reaction and at the time t, respectively. The fraction of bonds broken after the time t is then given by

$$1/P_{nt} - 1/P_{no}$$

and a first-order, random depolymerization follows the relation

$$1/P_{nt} - 1/P_{no} = kt. \qquad (1)$$

This relation holds[8] for P_n-values above approximately 50. The intrinsic viscosity and the number-average degree-of-polymerization of a polymer are related by the modified Staudinger equation

$$[\eta] = K(\lambda P_n)^a \qquad (2)$$

where K and a are constants and λ is the ratio between the viscosity-average and the number-average degree-of-polymerization. When the exponent, a, in the Staudinger equation is unity, and λ is constant throughout the degradation reaction, equations (1) and (2) give for a first-order reaction

$$1/[\eta]_t - 1/[\eta]_o = k't.$$

When the above conditions are fulfilled, the proportionality between $(\Delta\, 1/[\eta])/t$ and

the rate constant makes viscosity measurements a convenient method for following a depolymerization reaction.

In Fig. 1, the viscosity decrease of two alginate samples ($[\eta]=12.4$ and 4.2 *dl/g*, respectively) in phosphate–citrate buffer (pH 6) is given as $\Delta\ 1/[\eta]$ as a function of time. A straight line was obtained, fitting both samples. The result shows that the rate-of-depolymerization of alginate is independent of the degree-of-polymerization, in agreement with previous observations[6]. The decrease of specific viscosity, however, depends on the initial, intrinsic viscosity, and, after two hours, was 57 and 26% for the samples having the higher and lower intrinsic viscosity, respectively. A similar difference between two alginate samples was observed by Herp *et al.*[7]. Their conclusion, however, that the alginate sample having the higher intrinsic viscosity was more extensively degraded therefore only applies to the decrease of viscosity, and not to the actual rate of the depolymerization reaction (*i.e.*, the number of bonds broken in the polymer).

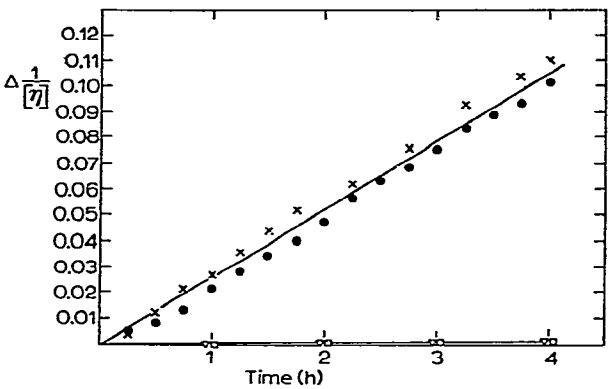
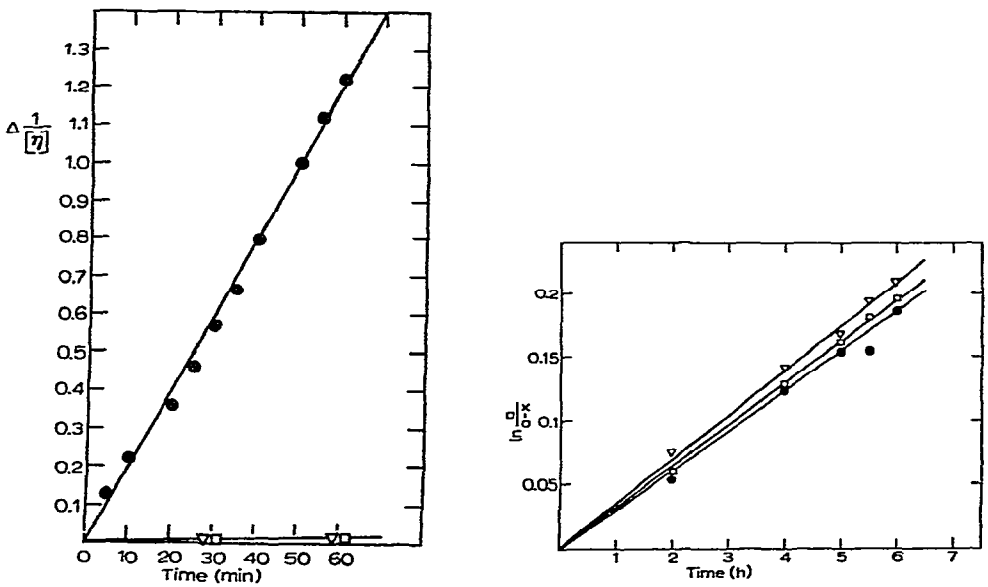


Fig. 1. Degradation of polysaccharides (0.6% solutions) in 0.001M ascorbic acid at pH 6. ● = Alginate, $[\eta]$ = 12.4 (100 ml/g); × = alginate, $[\eta]$ = 4.2 (100 ml/g); ▽ = soluble starch (Riedel-de Haën A.-G., Seelze by Hannover); □ = dextran (Pharmacia, Uppsala).

The viscometric method is a very convenient tool for following the degradation of such polymers as alginate or cellulose derivatives (*e.g.*, methylcellulose), where the exponent in the Staudinger equation is close to unity. Most linear polymers are less extended in solution, and consequently have a lower exponent (usually 0.5–0.8) in the Staudinger equation than have alginate and cellulose derivatives[9]. For such branched polymers as dextran and amylopectin, the exponent in the Staudinger equation is even lower[9,10] (for dextran of high molecular weight, a value of 0.1 has been reported[11]). For polymers of this type, the viscosity of the solutions depends only to a small extent on the degree-of-polymerization of the polymer, and therefore a depolymerization reaction cannot conveniently be followed by viscosity measurements. Consequently, and in agreement with Herp *et al.*[7], we found only a very small change in the viscosity of solutions of dextran and soluble starch under the same conditions used for alginate (Fig. 1). However, in order to reach any conclusion about the rate

of depolymerization, another method must be used. Determination of reducing end-groups is a convenient method for following the depolymerization of polysaccharides, but the presence of ascorbic acid and its degradation products interferes in this determination. We have previously examined the degradation of polysaccharides in solutions containing ferric chloride and hydrogen peroxide[12]. The reactions taking place in this system lead to the formation of hydroxyl radicals, causing degradation of the polysaccharides, and are thus of the same general type as the ORD reaction in the presence of such autoxidants as ascorbic acid. In this system, the depolymerization can be followed by determining the increase in reducing power of the solutions[13]. The degradation of alginate, soluble starch, and dextran was investigated in phosphate–citrate buffer (pH 6.0) containing 0.001M ferric chloride and 0.1M hydrogen peroxide. The decrease in viscosity, expressed as $\Delta 1/[\eta]$, is shown in Fig. 2, and the rate-of-depolymerization, determined by the increase in reducing power, is shown in Fig. 3. The reducing power was determined according to Nelson[14], in samples where the reaction had been stopped by adding catalase[12]. In calculating the degree-of-conversion, x, it was assumed that the degradation products had the same factor as the



Figs. 2 and 3. Degradation of polysaccharides (0.6%) solutions in 0.001M $FeCl_3$ and 0.1M H_2O_2 at pH 6 by viscosity determinations (Fig. 2) and determination of reducing power (Fig. 3). ● = Alginate; ▽ = soluble starch; □ = dextran.

monomer of the polysaccharide, and the result was plotted according to a first-order reaction with a limit, a, equal to the total amount of carbohydrate. The results show that, under the conditions used in our experiments, the rates of depolymerization (as measured by the increase in reducing power) are approximately the same for neutral polysaccharides and polysaccharides containing carboxyl groups, and

illustrate that conclusions about the relative rates of depolymerization of different polysaccharides cannot be drawn from viscosity data alone, without a careful consideration of the relation between the intrinsic viscosity and the degree-of-polymerization.

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Carbohyd. Res., 5 (1967) 482–485

Synthese eines *C*-methyl-verzweigten Inosamins[1]

Verzweigtkettige Inosite, wie Mytilit und Laminit, sind mehrfach aus natürlichem Material isoliert worden[2]. Verzweigtkettige Inosamine dagegen sind, trotz des relativ häufigen Vorkommens nicht verzweigter Vertreter in einer Reihe von Antibiotika[2,3], bislang nicht in der Natur gefunden worden. Eine synthetische Methode zur Darstellung von Verbindungen dieses Typs ergab sich in der Übertragung der Dialdehyd-Nitromethan-Cyclisierung[4] auf Nitroäthan, dessen Fähigkeit, mit Dialdehyden zu *C*-methylverzweigten Nitrodiolen zu kondensieren, bereits bei einer Reihe aliphatischer[5], aromatischer[5] und von Zuckern abgeleiteter Dialdehyde[6,7] gezeigt wurde.

xylo-Pentodialdose (**2**), aus 1,2-*O*-Isopropylidene-α-D-glucofuranose (**1**) durch Perjodatoxidation und nachfolgende saure Hydrolyse leicht zugänglich[8,9], ergibt bei Kondensation mit Nitroäthan–Natriummethylat in Methanol und anschließendem Ansäuern ein Gemisch von Cyclisierungsprodukten. Durch Dioxan-Extraktion läßt

illustrate that conclusions about the relative rates of depolymerization of different polysaccharides cannot be drawn from viscosity data alone, without a careful consideration of the relation between the intrinsic viscosity and the degree-of-polymerization.

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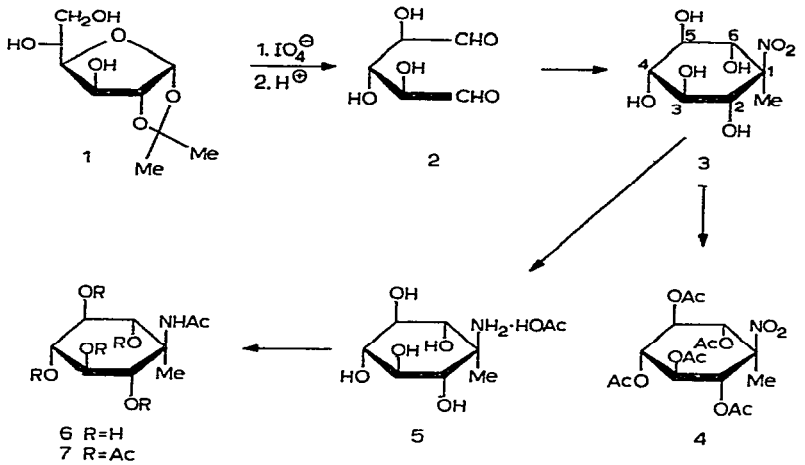
Synthese eines *C*-methyl-verzweigten Inosamins[1]

Verzweigtkettige Inosite, wie Mytilit und Laminit, sind mehrfach aus natürlichem Material isoliert worden[2]. Verzweigtkettige Inosamine dagegen sind, trotz des relativ häufigen Vorkommens nicht verzweigter Vertreter in einer Reihe von Antibiotika[2,3], bislang nicht in der Natur gefunden worden. Eine synthetische Methode zur Darstellung von Verbindungen dieses Typs ergab sich in der Übertragung der Dialdehyd-Nitromethan-Cyclisierung[4] auf Nitroäthan, dessen Fähigkeit, mit Dialdehyden zu *C*-methylverzweigten Nitrodiolen zu kondensieren, bereits bei einer Reihe aliphatischer[5], aromatischer[5] und von Zuckern abgeleiteter Dialdehyde[6,7] gezeigt wurde.

xylo-Pentodialdose (**2**), aus 1,2-*O*-Isopropylidene-α-D-glucofuranose (**1**) durch Perjodatoxidation und nachfolgende saure Hydrolyse leicht zugänglich[8,9], ergibt bei Kondensation mit Nitroäthan–Natriummethylat in Methanol und anschließendem Ansäuern ein Gemisch von Cyclisierungsprodukten. Durch Dioxan-Extraktion läßt

sich hieraus ein chromatographisch einheitliches Isomeres vom Schmp. 235° in 14-proz. Ausbeute isolieren, dem die Konfiguration eines 1-*C*-Methyl-1-desoxy-1-nitro-*scyllo*-inosit (1-Desoxy-1-nitromytilit, **3**) zukommt. Saure Acetylierung lieferte ein Pentaacetat (**4**), katalytische Hydrierung über Platin in methanolischer Essigsäure ergab das entsprechende Amin, 1-*C*-Methyl-*scyllo*-inosamin (**5**), das als Hydroacetat,



N-Acetyl Derivat (**6**) und Hexaacetat (**7**) charakterisiert wurde. Das Amin **5** stellt das 1-Amino-Derivat des natürlichen Mytilits dar, und kann somit als Mytilamin bezeichnet werden.

Durch Nitroäthan-Cyclisierung des Dialdehyds **2** können theoretisch sechs Diastereomere (2 meso und 4 racemische) entstehen. Daß der isolierte 1-*C*-Methyl-1-nitroinosit sowie dessen Folgeprodukte *scyllo*-Konfiguration besitzen, ergibt sich in eindeutiger Weise aus NMR-spektroskopischen Daten des Pentaacetats **4** sowie des Hexaacetats **7**. So wurde im Fall von **4** für die Methylprotonen der Acetoxygruppen ein 15H-Signal bei τ 8.02 erhalten, was auf Grund der für $CDCl_3$ festgelegten Signalbereiche axialer bzw. äquatorialer Acetoxygruppen[10] die äquatoriale Orientierung der Acetoxygruppen erweist.

Diese Zuordnung wird unterstützt durch die Kopplungsverhältnisse der Ringprotonen: bei τ 4.19 wird ein 2H-Dublett mit $J=8$ Hz erhalten, das für die axialen Protonen H-2 und H-6, sofern sie axiale Nachbarn besitzen, zu erwarten ist; entsprechend geben die Protonen H-3, H-4 und H-5 ein um τ 4.76 zentriertes 3H-Triplett mit $J=8$ Hz, das nur bei jeweils axialer Orientierung verständlich ist.

Das Amin-hexaacetat **7** zeigt im $CDCl_3$-Spektrum vier Signale im τ 8-Gebiet, und zwar bei τ 7.99 (6H), 8.04 (9H), 8.20 (3H) und 8.57 (3H). Hierbei ist letzteres durch die C-CH_3-Gruppe bedingt, während die ersteren den jeweils äquatorialen Acetoxy-Gruppen and C-2 and C-6 (τ 7.99) bzw. an C-3, C-4 und C-5 (τ 8.04) zuzuordnen sind, in Übereinstimmung mit den für äquatoriale Acetoxygruppen festgelegten Signalbereichen[10]. Das 3H-Signal bei τ 8.20 ist auf die Methylprotonen

der Acetamido-Gruppe zurückzuführen, die, wie der Vergleich mit analogen Verbindungen[10] ergibt, äquatoriale Anordnung besitzt, wodurch für die Verbindungen 3–7 die Konfiguration an der Verzweigungsstelle festgelegt ist.

EXPERIMENTELLER TEIL

xylo-*Pentodialdose* (2). — Die aus 30 g (0.135 mMol) 1,2-*O*-Isopropyliden-α-D-glucofuranose (1) hergestellte sirupöse 1,2-*O*-Isopropyliden-D-*xylo*-pentodialdose[9] wurde in 150 ml 0.1N Schwefelsäure gelöst und 1.5 Stdn. auf 80° erhitzt. Nach Abkühlung wurde mit N Natronlauge neutralisiert und i.Vak. (Badtemp. 40°) zur Trockne eingedampft. Der verbleibende Rückstand wurde in 150 ml Äthanol aufgenommen, vom ausgeschiedenen Natriumsulfat abfiltriert und erneut zur Trockne eingedampft. Es blieb ein farbloser Sirup zurück.

1-Desoxy-1-C-methyl-*1-nitro*-scyllo-*inosit* (3). — Die oben erhaltene, sirupöse *xylo*-pentodialdose (2) wurde in 250 ml Äthanol–Wasser (1:1) gelöst, 10.5 g (0.14 mMol) Nitroäthan zugegeben und unter Rühren im Verlauf von 15 Min. 50 ml einer N Natriummethylat-Lösung zugetropft. Es wurde 3 Stdn. bei Raumtemperatur stehengelassen, mit 2N HCl neutralisiert und i. Vak. (40° Badtemp.) zur Trockne eingedampft. Der verbleibende Rückstand wurde in einem Soxhlet-Extraktor 20 Stdn. mit 200 ml *p*-Dioxan extrahiert. Nach Abdampfen des *p*-Dioxans i. Vak. blieb ein kristallines Produkt zurück, das aus Äthanol–Methanol (1:1) umkristallisiert wurde; Ausbeute 5.1 g (14% d. Th. bezogen auf 1) farbloser Kristalle vom Schmp. 235°. Die Substanz war chromatographisch einheitlich, $R_F = 0.22$* und 0.77**.

Anal. Ber. für $C_7H_{14}NO_7 \cdot C_2H_5OH$: C, 40.2; H, 7.10; N, 5.20. Gef.: C, 40.52; H, 7.13; N, 5.32.

Penta-O-acetyl-1-desoxy-1-nitromytilit (4). — Nitromytilit (3, 200 mg) wurden in 7 ml Acetanhydrid suspendiert und unter ständigem Rühren und Eiskühlung 3 ml Acetanhydrid, die 2 Tropfen konz. Schwefelsäure enthielten, vorsichtig zugegeben. Die Lösung wurde kurze Zeit bei 0° gerührt und dann 4 Stdn. bei Raumtemp. stehengelassen. Es fiel ein farbloses, kristallines Produkt aus, das abfiltriert und aus heißem Äthanol umkristallisiert wurde; Ausbeute 300 mg (93%) vom Schmp. 212°.

Anal. Ber. für $C_{17}H_{23}NO_{12}$ (423.4): C, 47.11; H, 5.32; N, 3.23. Gef.: C, 47.24; H, 5.37; N, 3.23.

NMR-Daten. a) in $CDCl_3$: τ 4.19 (H-2 und H-6-Dublett, $J = 8$ Hz); τ 4.76 (3H-Triplett der H-3, H-4 und H-5-Protonen, $J = 8$ Hz); τ 8.02 (5 äquat. OAc an C-2 bis C-6); τ 8.71 (CH_3-Singulett an C-1); b) in Me_2SO-d_6: τ 8.05 (2 äquat. OAc an C-2 und C-6); τ 8.08 (3 äquat. OAc an C-3, C-4 und C-5); τ 8.21 (CH_3-Singulett an C-1).

Mytilamin-Hydroacetat-Monohydrat (5). — Nitromytilit 3 (1.5 g) wurden in 150 ml 10-proz. Methanol–Eisessig gelöst und in einer vorhydrierten Suspension von

*Chromatographie absteigend; Papier: Schleicher u. Schuell 2043b; Laufmittel: 1-Butanol–Essigsäure–Wasser (5:1:4, organische Phase); Entwicklung mit Silbernitrat–Natriumhydroxyd[11].

**Dünnschicht-Chromatographie aufsteigend auf Kieselgel $HF_{254+366}$ (Fa. E. Merck AG, Darmstadt). Laufmittel wie unter*. Entwicklung durch UV.

1 g PtO_2 in 30 ml Methanol bis zur Aufnahme der theoretischen Menge H_2 (405 ml in 22 Stdn.) hydriert. Die vom Katalysator befreite Lösung wurde i. Vak. (Badtemp. 40°) bis auf ein Volumen von 20 ml eingeengt. Mehrmaliger Zusatz von Äthanol und nachfolgendem Abdampfen i. Vak. bis zur Trockne ergab 1.4 g (93%) eines farblosen kristallinen Produkts vom Schmp. 245°. Im Papierchromatogramm erhielt man einen Fleck* mit $R_F=0.14$.

Anal. Ber. für $C_7H_{15}NO_{15}\cdot HOAc$ (253.2): C, 42.68; H, 7.56; N, 5.53; für Monohydrat (271.3): C, 39.85; H, 7.80; N, 5.16. Gef.: C, 40.12; H, 7.82; N, 5.19.

Hexaacetyl-mytilamin (7). — Amin **5** (500 mg) wurden mit 15 ml Acetanhydrid und 200 mg frisch geschmolzenem Natriumacetat 1 Std. unter Rückfluss erhitzt. Nach der Abkühlung rührte man die Lösung in Eiswasser ein. Hierbei fiel ein farbloses kristallines Produkt aus, das abfiltriert und aus heißem Methanol umkristallisiert wurde: 0.3 g farbloser Kristalle vom Schmp. 268°. Das Filtrat wurde mit Natriumhydrogencarbonat neutralisiert und mit 40 ml Chloroform extrahiert. Nach Abziehen des Chloroforms i. Vak. wurde der verbleibende Rückstand aus Methanol umkristallisiert: 0.4 g vom Schmp. 268°; Ausbeute: 0.7 g (85% d. Th.).

Anal. Ber. für $C_{19}H_{27}NO_{11}$ (445.5): C, 51.23; H, 6.11; N, 3.15. Gef.: C, 51.35; H, 6.36; N, 3.10.

NMR-Daten. a) in $CDCl_3$: τ 7.99 (6H-Singulett, OAc an C-2 und C-6); τ 8.04 (9H-Signal der C-3-, C-4- und C-5-OAc); τ 8.20 (3H-Singulett der NHAc-Gruppe); τ 8.57 (CH_3-Singulett an C-1); b) in Me_2SO-d_6: τ 8.03 (6H-Singulett, äquat. OAc an C-2 und C-6); τ 8.07 (9H-Singulett, äquat. OAc an C-3, C-4 und C-5); τ 8.37 (3H-Singulett, NHAc); τ 8.75 (CH_3-Singulett an C-1).

N-*Acetyl-mytilamin* (6). — Mytilamin-Hexaacetat (1.0 g) wurde in 80 ml an Ammoniak gesättigtem Methanol suspendiert und bei Raumtemperatur über Nacht (16 Stdn.) magnetisch gerührt. Das ausgefallene kristalline Produkt wurde abfiltriert und aus Äthanol–Wasser (4:1) umkristallisiert; Ausbeute 0.5 g (95% d. Th.); Schmp. 265°.

Anal. Ber. für $C_9H_{17}NO_6$ (235.2): C, 45.95; H, 7.28; N, 5.96. Gef.: C, 46.12; H, 7.36; N, 5.91.

NMR-Daten. a) in $CDCl_3$: τ 8.05 (3H-Singulett, C-1-NHAc); τ 8.94 (3H, C-CH_3); b) in Me_2SO-d_6: τ 8.22 und 9.03 (3H-Signale der 1-NHAc und C-CH_3).

DANK

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Laufmittel und Papier analog; Entwicklung durch Ninhydrin.

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Carbohyd. Res., 5 (1967) 485–489

Studies on uronic acid materials
Part XXIII. Possible degradations with the sodium hydride–methyl iodide–methyl sulphoxide methylation system

Anderson and Cree[2] have described the potential use of the sodium hydride–methyl iodide–methyl sulphoxide system for methylating acidic mono- and di-saccharides, and also for some acidic polysaccharides. The method appeared to offer advantages when compared with the classical Haworth and Purdie methods, but it was suggested[2] that the method should be evaluated with a wider range of polysaccharides before general conclusions could be reached.

Following a communication[3] that the sodium hydride system caused undesirable degradation of dextrans with the formation of abnormally high yields of 2,3,4,6-tetra-*O*-methyl-D-glucose, we have now compared the methylation products, obtained with sodium hydride, of a potato starch and of three different *Acacia* gum polysaccharides, with their respective methylated products obtained by treatments with the Haworth and Purdie reagents.

EXPERIMENTAL

Methylations with the sodium hydride–methyl iodide–methyl sulphoxide system, and the analyses by paper and gas chromatography, were carried out by the procedures and apparatus described previously[2].

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EXPERIMENTAL

Methylations with the sodium hydride–methyl iodide–methyl sulphoxide system, and the analyses by paper and gas chromatography, were carried out by the procedures and apparatus described previously[2].

RESULTS

Experiments with *Acacia nubica* gum showed that two additions of sodium hydride and methyl iodide were required to give complete methylation (OMe, 40.3%); the methoxyl content after 1 addition was only 16.3%. Lithium hydride was less effective (OMe after 1 treatment, 11.5%) than sodium hydride. Calcium hydride was ineffective when used to replace the sodium hydride, and stable emulsions were formed (*cf.* the effect of barium salts[2]).

Potato starch. — A laboratory sample of potato starch (var. Kerr's Pink) was methylated exhaustively with (*a*) the sodium hydride–methyl iodide–methyl sulphoxide system, and (*b*) several additions of the Haworth and the Purdie reagents. After methanolysis, the methyl glycosides of products (*a*) and (*b*) were examined by g.l.c.; the chromatograms were identical, except that product (*a*) showed a small peak caused by a trace of residual methyl sulphoxide.

Acacia drepanolobium *gum.* — This gum polysaccharide contains[1] D-galactose (38%), L-arabinose (52%), L-rhamnose (1%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%). The gum was methylated by methods (*a*) and (*b*), as above. After methanolysis of each product, the chromatograms for products (*a*) and (*b*) were identical, except for the presence in (*a*) of a trace of methyl sulphoxide.

Acacia campylacantha *gum.* — This gum polysaccharide contains[4] D-galactose (56%), L-arabinose (28%), L-rhamnose (7%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%). The chromatograms of the methanolysed, fully methylated gum obtained by methods (*a*) and (*b*) were similar in all respects other than that the proportion of 2,3,4-tri-*O*-methyl-L-rhamnose in product (*a*) was slightly less than in product (*b*).

Acacia senegal *gum.* — This gum polysaccharide contains[5] D-galactose (39%), L-arabinose (28%), L-rhamnose (14%), D-glucuronic acid (17.5%), and 4-*O*-methyl-D-glucuronic acid (1.5%). The gum was methylated by methods (*a*) and (*b*), as above. When the chromatograms of the methanolysate were compared, the proportion of 2,3,4 tri-*O*-methyl-L-rhamnose in product (*a*) was only about 33% of that in product (*b*). The proportion of 2,3-di-*O*-methyl-D-glucuronic acid also appeared to be less in product (*a*) than in (*b*).

DISCUSSION

Base-catalysed reactions proceed at greatly increased rates in methyl sulphoxide, in which the strongly basic methylsulphinyl carbanion is readily formed[6]; the methyl sulphoxide is a reactant, as well as being the solvent. There is no doubt that the sodium hydride–methyl iodide–methyl sulphoxide system is much more strongly alkaline in reaction[7] than a well-conducted Haworth methylation.

Hamilton and Thompson[8] have shown that alkaline hydrolysis of gum arabic (*Acacia senegal*) leads to the loss of residues of L-rhamnose and D-glucuronic acid. At least some of the L-rhamnopyranose residues in gum arabic were shown[9] to be

(1→4)-linked to D-glucuronic acid residues; subsequent structural studies[5,10] have confirmed earlier indications[11] that all of the L-rhamnopyranose residues are present as non-reducing end-groups.

It appears that those *Acacia* gum exudates (*e.g.*, *A. nubica* and *A. drepanolobium*) having low L-rhamnose and low methoxyl contents (and therefore having the majority of their D-glucuronic acid residues as non-reducing end-groups) are reasonably stable under the conditions described[2] for methylation by the sodium hydride–methyl sulphoxide–methyl iodide system. In contrast, those *Acacia* gum polysaccharides having significant proportions of L-rhamnopyranose residues as end groups attached to position 4 of D-glucuronic acid residues (*e.g.*, *A. senegal*) appear to be much less stable; loss of 2,3,4-tri-*O*-methyl-L-rhamnose, and also probably 2,3-di-*O*-methyl-D-glucuronic acid, occurs. This may be explained in terms of a β-elimination at C-4 of the glucuronic acid; such an elimination did not occur[2] with *A. nubica* gum.

Although our results show that the sodium hydride–methyl sulphoxide methylation system does offer certain advantages for some polysaccharides, we must repeat here the suggestion[2] that its performance should be assessed with care on a wider range of polysaccharides. All too frequently, structural studies are based on the product from only one methylation system; methylation by more than one method should be attempted, so that the results can be compared.

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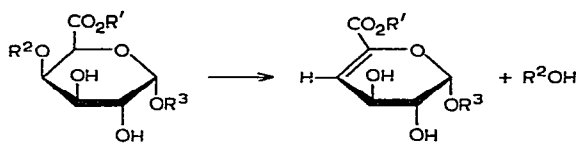
Preliminary communication

Selective splitting of polysaccharide chains at the linkage involving C-4 of uronic acid residues

We are prompted by a recent publication[1] to report on our attempts to devise methods for selective cleavage of polysaccharide chains at uronic acid residues. Because these residues very often carry glycosyl substituents at C-4, a selective method for splitting the chain by elimination (*e.g.*, **1**→**2** for D-galacturonic acid) might have many applications. Breakdown by this path occurs when pectins are heated in neutral aqueous solution[2], and this reaction has been elegantly exploited for structure analysis by Northcote and his group[3]. However, the extent of reaction is slight, partly owing to competing de-esterification, and, in our experience, it is variable. In this form, the reaction therefore has little scope. Enzyme-catalysed eliminations are also known, for example, for hyaluronic acid[4], chondroitin sulphates[5], alginic acid[6], and pectic substances[7]. Although the specificity of enzymic reactions is often helpful in structure investigations, at other times it may be inconvenient, and an efficient chemical method would be useful as a supplementary tool.



It was pointed out to us by Dr. G. O. Aspinall that if alkoxide were used as the base, non-competitive transesterification would occur instead of de-esterification, and reaction might then be more complete. Subsequently, model compounds of low molecular weight were shown to undergo this elimination when treated with sodium methoxide in methanol[8]. We first examined the reaction using 2-hydroxyethyl alginate, which seems[9] to consist entirely of (1→4)-linked uronic acid derivatives. When this ester was dried at 60°/14 mm and then treated heterogeneously with dry methanolic sodium methoxide, there was no visible reaction. However, if the ester was shaken for 1 h at room temperature with methanolic 2,2-dimethoxypropane (5% w/v) before addition of methoxide to a final concentration of 0.02M, part of it dissolved rapidly to give a solution having an absorption maximum at 235 nm in the ultraviolet spectrum; an insoluble portion *R* remained. Paper chromatography showed a series of spots that gave positive reactions with the thiobarbituric acid spray[6,10]. These products were separated on a cellulose column and found to be a series of

oligosaccharides (total yield of di- and higher saccharides, 30%) which showed u.v. and i.r. bands characteristic of $\alpha\beta$-unsaturated esters. The absorption at 235 nm disappeared on borohydride reduction of the saccharide mixture, or on ozonolysis (after which oxalic acid dihydrate was isolated), and treatment with 0.1N sulphuric acid at 80° converted it into a new band identical in position and pH dependence to that shown by 2-furoic acid[11]. Taken together, these observations indicate that fragmentation was by elimination, as with simpler substrates[9]. Partial de-esterification had also occurred, as shown by paper electrophoresis.

A diaxial arrangement of the eliminated groups is possible for a gulopyranosyluronate but not for a mannopyranosyluronate unit. Comparison of the behaviour of alginates that contained the two types of unit in different proportions suggested, however, that the reaction was not stereospecific. This was confirmed when the methyl ester–methyl ether[12] of leiocarpan A, in which all uronic acid residues have the D-*gluco* configuration, was found to undergo rapid reaction. The lack of stereospecificity is not surprising, because the glycosyloxy group is a poor leaving group, and ionisation of H-5 is probably easy, as shown by the ready racemisation of uronic acids[13]. On these grounds, a mechanism approximating to the *E1cB* type is to be expected. If the reaction was continued at room temperature for longer than 1 h, the peak at 235 nm progressively disappeared with the appearance of new absorptions at longer wavelengths, showing that 4,5-unsaturated units, analogous to structure 2, were not the end products. The release of these units as monomers would, of course, be followed by ketonisation, but no trace of α-keto acids could be found by paper chromatography or colour reactions[6].

The insoluble portion *R* (*ca.* 50% yield) appeared to be stable to further reaction and to consist of oligosaccharides terminated by unsaturated units. By comparison of the extinction coefficient with published values for similar compounds[7], the average chain-length of *R* was estimated as about 10–15 units. We conclude that (*i*) extensive breakdown occurs on heterogeneous treatment of 2-hydroxyethyl alginate with methanolic sodium methoxide, provided that a chemical method is used for removal of bound water from the polysaccharide; (*ii*) the rate of reaction is not usefully sensitive to the stereochemistry of the glycopyranosyluronate unit; (*iii*) the unsaturated product which is formed at first does not accumulate as either the keto or enol tautomer, but undergoes further reaction; (*iv*) fragmentation of the chain is incomplete, perhaps partly because of accessibility effects similar to those which limit heterogeneous hydrolysis[14] and heterogeneous reduction[15].

Because of the complications attending reaction, breakdown by base-catalysed elimination has not yet led to any new structural information about alginic acid. For two other polysaccharides, however, we have found application of the method to be more fruitful. The slime polysaccharide of *Escherichia coli* K 12 is a branched polymer[16], containing glucuronic acid, fucose, glucose, and galactose residues, in which the chains are terminated by a hitherto unknown and acid-labile sugar (*B*), which is still under investigation but might be a dicarbonyl sugar. It is sufficiently acid-stable as the methyl ether to permit isolation after hydrolysis of the methylated

polysaccharide, but attempts to isolate the parent sugar by acid hydrolysis before methylation have so far failed. Treatment of the 2-hydroxyethyl ester with dimethoxypropane–methanol–methoxide, followed by chromatographic purification, gave sugar *B* in fair yield. About 50% of the *B* units present could be isolated as monomer if ethyl acetate was included in the reaction mixture, presumably because the water, which is eliminated in side reactions and would normally cause de-esterification and stop glycoside cleavage, is removed by formation of acetate ion from ethyl acetate. Methylation analysis of the polymeric residue confirmed that there was little change in polysaccharide structure, apart from removal of *B* and destruction of glucuronic acid residues. This reaction provides the only method presently available for isolation of unsubstituted *B* for investigation of its structure. Most of the uronic acid may be recovered as a 3-*O*-(D-glucopyranosyluronic acid)-D-galactose (*cf.* ref. 17), and therefore the sequence at most (possibly all) of the chain ends is established as *B*-D-glucuronic acid-D-galactose.

A second example of the usefulness of this method of polysaccharide breakdown is in the structure investigation of mustard pectin, which has several types of side chain attached to a backbone of galacturonic acid and rhamnose units[18]. The side chains, which contain L-arabinofuranose residues, are acid-labile, and it is therefore difficult to establish their size and detailed structure by straightforward methods. However, by base-catalysed elimination, after esterification with ethylene oxide, it is possible to split the backbone to give side chains as separate molecules in combination with low proportions of backbone sugar units. Their characterisation by chemical[19] and physical[20] methods is proceeding. The conditions described for breakdown of alginates and the *E. coli* polysaccharide were ineffective, as were the aqueous conditions that have been used for some other pectins[2,3], but fragmentation occurred smoothly after dispersion in methyl sulphoxide.

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Announcement

CARBOHYDRATE DISCUSSION GROUP (A Chemical Society Subject Group). The fourth meeting of the Group will take place at Newcastle on April 8th–10th, 1968. The Programme will consist of general papers and a short symposium on stereochemical aspects of carbohydrate chemistry. Part of this symposium will be devoted to two Review Lectures: Professor S. J. Angyal (University of New South Wales) will speak on 'Composition and Conformation of Sugars in Solution', and Dr. L. N. Johnson (University of Oxford) will speak on 'The Interaction of Oligosaccharides with Lysozyme'. Further details of the meeting may be obtained from Dr. R. D. Guthrie, The Chemical Laboratory, The University of Sussex, Falmer, Brighton, Sussex BN1 9QJ.

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